final report

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Impacts on consumer acceptance of beef from interactions between pH, meat colour and packaging

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Executive summary

There is a recognised lack of scientific understanding of consumer colour preferences for retail beef products; a lack of scientific detail on ageing and colour stability; consistent observations of a mismatch between meat colour and pH, a desire for an objective instrumental colour measure linked to consumer responses and growing scientific evidence of a detrimental effect on eating quality of MAP packaging.

This trial was designed to improve understanding of the mechanisms involved in the above, their interaction and management approaches to overcome or prevent problems at a plant and retail level.

The design called for striploin, rump and tenderloins aged 5, 12 or 40 days in vacuum packaging to be packed into three retail packaging formats: Overwrap (OWP), Modified Atmosphere (MAP) and Vacuum Skin Pack (VSP). All retail packages were viewed and rated for colour appeal by beef consumers then fabricated into MSA consumer samples and sensory tested using MSA protocols. Further samples were put aside for flavour chemistry evaluation.

Scores from a 'Consumer Meat Colour Score' (CMC) developed from the 20,140 consumer observations from the trial showed results directly contrary to some accepted beliefs. The colour of the striploin surface at grading was found to relate poorly to the other cuts and to change with ageing. Furthermore consumer preference discounted light coloured 1C beef but did not discount darker meat colour 4 samples. Ultimate pH was found to be more aligned with ultimate retail colour acceptability than the grading assessment. Meat colour did not differ across dentition categories from 2 to 6.

The sensory results were also instructive confirming a 12 MQ4 point eating quality penalty for 80:20 MAP relative to OWP and VSP, which were similar. The penalty for MAP was consistent across the three cuts and all prior primal ageing periods. Meat colour was again confirmed as having no eating quality relationship. Flavour volatiles differed for each packaging type.

After evaluation of the data, the MSA Pathways Committee recommended meat colour be removed as an MSA grading criteria; a pH limit of 5.7 be retained and that a 12 MQ4 point deduction be applied to beef sold in MAP. These recommendations were presented to the MSA Taskforce and AUS-MEAT Language and Standards Committee. Subsequently meat colour has been removed as an MSA grading requirement.

This research has delivered significant industry value through improved understanding of meat colour and packaging relationships from both visual and sensory perspectives. The removal of meat colour as an MSA grading requirement will significantly increase the number of carcasses grading MSA and redress a current anomaly, common in grass fed groups, where carcasses with acceptable pH are excluded by slow developing meat colour at the time of grading.

The substantial negative effect of 80:20 MAP is of concern to retailers and raises substantial issues. Further research has been proposed to test how quickly eating quality deteriorates after packing and the potential to overcome the effect by utilising a different gas mix.

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1 Background

1.1 Project purpose

This project sought to address a number of industry and scientific concerns related to meat colour development and assessment in combination with consumer visual and sensory assessment of alternative packaging methods. Further related examination of potential objective meat colour assessment tools and flavour volatile relationships to packaging systems were also included within the project.

1.2 Background

This project followed a visit, hosted by Teys Australia, of Professor Melvin Hunt, Kansas State University, who visited a number of Teys Australia plants to review systems and provide expert advice in relation to meat colour management and the related science. During his visit, Professor Hunt presented to an industry seminar an extensive overview of colour chemistry and interactions with commercial practices. During this event he also reviewed the methodology of a proposed colour and packaging trial with a number of Australian meat scientists and MSA Pathways Committee members attending the seminar. The project design evolved to incorporate assessment of consumer evaluation of meat colour, potential objective measurement tools and interactions between pH, meat colour and packaging.

Retail meat colour is an important factor which directly impacts consumer purchasing (Mancini & Hunt 2005, McKenna *et al*., 2005) despite having no direct influence on eating quality. Colour also interacts with packaging and shelf life reflecting complex muscle and colour chemistry. Striploin/cube roll meat colour score ('AMC') is assessed by MSA graders against AUS-MEAT meat colour standards during grading, generally within 24 hours of slaughter. Only carcasses of AMC 3 and below are acceptable for supermarket customers with those of 4 and above discounted. This creates problems for plant disposal and relationships with producers. Prior to this project MSA grade requirements also required a AMC below 4 and an ultimate ph below 5.71. In practice a single colour assessment of the *M.longissimus dorsi et lumborum* muscle cross section at the quartering point resulted in acceptance or rejection of the entire carcase with all cuts included.

Some carcasses exhibit a classic dark cutting condition where pH is over 5.7, and up to 6.5, with consequent AMC of 4, 5 and 6. This is a result of low glycogen levels at slaughter (<57µm) with the typical cause related to stress through inadequate feed, weather, mixing etc. While other research work was planned to test potential dark cutting prediction tools it was not part of this trial.

Evaluation of meat colour issues by Teys over 2015 and 2016 identified a further condition where some carcasses with a pH <5.71 had MCSs of 4 or 5 at grading. These failed Meat Standards Australia (MSA) requirements on MCS but passed on pH. The problem was more pronounced in Northern grass fed cattle, varied widely between consignments and reduced with additional time between kill and grading. This indicated that pH decline and meat colour while aligned in a general sense were not directly related with colour continuing to change after ultimate pH had been attained. The biology underlying these observations was not understood but the observations were consistent with a number of published studies that reported poor relationships between colour at grading and during retail display (Mancini & Hunt 2005, Murray 1989, Holdstock *et al*., 2014, Mahmood *et al*., 2017). (The poor relationship between

grading and display colour is not just restricted to low pH high AMC cuts). The initial Teys studies had included instrumental colour measurement, providing more detailed colour data, in addition to MSA grader scoring.

There was also further interest in colour interactions across muscles, with animal dentition, packaging type and in relation to the period cuts were held as vacuum packed primals prior to final retail packing. In addition to the colour issues recent international research studies (Aaslyng *et al,* 2010, Lagerstedt et al 2011, and 2011a, Seyfert et al 2005, and Torngren 2003) reported significant eating quality reduction with high oxygen MAP (modified atmosphere packaging) relative to VSP (vacuum skin packing) or overwrap. This was also observed in a MLA funded lamb trial (Frank *et.al*. 2016). As the underlying biology of both colour and flavour development are closely related it was proposed that both aspects be evaluated in a detailed trial protocol.

This research project was designed to measure a number of these factors and to improve understanding of the mechanisms involved; their interaction; and management approaches to overcome or prevent problems at plant and retail level.

2 Project objectives

The project objectives are as follows:

1. To evaluate the alignment of pH at grading with ultimate retail beef colour, and in particular the relative correlation to visual AUS-MEAT colour assessment at grading.
2. To investigate the possibility of using an objective colour measurement at grading as an alternative to subjective human evaluation.
3. To examine the potential to correlate objective (HunterLab meter) output with subjective MSA grader assessments based on AUS-MEAT meat colour standards.
4. To relate objective and subjective meat colour measures recorded at grading with equivalent measures taken prior to packaging and after display in overwrap, MAP and VSP packaging after 5, 12 and 40 days in vacuum packaging as primal cuts.
5. To document the colour relationship over the grading to retail cycle between striploin (considered as high colour stability) and other selected muscles selected to represent low (tenderloin) and moderate (rump) colour stability.
6. To determine if ultimate retail colour can be accurately estimated from instrumental (pH and/or HunterLab) measures at grading.
7. To record progressive colour ratings during retail display in overwrap, MAP and VSP utilising untrained consumers.
8. Quantification of retail colour perception and acceptance standards for untrained consumers.
9. Consumer evaluation of samples to quantify any packaging effect on eating quality.
10. Analysis of flavour volatiles related to consumer response to identify flavour precursors and mechanisms that may explain consumer assessed response.
11. To develop practical plant and retail recommendations to minimise colour problems and maximise consumer satisfaction.

3 Methodology

3.1 Experimental Design and implementation

3.1.1 Selection of carcasses

The experimental design specified selection of carcasses against a matrix of meat colour and pH as shown in Table 1.

Table 1. Desired matrix of AUS-MEAT meat colour and pH for selected carcasses at grading

Number of Head per Cell					
AMC	1C	2	3	4	5
pH < 5.7	6	6	6	6	6
pH > 5.7			6	6	6

The design, and related planning, recognised that some cells within this matrix might be difficult or impossible to fill from a particular kill day or days due to their historic low incidence. It was agreed that should pH by colour combinations not be filled the missing numbers would be filled by carcasses with the desired meat colour.

Despite perceived difficulties the design also incorporated a further requirement for each cell of 6 to be sourced from a balanced number (two) of 2, 4 and 6 teeth carcasses where possible.

The experimental protocol specified that all carcasses be graded by a common senior MSA grader (Janine Lau) to ensure consistency and that in addition to collection of kill floor and MSA grade data a HunterLab colour reading be taken of the exposed rib eye (*M.longissimus thoracis*) surface at the time of grading.

Teys livestock staff provided outstanding assistance in arranging delivery of over 500 head of MSA eligible grass fed steers from selected properties for kill on the desired day to maximise the potential to collect the desired wide range of meat colour, dentition and pH. The entire kill of September 7th 2015 was available for selection during MSA grading on September 8th. This resulted in all designed cells being filled for meat colour and a majority filled in relation to pH and dentition specification. The only cell appreciably different to the ideal distribution was the 1C meat colour where four 0 teeth carcasses had to be selected from some young grain fed cattle to obtain the desired meat colour mix. The actual distribution of meat colour of the 48 carcasses by pH at grading and dentition is displayed in Table 2.

Table 2. Actual distribution of pH and dentition within meat colour and pH cell for 48 carcasses used in cut collection.

		AMC				
	Dentition	1C	2	3	4	5
5.71<	0T	4	0	0	0	0
	2T	2	3	3	2	2
	4T	0	2	4	2	2
	6T	0	1	2	3	2
5.7+	0T	0	0	0	0	0
	2T	0	0	1	2	2
	4T	0	0	2	2	2
	6T	0	0	0	1	2

Both sides of all carcasses selected were identified by large (100mm by 300mm) brightly coloured laminated tags as displayed in Figure 1 attached to the rump by 150mm stainless steel skewers to facilitate identification in the carcase chillers and during subsequent marshalling and boning. The tags were coloured coded to signify right or left sides and carried a prominent and unique CUD (Cut up developer) number together with a prominent L or R to designate a carcase side. The CUD number was used to enable tag production prior to carcase selection and used during the design and pre-operational phase to enable carcase and treatment allocation and unique ID. It was replaced by actual carcase number during subsequent data handling.

The 48 carcasses selected were graded by a senior MSA grader prior to ticketing. A HunterLab spectrophotometer reading was also taken of each carcase at the grading site as an objective colour measure to be evaluated against the MSA graded colour chip. The selected 96 sides were marshalled from over 600 bodies in multiple chillers to facilitate boning within a single run.

Figure 1: Example of carcase tag used for primary identification

3.1.2 Collection of cuts

The experimental design specified that the striploin, D rump and tenderloin be collected and identified from both carcase sides of the 48 selected carcasses. This was achieved with all cuts collected during standard commercial boning operations utilising the side chain system. Personnel were stationed at the

relevant tables where the specified cuts were removed plus an additional person above the chain in the area where the loin was removed and chined prior to slicing to ensure continuous visual identification of carcase (by reference to the large laminated tag CUD number and L or R designation) to cut relationships. Primal trimming was as designated as domestic supermarket specification.

Individual coloured and laminated tags (50mm x 38mm) were produced with unique identification (ID) numbers for all primal cuts. In addition to the unique primal ID each tag included the cut name and CUD number for the associated carcase side. These tags were colour coded to specify days ageing in vacuum packaging prior to retail packaging. (Blue for 5 days, Yellow for 12 and Green for 40) and packed within the vacuum bag with each individual cut. To facilitate identification the collected cuts were bagged at the slicing stations with one person allocated to observe the cut during slicing to retain visual identification to the source carcase side and transfer the label to the relevant cut immediately after slicing as shown in Figure 2. The bags were not sealed at this point and transferred by dolly to an open area within the boning room for further colour assessment.

Figure 2: Transfer of ID from carcase side to individual cuts with colour coded labels designating ageing.

Allocation of ageing treatments to sides and cuts was balanced within each meat colour by pH cell utilising MSA CUD (Cut up developer) software and manual intervention to achieve the experimental design. An example of cut by side allocation within one meat colour by pH cell of 6 head is displayed in Table 2.

Table 3. Allocation of days ageing prior to retail packaging for carcasses of AUS-MEAT meat colour 4 and pH less than 5.7.

	TENDERLOIN		RUMP		STRIPLOIN	
CUD No	Left	Right	Left	Right	Left	Right
25	40	5	5	12	12	40
26	12	5	40	12	5	40
27	5	12	12	40	40	5
28	40	12	5	40	12	5
29	12	40	40	5	5	12
30	5	40	12	5	40	12

The allocation ensured that each ageing by cut was evenly distributed between left and right sides within each meat colour by pH cell. Actual carcase numbers replaced the CUD No in Table 3 after collection. Primal numbers were drawn from an MSA reserve to ensure they were unique within the AUSBlue database.

The design resulted in the total 288 cuts (3 cuts from 96 sides) being evenly distributed by days ageing providing 32 of each cut within each of the 5, 12 and 40 day vacuum ageing periods with further balance of 2 replicates within carcase side within ageing period for each of the 8 meat colour by pH cells.

3.1.3 Colour measurement and packing post boning

After bagging each cut was scored for AUS-MEAT meat colour by the same grader that evaluated the source carcase without reference to the original ribbed LD (*M.longissimus thoracis*) MSA grade score. Standard muscle locations, being the anterior face of the striploin (LD), *M.gluteus medius* (GM) face of the rump and exposed side of the tenderloin (*M.psoas major*) near the head, were designated as was a minimum 20 minute bloom period. HunterLab readings were taken at the same time from the same cut surfaces. Time of assessment was recorded via the internal HunterLab time stamp to provide a time log from knock time to MSA grading to individual cut evaluation.

The methodology developed with works management provided an extensive bench area within the boning room to provide sufficient space to lay out all cuts post boning during blooming and colour measurement. The vacuum bags were rolled back sufficiently to allow air exposure and full bloom conditions. Figure 3 illustrates colour measurement of the cuts post boning and prior to vacuum packing.

Figure 3: Colour assessment of cuts post boning.

After colour assessment the primals were grouped by primal ID tag colour within cut prior to vacuum packing and boxing to facilitate subsequent procedures. New product codes were provided by the plant and each carton labelled with the relevant colour in addition to code. Cartons were chilled through the chill tunnel, palletised at the plant and transferred by normal company freight arrangements to the Teys Australia Food Service (TAFS) facility within 5 days of the slaughter date.

All cuts were transported without loss and maintained within their cut by ageing day's cycle. This reflected considerable effort and diligence from company staff who provided every possible assistance.

3.1.4 Fabrication and retail packaging at TAFS

The experimental design dictated three separate cycles, determined by the 5, 12 or 40 day vacuum packed ageing period, for fabrication into three retail packaging formats – overwrap (OWP), MAP (modified atmosphere packaging with an 80:20 oxygen:carbon dioxide gas mix) and VSP (vacuum skin pack using the Darfresh™ system). Standard retail tray sizes of 140 x 190mm (OWP), 170 x 220mm (MAP) and 190 x 230mm (VSP) were designated. MSA CUD software with manual intervention was utilised to develop a balanced design allocating cut position to packaging treatment for all cuts and further utilised to produce associated control documentation, unique sample ID (referred to as an EQSRef, a unique software allocated random four digit alphanumeric code) and final package ticketing.

On each of the retail fabrication days (12/9/15, 19/9/15 and 17/10/15) the allocated 96 primals (32 rump, 32 striploin and 32 tenderloin) were individually removed from vacuum packaging with their unique individual primal ID labels and each placed on a plastic tray. Each primal was then fully denuded including removal of epimysium and reduced to individual muscles. The major tenderloin portion (*M.poas major* Figure 4) and the head (*M.iliacis*) were retained as were the rostbiff (*M.gluteus medius* Figure 5), which was further separated along the internal seam into two heads, and rump cap (*M.biceps femoris* Figure 6) from the rump primal. Only the *M.longissimus dorsi* muscle was retained from the striploin (Figure 7).

Figure 4: Tenderloin (TDR062) after fabrication to single muscle and prior to slicing for retail packaging.

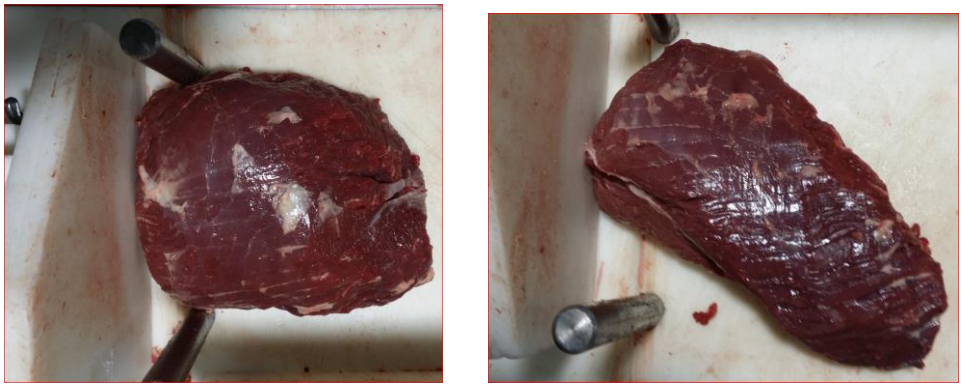


Figure 5: Rostbiff portions used in retail display. Note grain orientation for slicing in cutting jig for 2/3 – RMP131 (left) and 1/3 – RMP231 (right) portions of rostbiff separated along the seam.

Figure 6: Orientation of rump cap (RMP005) for slicing and fabrication.

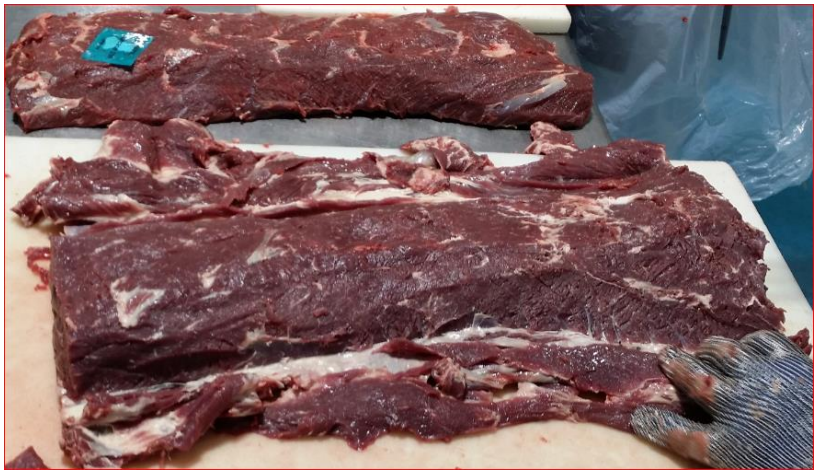


Figure 7: Striploin (STR045) showing fabrication detail. Note Blue label used to maintain Primal ID.

A second plastic serving tray was utilised for each primal to prepare and control subsequent packing and ID. A control file derived from MSA software specified a position within each muscle for the 3 packaging types (OWP, MAP and VSP). The design also rotated the packaging types through cut positions to ensure balance. A standard retail 140 x 190mm OWP black foam tray with soaker pad and a 170 x 220mm pre-formed clear plastic (MAP) tray with soaker pad were placed on the large plastic tray for each cut and a third space left for product to be placed in clear VSP (190 x 230mm) trays. A soaker pad was not used during the first round of VSP packaging but was utilised in the subsequent two rounds. The order of placement reflected the designated packaging by cut position order with the head of the cut aligned to the right hand edge. Pre-prepared laminated labels with unique 4 digit alphanumeric codes (EQSRef in MSA terminology) were removed from envelopes designated by primal number and placed within the retail trays or in the space designating the VSP sample. In each case there were two labels for each tray, identical other than by addition of an “F” suffix on one to designate a paired flavour chemistry sample.

Additional pre-printed Avery stick on labels were drawn from a further MSA software generated file linked to primal number and lightly attached along the top edge of the large tray in designated positions to indicate other samples required (Figures 8 and 9). In all cases an “Objective” label was produced to identify scrap from the primal suitable for laboratory testing for imf% or other analysis. For the rump a standard MSA grill label was produced for each rump cap and a fourth label associated with the posterior end of each striploin designated a further MSA grill. All labels used were then marked off on the control software.

Figure 8: Tray labelling and positioning of retail trays to control cut fabrication and position for each packaging type.

The packaging tray was then transferred to the cutting station and aligned with the denuded muscle and the primal ID cross-checked. Three further packs, one of each packaging type, were also located in the cutting area for flavour samples.

The source muscle was then sliced across the grain into 25mm slices from head to tail following MSA protocols and utilising a cutting jig to control thickness with the slices laid out in order. A suitable number of slices were then placed in the two retail trays (OWP and MAP) and in the VSP space with the slice order aligned with the tray positions to maintain the allocated cut position. The slices were placed to achieve a typical retail pack appearance (Figure 8) and the laminated EQSRef label placed in a designated corner of the pack. A further three small 50mm x 50mm x 25mm thick samples were prepared for use in flavour chemistry evaluation. The second laminated EQSRef label with the “F” suffix was placed on each flavour sample, then transferred to the appropriate paired packaging tray. Five flavour samples, each maintaining individual ID, were placed in each flavour tray to approximate the volume of meat in the retail packs used for consumer observation (Figure 10). For the rump primal two of the three packs were prepared from the larger 2/3 rostbiff portion (RMP131 MSA code) and the third from the smaller 1/3 portion (RMP231). The control software maintained balanced pack allocation across the two portions. The head portion of the tenderloin was not used unless the M.psoas major muscle was of insufficient size in which case it was drawn upon with the priority being retail pack then flavour sample.

Figure 9: Allocation of steaks to retail packages (furthest tray) and flavour (near tray and packs). Note cutting jig and steaks in cutting order. Note blue primal ID and stick on labels for other samples.

Figure 10: Example of flavour samples with EQSRef labels

The grill samples from the rump cap and posterior striploin portion were prepared as 5, 25mm thick, steaks approximately 40 x 65 mm. These were individually wrapped in freezer wrap then placed in a vacuum bag with the Avery label attached for ID and vacuumed. After vacuum packing they were frozen and retained for use as “Link” samples, served first to all consumers prior to six test samples.

Up to 250gms of surplus muscle was placed loose in a further vacuum bag with the objective label attached for ID as shown in Figure 13 and frozen to enable further proximate analysis if desired.

The tray with the filled OWP and MAP trays and slices for VSP was then transferred to a colour recording station as shown in Figures 11 and 12. The VSP slices and ID labels were placed in a VSP formed tray base and all samples bloomed for 20 minutes. The same MSA grader utilised in the initial grading and primal packing trial component then viewed each retail tray and assigned an AUS-MEAT meat colour score. Immediately following this HunterLab colour readings (CIE L*a*b* and individual wavelength values) were also taken using D65/10 light source from two steaks within each pack. These observations were then averaged for analysis.

Figure 11: Retail packs pre lidding with 20minutes bloom time prior to colour assessment. Note 3 packaging types with each tray derived from one primal. Unique pack EQSRefs relate to blue primal ID ticket from source primal vacuum bag.

In the final 40 days of primal ageing round a prospective new colour measurement device, the NIXPro, was added to the testing routine with duplicate readings taken immediately after and from the identical locations used for the HunterLab.

Figure 12: Colour assessment by MSA grader and AUS-MEAT chips (left) and spectrophotometer (HunterLab and NIX - right).

Immediately following colour analysis the trays were taken to the relevant retail packing line. The MAP packs were placed directly on a commercial packaging line for sealing while the VSP samples were transferred to the forming section of a commercial VSP (Darfresh™) packaging line. A typical retail butcher wrapping machine (Wedderburn N1793) was used to wrap the overwrap trays with oxygen permeable film.

The OWP trays were placed single depth in suitable containers and despatched via chilled transport to Charles Sturt University and placed in the cabinet on the day of packing. They were subsequently

viewed by consumers on the following day (24 hours post preparation from the primal) and the packs returned to the plant after viewing to enable the MAP and VSP product to be displayed.

The retail MAP and VSP packs were packed in standard cardboard outers and held within plant chillers at 1˚C following standard company procedures. To simulate typical distribution from central fabrication the MAP and VSP product was placed in the cabinet at 48 hours and first viewed on the third day post fabrication followed by two more viewings at two day intervals, the final viewing being 7 days from primal fabrication. Consequently the OWP product was viewed alone and served as a colour control whereas the MAP and VSP were co-located for viewing.

The flavour samples were held on plant in chilled storage and the objective samples frozen.

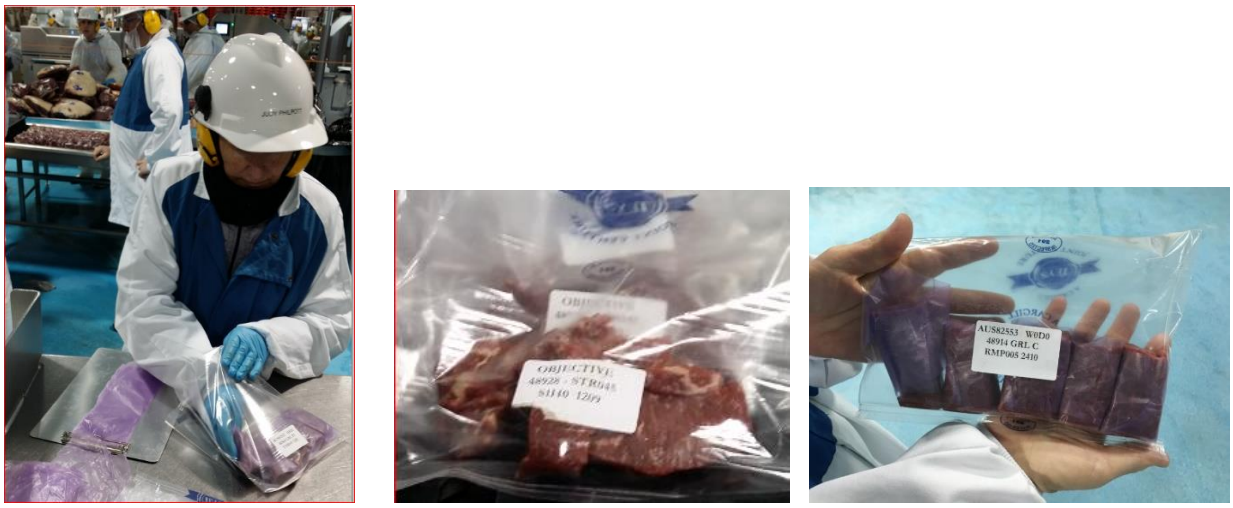


Figure 13: Packing grill for consumer sensory (right) and objective (left) samples.

3.1.5 Retail cabinet display at Charles Sturt University (CSU)

The experimental design specified that the retail display align with supermarket specifications which defined a retail case meeting M0 refrigeration standards of case temperature never exceeding 4˚C and never falling below -1˚C within a room environment where temperature was maintained below 25˚C with a maximum of 50% relative humidity. A suitable case was obtained and installed within an air-conditioned area at Charles Sturt University. The cabinet was fitted with temperature logging equipment throughout the trial period.

Calculations of pack combinations relative to individual shelf areas were made and suitable pack display templates developed to ensure controlled positions for individual packs. The relationship of pack and shelf sizes and required display numbers resulted in stipulated layouts involving a controlled mix of landscape and portrait pack alignment and single (portrait) and double (landscape) row presentation. MAP and VSP packs were specified to be arranged in alternate cabinet sides with the side rotated between display days.

Each display cycle included 96 OWP packs, being 32 of each of the three cuts, and a combined total of 192 MAP (96) and VSP (96) for these products. The protocol specified that the OWP product be displayed immediately after packing, with consumer viewing the day after packaging, and then removed from display to be replaced by the MAP and VSP product to be delivered two days post packing, reflecting normal distribution patterns.

Consequently the OWP product was viewed alone and acted as a colour control whereas the MAP and VSP were co-located for viewing. In accordance with the protocol all MAP and VSP product was viewed by consumers on three occasions, 3, 5 and 7 days post retail packing after which it was removed from display and returned to the plant for fabrication into consumer sensory samples.

The experimental design further specified that the 8 colour by pH cells within each cut be displayed as a group or set with the order of cabinet layout controlled to ensure that each cell was displayed in a balanced order relative to each other cell. In all there were 4 sets of 8 within each packaging type within each ageing cycle (5, 12 and 40 days in vacuum prior to retail packing).

To achieve this an 8 x 8 Latin square design as designated in Table 4 was employed with the Latin square columns rotated across cuts, packaging and ageing cycle to achieve a close to balanced meat colour presentation order within every cabinet plan. A further design requirement required dentition within meat colour to be as nearly as possible balanced within each of the 8 tray sets requiring individual allocation of all packs against the designated Latin square rows.

Table 4. Alternative order of retail pack display by AUS-MEAT meat colour and pH[1]

A	1C	2	3-	3+	4-	4+	5-	5+
B	2	3+	1C	4+	3-	5+	4-	5-
C	3-	1C	4-	2	5-	3+	5+	4+
D	3+	4+	2	5+	1C	5-	3-	4-
E	4-	3-	5-	1C	5+	2	4+	3+
F	4+	5+	3+	5-	2	4-	1C	3-
G	5-	4-	5+	3-	4+	1C	3+	2
H	5+	5-	4+	4-	3+	3-	2	1C

[1] + indicates pH over 5.7, - indicates pH of 5.7 or less

The shelf position of each set of 8 was also rotated between viewings to achieve a mix of front to back (of shelf), left to right and upper to lower shelf to minimise the risk of cabinet position bias in subsequent consumer evaluation. Further nuances were to rotate the positioning of cuts relative to each other and between landscape and portrait presentation. Separation of sets of 8 within the cabinet was reinforced by leaving a small space and by placement of a fresh green capsicum between sets.

Three cabinet plans, one per ageing cycle, were employed to achieve the design parameters for OWP whereas for the MAP and VSP display a different plan was utilised across the 3 display days within each cycle. The designated plans included rotation of product by shelf, by cabinet side and by row on shelf for each of the 4 sets of 8 within each cut by packaging type.

To facilitate analysis the position of every pack (by EQSRef) within the cabinet was recorded in combination with the pack order within the specific set of 8. Cabinet positions were designated by code within shelf with 1A the extreme left of the top shelf back row, 1B the extreme left of the front row and 1C and 1D the extreme right for example. This terminology was extended so that 1AC and 1BD designated central shelf placement in two rows and a 1E central shelf placement where a set was displayed as two rows of 4 and 1CD two rows of 4 at the extreme right. Example plans for OWP and MAP/VSP layouts are shown in Figures 14 and 15.

CABINET LAYOUT FOR OVERWRAP TO VIEW ON SUNDAY SEPT 20th

TOP SHELF

	Z4C8	U0Z9	L7E7	J0A6	L4Y5	W8S3	A4Q6	L7F8
1AC	T13	T13	T13	T13	T13	T13	T13	T13
	1	2	3	4	5	6	7	8
	B2D1	V8K2	E2A9	U9A7	S6U3	S0E7	L9Z9	L1R8
1BD	S13	S13	S13	S13	S13	S13	S13	S13
	1	2	3	4	5	6	7	8

2ND SHELF

	M2Z1	R2L9	X8B8	K3H8	G6E9	V0B6	M6Q4	A8Q9
2AC	S14	S14	S14	S14	S14	S14	S14	S14
	1	2	3	4	5	6	7	8
	P2P5	C1W8	F7R5	R9R5	D7A1	U5H1	H3G4	R9E9
2BD	R13	R13	R13	R13	R13	R13	R13	R13
	1	2	3	4	5	6	7	8

3RD SHELF

	D7Z5	P2N8	A8T3	X2C1	A0Y5	V0C7	U2L1	Y3E1								
3A	R14	R14	R14	R14	R14	R14	R14	R14	U5D5	W7V8	C3K9	M9B0	B7U4	B9V5	P9U7	F5H0	
	1	2	3	4	5	6	7	8									
									S15	S15	S15	S15	S15	S15	S15	S15	3CD
	X9J7	M4B5	K2L4	S7D0	X1Z3	G3E2	U5V6	D6W0									
3B	T14	T14	T14	T14	T14	T14	T14	T14	1	2	3	4	5	6	7	8	
	1	2	3	4	5	6	7	8									

4TH SHELF

	Q6X8	N7M0	C6Z7	M0Z2	Z8C9	V8U2	N5Q5	T6Q5
4AC	R15	R15	R15	R15	R15	R15	R15	R15
	1	2	3	4	5	6	7	8
	M6U3	R1A7	G0Q3	M8H2	E6B3	D0U1	R2A1	M1H3
4BD	T15	T15	T15	T15	T15	T15	T15	T15
	1	2	3	4	5	6	7	8

BOTTOM WELL

									P9S7	K7Z4	C9M1	M9J1	Z6A8	C7U5	J0M2	T6N0	
	G2U2	G5C0	H9Q6	R0E1	M5B6	U7U0	K4F9	G0N2	R16	R16	R16	R16	R16	R16	R16	R16	5C
									1	2	3	4	5	6	7	8	
5AB	T16	T16	T16	T16	T16	T16	T16	T16									
									E5Y5	E1S4	E0F8	W3P6	G8E5	R6Q7	R8Y7	L9C1	
	1	2	3	4	5	6	7	8	S16	S16	S16	S16	S16	S16	S16	S16	5D
									1	2	3	4	5	6	7	8	

NOTE: Packs designated in large vertical alignment are in portrait presentation. All others are landscape.

Figure 14: Example cabinet layout for overwrap samples (Sept 9th, 2015)

CABINET LAYOUT FOR VSP (Shaded) & MAP ON 220915

		T4X5	E0E9	Q9J8	F3G5	H9V9	P1P2	S8X0	J4A4			S5X1	L4N4	X9A2	T1E7	B4Q6	B8T6	G8D5	K0C2	
	1A	R17	R17	R17	R17	R17	R17	R17	R17			S21	S21	S21	S21	S21	S21	S21	S21	1C
		1	2	3	4	5	6	7	8			1	2	3	4	5	6	7	8	
	SHELF 1																			
		Q0S0	S3J7	T8X2	A6M2	B1L1	Q5E8	N2H7	E9C1			T0A6	C5B3	A1Y5	A4A1	A6R7	K6M4	S2V7	L1D8	
	1B	T17	T17	T17	T17	T17	T17	T17	T17			T21	T21	T21	T21	T21	T21	T21	T21	1D
		1	2	3	4	5	6	7	8			1	2	3	4	5	6	7	8	
		Blue with yellow dots										White Label								
										SECOND SHELF										
		U9K3	Y9E5	M0B6	Y1Q7	X4X6	N7K6	T1N8	X4D6		2E			K3D5	S8X3	N9M0	A0Q3	R2W2	B7R9	Q3Z1	A1E3	
2A		S17	S17	S17	S17	S17	S17	S17	S17	Q6T8	U6H1	U8Y9	L3F8	T24	T24	T24	T24	T24	T24	T24	T24	2C
		1	2	3	4	5	6	7	8	S24	S24	S24	S24	1	2	3	4	5	6	7	8	
										1	2	3	4									
		E1B2	L3H2	W6S6	U0W8	N1A8	N3E8	A1Q8	V1J8	Q1X0	P8L6	Y7E1	V9F5	F2K2	X9P8	K8S8	A5V5	K7T0	U0Q7	B2M6	Q2B4	
2B		R18	R18	R18	R18	R18	R18	R18	R18	S24	S24	S24	S24	R24	R24	R24	R24	R24	R24	R24	R24	2D
		1	2	3	4	5	6	7	8	5	6	7	8	1	2	3	4	5	6	7	8	
		Red Label								Yellow Label				White with red dots								
																			THIRD SHELF			
		D1K1	J1T9	S5T9	C4P6	U7X0	B8B6	J3Q1	W4S6		3E			X2S8	Y9V6	S9C5	Q6G6	N8A4	U4B2	B1M9	E8Z2	
3A		S18	S18	S18	S18	S18	S18	S18	S18	R5B8	D3X7	X3X3	N9J6	R21	R21	R21	R21	R21	R21	R21	R21	3C
		1	2	3	4	5	6	7	8	R19	R19	R19	R19	1	2	3	4	5	6	7	8	
										1	2	3	4									
		G6G2	J9J7	Y7D1	T5K1	Y3X4	C3P7	U0R6	P4F8	H5J1	U3J7	M7F6	J6D1	G4B7	X5L3	E9C9	R2N4	Z1H3	J8F0	A6E3	R7C9	
3B		T18	T18	T18	T18	T18	T18	T18	T18	R19	R19	R19	R19	S22	S22	S22	S22	S22	S22	S22	S22	3D
		1	2	3	4	5	6	7	8	5	6	7	8	1	2	3	4	5	6	7	8	
		Green with orange dots								Green Label				Blue Label								
		FOURTH SHELF										4EC										
		C8A8	M7U0	Y5L0	F4X5	Q1Z7	V4S4	P8S3	D4L3	X1T3	Z1G8	Y1H6	L6K0	Q1Z9	F8N3	Y2X7	S4C5					
4A		T19	T19	T19	T19	T19	T19	T19	T19	S23	S23	S23	S23	S23	S23	S23	S23	Z8C4	A2Y7	L0P4	W1P3	
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	R22	R22	R22	R22	
																		1	2	3	4	4CD
		C4G5	S4R0	E8Q6	R7H5	D8K4	M8E6	V2E1	Q8X9	D9S1	X5S2	J5Q9	K4F7	E0R2	V3X5	Y3F4	N9C4	U1B1	A4W0	R2H2	G8U2	
4B		S19	S19	S19	S19	S19	S19	S19	S19	T22	T22	T22	T22	T22	T22	T22	T22	R22	R22	R22	R22	
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	5	6	7	8	
		Yellow with pink dots								White with blue dots		4ED						White with green dots				
										5AE		BOTTOM WELL										
						W4R8	M8T5	N5V4	M9N3	V7L3	M6C9	N6A8	N0R0	X9V4	V6R5	A0S0	K7T5	G9A8	N5M1	F8D0	B7A3	
		F8N2	F0C2	M9K4	B8J4	S20	S20	S20	S20	S20	S20	S20	S20	T23	T23	T23	T23	T23	T23	T23	T23	5C
		T20	T20	T20	T20	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	
5AB		1	2	3	4																	
		W8Q6	C7Q4	V1J7	P4V7	R3P2	N0U3	K3N4	Y8A7	T1E3	W1H6	Q8Q2	S7W4	K6R0	X3V7	E6U5	K2P0	F9T2	N9E3	S5G6	Q0L2	
		T20	T20	T20	T20	R20	R20	R20	R20	R20	R20	R20	R20	R23	R23	R23	R23	R23	R23	R23	R23	5D
		5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	
		Orange Label				Red with yellow dots				5BE				Purple Label								

Figure 15: Example MAP (white) and VSP (shaded) cabinet plan for one viewing (Sept 22nd 2015). Note cabinet position designation, set numbers (T=tenderloin, R=rump and S=striploin) and EQSRef order within set representing Latin square column order.

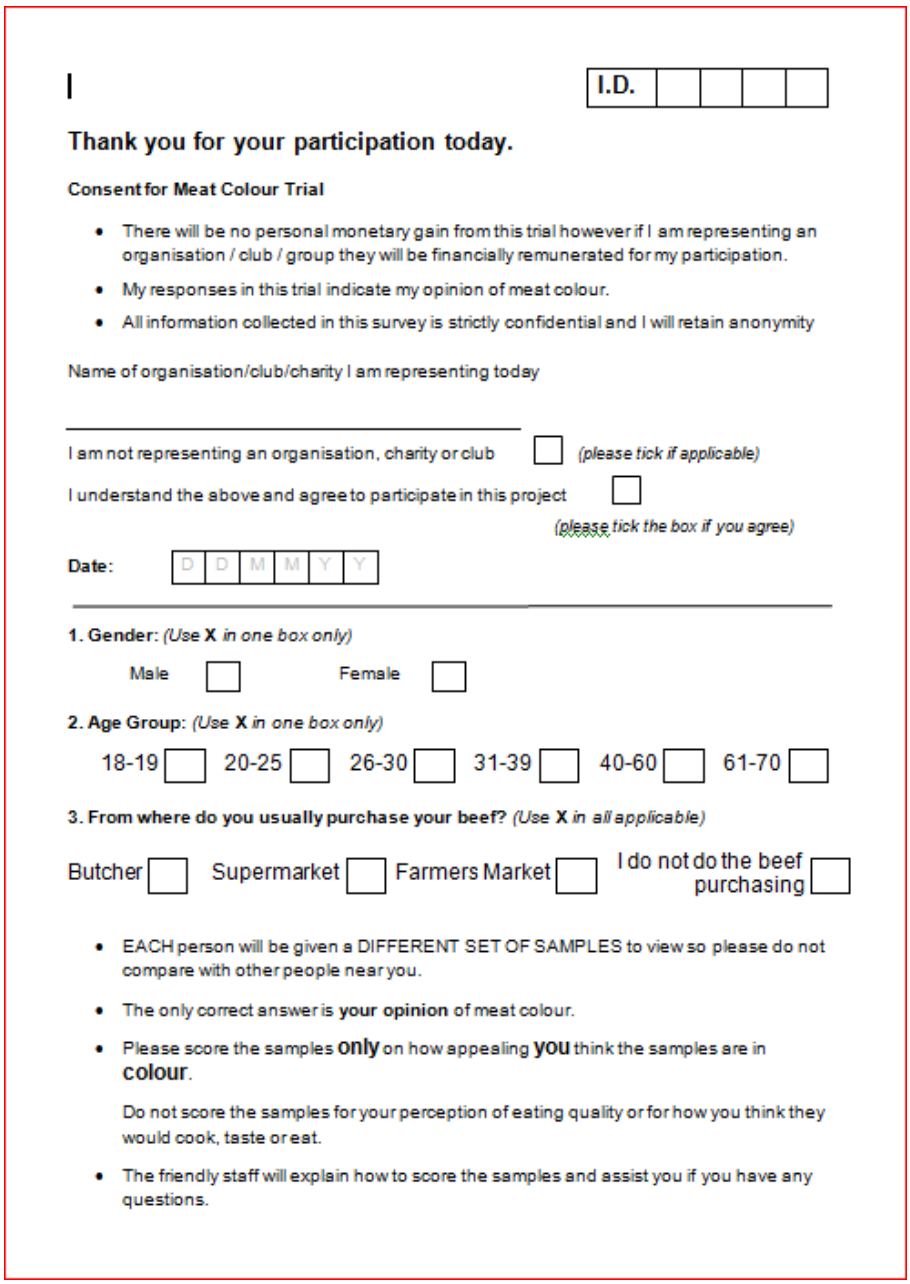
3.1.6 Consumer assessment of meat colour

In accordance with the experimental design each pack was assessed by 10 consumers with each consumer required to assess 24 packs being 3 sets of 8 from the 12 on display for OWP or from the 24 for the combined MAP and VSP display. A set comprised 8 retail packages of the same cut sourced from carcasses with 8 combinations of original MSA meat colour (1C, 2, 3, 4 and 5) and pH (above or below 5.7) and dentition from 0 to 6 teeth. This required 40 consumers to view the cabinet when evaluating OWP and 80 for each viewing day of MAP/VSP. Each ageing cycle required 280 consumers to be recruited, a total of 840 for the entire experiment resulting in 20,160 pack observations.

For all OWP observations and for 64 of 80 consumers viewing MAP/VSP the three sets allocated via the design were one of each cut. For 16 of the 80 MAP/VSP consumers only two of three cuts were viewed with the third set a repeat of one cut, the two common cut sets being one in MAP and the other VSP. In all 40 consumers were designated to view 2 MAP and 1 VSP pack and 40 the reverse.

Consumer recruitment was conducted by Charles Sturt University primarily via fundraising with community groups supported by the individual panellists. Where no affiliation was noted a movie pass was offered to the individual. Viewing times were scheduled across the day in 15 minute blocks between 8am and 8pm.

On arrival each consumer was briefed in regard to the scoring procedure and given a clipboard with one sheet of demographic questions as shown in Figure 16 followed by 3 pages, each relating to an assigned set of 8 retail packs identified by EQSRef number and display order within the relevant set of 8. Each set was preceded by an instruction defining the cabinet position such as “Blue Label, top shelf, back row” to assist in accurate location of the first sample.



I.D. ☐☐☐☐

Thank you for your participation today.

Consent for Meat Colour Trial

- There will be no personal monetary gain from this trial however if I am representing an organisation / club / group they will be financially remunerated for my participation.
- My responses in this trial indicate my opinion of meat colour.
- All information collected in this survey is strictly confidential and I will retain anonymity

Name of organisation/club/charity I am representing today

_____

I am not representing an organisation, charity or club ☐ *(please tick if applicable)*

I understand the above and agree to participate in this project ☐

(please tick the box if you agree)

Date: D D M M Y Y

1. Gender: *(Use* **X** *in one box only)*

Male ☐ Female ☐

2. Age Group: *(Use* **X** *in one box only)*

18-19 ☐ 20-25 ☐ 26-30 ☐ 31-39 ☐ 40-60 ☐ 61-70 ☐

3. From where do you usually purchase your beef? *(Use* **X** *in all applicable)*

Butcher ☐ Supermarket ☐ Farmers Market ☐ I do not do the beef purchasing ☐

- EACH person will be given a DIFFERENT SET OF SAMPLES to view so please do not compare with other people near you.
- The only correct answer is **your opinion** of meat colour.
- Please score the samples **only** on how appealing **you** think the samples are in **colour**.

  Do not score the samples for your perception of eating quality or for how you think they would cook, taste or eat.
- The friendly staff will explain how to score the samples and assist you if you have any questions.

Figure 16: Consumer demographic questionnaire

Each sample was scored via a 100mm line scale anchored by the words extremely unappealing and extremely appealing. For each sample the consumer was also asked to choose one of three category boxes described as definitely would buy, definitely would buy if discounted and definitely would not buy. An example scoring sheet is shown in Figure 17. The sample order on each sheet reflected the pack order in the case from left to right with the EQSRef pre-printed in the ID box.

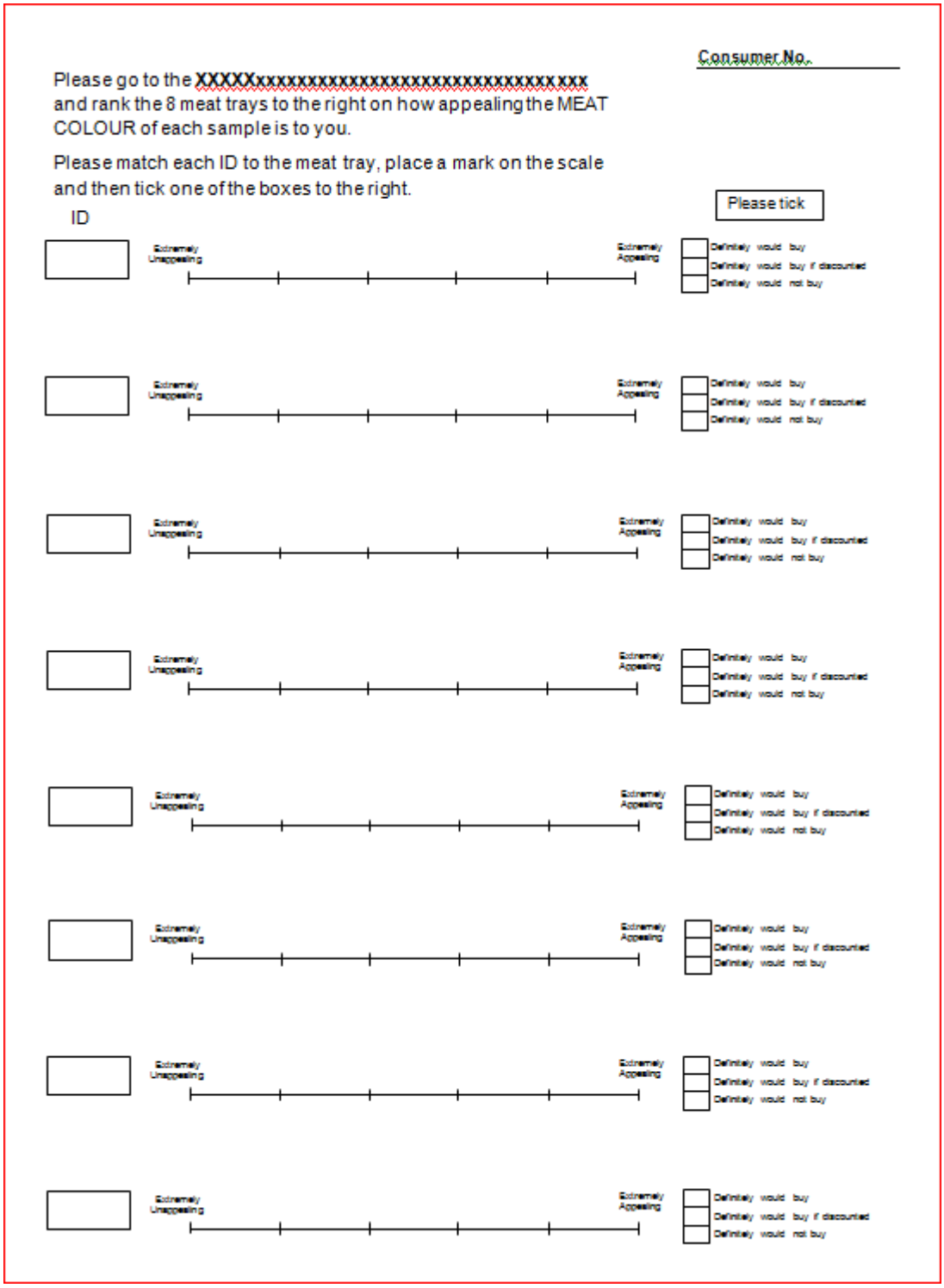


Figure 17: Consumer score sheet for one set of 8 packs (one page of 3)

The consumer was accompanied to the cabinet and assisted in locating the first starting position to ensure the instruction was understood. Figure 18 displays the cabinet and typical layout.

Figure 18: Retail cabinet display for MAP & VSP cycle. Note labels designating first pack in each set of 8.

Table 5. The number of consumers utilised on each viewing date.

Date	Prior Primal Ageing	Retail Pack	Days from kill	No of Consumers
13/09/2015	5	OWP	6	40
15/09/2015	5	MAP & VSP	8	80
17/09/2015	5	MAP & VSP	10	80
19/09/2015	5	MAP & VSP	12	80
20/09/2015	12	OWP	13	40
22/09/2015	12	MAP & VSP	15	80
24/09/2015	12	MAP & VSP	17	80
26/09/2015	12	MAP & VSP	19	80
18/10/2015	40	OWP	41	40
20/10/2015	40	MAP & VSP	43	80
22/10/2015	40	MAP & VSP	45	80
24/10/2015	40	MAP & VSP	47	80
			TOTAL	**840**

All consumer scores were measured twice and the data entered independently by two people. Any result where the two scores differed by more than 2 (mm) was resolved by re-checking the original sheet. A zero tolerance was applied to the 3 category boxes.

The consumer response files combined with source files detailing linkage of the EQSRefs to the packaging system, source muscle and carcase including MSA grading data and to the days aged in vacuum packaging prior to retail packing were forwarded to Dr Ray Watson and Dr Garth Tarr accompanied by an initial analysis brief.

All original questionnaires were filed and retained for reference.

On completion of the viewing period all samples were transported to the TAFS facility for final colour measurement and conversion to MSA sensory samples.

3.1.7 Preparation of MSA consumer samples and data recording post retail display.

The OWP trays were returned to the plant 2 days post original fabrication from the source primal whereas the MAP and VSP were returned 9 days after initial fabrication. The packs were progressively opened and allowed to bloom for a minimum of 20 minutes followed by colour assessment utilising the same MSA grader (who assessed colour at each earlier reading) using AUS-MEAT meat colour chips along with a matched HunterLab reading. A pH reading was also recorded for each sample.

A machine error after processing the MAP and VSP samples from the 49 day aged product after retail display resulted in HunterLab readings being lost for this date. Replacement readings were obtained by identifying individual samples during subsequent consumer testing.

The retail display samples were then fabricated into MSA grill sensory steaks following MSA protocol (Anon 2008). In brief this required fabrication of 5 steaks 25mm thick and approximately 50 x 38mm or equivalent area with each to be wrapped in freezer wrap prior to vacuum packing and freezing at the designated ageing days from slaughter. Each set of consumer sensory steaks were identified by attaching an Avery label incorporating the EQSRef and produced from the source CUD file to the vacuum bag during transfer from the retail tray and fabrication. This label replaced the laminated EQSRef tag used in each pack during retail observation.

To retain a constant ageing period prior to freezing the OWP consumer samples were held under chilled conditions, equivalent to the retail display case, until the ninth day post initial fabrication at which time the paired MAP and VSP samples were fabricated. All consumer sensory samples were then frozen. Equivalent treatments were adopted for the paired flavour samples, held as 5 per tray to reflect their paired retail case packs in chilled storage at the plant, and then transferred from the retail packaging to individual vacuum packaging at the same time as the matched sets displayed in the retail cabinet. The flavour samples utilised their original laminated EQSRef labels for identification.

3.1.8 MSA consumer testing

MSA sensory evaluation by untrained consumers was conducted on all retail pack samples to evaluate differences between the three cuts, the alternative ageing periods prior to retail packaging and the three packaging types. This required sensory evaluation of 10,080 grilled beef samples by 1,440 consumers which was achieved in full together with collection, entry and checking of consumer data

and collation with source cattle, grading, ageing, packaging and sample fabrication data from the AUSBlue data base. Further detail of the procedures follows.

3.1.8.1 Pick design

The individual "picks" each allocated 42 samples across 60 consumers, tested in three groups of 20 per session. All consumers received a "link" sample as the first of 7 samples with this product sourced from rump cap (*M.biceps femoris*) or the posterior end of the striploin (*M.longissimus dorsi lumborum*) prepared from the original trial primals and predicted to be of mid range eating quality. The allocation of samples followed MSA protocols (Watson *et.al*, 2008) where an input table with 6 samples in each of 6 products was accessed by software that allocated each sample to 10 consumers, treated as five pairs, to ensure a controlled allocation based on a 6x6 Latin square. Samples were allocated to the six products to achieve a transition from expected lowest to highest eating quality across the products. Each consumer was served one sample from each of the 6 product groups to ensure they each evaluated a wide and consistent quality range. This resulted in 420 individual consumer samples being evaluated in each pick.

For the six test samples following the standard link the 60 consumers are dispersed as 5 discreet groups of 12 (six pairs) within the software routines. Every sample was tested by 10 consumers (5 pairs). Each pair was allocated from a different subset of 12. The software allocated products to each consumer pair according to a 6×6 Latin square design of the form below with products allocated in the order designated by column.

1	2	3	4	5	6
2	4	1	6	3	5
3	1	5	2	6	4
4	6	2	5	1	3
5	3	6	1	4	2
6	5	4	3	2	1

Therefore consumer pair one was served a sample of product 1, followed by products 2, 3, 4, 5 and 6 whereas consumer pair two received products in the order 2, 4, 1, 6, 3, 5 and so on. A new Latin square was commenced for each sub group of 12 consumers. The 5 individual portions of each sample were allocated to 5 different order positions as they were dispersed across the 5 subsets of 12 consumers. The net effect was that every sample was tested in 5 of 6 possible different presentational positions by 5 consumer pairs from 5 sub groups.

As there are 6 Latin squares and 6 products, samples from every product occur an equal number of times (6) in each presentational position and before and after each other product in the MSA protocol. This provided a balance for frequency, order and carryover effects. The 5 pairs who tested any one sample were not combined again for any other sample.

The samples allocated to each pick were selected and allocated to prioritise packaging comparisons and to provide both statistical cut linkage within carcasses and connection between picks. A typical example is shown in Table 6.

Table 6. Typical product allocation within a pick

LINK	PRODUCT 1	PRODUCT 2	PRODUCT 3	PRODUCT 4	PRODUCT 5	PRODUCT 6
Rump Cap	**A**-RMP-MAP-14	**A**-STR-MAP-49	**B**-RMP-VSP-21	**C**-STR-OWP-21	**D**-TDR-MAP-14	**A**-TDR-VSP-14
Rump Cap	**A**-RMP-MAP-21	**C**-STR-MAP-21	**A**-RMP-VSP-21	**C**-STR-VSP-21	**D**-TDR-MAP-21	**A**-TDR-OWP-14
Rump Cap	**B**-RMP-MAP-14	**A**-RMP-VSP-14	**A**-RMP-OWP-21	**A**-STR-OWP-21	**A**-TDR-MAP-14	**D**-TDR-OWP-14
Rump Cap	**B**-RMP-MAP-21	**B**-RMP-VSP-14	**B**-RMP-OWP-21	**A**-STR-VSP-21	**A**-TDR-MAP-49	**D**-TDR-VSP-14
Rump Cap	**A**-STR-MAP-21	**A**-RMP-OWP-14	**C**-STR-OWP-14	**A**-STR-OWP-49	**A**-TDR-VSP-49	**D**-TDR-OWP-21
Rump Cap	**C**-STR-MAP-14	**B**-RMP-OWP-21	**C**-STR-VSP-14	**A**-STR-VSP-49	**A**-TDR-OWP-49	**D**-TDR-VSP-21

The columns represent the 7 products to be served with 6 individual samples within each. As described above the link is served first to all consumers with each individual then receiving one sample from each of the 6 product columns with order designated in accordance with the Latin square.

The detail within each cell, for example A-RMP-MAP-14, signifies the carcase (A in this case), the muscle (RMP), the retail packaging (MAP) and the days aged (14). The yellow shaded cells indicate that all 18 samples (MAP, OWP and VSP packed portions from the rump, striploin and tenderloin) produced from carcase “A” are included within this pick to provide a tight measure of cut x packaging within animal. An equal number of each cut are sourced from three different animals – “B”, “C” and “D” with each of these animals having their other two cuts in two further picks. In all this resulted in every pick being connected to 6 other picks creating strong linkage between picks. To ensure the strongest possible test of potential packaging differences the 3 packaging types and the two ageing variations within each muscle were always tested within a common pick (therefore evaluated by the same pool of consumers within the relevant 3 sessions).

3.1.8.2 Consumer recruitment

Consumer recruitment was managed by contractors, who utilised community groups for recruitment with the group rather than individual consumers paid for participation. Consumers were screened and selected within criteria of eating beef at least once per fortnight, preferring it cooked to medium doneness and being aged between 18 and 70.

3.1.8.3 Cooking, serving and data protocols

In accordance with MSA protocol samples for each pick being 7 “round sheets” each with 10 consumer steak samples vacuum packed on a sheet with coded sample numbers, were transferred from frozen storage to a 4˚C chilled area 24 hours prior to testing and allowed to thaw. Each sheet was laid on a plastic tray and the vacuum bag opened to allow the steaks to come to room temperature prior to cooking and to bloom for colour (20 minute time interval).

At this point two identified samples originating from the 49 days aged product where Hunterlab readings were lost were identified and new Hunterlab and NIX readings recorded. Due to each of the 5

steaks from any sample being dispersed across up to three sessions of consumers the two required steaks were often within different sessions requiring attendance with the colour instruments at multiple sensory sessions. To minimise the cost and difficulty efforts were made to identify round sheets where a maximum number of required samples were present and sessions containing these rounds allocated to a single location. While this resulted in a majority of required readings being obtained at that location further measurement from rounds allocated to Melbourne were also required and obtained.

MSA grill cooking and serving protocols were utilised for all sensory testing as described by Watson *et.al*, 2008a. In brief a 3 phase Silex (STronic 165) double sided grill was utilised with all cooking procedures regulated by count up timers. A first round of scrap meat was cooked to stabilise plate temperature recovery with the link and six sample rounds following at designated intervals. The round sheets were aligned beside the grill and steaks transferred onto the grill and after cooking to a cutting board for serving in a strict 3-4-3 left to right, top to bottom sequence to ensure ID was maintained.

10 steaks, approximately 50mm x 38mm by 25mm thick were cooked within each of the seven rounds, rested for 2 minutes after removal from the grill then halved with each served to 2 consumers. The ID on the consumer plates was further checked against the empty round sheet codes during cutting and serving. Allocation of steaks to rounds and to consumer ID was controlled by software in accordance with the design criteria described previously.

After an initial briefing each consumer completed a number of demographic questions followed by an individual scoring sheet for each of the 7 samples. Each sample was identified only by the 4 digit alphanumeric EQSref code and related Q code. The sample score sheets included four 100mm line scales for each of tenderness, juiciness, flavour and overall satisfaction followed by four category boxes labelled as unsatisfactory, good everyday quality, better than everyday quality and premium quality.

The tenderness scale was anchored with the words not tender and very tender, the juiciness scale with not juicy and very juicy and the flavour and overall scales with dislike extremely and like extremely. Consumers were instructed to make a vertical line across each scale at a point that reflected their judgement for each sample. They were also asked to mark one of the four category boxes.

Following serving and evaluation of the 7 samples each consumer was asked to mark a further line scale graduated in $10 increments from $0 to $80/kg representing the $/kg value they ascribed to each of the category boxes.

Each sheet was checked after completion by serving staff and scanned twice by the contractor with software that displayed the sheet and scores on screen while highlighting any score differences between scans. The mm to the consumer mark from the left end of the linescale was recorded as a score between 0 and 100. Each sheet required manual acceptance before writing to a sensory data file. The completed file for each pick (420 rows of data) was then emailed to the research manager who utilised further software to calculate both 10 consumer averages for each line scale and category score and a clipped score that removed the two highest and two lowest scores and averaged the remaining central six.

In addition a raw and clipped MQ4 score was calculated by multiplying the tenderness, flavour and overall scores by 0.3 and the juiciness scores by 0.1 before summing the results. The output was visually checked and raw product means calculated prior to uploading the sensory summary for each sample (a

single row with the 10 consumer averages and clipped scores) to the AUSBlue database where the sensory data was matched to the record of animal, carcase, grading, ageing and packaging detail.

Examples of the demographic, scoring sample and WTP (willingness to pay) sheets are presented in Appendices.

3.1.9 Flavour chemistry

Samples for flavour chemistry analysis were air freighted to Dr Linda Farmer at the Agri-Food and Biosciences Institute (AFBI) in Belfast, Northern Ireland. A selected sub-set samples representing each packaging method within cut and carcasses were processed through linked gas chromatograph and mass spectrometer (GCMS) instrumentation to evaluate flavour volatiles. The analysis utilised previously identified indicator compounds to examine the interactive roles of flavour precursors and formation pathways in modifying consumer flavour perception. The analysis was further targeted to include volatiles linked to breakdown of sugars, ribonucleotides, fatty acids, amino acids and antioxidants. AFBI were contracted to provide a written report detailing the experimental process adopted and a “plain English” discussion of the results.

3.1.10 Statistical analysis

Statistical analysis was applied to all trial data to examine a number of separate and interrelated factors. Primary issues investigated included relationships between cube roll colour and pH assessed during MSA grading compared to that of the three cuts from both sides post boning a short time later; the subsequent relationship of meat colour and pH prior and post retail display after primal ageing; the relationship between HunterLab measures and AUS-MEAT meat colour scores; consumer assessment of retail pack meat colour appeal including linkage to threshold meat colour scores; impact of cabinet position to consumer assessment; meat colour perception and packaging interaction; consumer evaluation of eating quality in relation to retail packaging and any ageing interaction plus evaluation of flavour chemistry elements in relation to consumer assessed eating quality and flavour ratings.

All trial data was recorded in Excel based files and a description of each (Attachment 9.9) and the analysis brief (Attachment 9.7) prepared and forwarded to Dr Garth Tarr and Dr Ray Watson for statistical analysis. Some files and other MSA colour data was also analysed by Dr Ian Lean and Dr John Thompson. Data analysis was subject to peer review at the MSA Pathways Committee meeting in March 31st 2016.

4 Results

To facilitate analysis and provide direct linkage to consumer outcomes a “Consumer Meat colour Score” (CMC) was developed from the consumer data linking the scores recorded to the category boxes selected across all 20,140 observations. Despite the overriding importance of consumer colour perception directly influencing purchase intent and pricing no previous consistent system of consumer reference was found with typical papers relating to instrumental, principally L*a*b* values or to hedonic scales particular to an experiment. While the majority of issues and recommendations within the American Meat Science Association (AMSA) Meat Color Measurement Guidelines (2012) were adopted or addressed in the study design a 100 mm line scale was utilised for consumer scoring rather than 7 or 9 point hedonic scales due to alignment with MSA sensory scale and category choice protocol.

The CMC was utilised as the benchmark measure for comparison of all visual colour comparisons.

4.1 Consumer perceptions of meat colour

The Consumer Meat Colour score (CMC) utilised for evaluation was the mean of the 10 individual consumer scores for each sample.

Prior to adopting this measure analysis was conducted on individual consumer data to determine the robustness of such a measure and its ability to define a purchase category. The dot plot in Figure 19 relates individual CMC to the category selected with 1 would definitely purchase, 2 would definitely purchase if discounted and 3 would definitely not purchase.

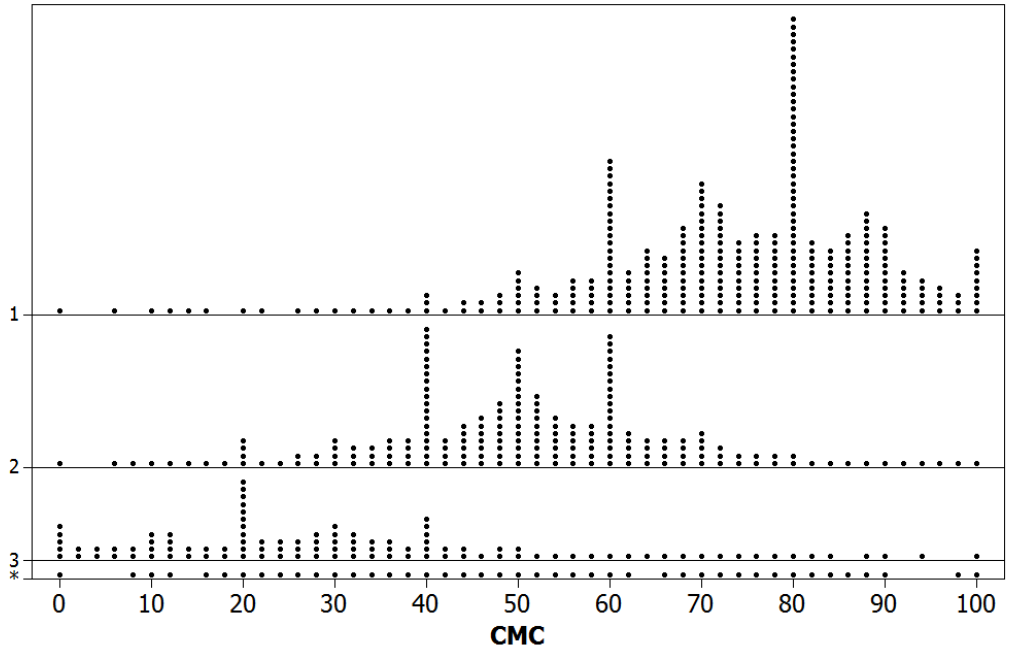


Figure 19: Consumer meat colour scores (CMC) in relation to purchase intent category.

The plots indicate a strong relationship and consistency between the two scales with a clear delineation of the purchase scale (PS) by CMC: higher CMC values are associated with PS=1, lower CMC values with PS=3. This, of course, is entirely in accordance with expectation indicating that consumers are relatively internally consistent: they tend to choose a CMC value that matches their choice of purchasing category. A discriminate analysis was conducted to evaluate optimal cut off scores and establish their effectiveness in defining purchase categories. This produced cut-off values of 38.5 and 62.5 for CMC, with a 75% success rate (over all individual consumers who gave a valid response) as shown in Table 8.

Table 8: Discriminate analysis of CMC as a predictor of purchase category.

	True Group			
Assigned Group	**1**	**2**	**3**	Total N
1 (CMC < 38.5)	8356	1093	82	9531
2 (38.5 < CMC < 62.5)	2204	4246	559	7009
3 (62.5 < CMC)	102	974	2349	3425
Total N	10662	6313	2990	19965
N correct	8356	4246	2349	14951
Proportion	0.784	0.673	0.786	0.749

N = 19965 N.Correct = 14951 %.Correct = 74.9%

Thus, CMC (consumer meat colour liking) and PS (purchasing category) are reasonably consistent; and it was concluded that CMC could be used as a predictor of PS. This result is comparable with the MQ4 (consumer meat quality) used as a predictor of MSA grades.

A further analysis question related to the use of mean versus clipped mean consumer scores for individual sample CMC arising from MQ4 practise in which the two highest and lowest scores are clipped to create a 10-4 clipped value from averaging the central 6. Table 9 and figures 20 and 21 provide descriptive statistics and dotplots for the observed values of the mean and standard deviation of CMC scores for each of the 840 consumers.

Table 9: Descriptive statistics for CMC and CMC standard deviation.

	n	**n***	**mean**	**sd**	**min**	**Q1**	**Med**	**Q3**	**Max**
consumer mean	839	1	59.2	13.0	15.0	50.6	58.5	68.5	94.6
consumer sd	839	1	17.8	6.5	0.8	13.1	17.3	22.1	40.2

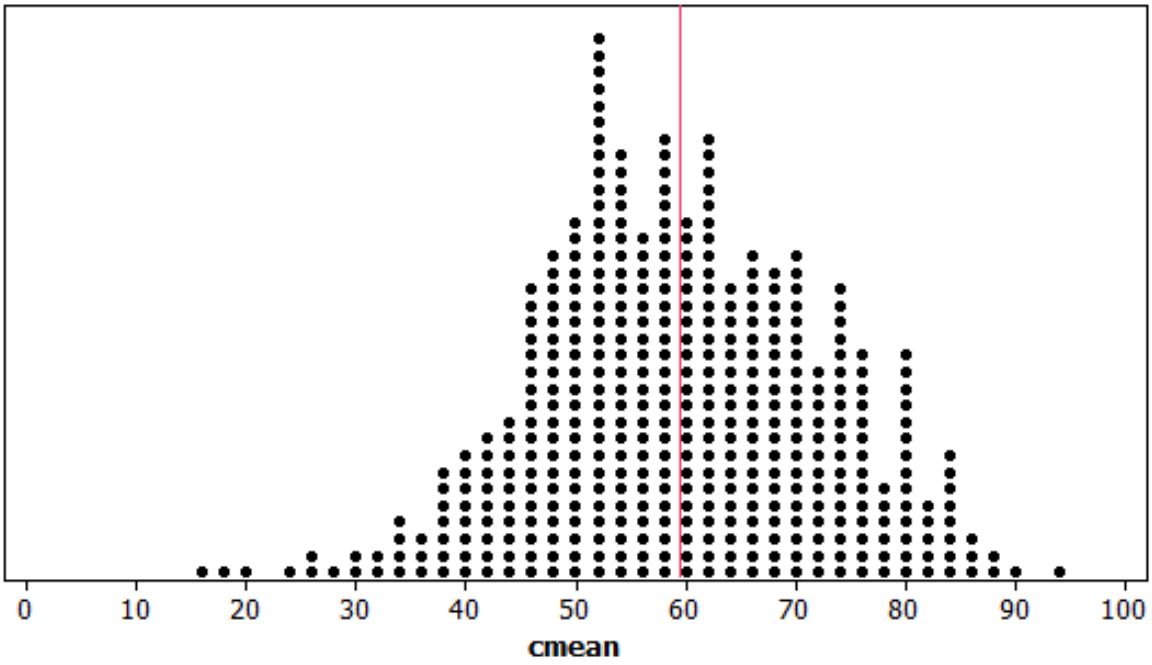


Figure 20: Dot plot of individual consumer CMC means (n=839)

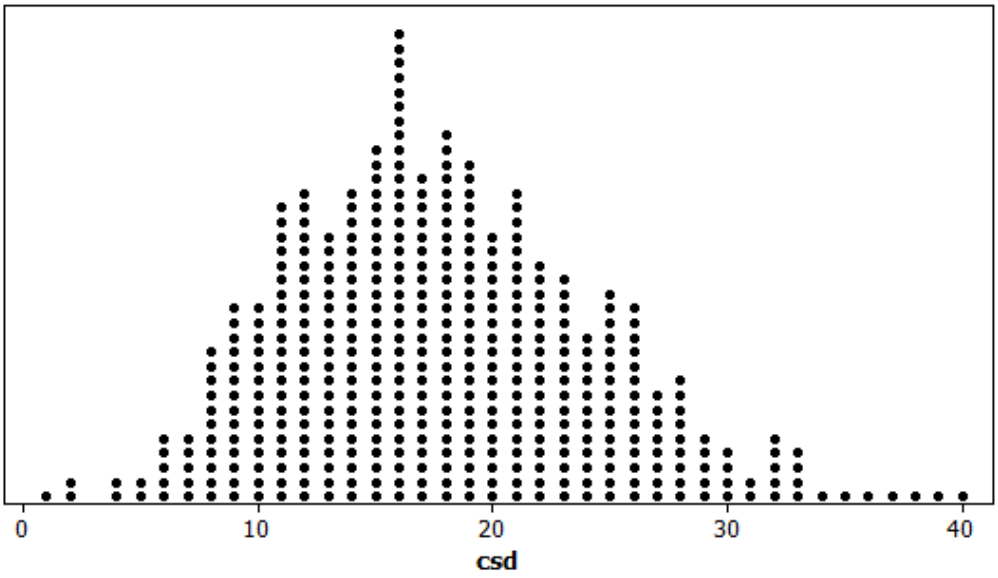


Figure 21: Dot plot of individual consumer CMC standard deviation (n=839).

The distribution of mean.deviation, displayed in Figure 22, reflected that of the consumer mean:

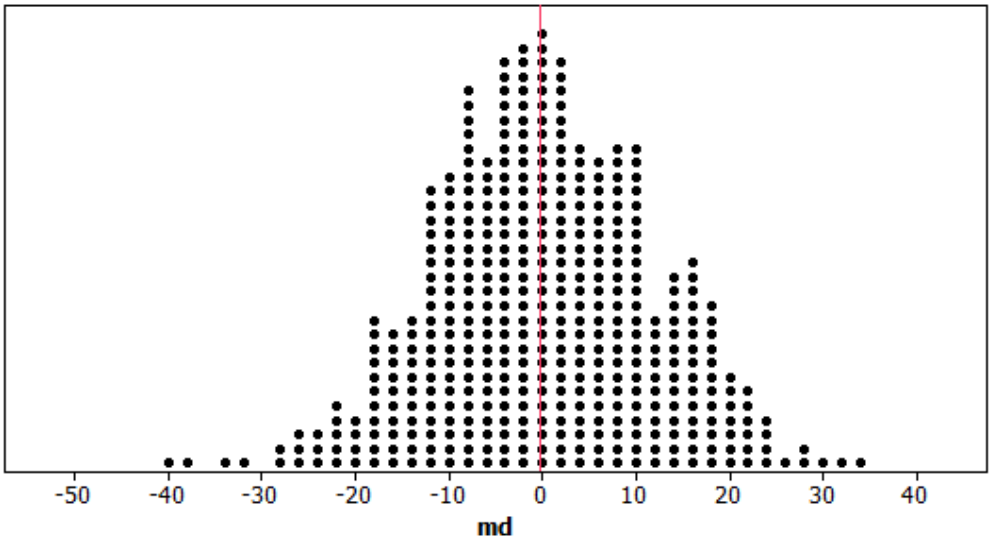


Figure 22: Distribution of the mean deviation.

While the analysis found a similar wide range of consumer variation to MQ4 the distributions appeared normal and it was elected to proceed with a simple mean value without clipping for the current project.

4.1.1 Consumer CMC relationship to demographic categories

The recorded demographic variables were:

1. Membership of organisation, charity or club (*46 groups, with 1-139 members*)
2. Gender (male, female)
3. Age group (*18-19, 20-25, 26-30, 31-39, 40-60, 61-70*).
4. Place of beef purchase (*butcher, supermarket, farmers' market, none*)**)**

Each sample (referenced by EQSref.day) was assessed by ten consumers. The average of these was computed and then the deviation of the value assigned by each consumer from this average computed. Thus if a consumer tended to rate high, they would have positive deviations (values above the average), while a consumer who rated low would tend to have negative deviations (values below the average).

While some demographic parameters were significant this was largely a consequence of the high number of observations and none were regarded as practically important given the typical small numerical difference and lack of convincing explanation for the minor charity group, gender, age or shopping habit differences. Table 10 provides gender data with the mean difference less than 2 CMC units with Table 11 summarising age categories, again with mostly overlapping distribution and relatively minor mean differences.

Table 10: Comparison of male and female CMC score distribution and standard deviation

gender	n	n*	mean	sd	Min	Q1	med	Q3	max
male	417	1	-0.91	10.86	-40.1	-8.3	-1.0	6.5	24.4
female	420	0	0.90	12.27	-38.7	-7.6	1.0	10.0	33.2
*	2	0	-1.70	15.70	-12.9		-1.7		9.4

Table 11: CRC score distribution by age category

```
Age Group    N   Mean  StDev   --+---------+---------+---------+-------
18-19       44  -0.99   9.90   (----------------*----------------)
2--25      164   1.65  12.76                       (--------*--------)
26-30      103  -2.17  13.35   (----------*----------)
31-39      199  -1.94  10.50       (-------*-------)
40-60      254   1.73  11.30                           (------*------)
61-70       74  -0.78  10.11        (------------*------------)
                        --+---------+---------+---------+-------
                       -4.0      -2.0       0.0       2.0
```

In response to the question **"From where do you usually purchase your beef?** *(Use* **X** *in all applicable)*" 627/836 (75%) bought beef at a supermarket at least some of the time; 332/836 (40%) from a butcher, and 37/836 (4.4%) from a farmer's market; while 51/836 did not purchase beef. While the mean scores were lower by several points for consumers purchasing from farmers markets this was also regarded as of limited importance and likely to reflect a general dislike of packaging.

This project utilised AMC and CMC to evaluate the impact of a number of factors that potentially might impact on consumer appeal in the retail case:

- AUS-MEAT meat colour assessed at MSA grading
- Individual cut AMC relationships at boning, prior to and post retail packing
- Dentition
- Effectiveness of AMC at boning versus pH as a predictor of CMC
- Packaging type (OWP, MAP and VSP)
- Days aged in vacuum packaging as a primal prior to retail packaging (5, 12 and 40)
- Days in retail packaging

4.1.2 AUS-MEAT meat colour assessed at MSA grading

MSA grade eligibility required an AUS-MEAT meat colour score (AMC) of 3 or less. This standard had been applied from the commencement of MSA and, while not used in MQ4 prediction as no relationship had been found, was adopted due to industry belief that consumers would not rate meat of AMC 4 and above as acceptable. The AMC grading score relates to a visual assessment of the quartered *M.longissimus* (LD) surface at the striploin/cube roll interface. A series of colour chips graduated from 1A, 1B, 1C (all light veal type colours) then 2 to 7 in whole number increments are viewed on the cut

surface with a standard light source to determine AMC. Assessment is only made by trained and certified graders.

Figure 23 graphically illustrates the observed CMC response to MSA grading by AMC chip. This graph includes all retail cut observations following industry practice and MSA requirements where the single LD reading is applied to the entire carcase and all cuts therein.

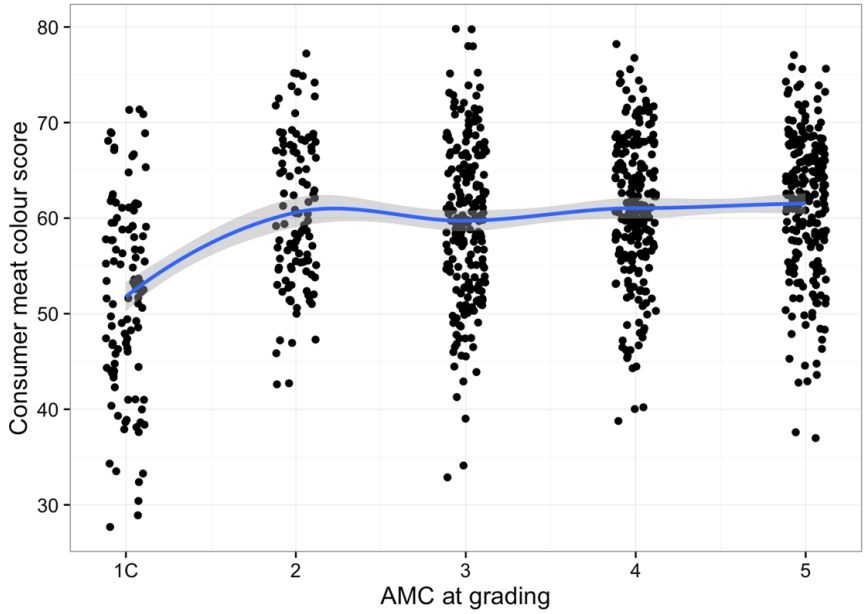


Figure 23: Consumer response to AMC

Contrary to expectation it can be clearly seen that consumers showed no discrimination between AMC 2, 3, 4 and 5 but discounted the lighter 1C. This finding was challenging but believed valid due to the strong balance within the experiment, large number of observations (20,140) and controlled display conditions where packs were displayed in an 8x8 Latin square relationship for all colour and pH combinations with tight control and balance of cabinet position for each cut set.

The result is further illustrated by the box plots in Figure 24.

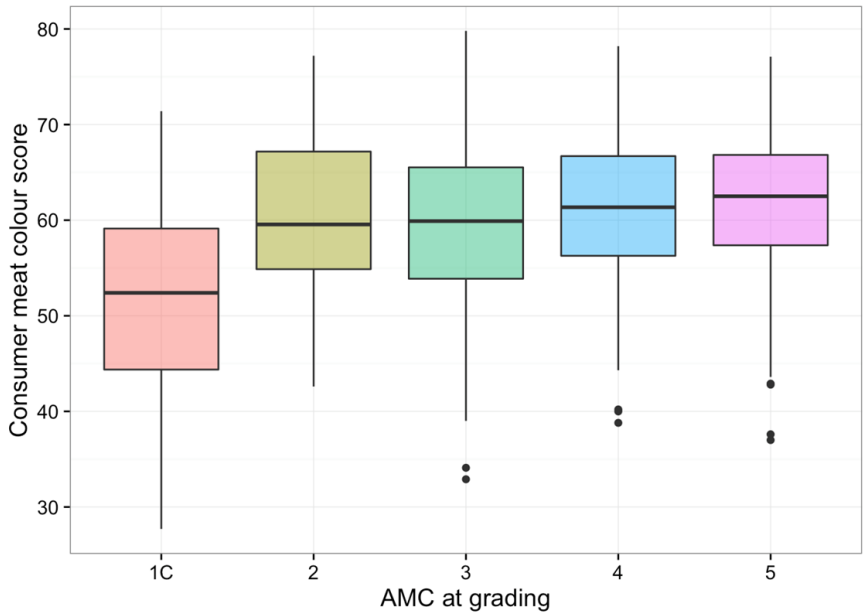


Figure 24: Consumer meat colour (CMC) score by AMC at grading

4.1.2 Individual cut AMC at boning, pre and post retail packing

The AUS-MEAT meat colour scores for every primal, comprising 2 tenderloins, 2 striploins and 2 rumps each replicated 3 times due to slicing and transfer to OWP, MAP and VSP packs was also evaluated against the consumer CMC scores. The AMC readings were assigned to each pack after a standard 20 minutes bloom period immediately before lidding or wrapping. The results, grouping each of the 3 primal ageing periods, are displayed graphically in Figure 25.

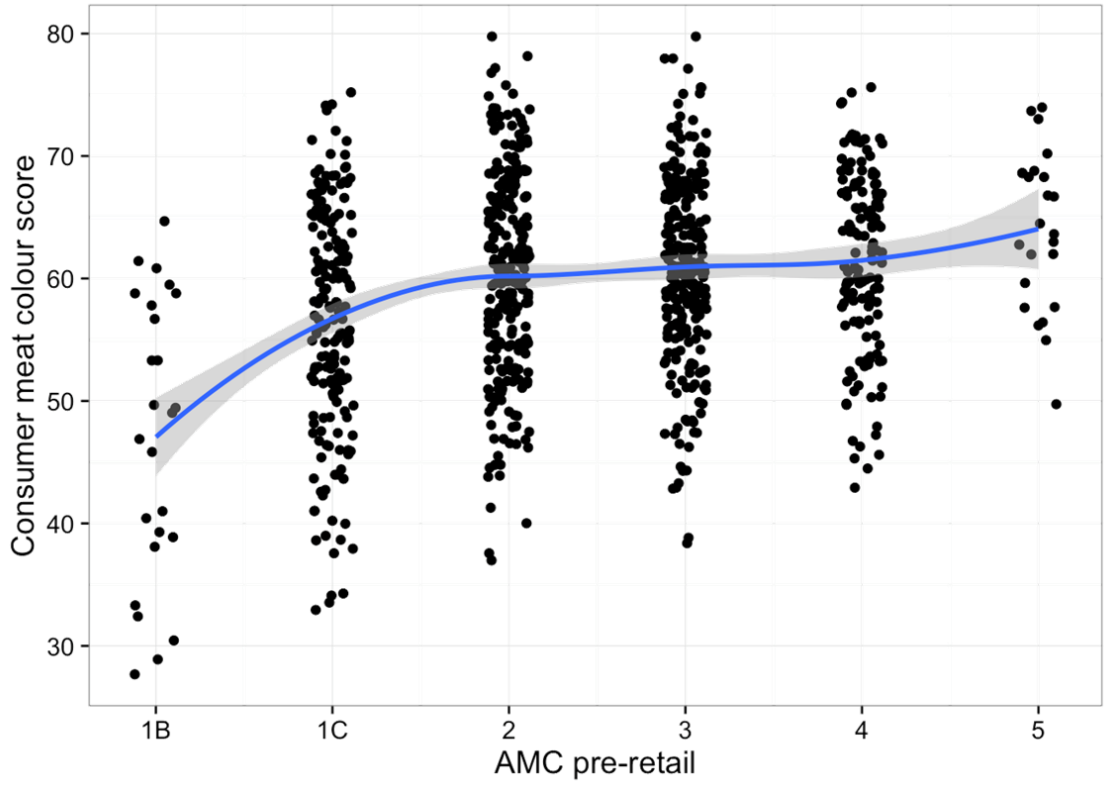


Figure 25: CMC and pre-retail AMC of individual retail trays incorporating 3 cuts and 3 replicates.

The graph indicates some movement in colour over the ageing period with a trend toward lighter colour seen in some 1B appearing and a reduction in AMC 5. The observed pattern between AMC and CMC remains however with the lighter colours scored substantially lower.

Clearly CMC could not be measured prior to retail display so only AMC or HunterLab values can be used to compare colour at grading (striploin only) and all cuts at boning, after each ageing period (pre-retail) and post retail. When AMC at grading was compared with AMC at boning it was found that while there was a relationship in striploin (STR) at boning no relationship was found in rump (RMP) or tenderloin (TDR) as illustrated in Figure 26.

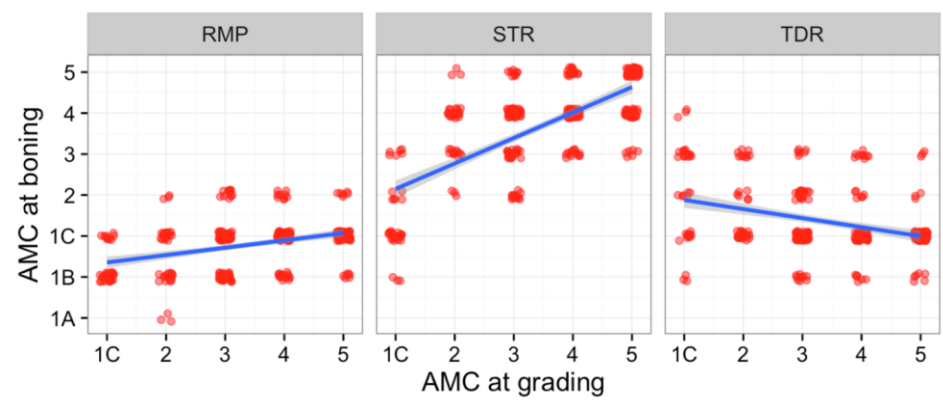


Figure 26: AMC at boning vs AMC at grading by cut

A further point noted was that despite the high proportion of carcasses selected as having meat colour above 3 and or pH greater than 5.7 no rumps and only two tenderloins from the same carcasses had an AMC above 3. This indicated that, for colour at least, the tenderloins and rumps excluded from MSA due to striploin meat colour in fact had acceptable AMC at boning. In essence there was practically no relationship between striploin AMC at grading and AMC for the other cuts at boning.

There was practically no relationship between AMC at grading and AMC post boning for all cuts selected from carcasses with a grading (striploin) pH >5.7 with the striploins constant around AMC 4 and the rumps and tenderloins around AMC 1C.

On the basis of these data AMC at grading was found to be ineffective as an indicator of AMC post boning with correlations of 0.391 (STR), 0.224 (RMP) and -0.248 (TDR).

When AMC at boning was compared with AMC pre-retail it was found that the striploin AMC tended to get lighter, while rump and tenderloin tended to get darker as illustrated in Figure 27 causing the individual cut meat colours to become more aligned on average after primal ageing. While the striploin correlation was strong at 0.768 it was moderate for rump at 0.409 and close to non existent (0.092) for tenderloin.

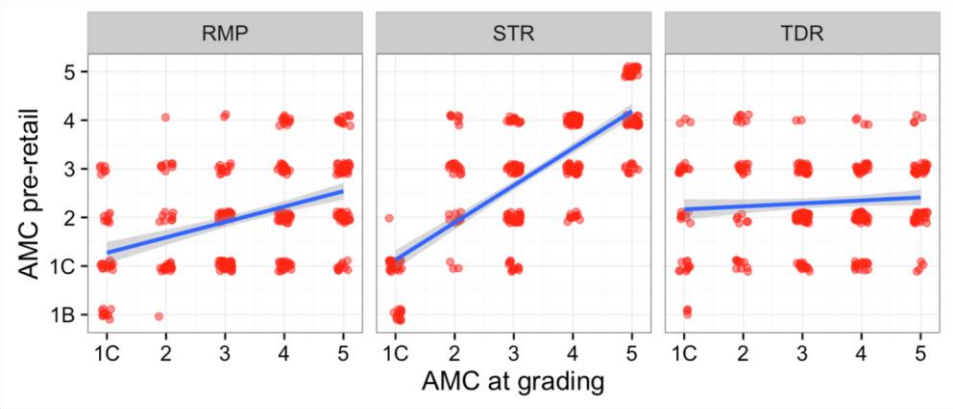


Figure 27: AMC pre-retail vs AMC at grading by cut

There was little difference between AMC at boning and AMC pre retail for groups either side of pH 5.7 although the two categories remained different.

Table 12 displays correlations between AMC at grading (striploin), boning, pre retail and post retail.

Table 12: Correlations by cut between AMC at grading, boning, pre and post retail packaging.

STR	AMCgrade	AMCbone	AMCpre-retail
AMCb	0.692		
AMCpre	0.768	0.638	
AMCpost	0.663	0.494	0.662

RMP	AMCgrade	AMCbone	AMCpre-retail
AMCb	0.360		
AMCpre	0.409	0.446	
AMCpost	0.362	0.279	0.359

TDR	AMCgrade	AMCbone	AMCpre-retail
AMCb	-0.341		
AMCpre	0.092	0.345	
AMCpost	0.123	0.188	0.225

It is seen that the correlations for STR are generally high and larger than for the other cuts: for STR they're around 0.65, for RMP around 0.35 and for TDR around 0.20, though with more variation. The strong negative correlation for TDR between AMC at grading & AMC at boning is unusual (partly explained by the odd result for one carcase). For each cut the correlation of AMC pre retail with AMC at boning is greater than the correlation of AMC post retail with AMC at boning. The post retail observation was impacted by packaging method and discussed further in section 4.1.5.

4.1.3 Relationship of dentition, AUS-MEAT meat colour and CMC scores

To objectively assess industry conjecture regarding whether, when corrected for pH and meat colour, consumers perceive a meat colour difference between cuts sourced from 6 teeth carcasses relative to 4 and 2 teeth a balanced design encompassing 2, 4 and 6 tooth cattle was employed across AUS-MEAT meat colours 1C to 5. Balance between pH above and below 5.7 was also sought for meat colours 3, 4 and 5. In practice the 1C were collected from four 0 and two 2 tooth carcasses with other cells well spread across dentition categories.

The results as shown in the box plot (figure 28) and further graphical representation (figure 29) indicate that for the 2, 4 and 6 teeth categories there is no sign of any dentition to colour relationship.

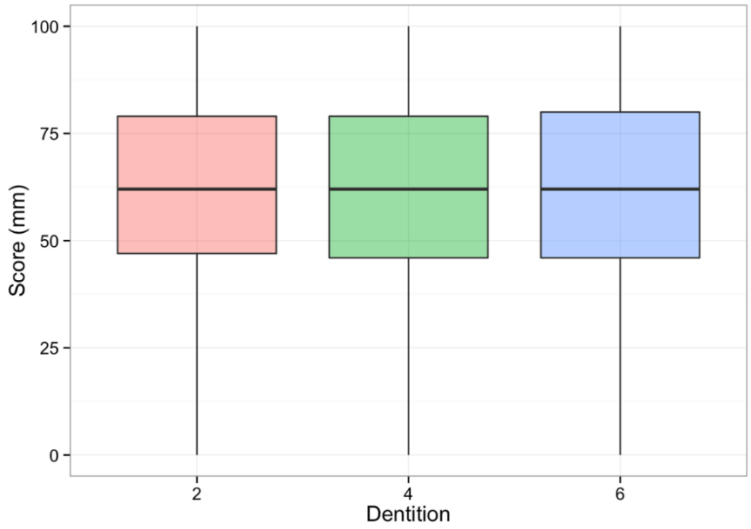


Figure 28: Box plot of consumer meat colour scores within dentition category.

More detailed analysis demonstrated that the lack of relationship extended across those with pH above and below 5.7 and across all days from slaughter to consumer observation variations and all packaging types, demonstrating that dentition has no effect on CMC.

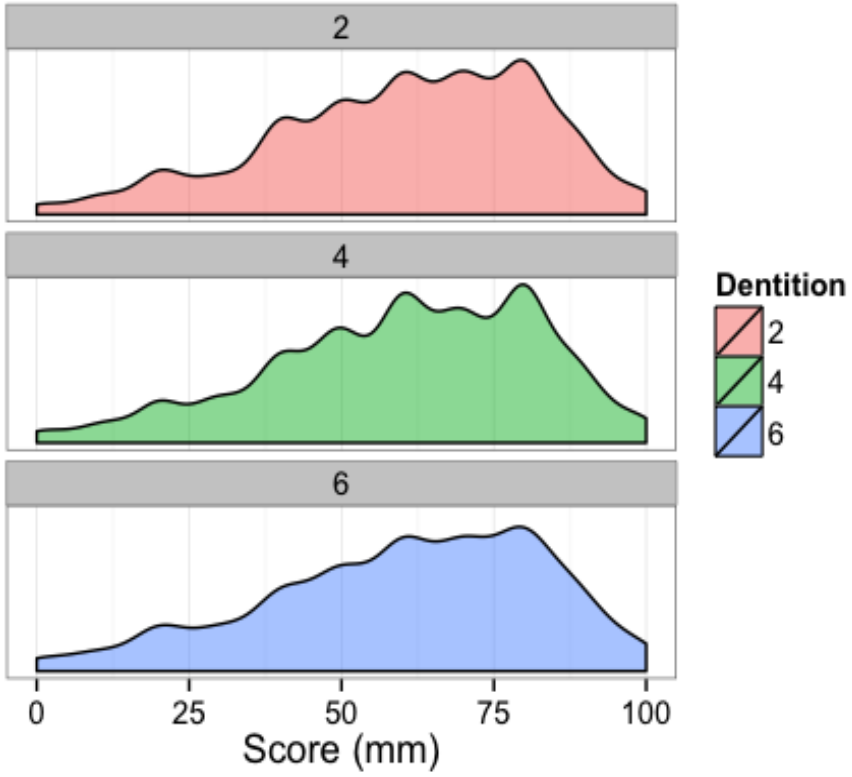


Figure 29: Distribution of CMC consumer scores within dentition categories.

4.1.4 Effectiveness of AMC at boning versus pH as a predictor of CMC

The CMC data were used to compare the effectiveness of the MSA grading standard of AMC less than 4 and pH below 5.71 to the use of ultimate pH alone, dentition having been ruled out by the results reported in 4.1.3. The analysis outcome is presented in Table 13.

Table 13: Effectiveness of MSA grading criteria (AMC <4 and pHu <5.71) relative to use of pHu, 5.71 alone.

	n	%
Total sample size	864	100%
pH < 5.71	666	77%
AMC < 4	432	50%
pH < 5.71 & AMC < 4	414	48%

	pH < 5.71 & AMC < 4		pH < 5.71	
	n	%	n	%
Definitely would buy	195	47%	357	54%
Definitely would buy if discounted	208	50%	297	44%
Definitely would not buy	11	3%	12	2%
Total	**414**	**100%**	**666**	**100%**

The analysis results clearly indicted pHu alone provided at least equal consumer protection while rejecting less carcasses. This result reflected the changes in AMC observed after the MSA grading assessment relative to pHu which remained stable from grading to post retail.

As shown pHu used alone removed an equivalent number of unsatisfactory (Definitely would not buy) samples while more accurately segregating the definitely would buy category. On the basis of this outcome the Pathways Committee recommended that AMC be dropped as an MSA grading criteria while pHu of <5.71 be retained. This recommendation was accepted by the MSA Taskforce and has since been endorsed by the AUS-MEAT Language and Standards Committee resulting in a change to the MSA standards.

4.2 Influence of Packaging type on Consumer Meat Colour scores

Figure 30 displays the CMC score distribution across all cuts, primal ageing and retail display periods for the three packaging systems. While OWP and MAP produced similar CMC score distributions the VSP boxplot is relatively, but not significantly, lower.

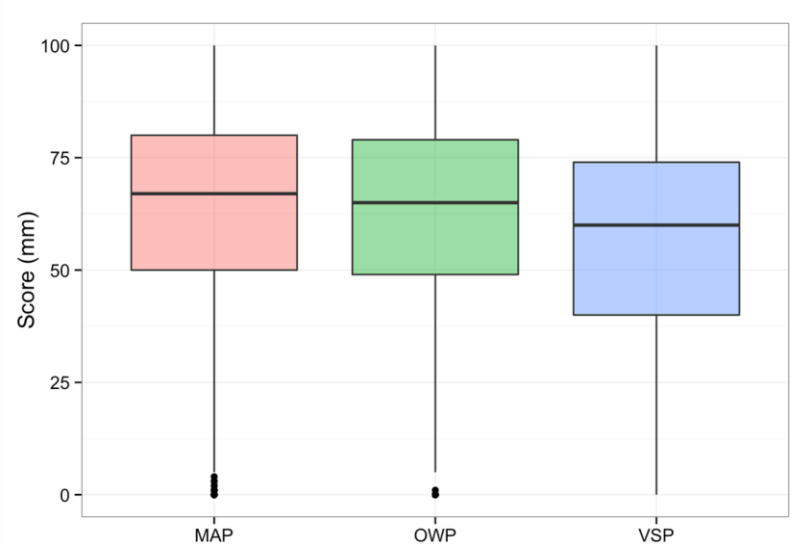


Figure 30: Consumer meat colour score (CMC) and packaging type

The OWP treatment was only displayed for one consumer observation period the day following retail packing whereas the MAP and VSP packs were observed at 3, 5 and 7 days post packing simulating retail distribution timing.

Figure 31 provides more specific data relating CMC to each packaging type in relation to the three purchase categories.

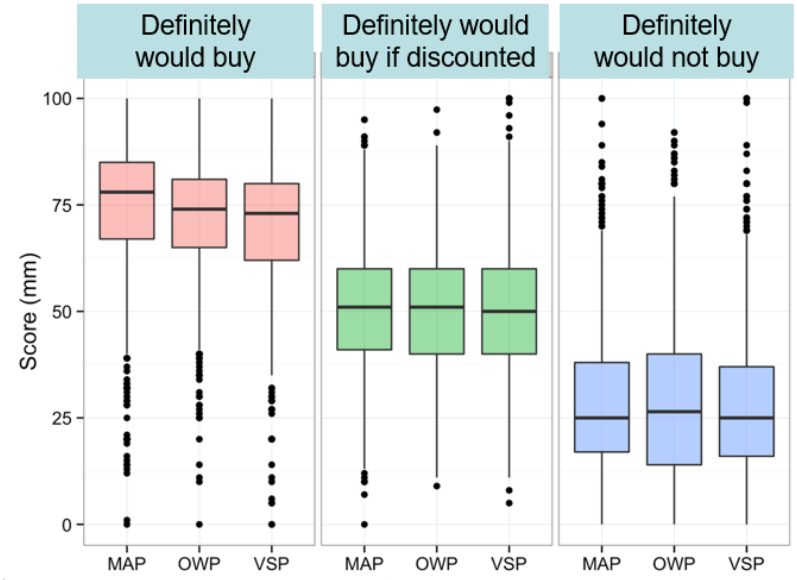


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Figure 31: Consumer CMC score relative to purchase intent and packaging type

While consumers returned similar scores for the categories 'Definitely would buy if discounted' and 'Definitely would not buy' across all packaging types the VSP and OWP 'Definitely would buy' thresholds had a lower trend. It was postulated that, despite each consumer being instructed to rate packs entirely on their colour without reference to a presumed eating experience, that the lower thresholds for OWP and VSP might reflect either prior experience with pleasing results from less optimal colour or alternatively a poor experience from attractive MAP product.

Figure 32 presents the relationship between CMC score at 3, 5 and 7 days after retail packaging for MAP and VSP product. It is evident that while the VSP product held constant over the 7 day period the MAP product decreased with the final 7 day CMC similar to the VSP.

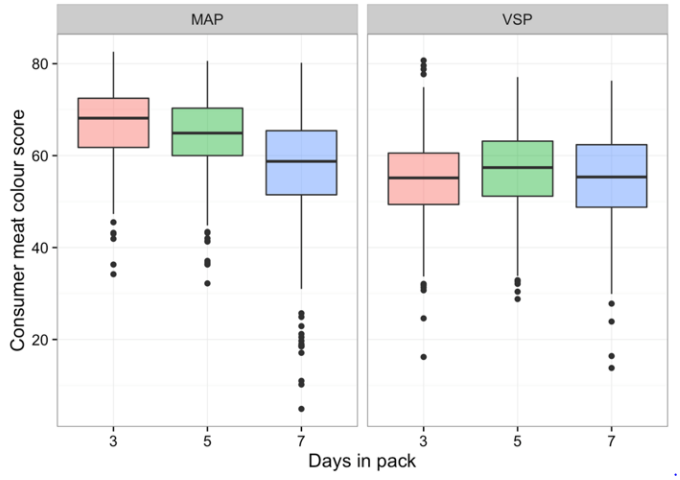

Figure 32: CMC by days in pack

Figure 33 presents further detail on the relationship between AMC at grading and CMC ratings of retail packs at 1 (OWP only) and 3, 5 and 7 days (MAP and VSP) post primal fabrication and retail packing.

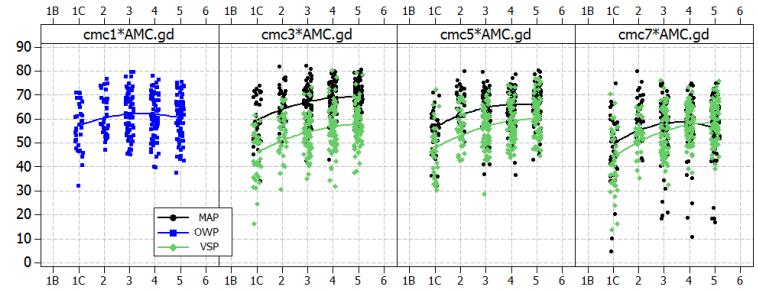

Figure 33: Consumer meat colour score (CMC) in relation to AMC at grading for 1 (OWP only) and 3, 5 and 7 days after retail packing (MAP and VSP).

A quadratic line is fitted to the Figure 33 plots and also to those in Figure 34 displaying comparisons of AMC at grading, boning, pre and post retail. A similar curvilinear pattern is observed with lesser CMC scores for the lighter 1B and 1C AMC values, higher values for AMC 2, 3 and 4 and a flattening out at AMC 5. The CMC deterioration for MAP product with increased days of retail display is evident in Figure 35.

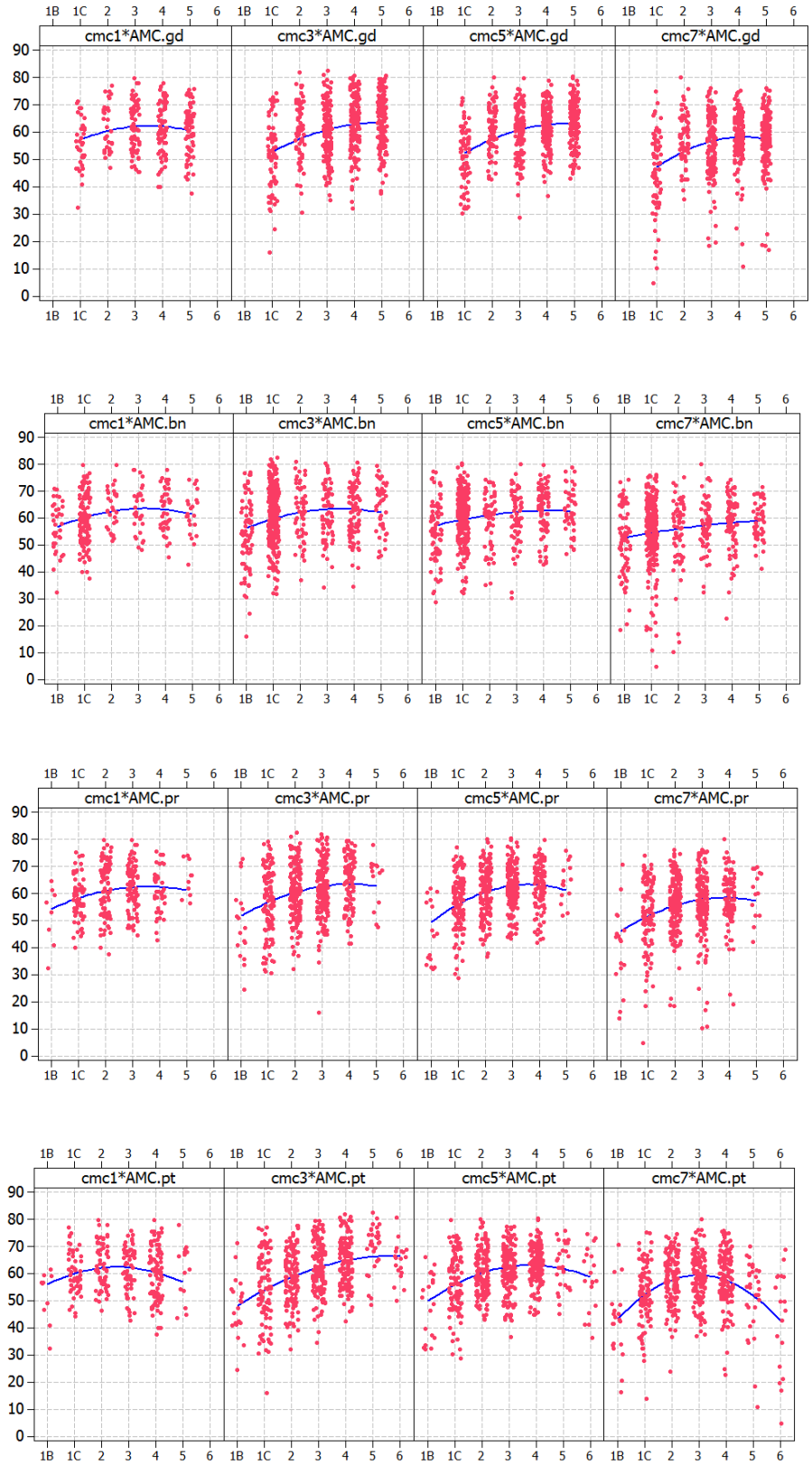

Figure 34: Relationship of CMC and AMC at grading, boning, pre and post retail display times of 1 (OWP), 3, 5 and 7 days (MAP and VSP)

The mean values for each pack method in relation to days of prior primal ageing, cut and days of retail display are presented in Table 14. As each cell contains 32 observations the data are balanced with the cell means effectively the interaction estimates.

While the VSP values are similar across both days of prior primal ageing and days of retail display the MAP values were found to significantly decline as retail pack days increased to a point where the less stable rump and tenderloin MAP scores were substantially below the VSP when packed after 40 days of primal ageing. An analysis of variance for CMC reduced to a final model of cut*days in retail pack+prior primal aged days for each cut found retail pack days and the cut*retail pack days interaction to be significant for MAP (P>0.01), cut, cut*retail pack days and primal days aged significant (P>0.01) for VSP with cut and primal days aged significant for OWP (P>0.01) which was only displayed for 1 day.

Table 14: Consumer colour score (CMC) means by pack, primal days ageing (horizontal axis), cut and retail display days (vertical axis).

		OWP				**MAP**				**VSP**			
		5	*12*	*40*		*5*	*12*	*40*		*5*	*12*	*40*	
RMP	*1*	63.2	56.9	55.5	*58.5*	*	*	*		*	*	*	
	3	*	*	*		64.6	69.1	66.5	*66.8*	50.2	51.3	52.9	*51.5*
	5	*	*	*		60.6	62.6	62.1	*61.8*	51.0	58.7	54.5	*54.7*
	7	*	*	*		57.6	55.4	52.3	*55.1*	50.8	58.0	57.7	*55.5*
		63.2	*56.9*	*55.5*	*58.5*	*60.9*	*62.4*	*60.3*	*61.2*	*50.7*	*56.0*	*55.1*	*53.9*
STR	*1*	66.7	61.7	59.2	*62.5*	*	*	*		*	*	*	
	3	*	*	*		66.3	64.7	68.2	*66.4*	54.6	55.5	58.9	*56.3*
	5	*	*	*		64.9	67.9	67.9	*66.9*	54.3	58.0	53.8	*55.3*
	7	*	*	*		59.2	60.4	61.4	*60.3*	55.6	53.6	52.8	*54.0*
		66.7	*61.7*	*59.2*	*62.5*	*63.5*	*64.4*	*65.8*	*64.6*	*54.8*	*55.7*	*55.2*	*55.2*
TDR	*1*	66.5	60.0	59.4	*61.9*	*	*	*		*	*	*	
	3	*	*	*		64.4	67.9	69.9	*67.4*	56.5	52.9	58.5	*56.0*
	5	*	*	*		65.6	63.5	61.6	*63.6*	59.2	62.7	59.2	*60.4*
	7	*	*	*		61.0	54.9	46.9	*54.3*	54.4	51.4	58.8	*54.9*
		66.5	*60.0*	*59.4*	*61.9*	*63.7*	*62.1*	*59.4*	*61.7*	*56.7*	*55.6*	*58.9*	*57.1*

The visual consumer CMC relationships were also reflected in the AMC values assigned by the MSA grader immediately prior to and post the retail pack periods for each primal ageing as displayed in

Figure 35. Again the relative stability of the VSP across cuts at all time periods contrasts with the deteriorating relationship evident in the MAP with increased days of primal ageing and increased retail display days. The OWP scores were also less stable for tenderloin and for rump and striploin after 12 and particularly 40 days of primal ageing.

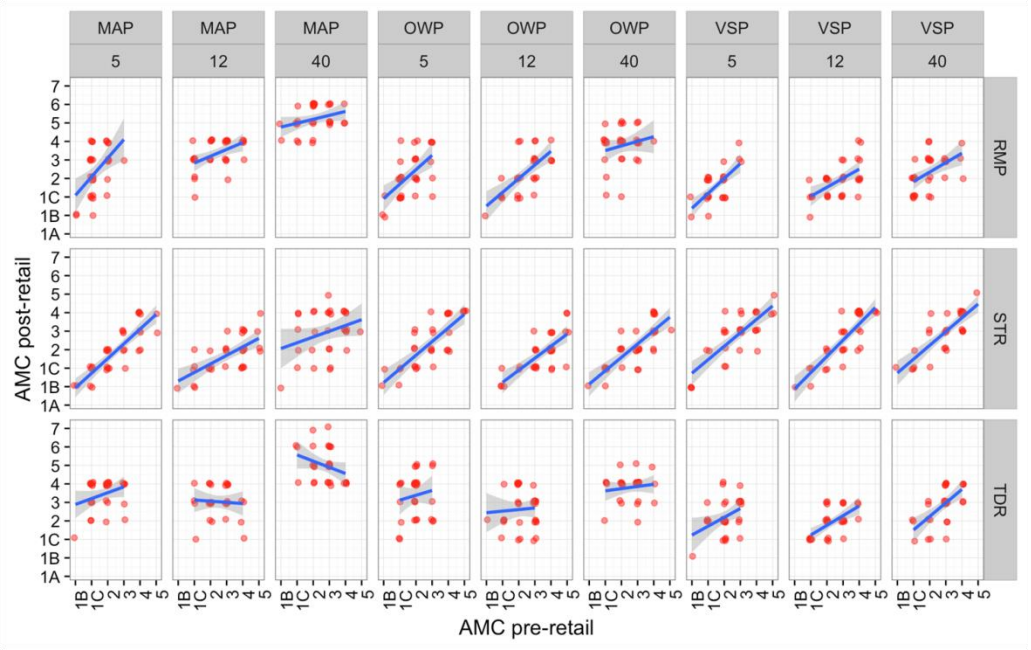

Figure 35: Pre and post-retail colour by pack and days aged

4.3 Cabinet position in relation to Consumer Meat Colour (CMC)

Cabinet position is often discussed as an important purchase driver of retail sales and routinely used to feature special offers and retail promotions. In relation to meat colour the impact of uniform and attractive colour is also often held to be important with any pack of different colour to adjacent packs regarded as likely to reduce merchandising impact. To ensure that the project evaluation was as fair as possible the trial protocols attempted to balance out these effects by displaying packs in an 8 x 8 Latin square sequence reflecting colour at grading and pH criteria within each cut and packaging type. Further protocol steps included displaying MAP and VSP product in separate “blocks” on a single cabinet side. The sides were rotated together with shelf and front or rear of shelf for each viewing day. As OWP product was displayed alone and for only one day in each ageing period the rotation related to cut and Latin square set across the 3 ageing periods.

An analysis of variance was run incorporating pack type, cut, days on shelf (pkd), shelf and position (Rpn - front, back and left, right or centre) and days aged was run and is displayed in Table 15. While all terms were significant (P>0.000 and P>0.002) other than days aged the numerical differences were slight and the practical consequences limited in regard to position with the largest estimates related to packaging type.

Table 15: Analysis of Variance for CMC in relation to cabinet cabinet display

Source	DF	SS	MS	F	P
cut	2	26949	13474	27.62	0.000
pkd	6	387496	64583	132.38	0.000
shelf	4	25320	6330	12.97	0.000
Rpn	4	8424	2106	4.32	0.002
dagd	2	631	316	0.65	0.524
Error	20085	9798794	488		
Total	20103	10259001			

S = 22.0877 R-Sq = 4.49% R-Sq(adj) = 4.40%

term	est	se
Constant	59.0143	0.2028
cut		
RMP	-1.6208	0.2220
STR	1.0882	0.2220
TDR	0.5326	0.2206
pkd		
OWP1	2.2991	0.4228
MAP3	7.5200	0.3882
MAP5	4.5580	0.3879
MAP7	-2.7156	0.3871
VSP3	-4.7167	0.3871
VSP5	-2.3879	0.3876
VSP7	-4.5569	0.3896
shelf		
1	1.9082	0.3405
2	-0.4672	0.3080
3	-0.3396	0.3139
4	-1.6505	0.3115
5	0.5490	0.3101
Rpn		
-1.0	0.9498	0.2924
-0.5	0.2953	0.5129
0.0	-1.0955	0.4189
0.5	-0.5117	0.5154
1.0	0.3621	0.2925
dagd		
5	0.1768	0.2205
12	0.0654	0.2216
40	-0.2422	0.2206

The analysis suggests that consumers give better ratings at the extremes of the display (top & bottom shelves, far left and far right). However, these differences are small and of doubtful consequence reflecting the mean shelf CMC scores displayed in Table 16.

Table 16: Average CMC for retail cabinet shelf and position.

Shelf	Average CMC
1	61
2	58
3	59
4	57
5	59

Position	Average CMC
Front	59.6
Back	60.2

It should be noted that the retail cabinet used was new and of high quality with LED shelf lighting. As such it was considered to have superior and more uniform lighting conditions than many commercial installations. Consumers were also directed to specific sets of 8 packs in nominated shelf positions ensuring that they would be viewed. While this resulted in the desired assessment of retail colour it should not be interpreted that shelf position has no influence on purchase behaviour. This was not tested within this project and is subject to many influences including line of immediate sight and merchandising cues.

4.4 Relationships between eating quality and meat colour

Consumer sensory testing data was analysed in conjunction with the consumer responses to colour and AMC at boning and grading. MSA grill protocols were used with consumers from Wagga Wagga and Melbourne participating in the sensory tests. Figure 36 displays a typical cooking and serving layout plus the 6 x 6 Latin square used to allocate sample order.

Figure 36: Consumer sensory testing

Data analysis found no relationship between consumer rated eating quality (MQ4) and consumer meat colour (CMC) scores, nor between eating quality and AMC at grading. This is illustrated in figures 37 and 38 below.

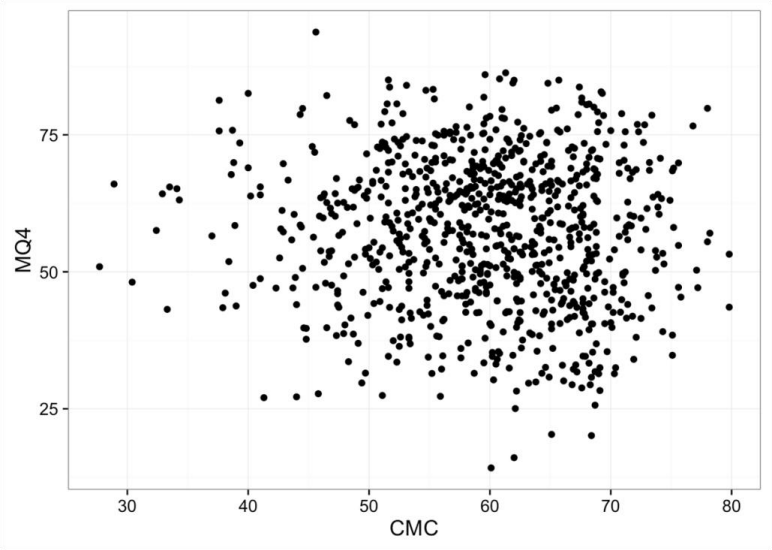

Figure 37: MQ4 and consumer meat colour

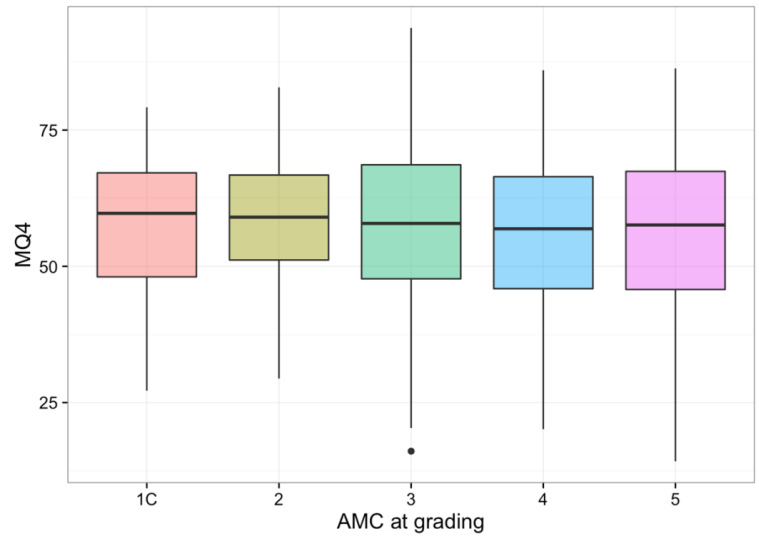

Figure 38: MQ4 and AMC at grading

4.5 Relationship between eating quality and packaging

In contrast to colour the relationship of eating quality to packaging type was highly significant with MAP MQ4 (Meat Quality 4 variables – Tenderness, Juiciness, Flavour and Overall Liking) scores substantially below those for OWP and VSP as illustrated in Figure 39.

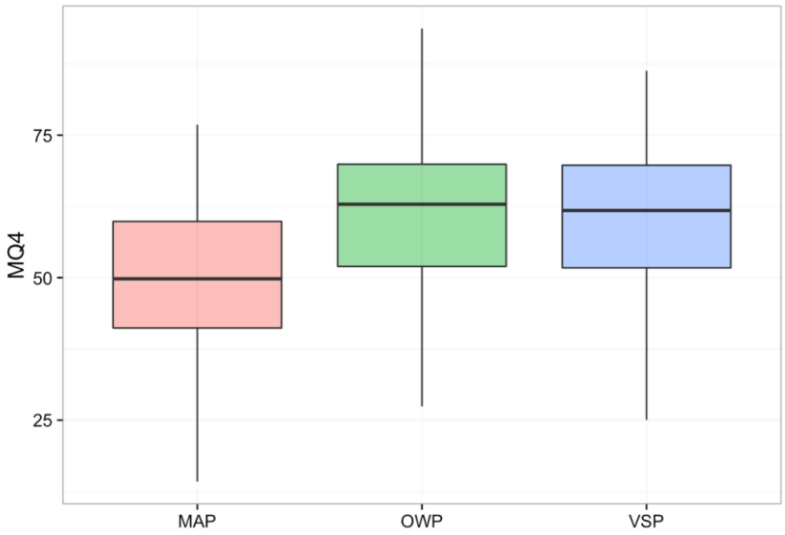

Figure 39: Eating quality (MQ4) scores by packaging

The same distinct pattern is evident within cut as illustrated by Figure 40.

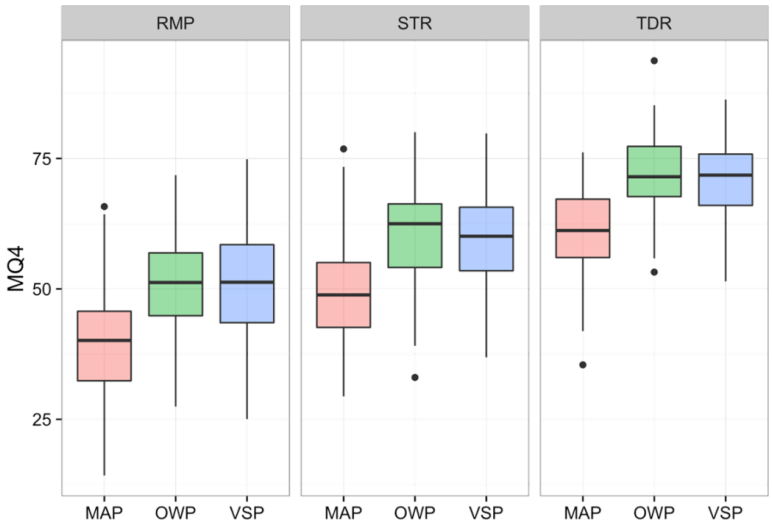

Figure 41: Eating quality (MQ4) scores by packaging and cut

A similar result was obtained within each of the three primal ageing periods as shown in Figure 42.

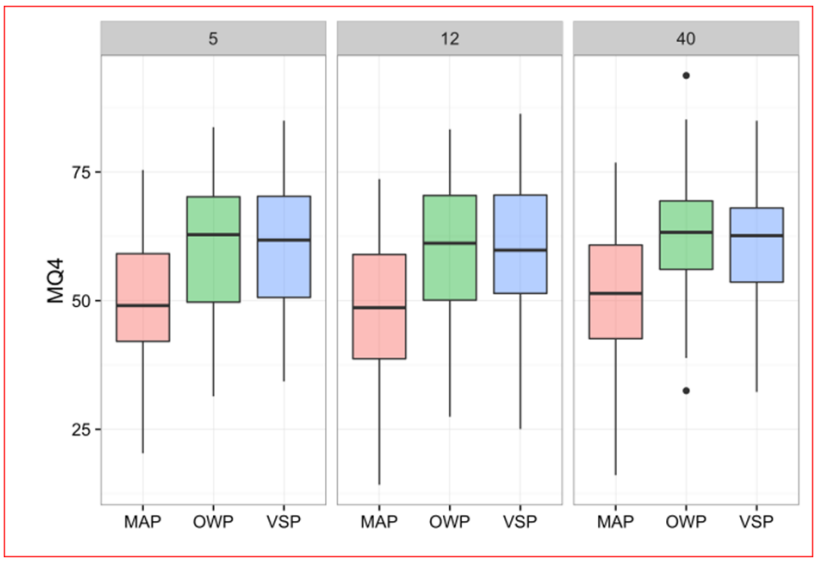

Figure 42: Packaging and MQ4 by prior primal days aged

The consistency of the MAP effect across cuts and ageing days indicates that it is a straight packaging effect rather than an interaction as further illustrated by Figure 43 which displays a consistent negative pattern across each cut by ageing cell.

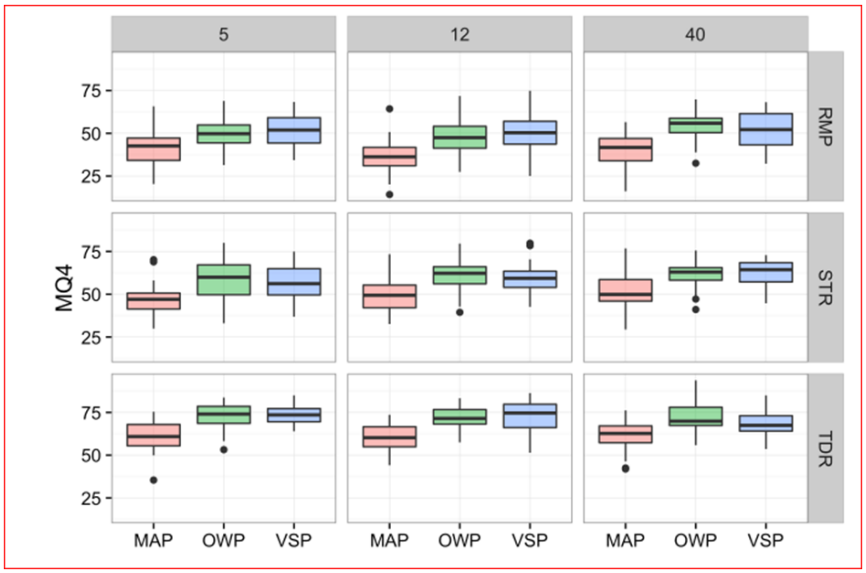

Figure 43: Packaging and MQ4 by cut and prior days aged

A number of analysis approaches were tested to confirm and cross check the magnitude of the detrimental MAP effect on MQ4. These included animal based models using all cuts, animal based using separate cuts and a covariate based model using separate cuts.

A final version of the animal based model with all cuts, considered applicable due to the balanced mix of cuts, ageing and packaging within each of the 46 animals, is displayed in Table 17. Terms for pack * days aged and cut * pack were removed after proving to be non significant (P>0.05) leaving a final model of MQ = animal + cut*days aged + Pack.

Table 17: Analysis of Variance for MQ4

Source	DF	SS	MS	F	P
animal	47	17122.0	364.3	5.61	0.000
dagd	1	351.3	351.3	5.41	0.020
cut	2	22225.7	11112.8	171.11	0.000
cut*dagd	2	2555.8	1277.9	19.68	0.000
pack	2	21949.6	10974.8	168.98	0.000
error	805	52282.1	64.9		
total	859	157261.4			

S = 8.059 R-Sq = 66.75% R-Sq(adj) = 64.52%

Term	est	se
constant	56.072	0.578
animal		
A5Q2	-5.555	1.999
B3F2	4.618	1.885
C7Q4	12.340	1.940
:		
Y5L0	-3.526	1.885
Z8W1	-2.746	1.885
dagd	0.04219	0.01814
cut		
RMP	-11.0326	0.8615
STR	-4.4842	0.8615
TDR	15.5168	0.8635
dagd*cut		
RMP	0.03035	0.02743
STR	0.13168	0.02744
TDR	-0.16203	0.02746
pack		
MAP	-7.1583	0.3897
OWP	3.8386	0.3882
VSP	3.3197	0.3882

The cut (muscle) and days aged estimates align in general with the MSA model estimates with the animal effects also within an expected range. The large packaging effect, driven by the strongly negative MAP estimate, was of concern and not included in model estimates. The alternative modelling approaches resulted in very similar packaging estimates of a 10 to 12 MQ4 point negative MAP effect.

The analyses were considered by the MSA Pathways Committee which recommended that a 12 MQ4 point penalty be applied to all cuts packed in 80:20 O2:CO2 gas mix. This recommendation was presented to the MSA Taskforce which endorsed the recommendation but elected to delay implementation to allow time for transition and potential further research that might address the problem. Two lines of research were proposed: a study to determine the effect development over the packaging period and changing the gas mix to 40:30:30 Nitrogen:Oxygen:Carbon Dioxide.

4.6 Objective measurement of colour

Preliminary analysis of HunterLab colour readings and limited NIXPro data was not encouraging in relation to predicting consumer CMC score. This finding added to earlier studies by Dr Ray Watson in which HunterLab output was examined in relation to the AUS-MEAT colour chips. His analysis indicated that there was significant variation in chips between plants/day/chip sets. He concluded that a score, Meat Red (MR) which is the average red wavelength (600 to 720) intensity, could be useful if the equipment had greater consistency. He presented data demonstrating that the current chips were not uniform in their progression with two serious anomalies between the 1C and 2 chips and between the 4 and 5 chips. He proposed a relationship of L* and chroma weighted 2:1 toward L* that could provide effective separation of the standard chips.

Analysis by Dr Garth Tarr evaluating the HunterLab wavelength and L*a*b* readings and consumer CMC scores indicated that consumer assessment was not fully explainable by pure colour measures but incorporated “something extra and not defined”.

The collected data is being combined with further data from other studies with additional analysis planned.

4.7 Flavour chemistry

A detailed flavour chemistry report has been completed by Dr Linda Farmer of the Agri-Food and Biosciences Institute of Northern Ireland (AFBI) and submitted within the contract. A birief outline of processes and results follows:

Flavour chemistry samples from each cut, primal ageing and packaging treatment were frozen (-21˚C) in Australia at the same time as their paired sensory samples and air freighted to AFBI where they were transferred to -80˚C storage. For the initial investigation, samples were selected as a 3 x 3 x 3 experiment for muscle, packaging and ageing in accordance with Table 18. Due to the number of samples available, RMP 231 and 131 were combined together to give a balanced design, but the data were also been analysed separately.

Table 18: Sample numbers by cut, packaging and ageing for initial analysis

Count		Packaging			
Muscle	Ageing	MAP	OWP	VSP	Grand Total
STR045	14	4	5	5	14
	21	4	5	4	13
	49	2	4	5	11
STR045 Total		**10**	**14**	**14**	**38**
TDR062	14	5	5	5	15
	21	5	4	5	14
	49	4	5	4	13
TDR062 Total		**14**	**14**	**14**	**42**
RMP131	14	2	4	3	9
	21	3	1	2	6
	49	2	2	3	7
RMP131 Total		**7**	**7**	**8**	**22**
RMP231	14	2	1	2	5
	21	2	3	2	7
	49	2	3	3	8
RMP231 Total		**6**	**7**	**7**	**20**
Grand Total		**37**	**42**	**43**	**122**

The beef was cooked in accord with the MSA protocol for grilled (medium), roast and casseroled beef. The following modifications were made to ensure that the cooking process was as similar as possible to that used for consumer panels:

Grill. Scrap spare meat was grilled before each test sample. When grilling each actual test sample was surrounded by standard portions of silverside beef.

Roasted. The samples received for roasting were 80 x 80 x 15 mm in dimensions, approximately 160g in total. The samples provided were “sandwiched” between two pieces of cheap local beef and netted to create a “roast” of similar size and weight to those usually roasted in the MSA roast cooking protocol. After cooking, samples were taken using a 12.5mm corer and internal samples chopped and analysed according to the grill protocol.

Slow Cook. The vegetable component of the casserole mix was formulated according to the MSA protocol for slow cook, and cubes of steak were cooked according to this protocol. A stock was prepared in equivalent proportions to those used in consumer panels. Mixed vegetables (450g: carrots parsley, celery and leek) plus 2.5l of water and 8.75g salt were simmered for 45 minutes.

Two sample cubes (approx 15mm x 15mm) were browned for 90 seconds in olive oil and then transferred to a bain marie steamer pan containing 27 ml of stock. Once cooked, samples were transferred to a sieve, gently shaken and two cores taken.

The volatile aroma compounds from the resulting samples were collected on to SPME fibres and analysed by GC-MS (Agilent 5973 MSD/ HP6890 GC) using a Zebron-5MS, 30m length, 0.32m diam.,

0.50µm column, according to the protocol prepared in 2009-10 on procedures for the analysis and quantification of odour volatiles from beef prepared according to the MSA cooking protocols.

The data was quantified automatically using an Agilent integration method based on three target ions and one quantification ion. The resulting data was combined into one spreadsheet using an AFBI "in-house" macro.

Quality assurance was conducted by checking the integration of 10% of the automatic quantifications, together with any results which appeared unusual. Actual linear retention times and mass spectra were checked against those of authentic standards of compounds and/or published values.

An external standard was run each day with n-alkanes to ensure reproducibility of instrument performance.

The compounds analysed in the headspace from each beef sample are listed in Table 19, together with their retention times (RT), linear retention indices (LRI, a standardised indicator of elution time relative to alkanes) and comments on their origin and relevance.

Table 19: List of compounds quantified

Compound	Mean RT (mins)	Mean LRI[a]	Comments
Alcohols			
1-Propanol	1.44	<600	
1-Hexanol	8.60	871*	Probably from thermal breakdown of lipids
2-ethylhexanol	12.95	1028*	Probably from thermal breakdown of lipids
Aldehydes			
Pentanal	2.95	??	
Heptanal	9.56	900	From thermal breakdown of lipids.
Octanal	12.32	1001	From thermal breakdown of lipids.
Nonanal	14.70	1105	From thermal breakdown of lipids.
(E)-2-Butenal	1.42	<600	
2-methylpropanal	1.42	<600	Strecker aldehyde from breakdown of amino acids. Marker compound for the Maillard reaction.
3-methylbutanal	2.25	<600	Strecker aldehyde. Marker for Maillard reaction.
2-methylbutanal	2.38	<600	Strecker aldehyde. Marker for Maillard reaction.
benzaldehyde	11.21		Strecker aldehyde. Marker for Maillard reaction.
Heterocyclic compounds			
2-methyl furan	1.49	<600	
2-methyl thiophene	4.73		From Maillard reaction
2-pentyl furan	7.99	989	From thermal breakdown of lipids, especially linoleic acid.

Compound	Mean RT (mins)	Mean LRI[a]	Comments
Ketones			
2,3-butandione	1.60	<600	From Maillard reaction
2-butanone	1.61	<600	From Maillard reaction
2-pentanone	2.71	<600	From Maillard reaction
2-heptanone	9.07	885	Probably from thermal breakdown of lipids
3-heptanone	9.07	883	From thermal breakdown of lipids
4-methyl-2-pentanone	4.17	<600	Probably from Maillard reaction
5-methyl-3-hexanone	8.84	888	
Sulphur compounds			
dimethyl disulphide	4.05	<600	From Maillard reaction
dimethyl trisulphide	11.38	967 *	From Maillard reaction
methyl propyl disulphide	5.58	<600	From Maillard reaction
Other			
ethyl acetate	1.71	<600	
Heptanes	2.98	700	
n-hexadecanoic acid	28.78	1944	

Differences between muscles and ageing periods were significant for a few compounds, while those caused by packaging and cooking were more extensive including a number of very highly and highly significant differences in volatile compounds, a relevant example being the pentanal difference illustrated in Figure 44 and the lowering of Strecker aldehydes shown in Figure 45.

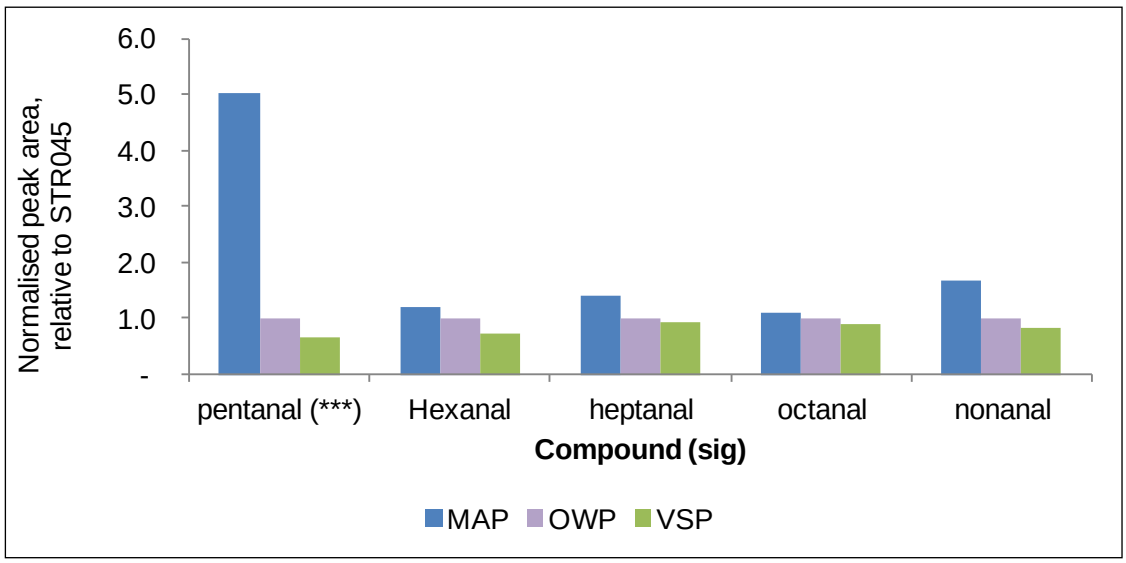

Figure 44: Effect of packaging on n-aldehydes (average of 4 muscles and 3 ageing periods shown relative to overwrap (OWP) = 1

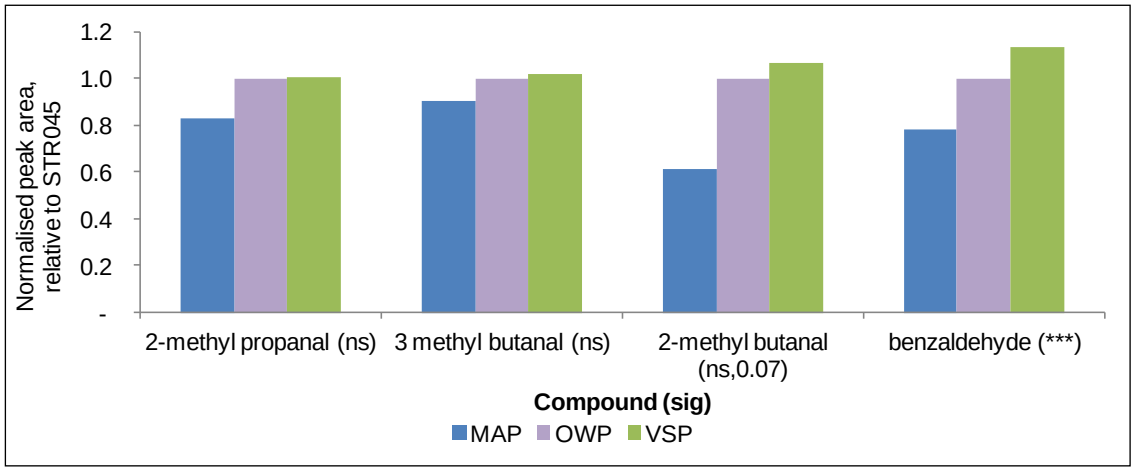

Figure 45: Effect of packaging on Strecker aldehydes (average of four muscles and three ageing periods, shown relative to OWP=1)

Data of this nature indicates that flavour can be altered by packaging processes and that the volatile compounds can act as marker compounds to indicate the changes occurring. They also have the potential to act as marker compounds for consumer liking. There were fewer differences between overwrapped and vacuum-packed beef. Vacuum-packed beef had significantly lower concentrations of two ketones, an alcohol and a furan. Further investigation will be needed to understand why these compounds alone show this effect, but all are likely to be formed via oxidation pathways and it is possible that the reduced oxygen in VSP compared with OWP has caused this effect. However, two of them were also reduced by MAP, which is less easy to understand.

The initial work focused on the volatile aroma compounds. Subject to a further contract, it is planned to conduct analyses of flavour precursors to determine whether the changes in volatile compounds can be related to changes in the composition of the meat. If this is so, then this may suggest which precursors need to be increased when using less favoured cuts.

The study to date has provided a new understanding of the factors affecting the formation of groups of flavour compounds in cooked beef. This evidence will enable new processing methods to be proposed to remedy flavour deficiencies and manage flavour formation in commercial beef products.

5 Discussion

5.1 Inferences and insights from the data relative to previous research

This research has significantly increased knowledge and understanding of: consumer assessment of beef colour and its relationship to eating quality; a greater understanding of consumer response to eating quality and colour preferences for beef packed in alternate packaging styles (OWP,VSP and VSP); of the alignment of pH at grading with ultimate retail beef colour, and the relative correlation to visual AUS-MEAT colour assessment at grading; pioneering studies around analysis of flavour volatiles related to consumer response to identify flavour precursors and mechanisms that may explain consumer assessed

response; the feasibility of using an objective colour measurement at grading as an alternative to subjective human evaluation; the potential to correlate objective (HunterLab meter) output with subjective MSA grader assessments based on AUS-MEAT standards and quantification of the serious detrimental effect of high oxygen MAP retail packaging on eating quality.

5.1.1 pH and colour

The results of this study demonstrate that, contrary to industry perceptions, consumers discounted lighter coloured (1C) meat colour but did not differentiate the higher scores. The evidence indicated that meat colour of the three cuts changed over time and did not relate well to other cuts at grading where the grading reference striploin score was universally higher than either the rump or tenderloin. Further examination indicated that a 5.7 pH cut off for MSA grading without any colour reference could yield a higher proportion of graded cuts with no loss in accuracy. Dentition was found to have no influence on consumer meat colour acceptability.

This trial provided strong evidence that striploin meat colour at grading above AMC 3, where pH is below 5.71, had no impact on consumer visual acceptance or eating quality. Consequently a recommendation to remove the screening requirement for carcasses to have an AMC 3 or less at grading to be eligible for MSA grading was removed and enacted by industry. Subject to the change being adopted in company and major customer specifications this change will increase the number of carcasses MSA graded and redress a current anomaly, common in grass fed groups, where carcasses with acceptable pH are excluded by slow developing meat colour of 4 at the time of grading.

5.1.2 Packaging

For several years there have been consistent research reports that high oxygen MAP packaging has a detrimental impact on the eating quality of beef. Many of these studies have been small and few included direct sensory appraisal. While the effect has been known “in the background” it has not been brought to industry attention and 80:20 MAP (80% Oxygen: 20% Carbon Dioxide) has continued to be widely used in many countries as a primary retail packaging. A 2015 MLA funded CSIRO trial evaluated lamb samples in MAP and VSP under MSA protocols and recorded a 5 point MQ4 reduction with MAP adding to concerns regarding potential effects on beef.

This project on beef colour, pH and packaging examined consumer visual appraisal of beef packaged in 80:20 MAP, VSP and overwrap as well as consumer rated eating quality utilising MSA consumer sensory protocols. The study quantified a 12 MQ4 point detrimental effect from MAP relative to both VSP and overwrap with the impact unaffected by muscle (striploin, tenderloin and rump tested) or by time in vacuum packaging prior to retail packing (5, 12 and 40 days tested).

The scale and consistency of this effect was unexpected and raised serious concerns. The MSA Pathways committee considered the data and recommended that MSA apply a 12 point discount to MAP packed product. The mechanism to apply this recommendation requires some time to develop. The MSA beef taskforce acknowledged the research outcomes and requested further investigation of alternative solutions before endorsing the implementation of the 12 point discount.

5.2 Challenges for project delivery

Difficulties encountered in completing milestone 2, and actions to address them, are reported in two areas. The first relates to HunterLab readings where a series of problems arose due to technical meter problems. The University of New England provided a HunterLab meter on loan for the initial round of the experiment but advised it was not available for the second round due to a conflict with a proposed sheep kill. This was addressed by arranging to borrow a second unit from the University of Melbourne. Prior to the third (40 day aged) round the UNE meter was reported as non functional and the Melbourne University meter was unavailable. A further meter was arranged via HunterLab in Hong Kong and subsequently used. This was then exchanged for the repaired UNE unit only to discover that in fact it was still inoperative. Being Saturday night the agent could not be contacted and a further solution was engineered by arranging to borrow a NSW Government unit from the Research station at Cowra. This was an old unit with battery pack problems but a complete final round of readings, in conjunction with NIXPro, was obtained on the 40 day packs after final consumer observation. Unfortunately however all readings were lost during the download procedure due to a power failure.

Following this series of challenges agreement was reached to enable purchase of a new HunterLab meter, subsequently delivered on December 9th. A workaround for the missed samples was initiated which required the missing 192 readings to be re-obtained by testing the MSA consumer sensory steaks after thawing and immediately prior to cooking. This in turn required individual steaks to be identified within all 24 sensory testing sessions, each comprising 3 sittings of 20 consumers with some in Wagga at CSU but a majority at multiple Melbourne venues. To reduce the complexity of achieving this consumer test sessions were re-allocated between CSU and Melbourne to maximise the number of HunterLab readings that could be taken in the single CSU venue and the loan unit from Hong Kong was re-acquired pending arrival of the new unit. Subsequent Melbourne based sessions were scheduled to avoid time conflicts with CSU and enable testing of designated samples with the single meter.

These actions resulted in a complete set of HunterLab readings for all retail packs and related initial carcase and grading readings. Replicated NIXPro readings were also taken of the sensory samples to further comparison of the two systems.

The second challenge related to examination of the duplicate consumer colour scoring data. Initial evaluation revealed an expected pattern of scattered differences of <2 mm across all consumer sessions but also an unexpected and unacceptable common set of large errors where the same obvious data entry errors – for example a score of 8078 on a 100mm line scale – was present in both files indicating that one was essentially a slightly modified copy of the first.

This issue was addressed by re-measurement and keying by a third independent party with rigorous comparison to the first data. The re-keyed data was forwarded for analysis replacing the original.

In addition to the challenges discussed above milestone 4, which required that the samples be shipped to Utah State University for GCMS flavour volatile analysis conducted by Dr Linda Farmer of AFBI and Dr Jerrad Legako of USU, was modified after establishing that samples for laboratory analysis could be shipped directly to Northern Ireland despite being despatched from a non EU registered facility.

This was contrary to early advice that this was not possible, resulting in the intention to ship to USA, conduct the analysis at Utah State University utilising Dr Legako who had previously conducted MSA

project work with Dr Farmer at Texas Tech University and then forward the data to AFBI for analysis. The change in protocol provided improved efficiency and benefits in utilising AFBI laboratory staff, in particular the chief chemist Dr Terrence Hagan, and equipment simplifying the project management and analysis.

In practice considerable delays were encountered despite prompt provision of an import permit from Northern Ireland. The trial protocol utilised an EU registered slaughter establishment with vacuum packed primal transferred to Teys Australia Food Service.

The on-site AQIS vet at Wagga interpreted the regulations as prohibiting export to the EU despite the provision of an import permit and refused to issue the required Australian export documentation. After protracted discussion, with the time delays requiring the reissue of the import permit on two occasions, Dr John Langbridge of Teys raised the matter with AQIS at a senior level in Canberra. An export permit was then issued and arrangements completed for air freight to Belfast.

Airfreight arrangements had to ensure that the product remained frozen during transit and that the port of entry was in the United Kingdom. This necessitated entry through London Heathrow rather than Dublin and transfer to Belfast. This was successfully arranged with the shipment held until after Easter to guard against possible disruption. Confirmation of arrival was received by email on June 17th, 2016 and GCMS analysis commenced shortly thereafter.

6 Conclusions/recommendations

The principle conclusions from the research were reviewed at the Pathways Committee meeting of March 31st and April 1st 2016 with agreement that:

1. The existing requirement that only carcasses with an ultimate striploin pH below 5.71 be eligible for MSA grading be retained.

   While the direct eating quality impact of slightly higher pH is slight at best there are significant detrimental effects in regard to microbiological growth and cooking with high pH meat that warrant exclusion.

2. The current screening requirement for carcasses to have an AMC 3 or less at grading to be eligible for MSA grading be removed.

   This recommendation was presented to Taskforce previously but not enacted with a request for further data. The recent extensive trial provides strong evidence that striploin meat colour at grading above AMC 3, where pH is below 5.71, has no impact on consumer visual acceptance or eating quality. Consequently it is regarded as superfluous and an unnecessary impost on industry.

3. The Taskforce and industry be advised that 80:20 high oxygen packaging has a serious detrimental effect on beef eating quality relative to overwrap and VSP. This effect is estimated at 12 MQ4 points and is constant across days aged prior to packing and across the three muscles evaluated.

This finding is a result of this project and is consistent with other published work for beef and lamb.

4. Mechanisms be developed within the MSA grading structure to apply a 12 MQ4 point deduction to any MSA graded MAP packaged product.

   In effect this would reduce all 5 product to 4*, all 4* product to 3* and all lower 3* to ungraded. There are operational issues in that the MSA output would require modification to designate two cut-off points within 3* to enable MAP product to be differentiated.*

On 6th May 2016 Taskforce endorsed the Pathways recommendations and resolved to forward recommendations for a change in MSA grading standards to the AUS-MEAT Language and Standards Committee.

7 Key messages

- AUS-MEAT meat colour (AMC) measurement at MSA grading is a poor indicator of meat colour of rump and tenderloin at boning and after subsequent ageing. It has no relationship to eating quality.
- A consumer meat colour score (CMC) was developed and shown to provide an excellent estimate of colour acceptability across cuts, packaging and days ageing.
- A maximum ultimate pH of 5.7 should be retained for MSA due to microbial and stability issues and provides an effective screen to remove unacceptable consumer CRC scored beef.
- Meat colour should be removed as an MSA requirement.
- While CMC is higher for MAP packaging in the initial display period it deteriorates rapidly reaching parity with VSP packs by 7 days.
- MAP has a highly detrimental effect on eating quality at or before 9 days of packing relative to OWP and VSP. This effect is constant across the 3 muscles tested and prior primal ageing periods.
- An MQ4 point deduction of 12 points should be applied to all 80:20 MAP product that is MSA graded.

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9 Appendix

9.1 Consumer demographics input – sensory testing

TPB TPB

Please use a black pen to fill in the form and write crosses in boxes like this ☒

5. How often do you eat Beef?
(in any form such as steaks, roasts, stews, casseroles, kebabs, BBQ etc.?
*(Use **X** in one box only)*

- ☐ Daily
- ☐ 4-5 times a week
- ☐ 2-3 times a week
- ☐ Weekly
- ☐ Fortnightly
- ☐ Monthly
- ☐ Never eat beef

6.1. How many adults (18 and over) normally live in your household ?
*(Use **X** in one box only)*

- ☐ 1 Adult
- ☐ 2 Adults
- ☐ 3 Adults
- ☐ 4 Adults
- ☐ 5 Adults
- ☐ 6 Adults
- ☐ 7 Adults
- ☐ 8 and over adults

6.2 How many children under 18 years normally live in your household??
*(Use **X** in one box only)*

- ☐ 0 Children
- ☐ 1 Child
- ☐ 2 children
- ☐ 3 Children
- ☐ 4 Children
- ☐ 5 Children
- ☐ 6 Children
- ☐ 7 and over children

7 Please read the following statements
*and use **X** in one box only for the **one statement** that applies to you*

- ☐ I enjoy red meat. It's an important part of my diet
- ☐ I like red meat well enough. It's a regular part of my diet
- ☐ I do eat some red meat although, truthfully it wouldn't worry me if I didn't
- ☐ I rarely / never eat red meat

8. When you eat beef, such as steaks, what level of cooking do you prefer?
*(Use **X** in one box only)*

- ☐ Blue
- ☐ Rare
- ☐ Medium / Rare
- ☐ Medium
- ☐ Medium / Well done
- ☐ Well done

9. What level of income best categorises your combined household income ?
*(Use **X** in one box only)*

- ☐ Below $ 25,000 per year
- ☐ $ 25,001 - $$ 50,000 per year
- ☐ $ 50,000 - $ 75,000 per year
- ☐ $ 75,001 - $ 100,000 per year
- ☐ $ 100,000 - $ 125,000 per year
- ☐ $ 125,000 - $ 150,000 per year
- ☐ More than $ 150,000 per year
- ☐ Prefer not to say

10. What level of education have you reached? *(Use **X** in one box only for the highest level achieved)*

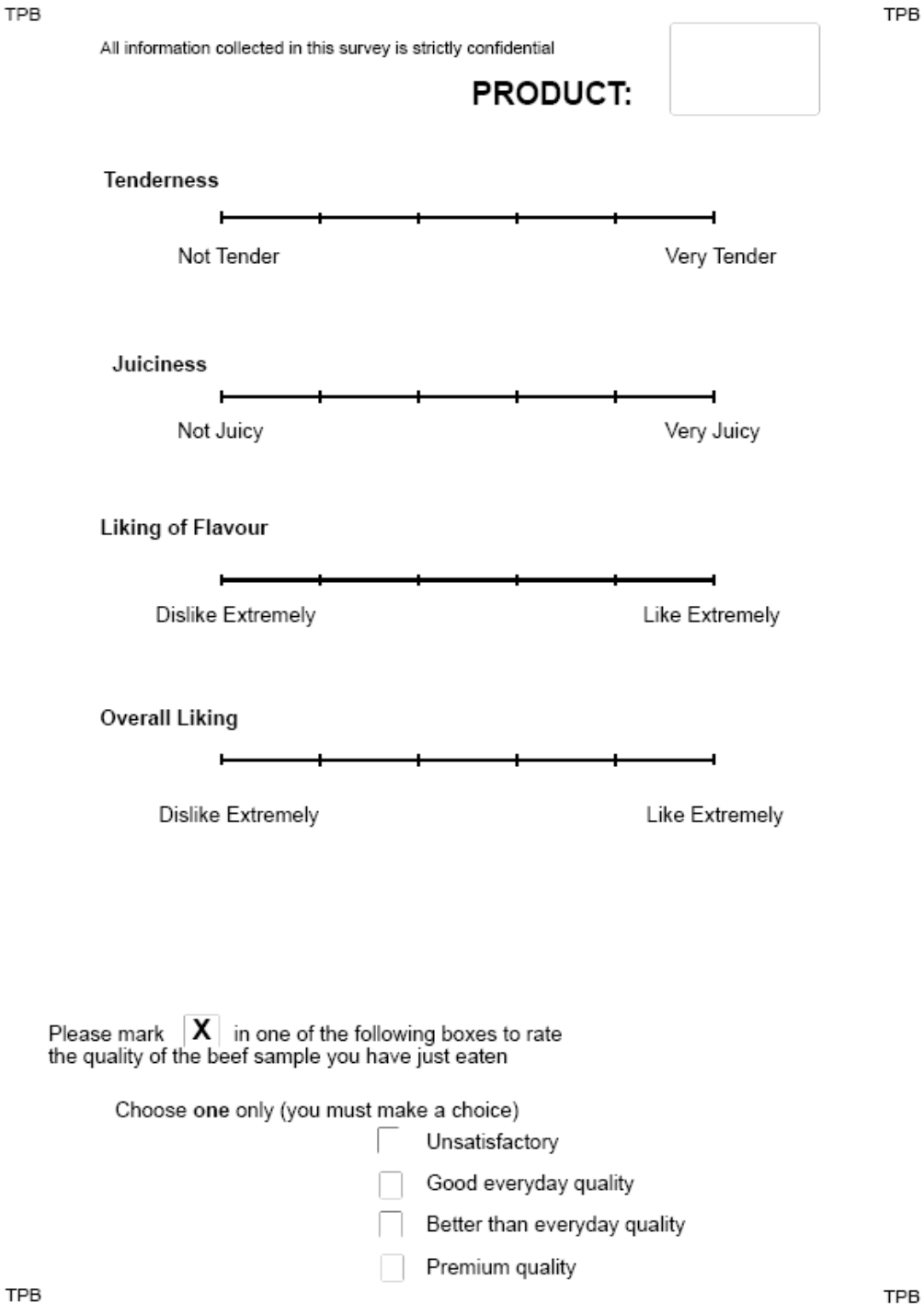
- ☐ Did not complete Secondary School
- ☐ Completed Secondary School
- ☐ A College/ TAFE course
- ☐ University Graduate

11. What is your cultural heritage ?
*(Use **X** in one box only)*

- ☐ Australian
- ☐ British descent
- ☐ European descent
- ☐ Asian descent
- ☐ Other
- ☐ Prefer not to say

TPB TPB

9.2 Consumer sensory scoring sheet


TPB TPB

All information collected in this survey is strictly confidential

PRODUCT:

Tenderness

Not Tender Very Tender

Juiciness

Not Juicy Very Juicy

Liking of Flavour

Dislike Extremely Like Extremely

Overall Liking

Dislike Extremely Like Extremely

Please mark **X** in one of the following boxes to rate the quality of the beef sample you have just eaten

Choose **one** only (you must make a choice)

- ☐ Unsatisfactory
- ☐ Good everyday quality
- ☐ Better than everyday quality
- ☐ Premium quality

TPB TPB

9.3 Consumer willingness to pay sheet

TPB TPB

Based on the beef you have just consumed:
Please mark the line at the price per Kg you believe best reflects the value for each category.

Unsatisfactory Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Good Everyday Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Better Than Everyday Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Premuim Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Are you the regular purchaser for your family ? *(Use* **X** *in one box only)*

- ☐ Yes
- ☐ No

TPB TPB

9.4 Analysis brief

ANALYSIS BRIEF FOR MEAT COLOUR AND PACKAGING TRIAL – 150116

Data and brief description of the related files is provided in the accompanying "Description of files for analysis of Teys meat colour trial" document. Analysis is requested of a number of core issues addressed through the trial work and summarised below:

1. **The relationship of AUS-MEAT meat colour (AMC) scores at MSA grading to subsequent AMC observations of the striploin, rump and tenderloin** as primals at boning, immediately prior to retail packaging after three ageing periods (5, 12 and 40 days) in vacuum packaging and after retail display in three alternative packaging systems – Overwrap (OWP), Modified atmosphere (MAP) with a gas mix of 80% O_2, 20%CO_2, and Vacuum skin packaging (VSP).

   The initial AMC score at grading is taken by viewing the surface of one cube roll at the carcase quartering site which is the lower side of the cut between the cube roll and striploin. Consequently this can be regarded as identical to the anterior end of one striploin. At boning the anterior end of both striploins were scored as were both rumps and both tenderloins, also at standard muscle positions. At retail pack fabrication each primal was sliced into 25mm slices with several slices from each transferred to OWP, MAP and VSP packaging. After 20 minutes of bloom time each pack was independently scored for AMC prior to lidding. The position of each pack type within muscle was rotated.

   The 48 source carcasses were selected to provide a range of meat colour and pH at MSA grading together with a mix of dentition within cells. The table below shows the actual dentition, pH and meat colour combinations collected. Note that the 0 teeth are all AMC 1C and also that all 0 teeth are from grain fed cattle as is one 2 tooth (carcase 1034). All other cattle are grass fed. The colour comparisons should be valid – colour being colour – but there could be potential interaction with dentition or other grading inputs.

   Actual distribution of pH and dentition within meat colour cell for carcasses used in cut collection.

		AMC				
	Dentition	1C	2	3	4	5
5.71<	0T	4	0	0	0	0
	2T	2	3	3	2	2
	4T	0	2	4	2	2
	6T	0	1	2	3	2
5.7+	0T	0	0	0	0	0
	2T	0	0	1	2	2
	4T	0	0	2	2	2
	6T	0	0	0	1	2

Under current retailer driven MSA rules any carcase with an AMC>3 or pH>5.7 is excluded from MSA grading. Further restrictions relating to dentition are often added by the retailer, typically excluding 6 and sometimes 4 teeth and above. Removal from MSA results in substantial financial penalties (around $300 per head) for the producer and discounts around 20% of wholesale price to the processor. The validity of utilising the grading AMC, pH or dentition as a proxy for subsequent performance in consumer visual appeal or sensory rating is consequently of critical interest.

1.1 How do the original STR045 MSA meat colour scores used for carcase grading compare to the MSA STR045 meat colour scores taken on the boned STR045 primals? (These were taken approx 2 to 5 hours later. One primal is the same as that used for carcase grading and a second from the other side)

- **1.1.1** Is there any difference in the relationship in 1.1 relative to pH of 5.7 and less versus >5.7?
- **1.1.2** How do the TDR062 and RMP131 MSA meat colour scores taken at boning compare to the STR045 score at grading and at boning?
- **1.1.3** Is there any difference in the relationship in 1.1.2 relative to pH of 5.7 and less versus >5.7?
- **1.1.4** Is there a difference in the relationship in 1.1.2 or 1.1.3 relative to dentition?

1.2 How do the MSA grader (AMC) scores at grading align with the MSA grader scores during retail fabrication by cut within ageing period for vacuum packed primals? (There are an even number of each cut (96) distributed across 5, 12 and 40 days post kill with time in vacuum packing 5, 21 and 40 days respectively. Two of the three ageing periods apply to each of the 3 cuts within each carcase). As each cut was packed in OWP, MAP and VSP there are three readings for each muscle at this point.

- **1.2.1** Do any relationships in 1.2 differ with grading pH at pH 5.7 and below versus >5.7?
- **1.2.2** Do any relationships in 1.2 differ with dentition?

1.3 How do MSA grader (AMC) scores post retail fabrication compare with prior AMC scores?

- **1.3.1** How do the post retail AMC scores relate to the original AMC (striploin) grading score?
- **1.3.2** How do the post retail AMC scores relate to the original individual primal AMC scores at boning – a few hours post grading?
- **1.3.3** How do the post retail AMC scores relate to the AMC scores at retail packing? (2 days prior for OWP and 9 days prior for MAP and VSP).
- **1.3.4** Do the relationships in 1.3.1, 1.3.2 and 1.3.3 vary with initial pH at grading?
- **1.3.5** Do the relationships in 1.3.1, 1.3.2 and 1.3.3 vary with dentition?
- **1.3.6** Do the relationships in 1.3.1, 1.3.2 and 1.3.3 vary with the ageing period as vac packed primals?
- **1.3.7** Do the relationships in 1.3.1, 1.3.2 and 1.3.3 vary with retail packaging?
- **1.3.8** Are there significant interactions between the factors above and AMC? (initial pH, dentition, ageing period and retail pack type)

Definition of beef colour appeal to consumers during retail display.

In all 840 consumers each evaluated 24 retail beef packs during the trial period which incorporated three cuts and three packaging types, three prior primal ageing periods and one (OWP) or three (MAP & VSP) time periods in the retail case. After viewing each pack they marked a 100mm line anchored by the descriptions “extremely unappealing” and “extremely appealing”. In addition to the line scale they selected one of three boxes for each sample labelled “definitely would buy”, “definitely would buy if discounted” and definitely would not buy”. Other than a small subgroup of consumers that viewed the same cut in MAP and VSP packaging the majority viewed one set of 8 packs from each of the three cuts on each viewing occasion. The order of cut viewing was rotated as was the presentational order of the eight within each cut, this being derived from assigning the initial carcase graded AMC and pH in accordance with the following table.

Alternative order of retail pack display by AUS-MEAT meat colour and pH[1]

A	1C	2	3-	3+	4-	4+	5-	5+
B	2	3+	1C	4+	3-	5+	4-	5-
C	3-	1C	4-	2	5-	3+	5+	4+
D	3+	4+	2	5+	1C	5-	3-	4-
E	4-	3-	5-	1C	5+	2	4+	3+
F	4+	5+	3+	5-	2	4-	1C	3-
G	5-	4-	5+	3-	4+	1C	3+	2
H	5+	5-	4+	4-	3+	3-	2	1C

[1] + indicates pH over 5.7, - indicates pH of 5.7 or less

The Latin square row is indicated in the “Consumer colour appeal scores – Q” file as a Set no with the letters R, S and T denoting the cut. The table above was applied prior to collection on the assumption that all desired pH and AMC combinations would be filled. There were minor variations to this in some cells (under AMC 3) with the substituted samples allocated against the original designation. The position of cut sets (shelf and position on shelf) was also rotated within the cabinet, between primal ageing days for OWP and for each of the three consumer viewing days within each ageing cycle for MAP and VSP packs.

1.4 Can a “consumer colour appeal scale” be derived from analysis of the line scale markings and associated category boxes? (I have used Consumer Colour Score – CCS below but am open to any abbreviation)

1.4.1 Can the categories be delineated by the scale? If so what are the appropriate values to best differentiate product assessed as “would definitely buy” from that marked as “definitely would but if discounted” and that marked “definitely would not buy”.

1.4.2 How consistent were consumers in their categorisation and how does this compare to prior data on sensory response?

1.4.3 Should outliers be adjusted or removed from analysis and if so what is the recommended method?

1.4.4 Are any of the recorded demographic variables significant in regard to colour scoring?

2.2 Please calculate mean scores for each EQSRef on each viewing occasion for all consumers and after recommended outlier treatment.

2.2.1 In evaluating potential cut, ageing, packaging, initial AMC and pH and cabinet position interactions with the retail colour score **(CCS)** and category selection should all individual scores be utilised in analysis, raw EQSRef means or adjusted EQSRef (Pack) means after outlier treatment? Please nominate the preferred approach and which is used in subsequent analysis.

2.3 How consistent is the CCS across cuts, ageing periods, packaging types and days of retail display? The core question is could a universal CCS be utilised regardless of these factors.

2.3.1 Does the CCS interact with cut?

2.3.2 Does the CCS interact with days aged in vacuum packaging as a primal prior to retail packaging?

2.3.3 Does the CCS interact with packaging type (OWP, MAP and VSP)?

2.3.4 Does the CCS interact with the days in retail packaging (1 for OWP and 3, 5 and 7 for MAP and VSP)?

2.3.5 Are there further interactions of CCS with days aged as a primal x days in retail packaging, days in retail packaging x cut, days aged in primal x cut x days in retail packaging x packaging type or other interactions between the variables in 2.3.2 to 2.3.5?

2.4 How can a recommended CCS be utilised for evaluation of beef in retail display? Can a single scale and related cut-offs be utilised across the retail case independent of other factors?

2.4.1 Are there different cut-off values for categories related to packaging type?

2.4.2 Are there different cut-off values for categories related to cut?

2.4.3 Are there different cut-off values for categories related to prior primal ageing periods?

3. Do CCS scores align with MSA grader assigned AMC?

3.1 How strong is any relationship between AMC at the point of retail packaging and consumer observed CCS?

3.1.1 How strong is any relationship between AMC after retail display and consumer observed CCS?

3.1.2 Do 3.1 and 3.1.1 have any difference in relation to viewing after various days in retail packaging? (OWP has only 1 day and MAP and VSP 3, 5 and 7 day from packaging observations)

3.1.3 Do any relationships in 3.1 to 3.1.2 differ between packaging types?

3.1.4 Do any relationships in 3.1 to 3.1.2 differ between periods of prior primal ageing?

3.2 How strong is any relationship between AMC taken on primal cuts at boning (file B) and CCS?

3.2.1 Do any relationships vary by cut?

3.2.2 Do any relationships vary with the subsequent period of ageing as vac packed primals prior to retail packing?

3.2.3 How strong is the relationship of STR045 AMC at boning and other muscles (RMP131, RMP231 and TDR062)?

3.2.4 Are there significant interactions of importance?

3.3 How strong is any relationship between the carcase MSA Grading AMC (file A) and CCS?

This is the critical question in regards to ultimate decisions in regard to MSA grading regulation

3.3.1 Do any relationships vary by cut?

3.3.2 Do any relationships vary with the subsequent period of ageing as vac packed primals prior to retail packing?

3.3.3 Do any relationships vary with packaging type?

3.3.4 Do any relationships vary with days of retail display when viewed?

4 How does pH at grading influence AMC and subsequent CCS?

This is a further critical input to MSA grading regulation.

4.1 How strong is the relationship between AMC and pH at grading and subsequently?

4.1.1 Does the (striploin) pH and AMC relationship at grading to boned primal AMC vary across the three cuts?

4.1.2 Does the grading pH to AMC relationship change with different periods of vacuum packed primal ageing?

4.1.3 How does pH at grading relate to pH after retail display?

4.1.4 Is pH at MSA grading a better or equal indicator of AMC during retail display than the AMC at grading?

5 How do instrumental colour measurements relate to CCS?

5.1 Can HunterLab or NIX outputs predict CCS?

5.1.1 Which HunterLab outputs, produced immediately prior to retail packing, best describe CCS and how effective is this prediction?

5.1.2 How does the HunterLab relationship to CCS compare to AMC taken at the same time?

5.1.3 How do the HunterLab values prior to and post retail display compare?

5.1.4 Does any change in 5.1.3 relate to a change in CCS during the viewing period?

5.1.4 What is the relationship between HunterLab values at prior periods (MSA grading (striploin) and boning (all cuts)) and CCS?

5.1.5 How does the relationship vary for cut, days aged in vac and packaging type?

5.1.2 Which NIX outputs best describe CCS?

5.1.3 How well does NIX output immediately prior to retail packing relate to CCS?

5.1.4 How do NIX values taken during retail display relate to CCS and does this vary by packaging type?

5.1.5 How does the NIX relationship to CCS compare to AMC taken at the same time?

5.1.3 How does the prediction potential of either instrument compare?

5.2 Can HunterLab or NIX outputs predict AMC?

All current MSA and AUS-MEAT colour standards are defined by the chips. If instruments are to be used for colour assessment they may be more readily accepted if the output relates to the existing chip descriptions, but hopefully with finer definition; 3.2 or 3.9 rather than 3 for example. The alternative of description solely by instrumental output – an L*a*b* definition for example – may be possible but is likely to be more difficult to implement.

5.2.1 Building on previous work how well can HunterLab output be utilised to estimate the AUS-MEAT chips directly?

5.2.2 How well can HunterLab output estimate the chip based grader AMC scores?

5.2.3 Can Hunterlab output be utilised to describe subdivision of AMC scores (3.1, 3.9 etc)?

5.2.4 Are these predictions influenced by time from kill or by muscle?

5.2.5 Building on previous work how well can NIX output be utilised to estimate the AUS-MEAT chips directly?

5.2.6 How well can NIX output estimate the chip based grader AMC scores?

5.2.7 Can NIX output be utilised to describe subdivision of AMC scores (3.1, 3.9 etc)?

6. Retail display interactions with CCS?

6.1 Does cabinet position affect the CCS for individual retail packs?

6.2 Does any such relationship differ by cut?

6.3 Does any such relationship differ by packaging type?

6.4 Do any relationships change over the display period or with prior primal ageing?

6.5 Are there significant interactions?

6.6 Are there useful guidelines for optimal display arising?

6.7 Does the AMC of adjacent packs influence CCS?

6.8 Does this interact with cut?

6.9 Does this interact with pack type?

6.10 Are there useful guidelines for optimal display arising?

7. Are there retail packaging effects on eating quality?

The current data is preliminary being for 10 consumer picks from a final 24 to be completed by early February.

7.1 Is there an effect of packaging on eating quality scores?

7.2 Does any effect vary with prior primal ageing period?

7.3 Does any effect vary with muscle/cut?

7.4 Does any effect relate to initial pH?

7.5 Are there any significant interactions?

9.5 Description of files for analysis of Teys meat colour trial

Prepared by Rod Polkinghorne for Dr Ray Watson and Dr Garth Tarr

The data to be analysed is dispersed across a number of files which require linkage via carcase, primal and EQSRef ID. A brief description of each follows:

1. The file "ANALYSIS FILE – Diagram of colour measures" provides a diagrammatic example of the samples, identified by unique EQSRef, derived from one of the 48 trial carcasses. The progression is from the MSA carcase grading, a single reading of one muscle only (striploin) and a single carcase no ID, to individual readings of three pairs of cuts with each within a pair aged a different period prior to retail packing, identified by primal no, to three retail packs within each muscle identified by EQSRef.
2. The file "Analysis file – AMC colour measurements from grading to sensory with linkage to HunterLab and NIX files" tracks each EQSRef derived from every carcase detailing the appropriate file (by alpha letter) and indicating the occasions that Hunterlab or NIX measures were recorded for each and their source primal and carcase. The notes page of this file includes a timeline of events and references to related files.

Note: There are a series of related ageing measures. The Days aged in AUSBlue refer to total days from kill to freezing of the consumer samples per standard MSA practice. Within the files are also 3 periods of primal ageing within vacuum packing (5, 12 or 40 days) prior to fabricating into retail packs (9 days prior to final freezing). A "Days from Kill" measure is also shown to reference dates and in particular relative time for packing, consumer viewing and final freezing.

3. The file "Base Linkage from carcase data through primal to EQSref and pack 301215" repeats the initial steps of (2) to provide the linkage from carcase to primal to EQSRef and associated retail pack type.
4. The file "AUSBlue data related to EQSRef – AB" has the standard AUSBlue data for all samples with the last column listing the pack type – OWP (overwrap), MAP (modified atmosphere) or VSP (vacuum skin pack). The link samples were prepared as standard MSA samples and were not retail packed.
5. The file "HunterLab file at MSA Grading – A" has HunterLab readings for each carcase at the time graded plus summary grade data.
6. The file "HunterLab file at Boning – B" has HunterLab readings for each primal after boning – loosely 2 to 5 hours after initial grading with AUS-MEAT colour and pH.
7. The file "HunterLab file at retail packing 5 days post kill - C" has the HunterLab readings and summary grade data for all retail packs prepared from 5 day aged primals.
8. The file "HunterLab file for 5 day OWP post retail 7 days post kill – D" has HunterLab and AUS-MEAT colour and pH data for each of the OWP packs from the 5 day primals – taken when removed from the retail cabinet after observation and prepared as standard MSA consumer samples at 7 days post kill. These were held chilled until frozen at 14 days in conjunction with their MAP and VSP pairs.
9. The file "HunterLab file for 5 day MAP and VSP product produced post retail 14 days post kill – E" contains the HUnterLab and summary grade data for all the MAP and VSP packs (pairs

of the OWP in (8)) after 3 consumer viewings (at 3, 5 and 7 days post retail packing) and immediately prior to fabricating as MSA consumer samples and freezing.

10. The file "HunterLab file at retail packing 12 days post kill – F) has basic grade data and HunterLab output for all the retail samples prepared from product aged 12 days in vac packed primal form.
11. The file "HunterLab file for 12 day OWP post retail 14 days post kill – G) has HunterLab output and grading links for the "12 day" OWP packs after retail observation and immediately before conversion to MSA consumer samples.
12. The file "HunterLab file for 12 day MAP and VSP product post retail 21 days post kill – H) has the colour and HunterLab values for retail packs after three consumer visual observations and immediately before conversion to MSA sensory samples and freezing at 21 days from kill. These samples were frozen in conjunction with the OWP packs in (11).
13. The file "HunterLab file at retail packing 40 days post kill – I) has graded colour plus HunterLab and NIX values for all retail packs prepared from product aged 40 days as vac packed primal. THIS IS THE FIRST USE OF THE NIX METER for colour evaluation.

Where there are HunterLab and NIX readings they were taken at identical sites using the 35mm circular imprint left by the HunterLab to position the NIX. Two readings were taken with each instrument from two separate steaks within each pack and averaged. The NIX readings were taken within a minute of the HunterLab.

14. The file "NIX file for OWP in retail cabinet on 181015 – J" provides NIX readings for all the OWP retail packs taken through the packaging film while on display within 30 minutes of the last consumer viewing (all previous HunterLab and NIX readings taken at retail packing and post retail display were taken either prior to applying the packaging film or after its' removal and allowing 20 minutes to bloom). It is claimed that the NIX could provide effective colour measurement through film whereas the film was thought to confound HunterLab readings.
15. The file "HunterLab and NIX file for 40 day OWP post retail – K" has HunterLab and NIX values for the OWP trays after removal from the cabinet and immediately prior to conversion to MSA sensory samples (readings after film removal) together with AMC and pH readings.
16. The file "NIX file for MAP and VSP in retail cabinet on 201015 – L" has NIX values taken for all retail packs in the cabinet within an hour of the last consumer viewing.
17. The file "NIX file for MAP and VSP in retail cabinet on 221015 – M" has NIX values taken for all retail packs in the cabinet within an hour of the last consumer viewing.
18. The file "NIX file for MAP and VSP in retail cabinet on 241015 – N" has NIX values taken for all retail packs in the cabinet within an hour of the last consumer viewing.
19. The file "NIX file for 40 day MAP and VSP post retail on 261015 – O" has NIX readings plus AMC and pH for all packs after removal from the cabinet and immediately prior to conversion to MSA sensory samples (lidding removed). The corresponding HunterLab readings were lost.
20. The file "HunterLab and NIX file for 40 day MAP and VSP post retail after thawing to 151215 – P" represents an effort to compensate for the lost HunterLab values in (19) by tracking and recording 40 day MAP and VSP samples during consumer sensory testing. This timing meant that readings were taken after freezing and thawing for sensory on a large number of dates

up to 15th Dec 2015 (more are scheduled as sensory is completed). All samples were however frozen at a constant 49 days ageing including the OWP samples from (15 - K).

Consequently files I, K and P provide direct comparisons of the HunterLab and NIX meters in identical positions and times across significant sample numbers.

21. The file "Consumer colour appeal scores – Q" provides the individual consumer ratings (score mm) for colour appeal and a choice of 3 regarding purchase intent (box). Ten consumers viewed each retail pack (EQSRef) on any given observation day. As a consequence there are 10 consumer observations for OWP packs (only viewed on one day) and 30 observations for the MAP and VSP packs due to their viewing on three days, each 2 days apart. (**Note exception for the 8 packs in sets R19 and R20 in note below) All the retail packs were displayed in sets of 8, representing different original carcase graded meat colour (AMC) and pH categories, within each of the three cuts with order within the 8 rotated in accordance with an 8x8 Latin square. Every consumer viewed three sets of 8, one from each cut in the majority of cases. Each set of 8 EQSRef numbered packs were further described by a set number and order within the set. The position of each set was allocated to a specific cabinet position on each viewing occasion, with the MAP and VSP position being rotated between each of the three viewings. File "Q" includes Set, Order and Cabinet Position for each pack (EQSRef) for each viewing. Two example cabinet plans are shown on separate sheets within the file to provide detail of the cabinet position. The file also shows the muscle, days from kill, vac pack primal ageing days, days in the retail pack when viewed and pack type.

    **** There is an offsetting error in each of 2 sets of 8 and the associated EQSRef's, on the 24th Sept (17 Days from Kill). These are R19 which has 11 observations (31 in total) and R20 which has 9 observations (29 in total). Also a few cases of missed observations including 2 consumers.**

22. The file "Consumer demographics file – R" provides basic demographic data for each consumer.

9.6 Pathways Committee Recommendations

Pathways Committee Recommendations

Rod Polkinghorne

22nd April 2016

It was resolved at the Pathways Committee meeting of March 31st and April 1st 2016 that:

1. The existing requirement that only carcasses with an ultimate striploin pH below 5.71 be eligible for MSA grading be retained.

   While the direct eating quality impact of slightly higher pH is slight at best there are significant detrimental effects in regard to microbiological growth and cooking with high pH meat that warrant exclusion.

2. The current screening requirement for carcasses to have an AMC 3 or less at grading to be eligible for MSA grading be removed.

   This recommendation was presented to Taskforce previously but not enacted with a request for further data. The recent extensive trial provides strong evidence that striploin meat colour at grading above AMC 3, where pH is below 5.71, has no impact on consumer visual acceptance or eating quality. Consequently it is regarded as superfluous and an unnecessary impost.

3. The Taskforce and industry be advised that 80:20 high oxygen packaging has a serious detrimental effect on beef eating quality relative to overwrap and VSP. This effect is estimated at 12 MQ4 points and is constant across days aged prior to packing and across the three muscles evaluated.

   This finding is a result of the recent meat colour and packaging trial and is consistent with other published work for beef and lamb.

4. Mechanisms be developed within the MSA grading structure to apply a 12 MQ4 point deduction to any MSA graded MAP packaged product.

   In effect this would reduce all 5 product to 4*, all 4* product to 3* and all lower 3* to ungraded. There are operational issues in that the MSA output would require modification to designate two cut-off points within 3* to enable MAP product to be differentiated.*

9.7 Proposed additional research to quantify MAP effects on EQ

Item for Discussion

Additional research to quantify the development of MAP packaging effects on eating quality.

Author: Rod Polkinghorne

Date: 19th October 2016

Purpose

To advise of a planned trial to evaluate the Modified Atmospheric Packaging (MAP) effect on eating quality at 3, 5, 7 and 9 days post packaging including trial of an alternative gas mix, comparison to Vacuum Skin Packaging (VSP) and consumer visual evaluation.

Recommendations

That the Taskforce endorse the proposed trial and note that it is designed to clarify the impact of current commercial high oxygen MAP packaging on eating quality over the retail display period and to compare an alternative gas mix.

Background:

For several years there have been consistent research reports that high oxygen MAP packaging has a detrimental impact on the eating quality of beef. Many of these studies have been small and few included direct sensory appraisal. While the effect has been known “in the background” it has not been brought to industry attention and 80:20 MAP (80% Oxygen: 20% Carbon Dioxide) has continued to be widely used in many countries as a primary retail packaging.

A 2015 MLA funded CSIRO trial evaluated lamb samples in MAP and VSP under MSA protocols and recorded a 5 point MQ4 reduction with MAP adding to concerns regarding potential effects on beef. As an adjunct to a major 2015 study of beef colour and pH funded by Teys Australia, MLA and AMPC, retail packaging was examined utilising MSA consumer sensory protocols and direct consumer visual appraisal of beef packaged in 80:20 MAP, VSP and overwrap. This study quantified a 12 MQ4 point detrimental effect from MAP relative to both VSP and overwrap with the impact unaffected by muscle (striploin, tenderloin and rump tested) or by time in vacuum packaging prior to retail packing (5, 12 and 40 days tested). The scale and consistency of this effect was unexpected and raised serious concerns. The MSA Pathways committee considered the data and recommended that MSA apply a 12 point discount to MAP packed product. The mechanism to apply this recommendation requires some time to develop. The MSA beef taskforce acknowledged the research outcomes and requested further investigation of alternative solutions before endorsing the implementation of the 12 point discount.

Unresolved issues:

The recent Australian study only sensory tested product at 9 days post retail packaging, approximating typical Use By date coding. Major retailers report that they have not experienced consumer complaints relating to MAP indicating either that their product may be of sufficiently high standard that a 12 point deduction still produces a satisfactory eating experience or that the effect is less at earlier days post packing. Several research studies have also indicated that the 80:20 MAP problem may be reduced or eliminated by utilising a 40%N:30%O_2:30%CO_2 gas mixture (Trigas).

Proposed Study:

To resolve the issues above it is proposed that a well controlled within animal study be conducted in which Trigas, 80:20 MAP and VSP treatments are tested by consumer visual appraisal and sensory testing at 3, 5, 7 and 9 days post packaging. It is proposed that left and right cube rolls and striploins be collected from 18 MSA graded domestic carcasses. The use of both cube roll and striploins from both sides would enable 12 samples (3 packaging types x 4 days in pack times) to be prepared from each carcase which provides a very strong comparison as all treatments are applied within each animal. The position within muscle would be rotated evenly to ensure any side or position effect is also balanced.

Ideally the product should be packed on standard commercial packing lines and viewed in a commercial meat case by untrained consumers. This would utilise 360 consumers for sensory appraisal and 450 for visual appraisal of the samples across the four periods.

9.8 The Effect of high oxygen packaging on beef eating quality

John Thompson

May 2016

1. Introduction

MAP with a high oxygen (70–80%) is increasingly used at retail to preserve the bright red colour of fresh meat and increase shelf-life by reducing microbial growth. However, high concentration of oxygen also increases the levels of oxidation in meat which has been shown to cause rancidity for lipids and increased toughness of myofibres. A number of reviews have clearly stated that high oxygen packaging should be avoided wherever possible because of the negative impact on eating quality (Mathews 2011, Scetar et al 2010).

2. What is the evidence for an increase in toughness with high oxygen packaging

One of the earliest reports of packaging effects on palatability of beef was by Torngren (2003) presented at the International Congress of Meat Science and Technology (ICoMST) in Brazil. He reported that high oxygen (80% O_2/20% CO_2) packaging beef cuts resulted in poorer eating quality (ie lower tenderness and flavour scores) compared with vacuum packaging followed by overwrap.

Following the publication of the Brazilian results Seyfert et al (2005) reported the effect of high oxygen packaging on sensory scores in beef. Their study compared sensory traits in four muscles (*mm. biceps femoris, semimembranosus, rectus femoris, vastus lateralis*) from the round which were harvested from warm or cold boned sides and subjected to either high (80 O_2:20 $C0_2$) or low (80 N_2:20 $C0_2$) oxygen packaging before storing for 14 days and sensory testing. Two experiments were conducted which overlaid injection of enhancement solutions at 6 or 10%. The sensory testing was conducted using an 8 point scoring system which was rescaled to the MSA 1to 100 scale. In experiment 1 both the hot and cold boned treatments steaks stored in the high oxygen atmosphere were tougher than those in the low oxygen atmosphere. With the lower level of enhancement (ie 6% w/w) the average tenderness scores were 60 versus 67 in the cold boned sides and 62 versus 67 in the warm boned sides for high oxygen packaging versus low oxygen packaging, respectively. At the higher level of enhancement (ie 10% w/w) a similar trend was evident but the differences were slightly smaller at 65 versus 67 for the high versus low oxygen packaging in both warm and cold boned carcasses. The authors concluded that high oxygen packaging systems had a detrimental effect on sensory.

The study by Lund et al (2007) described the consequences for tenderness and protein oxidation of the use of commercial high oxygen atmosphere packaging compared to VSP for storage of pork loin for up to 14 days at 4^{o}C. They found that a high oxygen atmosphere resulted in tougher meat and lower juiciness scores. Protein gels revealed the high oxygen atmosphere increased cross-linking of myosin heavy chain through disulfide bonding, and

the content of protein thiols was reduced indicating protein oxidation. Lower proteolysis and/or cross liking of proteins in the high oxygen atmosphere was indicated by lower myofibril fragmentation. They concluded that packaging pork loin in modified atmosphere containing a high level of oxygen resulted in protein cross-linking and reduced tenderness and juiciness of the meat. In their study tenderness was scored using a 15 point scoring system which when rescaled to 1 to 100 scale showed that at 8 and 10 weeks post-mortem the high oxygen resulted in a 10 or 15 point decline in tenderness compared to the VSP packaging system.

Lagerstedt at al (2011a) compared high O_2 MAP with vacuum pack and skin beef packed steaks at 14 and 21 days ageing. As expected the MAP product was tougher at both 14 and 21 days ageing. When the sensory scores were rescaled MAP showed a 14 point decrease in tenderness at 14 days compared to the skin or vacuum packed product. This difference between MAP and vacuum packed increased to 20 points at 21 days ageing. The increase in the magnitude of the treatment effect occurred because the MAP product did not age, whilst the vacuum packed steaks did.

In another experiment Lagerstedt et al (2011b) examined different combinations of ageing in MAP and vacuum packing. When the sensory scores were rescaled the MAP product was scored 7 units lower than vacuum packed product after 5 days ageing. This increased to 12 points after 15 days ageing. Again the increase in the magnitude of the MAP effect was because once placed in the pack the MAP product did not age compared to the vacuum packaged product.

An interesting study was reported by Aaslyng et al (2010) where different forms of low oxygen packaging was compared with high oxygen packaging using consumers who were asked to cook and consume the steaks in the home. Approximately 400 consumers from each of three countries (Norway, Sweden and Denmark) were given two packs of steaks to be cooked at home and consumed by two consumers in the household. Consumers in all three countries clearly preferred steaks packed without oxygen, in terms of overall liking, willingness to pay and their preferred choice of one steak from the pack of four taken home. Furthermore they showed a clear preference for oxygen free steaks in terms overall liking and liking of tenderness juiciness and flavour scores. Consumer scores showed on average a 5 point penalty for high oxygen packaging compared to vacuum, or other forms of packaging without oxygen.

The most recent results are from Australian studies. Using lamb backstrap and topside cuts Franks et al (2016) showed that high oxygen packaging resulted in a 4 and 9 point decrease on a 100 point scale in tenderness compared to vacuum packed cuts when aged for 5 or 10 days. As shown previously the larger difference in the longer aged product arose because the MAP product failed to age, whereas the vacuum packed product continued to improve with ageing.

A recent beef experiment undertaken by Polkinghorne (unpublished data) compared high oxygen MAP with overwrap and vacuum packed samples using striploin, rump and tenderloin beef steaks. In the three muscles aged for 5, 12 or 40 days the high oxygen MAP samples had a lower MQ4 score than the overwrap or vacuum packed product. The magnitude of the MAP penalty was 10 to 12 points but unlike other studies it did not appear to increase with ageing. The MSA Pathways Committee reviewed the results and recommended that MSA adopt a 12 point penalty for MAP packed product regardless of cut.

3. What are the mechanisms that cause the increase in toughness to occur?

A number of studies have been carried out to understand the mechanism by which post-mortem oxidation in muscle proteins impact on eating quality. These proteins include both functional proteins (calpain system) and myofibrillar proteins (myosin and actin). Several researchers reported that oxidation processes in post-mortem muscle converts some amino acid residues (especially histidine) to carbonyl derivatives, free thiol groups in cysteine residues to disulfide bonds, tyrosine groups to dityrosine bonds, and fatty acids to hydroperoxides (Martinaud et al., 1997; Xiong, 2000). These oxidative changes in proteins cause protein fragmentation, cross-linking, and aggregation. Those changes can alter activities of proteolytic enzymes and myofibrillar proteins in the muscle microstructure and eventually reduce post-mortem ageing.

In summary the two main theories are that i) the enzymes involved in the tenderisation process might be oxidized by the high-oxygen content leading to a slower or interrupted tenderisation process (Rowe et al., 2004) and that ii) intermolecular cross-links involving disulfide bonds are formed in myosin leading to a tougher meat.

Premature browning is another side effect of packing in high oxygen atmosphere. The high oxygen atmosphere results in oxidation of oxymyoglobin to metmyoglobin. The produces a brown pigment in the pack which denatures at a lower temperature during cooking producing a well done appearance at temperatures which normally produce a medium degree of doneness (Torngren 2003).

4. Are there any techniques that could moderate the magnitude of the effect?

The literature showed that clearly high oxygen packaging systems had a detrimental effect on eating quality. The question arises as to whether this negative impact can be ameliorated by pre or post-slaughter interventions.

The effect of feeding animals antioxidants (AGRADO-PLUS) during the final stages of finishing was investigated by Senaratne (2012). The feeding of AGRADO-PLUS showed positive antioxidant effects against colour and lipid oxidation. The antioxidant effect was less effective when fed a diet containing wet distillers grain. Unfortunately no eating quality measurements were reported in this study. In the same laboratory steaks were dipped in a solution of antioxidant prior to packaging (Bolte et al 2012). As expected their results

showed that the steaks packed in the high oxygen product were tougher and did not age, but there was no effect on shear force by treating the steaks with the antioxidant prior to packaging.

Seyfert et al (2005) used two levels of enhancement solution which had an anti-oxidant component to treat steaks prior to packaging in high and low oxygen systems. Whilst the enhancement solution had an inhibitory effect on lipid oxidation it had no effect on tenderness.

An obvious question arises as to whether the magnitude of the effect is relation to the concentration of oxygen in the packaging. A number of systems have been\ developed which use different gas ratios for the packaging environment by the Ireland at the University College Cork. The first was an experiment which compared MAP with 0%, 10%, 20%, 50% and 80% O_2 after striploins had been aged on the carcass for 14 days (Zakyrs et al 2008). There was 20% CO_2 in all treatments with the balance made up with nitrogen. Unfortunately, the number of striploins or total number of samples used were not cited. Panellists were scored sensory attributes at days 0, 3, 6, 9 and 9 post MAP and were asked to identify the most 'preferred' sample out of a random selection of 5 steaks. Results were expressed as regression coefficients with time within treatments, which made it difficult to assess differences between the different oxygen treatment. The treatment means were not presented but the comment made that the different oxygen treatments did not differ significantly in tenderness or juiciness. The authors state there was a trend for the 50% O_2 to be more acceptable than the 80 O_2 treatment but the results were only given as part of a principal component analysis. This conclusion that 50%O_2 was the most acceptable treatment was supported by the preference analysis which showed a trend for the 50% O_2 treatment to be the optimal treatment.

A subsequent experiment examined the effect of different oxygen concentrations on consumer acceptance of beef (Zakrys et al 2009). The design only included oxygen concentrations of 40, 50, 60 70 and 80% all with 20% CO_2 with the balance made up with nitrogen. The experiment used striploins from nine beef heifers carcasses with 4 steaks from each animal used for sensory. The focus of the study was flavour or rancidity changes over time with sensory tests undertaken at 0, 4, 8 and 12 days after placing in the MAP. The statistical analyses were similar to the previous study so again it was difficult to quantify the magnitude of the treatment effects. As before the treatments differences were not significant, but there was a trend for the 40% O_2 samples to be the most acceptable.

The final experiment (Zakrys et al 2011) was a similar design using striploins from five heifers whose carcasses were boned at 4 days post-slaughter. The packaging treatments of 50% O_2, 70% O_2, 80% O_2 and a commercial pack which had 75% O_2. Samples were evaluated at 1, 4, 7, 10 and 12 days. Again treatment difference were not significant, except that the commercial treatment were less acceptable than the treatment prepared in the laboratory.

Using a similar design O'Sullivan et (2015) tested whether the penalty associated with high oxygen packaging was maintained if the product was allowed to stabilise in air for 30 minutes before cooking. They showed that exposing MAP to air prior to cooking decreased sourness and juiciness scores but had little effect on tenderness scores with the exception of the 70% O_2 which became slightly tougher when rested in air prior to cooking. No reason for this was put forward.

Conclusion

The literature is clear that there is a large sensory penalty associated with high oxygen atmosphere packaging. What is less clear is the mechanism, although as discussed by Lund et al (2007) a number of mechanisms are potentially operating to give the effect of an increase in off-flavours along with increased toughness with high oxygen packaging preparations.

Commercially the problem can be overcome by moving towards 0% oxygen, or a VSP pack, although this would have repercussions on colour. It was disappointing that the Irish experiments did not show clear effects on optimising the gas mix. I suspect that the reason for this lies in the experimental design with a large number of oxygen mixes and ageing times resulting in low sample numbers within each treatment cell making it difficult to clearly distil packaging and ageing trends. It is also worth noting that for MSA purposes a VSP control should be part of all experiments.

Given the benefits of high oxygen packaging in terms of meat colour and shelf life further research into the effect of other gas mixtures is warranted as a potential tool to ameliorate the negative effects on eating quality. Alternatively the recent results of Polkinghorne (unpublished data) which show consumers do not discriminate between different meat colour scores at retail suggest that either nitrogen/carbon dioxide gas or a vacuum systems may provide an alternative to high oxygen packaging for fresh meat without the negative impact on eating quality.

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