final report

Project code: V.EQT.1313

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Date published: February 2014

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

1. The impact of processing conditions and packaging methods on beef quality

2. Development of an assay to quantify post mortem proteolysis

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

The present report describes the results of three projects which have been largely conducted by Joanna Robertson as research projects within her DPI graduate program rotation at Meat Science UNE. The different projects are described in this report under the titles:

1. The effect of processing conditions on beef quality
2. Development of an assay for post-mortem proteolysis
3. The effect of retail packaging method on meat quality traits

For project 1, objective meat quality traits (shear force, colour, water-holding capacity, sarcomere length, p.m. proteolysis) were determined on bovine M. longissimus lumborum et thoracis (striploin and cube roll; n = 189) and M. semimembranosus (topside; n = 144) samples aged for 5 or 26 days. These samples originated from an experiment conducted by John Thompson and Rod Polkinghorne at ACC (Brisbane), investigating the impact of cold-, and heat-shortening conditions and carcass hanging method on sensory beef quality. The objective meat quality traits determined in the present project corroborated the results of the sensory trials, in the sense that the cold-, and heat shortening conditions applied in this experiment did not significantly affect tenderness (shear force), muscle contraction (sarcomere length), or measures of post-mortem proteolysis. Tenderstretching, however, did result in an increase in sarcomere length and a reduction in shear force. Moreover, tenderstretching resulted in a significant improvement in water-holding capacity (thaw loss). These results indicate that tenderstretching is not only an effective way to improve meat tenderness, but can also have a significant impact on yield.

Within project 2 it was investigated whether potential indicators of post mortem proteolysis (ageing) in the soluble muscle fraction correlated with the improvement in tenderness (shear force) during ageing and well-established measures of post-mortem proteolysis (Myofibrillar Fragmentation Index (MFI) and quantification of desmin degradation after SDS-PAGE and Western blotting). For the purpose of this experiment striploins (n = 10) were divided into to 4 subsamples and allocated to ageing for 1, 7, 14 and 21 days. After the respective ageing periods, samples were analyzed for shear force and measures of post-mortem proteolysis. The amount of degradation products of titin in the soluble muscle fraction proved to be a useful measure of post mortem proteolysis. On the basis of this, a novel assay for p.m. proteolysis was developed which allows for a faster and more cost-effective quantification of the impact of ageing on p.m. proteolysis. As discussed, the principle that the ageing response can be assessed on indicators in the soluble muscle fraction, would lend itself to the development of assays suitable for screening of, for instance, purge/weep in vacuum packaged meat.

Project 3 addressed the issue whether retail packaging method (MAP [80% O_2, 20% CO_2] vs skinpack [Darfresh®]) affects sensory and objective meat quality traits. To this end, steaks from 1 day p.m. (n = 10) and 7 day p.m. (n = 10) striploins were MAP- and skin-packaged and stored under refrigeration for 7 days. After the storage period, steaks were sampled for sensory analysis and objective meat quality traits. MAP packaging resulted in an average 8 point decrease in MQ4 score and a 0.5 kgF increase in shear force. However, there was no significant effect of packaging method on indicators of post-mortem

proteolysis. These results indicate that the decrease in MQ4 score is, at least partly, due to a physical effect of the packaging method on the muscle structure, other than inhibition of p.m. proteolysis. Results from other studies on this subject suggest that oxidative cross-linking of proteins may explain this effect, but thus far, quantitative data regarding this hypothesis are lacking. The results from this study indicate that retail packaging method represents a major factor affecting the sensory quality of beef, which at present, is not captured within the MSA grading system.

The major conclusions of these projects can be summarized as follows:

1. Tenderstretching of carcasses may result in a significant increase in water-holding capacity and therefore yield. Further research on the magnitude of this effect, may aid in the adoption of tenderstretching for reasons additional to its well-established beneficial effect on tenderness.
2. The impact of ageing on p.m. proteolysis of muscle proteins can be assessed on soluble muscle protein samples. This observation led to the development of a more cost-effective laboratory method to quantify p.m. proteolysis. Further development of this principle may allow for the development of at-line methods to assess the impact of ageing on p.m. proteolysis.
3. Retail packaging method (MAP vs skinpack) has a major effect on both sensory and objective meat quality traits. However, the effect of packaging method is currently not captured in the MSA grading system, and the physical mechanisms responsible for this effect are not fully understood and/or quantifiable.

Acknowledgements

We gratefully acknowledge the financial support of MLA, the input of Alex Ball (MLA) in facilitating and directing the experiments covered in this report, Bindaree Beef (Inverell) for supplying and packaging samples, the technical assistance of Xuemei Han, Colin Starkey and Annie Baldwin, and UNE ANPR211 students for participating in the sensory trial.

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The effect of processing conditions on beef quality

Background

Recently, the impact of processing conditions (heat- and cold-shortening, hanging method and subsequent aging potential) on sensory meat quality traits were investigated in a trial conducted at ACC (Brisbane) by John Thompson and Rod Polkinghorne (see appendix 1). Surprisingly, the results of this experiment indicated that heat- and cold-shortening conditions had little impact on sensory quality traits. As mentioned, in appendix 1, this would suggest that the importance of rigor temperature in the MSA grading system may need to be reviewed.

Despite the limited effect of the processing conditions on sensory meat quality (MQ4 score), it cannot be concluded that the treatments did not affect separate aspects (tenderness, juiciness, flavor, etc.) of meat quality. An unavoidable aspect of using an untrained panel is that the average consumer is not able to score different sensory traits independently, and as a result, the different sensory traits are highly correlated. This implies that objective meat quality measurements are needed to quantify treatment effects that specifically affect the different meat quality traits.

In the present study, objective meat quality traits were assessed to allow for a more in-depth analysis of the effects of rate of pH-decline, carcass hanging and aging treatments. For the purpose of this study two muscles were selected for analysis of objective meat quality traits. These were the *M. longissimus lumborum et thoracis* (striploin and cube roll: EYE075 and CUB045) and the M. semimembranosus (topside: TOP073). The decision to use these muscles for further analysis was based on the availability of samples that were large enough to lend themselves for further analysis, a direct link with pH and temperature decline measurements (loin samples), and the choice of a muscle that chills relatively slowly, and therefore likely exposed to heat-toughening conditions in this experiment (topside).

Experimental design

The experimental design is described in Table 1, and in more detail in appendix 1. The no stimulation group was intended to result in a low rigor temperature (t@pH6<15^{o}C), whereas the Smart Stim and LVES 10 secs treatments were aimed at optimal rigor temperature (t@pH6 = 20-30^{o}C). For the high rigor temperature treatment (t@pH6>30^{o}C) the LVES system was applied for 40 secs.

Table 1. The number of sides allocated to the different carcass hanging and electrical stimulation treatments

	Low rigor temp	Optimal rigor temperature	High rigor temperature

Hang	No stim	LVES 10 secs	Smart Stim	LVES 40 secs
Achilles hung (AT)	10	10	10	10
Tenderstretch TX)	10	10	10	10
SuperStretch (SS)		10	10	

Stimulation treatments were cycled between no stimulation, LVES (10 s), Smart Stim and LVES (40 s) treatments every half hour. The stimulator was turned off for the no stimulation treatment. The carcass hanging treatments (AT, TX and Super Stretch), for the different stimulation treatments, were rotated between left and right sides.

Pre-rigor measurements of pH, temperature and time were recorded every half hour until carcasses reached pH6. At boning, nine primals from each side were collected and held at 1^{0}C for sample preparation. Samples were aged at 1^{o}C and after the designated ageing period, frozen down and stored at -20°C until analyses.

Results and discussion

Relevant data of the initial experiment pertaining to the selected samples in the present study

An inventory of the collected samples resulted in the identification of 189 samples of the M. longissimus lumborum et thoracis (striploin and cube roll) and 144 samples of the M. semimembranosus (topside) aged for either 5 or 26 days. Relevant summary statistics for the carcasses and selected muscles from the original experiment are given in tables 2, 3 and 4.

Table 2. Summary statistics for the effect of the electrical stimulation treatments on the pH and temperature decline (T@pH 6) in the *M. longissimus lumborum* (striploin) of selected carcasses.

Treatment	n	T@pH 6	S.D.
Cold	10	16.7	3.7
Control	15	24.9	5.3
SmartStim	15	34.0	3.8
Hot	10	37.2	1.5

Table 3. The effect of electrical stimulation method, carcass hanging method and ageing period on the MQ4 score of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												

AT	46.7	43.6	47.1	48.0	54.4	52.2	55.9	51.8	4.1	0.02	0.80	0.96
XT	55.4	46.7	51.1	53.1	64.0	58.0	56.4	64.6	3.3	0.00	0.07	0.08
SS	-	53.4	55.1	-	-	58.1	58.5	-	2.9	0.18	0.73	0.83
SEM		3.35				3.25						
P-value												
Stim		0.60				0.22						
Stretch[a]		0.01				0.00						
Stim*Str		0.96				0.90						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Table 4. The effect of electrical stimulation method, carcass hanging method and ageing period on the MQ4 score of the M. semimembranosus (topside).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	36.7	33.4	34.7	32.3	44.5	39.7	41.6	45.3	3.3	0.00	0.67	0.77
XT	43.7	41.5	40.3	39.2	47.8	49.0	45.0	46.4	2.8	0.01	0.59	0.90
SS	-	39.0	41.4	-	-	46.8	47.1	-	2.7	0.08	0.71	0.78
SEM		2.74				3.32						
P-value												
Stim		0.51				0.83						
Stretch[a]		0.00				0.08						
Stim*Str		0.99				0.79						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Shear force

The shear force results for the loins (Table 5) and topsides (Table 6) indicated that stimulation method did not significantly affect the shear force of either muscle. Carcass hanging method did have a significant effect on the shear force of both muscles after 5 days of ageing, but not after 26 days of ageing. Ageing time had a highly significant effect on the shear force of both the loin and topside of AT suspended carcasses, but less so on the shear force of TX and SS carcasses. These results confirm the common view that muscle stretching negates the importance of ageing to produce a tender product.

Overall, the shear force results corroborate the sensory analysis results (Tables 3&4; appendix 1) in the sense that the rate of pH decline did not significantly impact tenderness.

Table 5. The effect of electrical stimulation method, carcass hanging method and ageing period on shear force (kg) of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	5.7	5.2	4.6	4.5	3.8	4.0	3.7	3.8	0.4	0.00	0.31	0.42

TX	3.9	4.6	4.5	4.0	3.5	3.6	3.9	4.0	0.3	0.04	0.48	0.52
SS	-	4.0	4.3	-	-	3.1	3.1	-	0.2	0.00	0.39	0.47
SEM		0.3				1.0						
P-value												
Stim		0.44				0.73						
Stretch[a]		0.00				0.22						
Stim*Str		0.11				0.45						

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

Table 6. The effect of electrical stimulation method, carcass hanging method and ageing period on shear force (kg) from the M. semimembranosus (topside).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	5.6	5.2	5.0	5.1	4.2	4.3	4.4	4.7	0.4	0.01	0.94	0.75
TX	4.6	4.2	4.8	5.2	4.3	4.0	4.2	4.2	0.3	0.02	0.21	0.58
SS	-	4.4	4.0	-	-	4.4	4.5	-	0.3	0.44	0.58	0.38
SEM		0.2				0.3						
P-value												
Stim		0.39				0.93						
Stretch[a]		0.02				0.60						
Stim*Str		0.88				0.91						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Muscle contraction (sarcomere length)

Given the design of this experiment and the effect of the treatments on the rate of pH decline, it was anticipated that some of the treatments would result in either cold- or heat-shortening in muscles from the AT suspended carcasses. However, neither significant cold- or heat-shortening was observed in the loin from AT suspended carcasses (Table 7). Tenderstretching of the carcasses (TX and SS) did have a significant effect on sarcomere length and consequently the shear force of the loin (Table 5).

Table 7.The effect of electrical stimulation method, carcass hanging method and ageing period on sarcomere length (µm) of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	1.74	1.84	1.88	1.78	1.87	1.65	1.73	1.75	0.07	0.18	0.75	0.08
TX	1.92	2.13	1.97	2.20	2.03	1.88	2.20	1.95	0.11	0.61	0.69	0.06
SS	-	2.17	2.35	-	-	2.31	2.16	-	0.11	0.84	0.89	0.15
SEM		0.08				1.0						
P-value												
Stim		0.15				0.67						

Stretch[a]	0.00	0.00
Stim*Str	0.65	0.19

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

Water-holding capacity

The ability of muscles to retain their fluids during chilled storage, after defrosting or during cooking is affected by contraction status and protein denaturation (heat-toughening conditions). To assess the impact of the experimental conditions on water-holding capacity, thaw loss and cooking loss were determined.

The stimulation treatments did not result in significant differences in thaw loss for either the loin or the topside (Tables 8&9). However, tenderstretching (TX and SS) resulted in a highly significant difference in thaw loss, indicating that sarcomere length is an important determinant of water-holding capacity. Also, these results indicate that tenderstretching is not only an effective way to improve meat tenderness, but can also have a significant impact on yield.

Table 8.The effect of electrical stimulation method, carcass hanging method and ageing period on thaw loss (%) from the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	12.8	12.1	11.9	11.1	11.3	11.7	11.3	12.7	0.9	0.72	0.97	0.45
TX	10.5	10.5	9.0	10.1	10.3	10.5	9.2	10.8	0.7	0.73	0.18	0.90
SS	-	8.2	8.5	-	-	8.6	7.9	-	0.5	0.85	0.82	0.36
SEM		0.8				0.7						
P-value												
Stim		0.40				0.09						
Stretch[a]		0.00				0.00						
Stim*Str		0.52				0.61						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Table 9.The effect of electrical stimulation method, carcass hanging method and ageing period on thaw loss (%) from the M. semimembranosus (topside).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	11.9	10.0	10.5	9.3	10.7	10.4	9.4	8.7	0.9	0.32	0.10	0.79
TX	9.9	7.5	7.8	7.4	7.9	7.3	7.5	6.8	0.7	0.11	0.04	0.50
SS	-	7.5	7.7	-	-	7.8	6.9	-	0.6	0.66	0.59	0.35
SEM		0.5				0.6						
P-value												
Stim		0.00				0.07						

Stretch[a]	0.00	0.00
Stim*Str	0.90	0.86

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Neither stimulation method, ageing period, nor carcass hanging method had a significant effect on cooking loss from either muscle (Tables 10&11). This result indicates that the improvement in yield as a result of tenderstretching is maintained during cooking.

Table 10.The effect of electrical stimulation method, carcass hanging method and ageing period on cooking loss (%) from the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	19.7	19.5	20.3	19.7	19.8	18.8	21.0	20.8	0.9	0.64	0.33	0.77
TX	18.5	18.1	18.8	20.4	19.4	19.7	19.6	19.0	1.0	0.51	0.85	0.43
SS	-	19.7	19.7	-	-	19.3	20.7	-	1.0	0.77	0.49	0.51
SEM		0.8				1.0						
P-value												
Stim		0.64				0.44						
Stretch[a]		0.38				0.37						
Stim*Str		0.63				0.62						

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

Table 11. The effect of electrical stimulation method, carcass hanging method and ageing period on the cooking loss (%) of the M. semimembranosus (topside).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	23.2	24.5	24.1	23.7	22.9	23.2	25.3	23.3	1.1	0.82	0.70	0.63
TX	22.0	22.7	23.9	24.4	23.7	23.9	24.4	22.5	1.0	0.62	0.67	0.31
SS	-	23.4	23.9	-	-	24.4	24.3	-	0.7	0.37	0.78	0.68
SEM		0.6				0.9						
P-value												
Stim		0.55				0.93						
Stretch[a]		0.75				0.99						
Stim*Str		0.84				0.22						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Colour

The colour effects associated with the experimental conditions in this experiment are an increase in "paleness" as a result of heat-toughening conditions. The explanation for this phenomenon is that heat-toughening conditions lead to protein denaturation and, as a result, a decrease in water-holding capacity. This in turn leads to an increase in the amount of fluid on the muscle surface which reflects light, and thus the impression of colour. This effect is most notable from an increase in L*-values in the L*, a*, b* colour scheme. For this reason only the effects of the experimental treatments on L*-values are reported.

For the loin neither the electrical stimulation treatments nor the hanging treatments had a significant effect on the L*-value (Table 12). Only ageing period had a significant effect, with 26 days aged samples having slightly higher L*-values than 26 days aged samples.

Similarly, for the topside no clear effects of heat-toughening conditions on the L*-value were observed (Table 13). However, tenderstretching (TX and SS) did lead to a decrease in L*-values, which may be explained by an increase in water-holding capacity (Table 9).

Table 12.The effect of electrical stimulation method, carcass hanging method and ageing period on the L*-value (lightness) of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	38.8	39.1	39.7	40.2	41.2	40.7	41.5	40.3	0.8	0.01	0.84	0.60
TX	41.0	37.1	38.5	38.9	40.1	39.8	40.8	39.9	0.9	0.05	0.15	0.16
SS	-	37.4	39.2	-	-	40.6	40.6	-	0.8	0.01	0.26	0.24
SEM		0.8				0.8						
P-value												
Stim		0.10				0.65						
Stretch[a]		0.44				0.19						
Stim*Str		0.06				0.97						

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

Table 13.The effect of electrical stimulation method, carcass hanging method and ageing period on the L*-value (lightness) of the M. semimembranosus (topside).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	39.7	40.0	42.3	39.9	40.2	40.7	40.6	40.3	0.9	0.93	0.38	0.51
TX	36.3	36.6	38.2	37.7	39.4	38.8	37.9	40.4	0.9	0.01	0.35	0.44
SS	-	38.7	39.8	-	-	38.1	40.0	-	0.9	0.85	0.11	0.63
SEM		0.6				0.3						
P-value												
Stim		0.04				0.50						
Stretch[a]		0.00				0.02						
Stim*Str		0.96				0.49						

[a]For this analysis results for the stretched samples (XT and SS were pooled).

Post-mortem proteolysis

In this study, the impact of ageing was assessed by quantifying degradation of the structural myofibrillar protein desmin. The working hypothesis for this experiment was that post-mortem proteolysis of myofibrillar proteins commences at the onset of rigor mortis (pH about 6). Thus, a rapid pH-decline results in an early start of post-mortem proteolysis at a relatively high temperature. Given that the activity of proteolytic enzymes is temperature dependent, this would result in a relatively rapid tenderization. However, given that low pH and high temperature conditions favour protein denaturation, and thus denaturation (inactivation) of proteolytic enzymes, the extent of protein degradation (tenderization) after complete ageing (21-28 days?) may be less under heat-toughening conditions. As mentioned in appendix 1, according to an analysis of the BLUE database, the cross-over point in tenderness between loin samples that had a high T@pH6 and an intermediate T@pH6 is estimated to be at 14 days of ageing. This would imply for the present experiment that measures of post-mortem proteolysis (degradation of desmin) would be lower at 5 days p.m. in the COLD and CONTROL groups in comparison with the SmartStim and HOT groups, and that it would be the inverse at 26 days p.m. Therefore, a significant interaction of ageing period and stimulation treatment would be expected.

Degradation of desmin was quantified as the ratio of desmin degradation products and intact desmin (Table 14) and the amount of intact desmin relative to a standard (Table 15). In both cases, ageing period had a highly significant effect on desmin degradation. However, stimulation treatment, the interaction between stimulation treatment and ageing period, and the carcass hanging treatment did not affect post mortem proteolysis as assessed by desmin degradation.

Table 14.The effect of electrical stimulation method, carcass hanging method and ageing period on desmin degradation (ratio of desmin degradation products and intact desmin) of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	0.09	0.07	0.08	0.12	0.22	0.22	0.17	0.22	0.04	0.00	0.74	0.83
TX	0.06	0.05	0.05	0.09	0.22	0.15	0.19	0.21	0.03	0.00	0.74	0.65
SS	-	2.17	2.35	-	-	2.31	2.16	-	0.03	0.00	0.89	0.37
SEM		0.03				0.04						
P-value												
Stim		0.47				0.97						
Stretch[a]		0.20				0.89						
Stim*Str		0.88				0.59						

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

Table 15. The effect of electrical stimulation method, carcass hanging method and ageing period on desmin degradation (ratio of intact desmin and a desmin standard) of the M. longissimus lumborum et thoracis (striploin and cube roll).

Age	Day 5 p.m.				Day 26 p.m.				SEM	P-value		
Stim	Cold	Cont.	SmSti	Hot	Cold	Cont.	SmSti	Hot		Day	Stim	Day*Stim
Hang												
AT	1.01	0.98	1.04	1.00	0.79	0.86	0.89	0.92	0.06	0.00	0.61	0.75
TX	0.95	1.05	0.95	1.09	0.81	0.83	0.84	0.85	0.06	0.00	0.32	0.60
SS	-	0.95	0.99	-	-	0.82	0.80	-	0.05	0.00	0.89	0.56
SEM		0.05				0.05						
P-value												
Stim		0.64				0.54						
Stretch[a]		0.85				0.28						
Stim*Str		0.39				0.75						

[a]For this analysis results for the stretched samples (XT and SS) were pooled.

General discussion

The results regarding shear force analysis largely confirm the results of the sensory analysis (appendix 1) with regard to the limited effect of rate of pH decline on beef tenderness in the present experiment. In addition, these results are supported by non-significant effects of stimulation treatment (T@pH6) on either muscle contraction (sarcomere length) or post –mortem proteolysis (degradation of desmin). On the other hand, carcass hanging method (AT vs TX and SS) had a highly significant effect on both sensory quality and shear force. In addition, tenderstretching (TX and SS), significantly reduced thaw loss of both the loin and topside.

Regarding the recommendation that “the importance of rigor temperature in the MSA grading system may need to be reviewed” (appendix 1), it is important to realize that the present experiment only involves measurements on 50 carcasses and that the rate of pH decline (T@pH6) within the MSA grading system only serves as a criterion for MSA compliance for abattoirs. Given that the T@pH6 compliance requirements are based on a much larger body of research than the present experiment and that this measure does not play a role in grading of individual carcasses, a review of the impact of rigor temperature on meat quality may be not worth the effort in the sense of a return of money for investment. On the other hand, the observation in the present experiment that tenderstretching of carcasses results in a highly significant increase in waterholding capacity (a decrease in thaw loss for both the loin and the topside), may be a powerful incentive to adopt tenderstretching as a routine processing procedure to improve yield as well as sensory quality.

Development of an assay for post-mortem proteolysis

Background

Standard methods to assess the impact of ageing on degradation of muscle proteins include methods to determine the fragility of the myofibrillar structure upon homogenization (Myofibrillar Fragmentation Index [MFI], Particle Size Analysis [PSA]), or determination of the extent of degradation of specific myofibrillar proteins (desmin, troponin-T, etc) using SDS-PAGE, Western blotting and densitometry. In general, these methods are quite labour intensive and consequently, for economic and/or time constraint reasons do not lend themselves to large scale screening of muscle samples for quantification of p.m. proteolysis.

A possibility to assess degradation of specific myofibrillar proteins presents itself when the intact proteins are insoluble, but their degradation products are soluble and can be detected and quantified in the soluble muscle fraction. Two *in vitro* studies on degradation of myofibrillar proteins by μ-calpain suggest that this may be the case for the proteins titin and myosin light chain (Geesink and Koohmaraie, 1999; Anderson et al, 2012).

The purpose of the present study was to determine whether the impact of ageing of p.m. muscle on proteolysis of muscle proteins can be assessed by quantification of titin degradation products and/or myosin light chain in the soluble muscle fraction. Further, if this is the case, whether this lends itself to the development of a high throughput screening method to determine the extent of proteolysis in p.m. muscle.

Experimental design

For the purpose of this experiment, ten striploins (M. longissimus lumborum) of 1 day p.m YG MSA carcasses were obtained from Bindaree Beef (Inverell) . The anterior end of these striploins was sampled as described for experiment 3. The posterior end of the striploin was subdivided into equal sections, vacuum packed and randomly assigned to 1, 7, 14 or 21 days of ageing. Muscle sections were frozen and stored at -20°C after their allocated ageing period and until further sampling.

Results and discussion

The possibility to use the accumulation of titin degradation products and/or myosin light chain in the soluble muscle fraction was initially explored using SDS-PAGE followed by Western blotting (Figure 1). The examples for two samples presented in figure 1B show an increase in the abundance of immuno-reactive titin degradation products over the ageing period. In contrast, the amount of detectable myosin light chain did not appear to change over the ageing period (Figure 1C).

Based on these results it was decided to investigate whether direct quantification of titin degradation products in the soluble muscle fraction is an efficient method to quantify the extent of post-mortem proteolysis. For the purpose of this study, this involved extraction of the soluble muscle fraction, loading of the samples onto Western blotting membranes using a slot-blot manifold, and immunological detection and quantification of titin degradation products (see Figure 2, for an example). After

development of an assay based on this principle (Appendix 2), results using this assay were compared with standard assays to assess post mortem proteolysis (MFI and desmin degradation) and its relationship with the improvement in tenderness during ageing (Tables &). Quantification of the intensity of the bands corresponding to myosin light chain was conducted using SDS-PAGE and Western blotting.

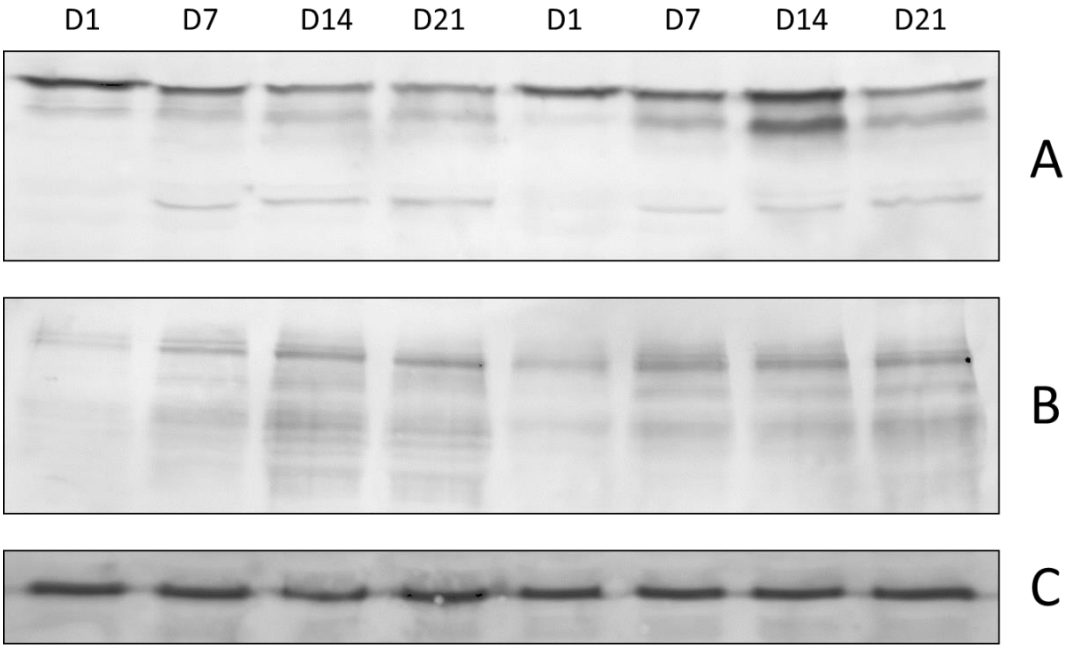

Figure 1. Western blot analysis against desmin (A) in whole muscle extracts, and titin degradation products (B) and myosin light chain (C) in soluble muscle extracts after 1, 7, 14 and 21 days of ageing of beef M. longissimus.

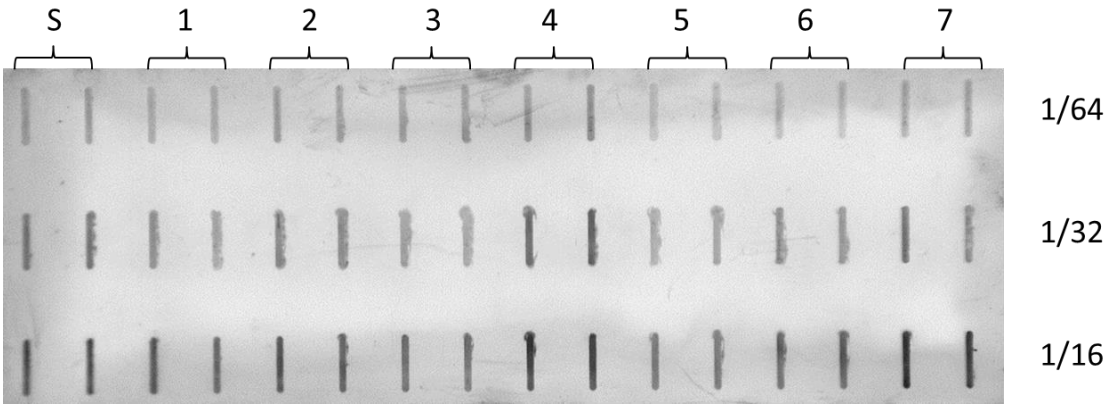

Figure 2. Representative example of a slot-blot against titin degradation products in the soluble muscle fraction of beef M. longissimus. (S = standards, 1-7 = sample number, 1/16 – 1/64 = sample dilution).

Table 16. The effect of ageing period on shear force and indicators of post mortem proteolysis of bovine M. longissimus.

Trait	Day 1	Day 7	Day 14	Day 21	SEM	P-value
Shear force (kg)	6.8	4.8	3.6	3.6	0.35	0.00
MFI	135	143	154	165	5.5	0.00
Desmin	0.28	0.44	0.54	0.56	0.04	0.00
Titin	0.63	0.89	0.96	1.03	0.04	0.00
Myosin light chain	1.07	1.02	1.00	1.05	0.03	0.38

Table 17. Correlations between shear force and ageing indicators (Pearson correlation coefficients and P-values).

Trait	MFI	Desmin	Titin	MLC
SF (kg)	-0.49 (0.001)	-0.51 (0.001)	-0.57 (0.000)	0.07 (0.685)
MFI		0.56 (0.000)	0.47 (0.002)	-0.19 (0.253)
Desmin			0.637 (0.000)	-0.08 (0.611)
Titin				0.11 (0.494)

The results presented in Table 16 show that ageing time had a significant effect on shear force, MFI, desmin degradation and titin degradation products in the soluble muscle fraction. However, the amount of myosin light chain (MLC) in the soluble muscle fraction was not affected by ageing time. Shear force was highly significantly correlated with MFI, desmin degradation and titin degradation, but not with the abundance of MLC in the soluble muscle fraction. (Table 17). Also, results regarding quantification of titin degradation products were significantly correlated with the standard indices of p.m. proteolysis (MFI and desmin degradation). Therefore, this method appears to be a useful alternative to the well-established methods to quantify the impact of ageing on degradation of muscle proteins.

A synopsis of the different steps in obtaining results for the different measures of p.m. proteolysis in the present study is listed in Table 18. Based on the experience with the different methods, it is estimated that the slot blot assay is at least twice as efficient regarding sample throughput as the other methods.

At the same time, it has to be realized that the materials and methods used for the development of this assay have their limitations and leave ample room for improvement. The limitations regarding the use of this assay include:

1. The use and binding capacity of PVDF membranes to capture the proteins of interest

2. The sensitivity and linearity of the detection method (alkaline phosphatase labeled secondary antibodies)

Given that this assay is based on detection of protein degradation products in the soluble muscle fraction, development of an ELISA or a dipstick (pregnancy test) type of assay for analysis of soluble muscle extracts, or even weep/purge appears feasible. Regarding the detection method, a good alternative is chemiluminescence based detection. This detection method is much more sensitive and has a much larger linear range than alkaline phosphatase based (colorimetric) detection methods. However, the use of this detection method requires rather expensive detection/image capture equipment.

As it is, the developed method lends itself to a more cost-effective analysis of the impact of ageing on p.m. proteolysis than standard methods. As such, this method may present a way to quantify the ageing response, without the interference of factors like muscle contraction status or connective tissue characteristics which are inherent in using tenderness measures.

Table 18. A synopsis of the steps involved in quantification of p.m. proteolysis of muscle proteins during ageing using different methods.

	Method		
Step	**MFI**	**SDS-PAGE +WB**	**Slot blot**
1	Weigh	Weigh	Weigh
2	Homogenise	Homogenise	Homogenise
3	Centrifuge	Solubilise protein	Centrifuge
4	Resuspend	Centrifuge	Dilute samples
5	Centrifuge	Protein determination	Load unto membranes*
6	Resuspend	Adjust protein content	Develop membranes
7	Protein determination	Run SDS-PAGE gels**	Quantify band intensities
8	Adjust protein content	Transfer to membranes	
9	Determine MFI	Develop membranes	
10		Quantify band intensities	

*48 samples per membrane. **13 samples per gel/membrane

The effect of retail packaging method on meat quality traits

Introduction

Modified atmosphere packaging (MAP) is the removal (vacuum packaging) or replacement of the atmosphere surrounding a product before sealing. Regarding the packaging of fresh beef, the use of a high oxygen and carbon dioxide gas mixture (typically 80% O_2 and 20% CO_2) is currently common practice. The advantage of the use of this gas mixture is that the high oxygen content improves colour and colour stability (shelf life) and the high carbon dioxide content has a bacteriostatic effect.

As reviewed by McMillan (2008), the use of high oxygen MAP may have a negative effect on some sensory quality traits. Given that the formation of off-flavours is frequently due to lipid oxidation, it is not surprising that high oxygen MAP has been associated with an increase in off-flavours and thus, lower consumer acceptability. Similarly, given that browning of meat (denaturation of myoglobin) during cooking is a function of temperature, time and oxidative status, it is not surprising that high oxygen MAP is associated with "premature browning". For minced beef this has been identified as a health risk, since beef patties produced from high oxygen packaged beef mince may appear fully cooked at temperatures insufficient to eliminate important pathogens.

Interestingly, a number of studies have also observed that high oxygen MAP may have a detrimental effect on meat tenderness. This effect has been attributed to a reduction in post mortem proteolysis, since the calpain system needs a reducing environment to be active, and/or cross-linking of proteins due to protein oxidation (Lund et al., 2007). However, a recent study on this topic did observe a decrease in tenderness and juiciness, an increase in oxidative cross-linking of proteins, but no effect on post mortem degradation of the myofibrillar proteins desmin and troponin-T (Kim et al., 2010). From a theoretical point of view, the latter result implies that research regarding the effect of ageing on meat tenderness has to go beyond consideration of the combined effects of connective tissue characteristics, muscle contraction (sarcomere length) and postmortem proteolysis, but also evaluate the effect of oxidative cross-linking of proteins on meat tenderness.

The MSA beef grading system is based on a Palatability Analysis of Critical Control Points (PACCP) that impact on eating quality (Thompson and Polkinghorne, 2008). However, the wide-spread use of high oxygen MAP of beef occurred after the main research efforts on which the current MSA model is based. Consequently, the current MSA model does not include the possible impact of packaging method.

Given the information above, it was decided to perform a pilot study to determine whether packaging method (high oxygen MAP vs vacuum storage) affects sensory quality as determined using an MSA-style protocol, objective meat quality traits and post mortem proteolysis during ageing.

Experimental design

For the purpose of this experiment, twenty striploins (M. longissimus lumborum) were selected at Bindaree Beef (Inverell). Of these, ten were obtained from 1 day p.m YG MSA carcasses (females or steers up to 30 months old that have passed MSA grading). The other ten striploins (7 day vacuum aged)

originated from PR carcasses (up to 42 months old animals with no more than 7 permanent incisor teeth).

Four adjacent steaks (about 2.5 cm thick) were cut from the anterior end of the primal of which 2 were randomly assigned to MAP packaging (80% O_2, 20%CO_2) and the remaining 2 to Darfresh® (skinpack) packaging. Samples were transported to UNE and stored for an additional 7 days at about 3°C before further sampling.

After the 7 day ageing period, the packages were opened and the steaks were sampled for colour measurements, sensory analysis, shear force and degradation of myofibrillar proteins. Regarding the latter, a sample of about 4 g was cut from each steak, placed in 5 mL tubes and frozen at -20°C until analysis.

Results and Discussion

Sensory analysis

From the results presented in Table 19, it is evident that the source of the samples had a large effect on the sensory quality traits. As mentioned in the material and methods section, the samples packed 1 day p.m. originated from YG MSA carcasses, whereas the samples packed at 7 days p.m. originated from PR carcasses.

Regarding the effect of packaging method *per se,* skin packaging resulted in a significantly higher quality scores for juiciness, flavour and overall liking, and as a result, the MQ4 score as well (Table 19). Overall, packaging method accounted for an almost 8 point difference in MQ4 score.

Degree of doneness in this experiment was evaluated from rare (1) to well done (5) (see appendix 3). From the results presented in Table 19, it appears that both the source of the samples and the packaging method had an effect on the degree of doneness. The effect of the source of the samples can be explained by a difference in ultimate pH (Table 20), where the samples obtained from 7 day p.m. striploins had a significantly higher pH. The effect of packaging method is likely explained by a more advanced stage of oxidation of myoglobin in the MAP packed samples.

Table 19. The effect of retail packaging day and packaging method on sensory meat quality traits (Least square means and pooled standard errors of the measurements).

Pack Day	Day 1 p.m.		Day 7 p.m.		SEM	P-value		
Pack Method	MAP	Skin	MAP	Skin		Day	Pack	Day*Pack
Tenderness	64.5	64.3	39.4	52.0	4.05	0.000	0.135	0.123
Juiciness	71.4	75.2	56.3	65.3	2.62	0.000	0.019	0.327
Flavour	57.4	66.6	52.5	59.5	3.12	0.066	0.015	0.730
Overall	57.9	66.5	47.6	59.3	3.81	0.028	0.011	0.687
MQ4	61.1	66.8	47.6	57.8	3.20	0.001	0.018	0.487
Doneness	3.1	2.7	2.6	2.1	0.23	0.020	0.054	0.826

Objective meat quality traits

Corroborating the results regarding sensory quality, the source of the samples also had a large effect on the objective quality traits (Table 20). As mentioned in the material and methods section, the samples packed 1 day p.m. originated from YG MSA carcasses, whereas the samples packed at 7 days p.m. originated from PR carcasses. Sensory tenderness scores were highly correlated with shear force measurements (Figure 3).

Regarding the effect of packaging method *per se,* skin packaging resulted in a significantly lower shear force than MAP packaging for both samples packed at 1 and 7 days p.m. (Table 20).

Table 20. The effect of retail packaging day and packaging method on objective meat quality traits.

Pack Day	Day 1 p.m.		Day 7 p.m.		SEM	P-value		
Pack Method	MAP	Skin	MAP	Skin		Day	Pack	Day*Pack
pH	5.48	5.47	5.62	5.61	0.04	0.001	0.855	0.938
L*	40.92	41.37	38.24	36.16	0.75	0.000	0.288	0.101
a*	21.46	24.13	23.74	22.37	0.70	0.712	0.366	0.007
b*	11.05	11.93	11.66	10.62	0.46	0.455	0.876	0.045
SF	3.94	3.53	4.81	4.30	0.14	0.000	0.003	0.736

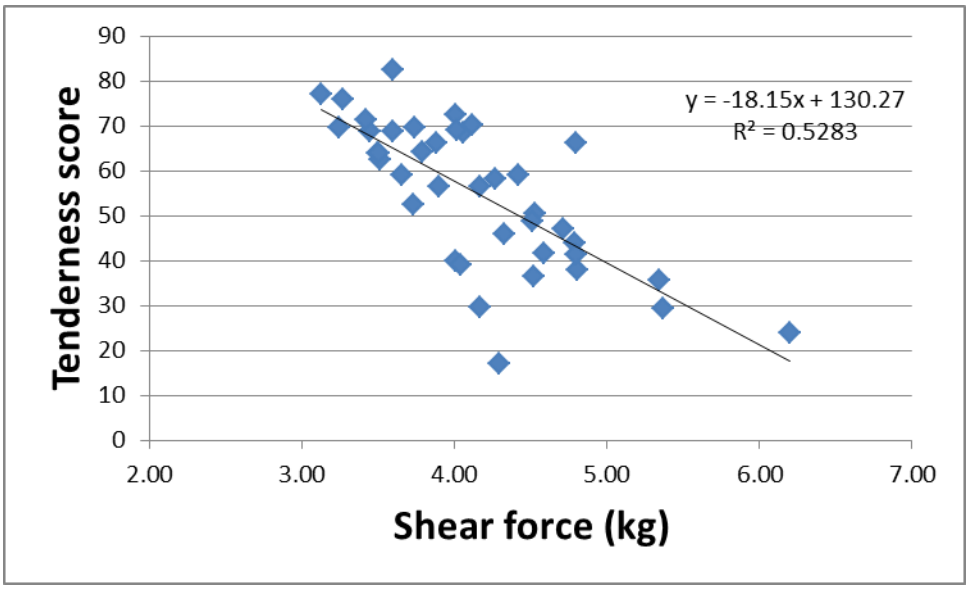

Figure 3. The relation between sensory tenderness scores and shear force.

Post mortem proteolysis

To assess the effect of packaging day and packaging method on post mortem proteolysis the amount of intact desmin and its degradation products was quantified using Western blotting (Figure 4). The results presented in Table 21 suggest that packaging method did not affect degradation of desmin.

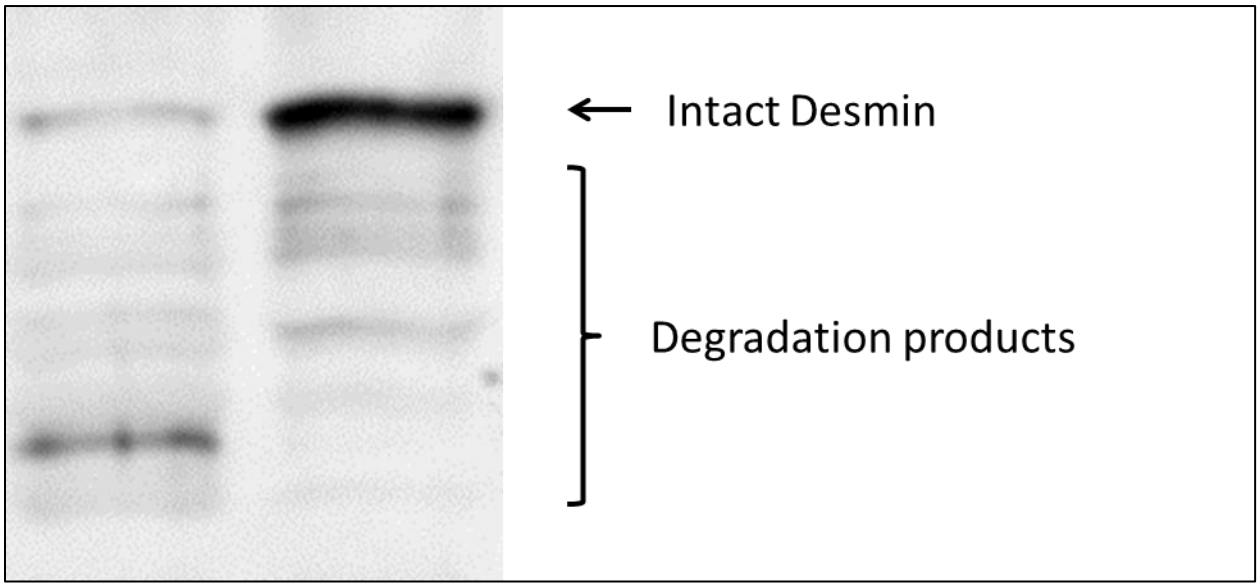

Figure 4. Western blot against intact desmin and desmin degradation products.

Table 21. The effect of retail packaging day and packaging method on proteolysis of desmin.

Pack Day	Day 1 p.m.		Day 7 p.m.		SEM	P-value		
Pack Method	MAP	Skin	MAP	Skin		Day	Pack	Day*Pack
Desmin*	0.43	0.32	0.43	0.43	0.04	0.205	0.197	0.262

*Ratio of degraded desmin and intact desmin.

General discussion

The results of this experiment indicate that retail packaging method can have a significant effect on sensory meat quality traits as exemplified by an almost eight point difference in MQ4 score between high-oxygen MAP and skin-packed samples. Within the MSA grading system, an eight point difference in MQ4 represents a relatively large difference in sensory quality. Yet, retail packaging method is not a factor incorporated in the current MSA grading system.

The MQ4 score reflects an evaluation of the weighted scores for tenderness, juiciness, flavour and overall quality and these scores are often highly correlated because of the use of un-trained consumer panels. On a theoretical basis, a decrease in flavour score as a result of high-oxygen MAP is to be

expected, since the formation of off-flavours may be due to oxidative processes. An effect of packaging method on sensory tenderness scores, however, may be simply due to a "halo-effect" of flavour scores on tenderness scores, inhibition of post mortem proteolysis (ageing response), or, as suggested in previous studies on the subject, oxidative cross-linking of structural muscle proteins. To address this issue we determined the shear force of all the samples presented to the sensory panel, and assessed degradation of desmin as an indicator of post mortem proteolysis. Packaging method had a significant effect on shear force values and the shear force values where strongly correlated with sensory tenderness scores. Yet, post mortem proteolysis, as assessed by desmin degradation, was not affected by packaging method. Thus, the detrimental effect of high-oxygen MAP on meat tenderness appears to be due to a physical effect on the muscle structure other than inhibition of post mortem proteolysis of muscle proteins. As suggested by work of others, a possible explanation for this may be oxidative cross-linking of proteins. Thus far, the contribution of oxidative cross-linking of proteins to meat toughness has only been evaluated in a qualitative fashion to explain the detrimental effects high-oxygen MAP on meat tenderness. Given the magnitude of the effect of high-oxygen MAP on tenderness, further research to quantify the impact of oxidative cross-linking of proteins on tenderness appears warranted.

Conclusions and recommendations

The results of this experiment allow for the following main conclusions:

- High-oxygen MAP can have a significant negative impact on beef quality.
- The adverse effect of high-oxygen MAP on meat tenderness may be due oxidative cross-linking of proteins.

Based on these conclusions it is recommended that additional research is performed to quantify the effects of retail packaging method on beef quality to the extent that it can be incorporated in the MSA grading system. In addition, to further our understanding of the effects of retail packaging method on meat quality, the recommended research effort should include objective measures that may explain effects on flavour (lipid oxidation) and tenderness (oxidative protein cross-linking and/or inhibition of post mortem proteolysis).

Material and Methods

Sampling

Project 1

As described in appendix 1, the samples originated from an experiment conducted at ACC (Brisbane). Samples for analysis of objective meat quality traits were vacuum packed and stored frozen at -20°C at UNE until analysis. An inventory of the collected samples resulted in the identification of 189 samples of the M. longissimus lumborum et thoracis (striploin and cube roll) and 144 samples of the M. semimembranosus (topside) aged for either 5 or 26 days. Samples were defrosted for 48 hours in the cold room and processed for further analysis as described below.

Project 2

For the purpose of this experiment, ten striploins (M. longissimus lumborum) of 1 day p.m YG MSA carcasses were obtained from Bindaree Beef (Inverell) . The anterior end of these striploins was sampled as described for experiment 3. The posterior end of the striploin was subdivided into equal sections, vacuum packed and randomly assigned to 1, 7, 14 or 21 days of ageing. Muscle sections were frozen and stored at -20°C after their allocated ageing period and until further sampling.

Project 3

For the purpose of this experiment, twenty striploins (M. longissimus lumborum) were selected at Bindaree Beef (Inverell). Of these, ten were obtained from 1 day p.m YG MSA carcasses (females or steers up to 30 months old that have passed MSA grading). The other ten striploins (7 day vacuum aged) originated from PR carcasses (up to 42 months old animals with no more than 7 permanent incisor teeth).

Four adjacent steaks (about 2.5 cm thick) were cut from the anterior end of the primal of which 2 were randomly assigned to MAP packaging (80% O_2, 20%CO_2) and the remaining 2 to Darfresh® (skinpack) packaging. Samples were transported to UNE and stored for an additional 7 days at about 3°C before further sampling.

After the 7 day ageing period, the packages were opened and sampled for colour measurements, sensory analysis, shear force and degradation of myofibrillar proteins. Regarding the latter, a sample of about 4 g was cut from each steak, placed in 5 mL tubes and frozen at -20°C until analysis.

Sensory analysis (project 3)

The sensory panel (40 persons) allowed for the evaluation of 80 steaks. These steaks were:

- No ageing in vacuum, Darfresh® packed and aged for 7 days (n = 20) (treatment A)
- No ageing in vacuum, MAP packed and aged for 7 days (n = 20) (Treatment B)

- Aged for 7 days in vacuum, Darfresh® packed and aged for 7 days (n = 20) (Treatment C)
- Aged for 7 days in vacuum, MAP packed and aged for 7 days (n = 20) (Treatment D)

Samples were grilled on a hot plate at 220°C for 5 min (samples were turned after 2.5 minutes). Each grill session contained 2 steaks of each treatment. A 6x6 cm sample was cut from the grilled steaks for sensory evaluation. The remainder of the samples were packed in ziplock bags and stored in the chiller until the following day when the shear force was determined.

Samples were placed on disposable plates and served to the sensory panel (4 panelists per group) for evaluation. The sensory evaluation session was started by serving all panelists a starter sample. Samples that were part of this trial were evaluated subsequently over 8 rounds (Table 22). Each group was instructed to cut the samples into four portions, and the individual members were instructed to score the samples MSA style (tenderness, flavor, juiciness and overall liking). In addition, the panelists were instructed to evaluate the degree of doneness by visual appraisal (rare, medium rare, medium, medium-well done, well done).

Table 22. Cooking/serving schedule (S = Starter, A = MAP packed, B = Skin packed, 1-10 = Day 1, 11-20 = Day 7).

Round	1	2	3	4	5	6	7	8	9
Group									
1	S	A1	B12	A3	B14	A6	B17	A8	B19
2	S	B1	A12	B3	A14	B6	A17	B8	A19
3	S	A11	B2	A13	B5	A16	B7	A18	B10
4	S	B11	A2	B13	A5	B16	A7	B18	A10
5	S	A1	B12	A4	B15	A6	B17	A9	B20
6	S	B1	A12	B4	A15	B6	A17	B9	A20
7	S	A11	B3	A14	B5	A16	B8	A19	B10
8	S	B11	A3	B14	A5	B16	A8	B19	A10
9	S	A2	B13	A4	B15	A7	B18	A9	B20
10	S	B2	A13	B4	A15	B7	A18	B9	A20

Colour measurements

Colour values (L*-, a*- and b*-values) were determined about 1 hour after opening of the packages using a Minolta CR-300 Chromameter. The light source of the color meter was D65 and the instrument was calibrated on a white tile according to the manufacturer's specifications.

Shear force and cooking loss

Shear force and cooking loss were determined on cooked (study 1 and 2) and grilled (study 3) samples as described by Perry et al. (2001).

Sarcomere length

Sarcomere length determination was similar to the filar micrometer method described by Cross et al. (1981). Briefly, samples were fixed using a glutaraldehyde fixative and subsequently homogenised in a sucrose solution. A drop of the homogenate was placed onto a microscope slide and covered with a coverslip. Samples were evaluated using a microscope at 100X magnification. Once a suitable myofibril was located, a digital image was recorded and the length of a 10 sarcomere segment was recorded and used to calculate the sarcomere length using an image analysis program (NIS-Elements version 3.13, Nikon Corporation, Japan). For each sample, images for ten different fields of view were recorded and the average sarcomere length is reported.

SDS-PAGE and Western blotting

A portion of diced muscle tissue (approximately 1 g) was extracted in 5 mL of ice-cold extraction buffer (50 mM Tris/HCl, 10 mM EDTA, pH 8.3). Tissue was homogenized using a polytron on high speed. Total tissue homogenate (0.5 mL) was mixed with an equal volume of 2x SDS-PAGE sample buffer (0.125 M Tris/HCl, 4% SDS, 20% glycerol, pH 6.8) and heated at 50°C for 20 min. After centrifuging the solution at 16,000 x g_{max} for 5 min at room temperature, the supernatant was collected and the protein concentration was determined using a Pierce BCA protein assay kit (Pierce Laboratories, Rockford, IL). For collection of soluble muscle protein, the remaining homogenate was centrifuged at 2,000 x g_{max} and 2 x 0.75 mL of the supernatant was collected and frozen at -20°C until use.
For SDS-PAGE and Western blotting of soluble muscle proteins, samples were mixed with an equal volume of 2x SDS-PAGE sample buffer and processed as described above for total tissue homogenates.
For detection of titin degradation products in the soluble muscle fraction, samples were processed described in the developed protocol (appendix 2).
For SDS-PAGE, samples were diluted to the appropriate protein concentration using SDS-PAGE sample buffer containing 0.5% (v/v) 2-mercapthoethanol and bromophenol blue (0.04% v/v). SDS-PAGE was performed using 8 x 10 x 0.075-cm 7.5 or 12% acrylamide minigels using a 37.5:1 acrylamide to bisacrylamide stock solution.
After electrophoresis, at 200 V, gels were transferred onto Hybond-P polyvinylidine fluoride membranes (Amersham Biosciences, Uppsala, Sweden) at 200 mA for 1 h using a wet transfer apparatus (BioRad Laboratories, Hercules, CA). All the following steps were performed at room temperature. Membranes were blocked with 3% (wt/vol) nonfat dry milk in Tris-buffered saline containing Tween (TTBS: 20 mM Tris/HCl, 137 mM NaCl, 5 mM KCl, 0.05% Tween, pH 7.5). After blocking for 1 h, the membranes were exposed to mouse monoclonal anti-desmin (DE-U-10; dilution 1:2,500; Sigma-Aldrich, St. Louis, MO), mouse monoclonanal anti-titin (9D10; dilution 1:250; DSHB, Iowa City, IA), or mouse monoclonanal anti-myosin light chain (F310; dilution 1:500; DSHB, Iowa City, IA) . Blots were incubated for 1 h before being washed with TTBS. The secondary antibody used was alkaline phosphatase conjugated antibody against total mouse IgG (A3562; dilution 1:30,000; Sigma-Aldrich, St. Louis, MO). Blots were exposed to the secondary antibody for 1 h before being extensively washed with TTBS. Antibody binding was visualised using an alkaline phosphatase conjugate substrate kit (BioRad Laboratories, Hercules, CA).
Digital images of the developed Western blots were recorded and optical density of the bands was analysed using an imaging system(Bio 1-D, Vilbert Lourmat, Marne-La-Vallee, France).

Statistical analysis

Calculation of summary statistics, analysis of variance of main effects and their interaction and correlation analysis were performed using Minitab-14.

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Appendix 1. A summary of the gene marker effects from the ACC experiment. Prepared for the Pathways meeting Jul 2012. John Thompson and Rod Polkinghorne

Background

Recent analyses of the BLUE data base showed an interaction between days aged, rigor temperature and hanging method for MQ4 score (Warner et al *submitted*). In AT carcasses high rigor temperatures (Temp@H6 of 35^{o}C) resulted in reduced MQ4 scores at 35 days ageing compared to carcasses which had been processed at lower rigor temperatures (temp@pH6 15^{o}C). At lower rigor temperatures this effect was reversed with a crossover at day 14 ageing where there was no effect of the rigor temperature on MQ4. Effectively tenderstretching the sides eliminated the significance of the interaction.

This raised the question as to the transportability of these results and the implications for long aged product. Are the differences in eating quality in carcasses exposed to high rigor temperatures maintained as carcasses are aged for extended times? Most of the consumer data in BLUE was for samples aged less than 35 days. In the early days of MSA there was less interest in extended ageing, although more recently there has been interest in using ageing as a tool to achieve higher grades. Longer ageing times also has implications for export product.

Long aged samples tended to decrease in palatability but there is little information as to the magnitude of this decline in palatability at extended ageing times. Any decline in palatability with extended aging has links to the research being done on flavor as it is unlikely to be due to tenderness.

Objectives

The experiment was set up with several objectives.

a) The major objective was to investigate the interaction between rigor temperature and days ageing in normally hung and tenderstretched carcasses for a range of muscles.

b) The second objective was to investigate whether Smart Stim had an effect on eating quality and if so whether this effect simply mediated via temperature at pH6.

c) The third objective was whether Super Stretch (ie suspending the carcass via the aitch bone and pulling the hind leg down towards the thorax and securing it prior to the onset of rigor) had an effect on eating quality.

d) Finally there was an opportunity to overlay a gene marker (GM) treatment across sub groups. This involved obtaining GM status on a large number of animals prior to slaughter and then selecting individuals which were divergent in GM status for the different treatment groups.

Materials and methods

A month before the experiment was run the two pens of cattle (ca. 700 head) from Brinderly Park which were going to be killed on the trial day were identified and tail hair pulled. Hair samples were submitted to Pfizer Genetics for marker analysis and the results returned a week prior to the slaughter day. Cattle were sorted on their tenderness MVP and the high and low percentiles identified and booklets printed.

A day prior to slaughter a trial run was conducted which measured glycolytic rate using the LVES and no stimulation systems were run to ensure that carcasses would achieve the desired outcomes in terms of rigor temperature. Based on these results the LVES treatment was reduced from 20 to 10 seconds and also fan speed was increased for the no stimulation group.

The experimental design is described in Table 1. Within each of these groups animals of low and high GM status were selected at the knocking box. The no stimulation group was intended to result in a low rigor temperature (t@pH6<15oC), whereas the Smart Stim and LVES 10 secs were aimed at optimal rigor temperature (20 to 30oC). For the high rigor temperature treatment (t@pH6>30oC) the LVES system was applied for 40 secs (the maximum possible with the chain speed at ACC).

Table 1 The number of sides allocated to the different hang and stim treatments

	Low rigor temp	Optimal rigor temperature		High rigor temperature
Hang	No stim	LVES 10 secs	Smart Stim	LVES 40 secs
Achilles hung (AT)	10	10	10	10
Tenderstretch TX)	10	10	10	10
SuperStretch (SS)		10	10	

Stimulation treatments were cycled between no stimulation, LVES (10 secs), Smart Stim and LVES 40 secs treatments every half hour. The stimulator was turned off for the no stimulation treatment. If when applying the LVES 10 secs, Smart Stim and no stimulation treatments, OH&S required the immobilser to the applied during shackling this animal was discarded and another of the correct GM status found from the remaining animals. Animals for all treatments were assigned to treatments before lunch time and so all carcasses had adequate time to reach ultimate before grading the next morning. The hang treatments (AT, TX and Super Stretch) for the different stimulation treatments were rotated between left and right sides. Within each of the hang and stimulation treatments shown in Table 1 carcasses were selected from the high and low percentiles for the tenderness MVP.

Glycolytic rate was measured in the chiller by MSA. Pre-rigor measurements of pH, temperature and time were recorded every half hour until carcasses reached pH6. Temperature at pH6 was estimated using both the exponential functions of pH versus time and temperature versus time and interpolation. The correlation between these 2 methods was 0.98. For consistency with other MSA results the interpolation estimate for rigor temperature was used in the following analyses.

At boning nine primals from each side were collected and held at 1^0C for sample preparation. The primal and subsequent breakdown to ageing treatments are shown in Table 2. Given the large number of samples (5,000) sample preparation was undertaken over 4 days. Samples were aged at 1^oC and then frozen down in boxes at the designated days. To ensure rapid freezing boxes were restricted to having only 3 layers of samples.

Table 2 Ageing (in days) and cooking treatments (Grill and Yakiniku) for the individual samples collected from each of the 100 sides.

Primal	MSA code	Cook	Ageing times
Tenderloin	TDR062	GRL	5,26,47
Cube Roll	CUB045	GRL	5, 26, 47, 68
	SPN081	GRL	5,26,47
Striploin	STR045	GRL	5, 26, 47, 68
Oyster	OYS036	GRL	5,26,47
Rump	RMP005	GRL	5,26,47
	RMP131	GRL	5,26,47
	RMP231	GRL	5,26,47
	RMP087	YAK	5,26,47
Topside	TOP001	YAK	5,26,47
	TOP033	YAK	5,26,47
	TOP073	GRL	5,26,47
Eye Round	EYE075	GRL	5,26,47
Outside Flat	OUT005	GRL	5,26,47
Knuckle	KNU066	GRL	5,26,47
	KNU099	GRL	5,26,47

Sensory testing was undertaken using the MSA protocol over the next 12 months.

Results

Table 3 means, variance and range for carcass traits of the 50 carcasses in the ACC experiment.

Carcass traits	Mean	Stdev	Min	Max
Carcass weight	225.8	16.5	197	279
UMb	287	64.2	140	430
Uoss	169	40	110	380
Ribfat	4.5	3.2	1	20
EMA	65.7	6.2	50	80
T@pH6	28.8	8.7	10.5	39.6
Tend MVP	0.21	0.35	-0.38	0.70

Carcasses were typical of domestic bodies processed for the supermarkets trade. Rigor temperature ranged from ranged from 10 to 40^{o}C (Table 3, Figure 1). The aim of the experiment in terms of achieving a wide range of rigor temperature was certainly achieved which reflects the excellent co-operation we obtained from the abattoir.

Similarly MVPs for tenderness ranged from -0.4 to 0.7. Distribution of MVPs was shown in Figure 2. The mean MVP for the tender group (ie Low MVPtender) was -0.12 and the tough group (high MVPtender) was 0.55.

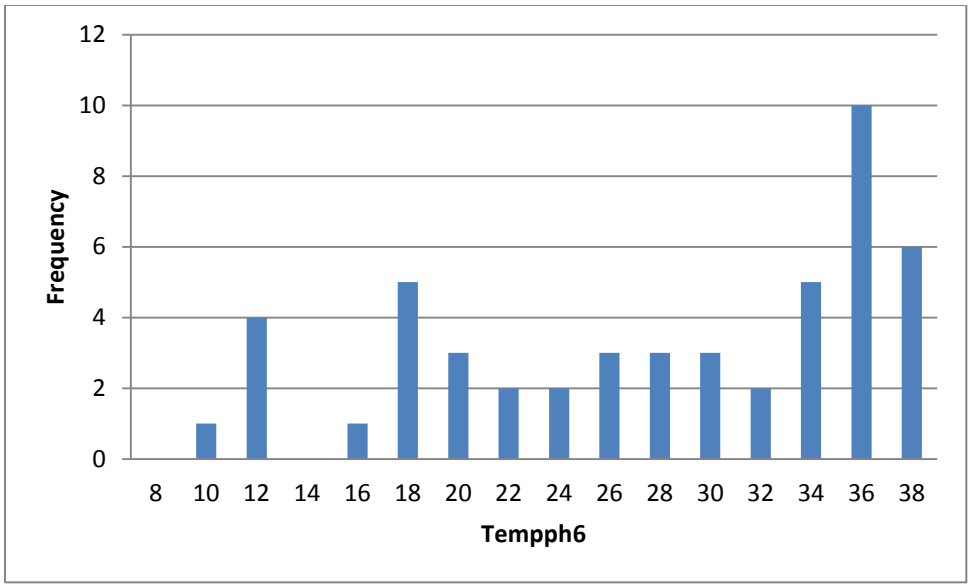


Figure 1 The distribution of rigor temperature for the 50 carcasses.

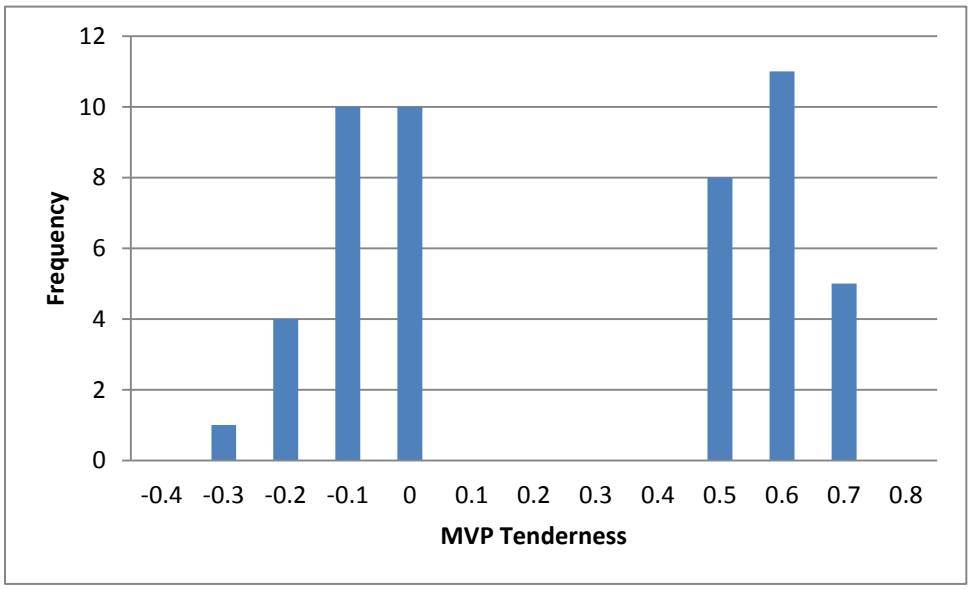


Figure 2 The distribution of MVP for tenderness

Analysis

These initial analyses were targeted at summarizing the rigor temperature, ageing and gene marker effects. MQ4 scores for individual muscles were analysed using mixed models which contained fixed effects of hang (AT, TX and SS), muscle position, days aged, gene marker status (Low and high MVP tenderness) with covariates for T@pH6. The mixed model also included a random term for animal nested within GM group. First order interactions hang X days aged, GM*hang, GM*Daged, GM*T@pH6, days aged*t@pH6 were also tested and non-significant interactions discarded (P>0.05). In

analyses not shown here both linear and curvilinear terms for rigor temperature were tested but found to be not significant.

Only MQ4 results are shown in this summary. The correlation between MQ4 and other sensory scores ranged from 0.92 to 0.99. Analysis of the four individual sensory scores showed very similar outcomes in terms of final models.

Table 4 a) Significance of terms in the initial models used to investigate the effects of Hang days aged, t@pH6 and GM status on MQ4 score.

	Cook/Muscle combination							
Independent Variables	GRL CUB 045	GRL SPN 081	GRL EYE 075	GRL KNU 066	GRL KNU 099	GRL OUT 005	GRL OYS 036	GRL RMP 005
Hang	NS	0.07	*	***	***	***	NS	NS
Position			***			***		
Daged	NS	NS	NS	NS	NS	NS	NS	NS
Hang*Pos			NS			*		
Hang*daged	NS	NS	NS	*	NS	NS	NS	NS
t@pH6	NS	NS	NS	NS	NS	NS	NS	NS
T@pH6*daged	NS	NS	NS	NS	NS	NS	NS	NS
GM	NS	NS	NS	NS	NS	NS	NS	NS
Hang*GM	NS	NS	0.06	NS	NS	NS	NS	NS
Daged*GM	NS	NS	NS	NS	NS	NS	NS	NS
T*pH6*GM	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, *** was P>0.10, <0.05, 0.01 and 0.001, respectively

Table 4 b) Significance of terms in the initial models used to investigate the effects of Hang, days aged, t@pH6 and GM status on MQ4 score.

	Cook/Muscle combination							
Independent Variables	YAK RMP 087	GRL RMP 131	GRL RMP 231	GRL STR 045	GRL TDR 062	YAK TOP 001	YAK TOP 033	GRL TOP 073
Hang	NS	***	***	***	***	***	NS	NS
Position				***	NS			0.09
Daged	NS	NS	NS	***	NS	NS	NS	NS
Hang*Pos				*	NS			NS
Hang*daged	NS	NS	NS	NS	*	NS	NS	NS
t@pH6	NS	NS	NS	NS	NS	NS	NS	NS
T@pH6*daged	NS	NS	NS	NS	NS	NS	NS	NS
GM	NS	NS	NS	NS	NS	**	NS	NS
Hang*GM	NS	NS	NS	NS	*	NS	NS	NS
Daged*GM	NS	NS	NS	NS	0.09	NS	NS	NS
T*pH6*GM	NS	NS	NS	NS	NS	*	NS	NS

The only interactions to achieve significance were t@pH6 X GM for the TOP001 and hang *GM for the TDR062.

It was acknowledged that the presence of non-significant interactions with covariates may have masked the significance of main effects and therefore the models were rerun with non-significant (P>0.05) interactions sequentially removed.

Despite several significant interactions shown in Tables 4 a) and b) the results in Tables 5 a) and b) clearly showed that for the majority of muscles the importance of interactions between days aged, hang, t@pH6 and GM status were small and unlikely to be important.

Table 5 a) Significance of final models to investigate the effects of Hang days aged, t@pH6 and GM status on MQ4 score.

Independent Variables	GRL CUB 045	GRL SPN 081	GRL EYE 075	GRL KNU 066	GRL KNU 099	GRL OUT 005	GRL OYS 036	GRL RMP 005
Hang	NS	NS	*	***	***	***	NS	NS
Pos			***			***		
Daged	**	0.08	***	0.06	*	*	*	**
Hang*Pos			NS			*		
Hang*daged	NS	NS	NS	NS	NS	NS	NS	NS
t@pH6	NS	NS	NS	*	NS	NS	NS	NS
GM	NS	*	*	*	NS	*	NS	NS

Table 5 b) Significance of final models to investigate the effects of Hang days aged, t@pH6 and GM status on MQ4 score.

Independent Variables	YAK RMP 087	GRL RMP 131	GRL RMP 231	GRL STR 045	GRL TDR 062	YAK TOP 001	YAK TOP 033	GRL TOP 073
Hang	***	***	***	***	***	***	NS	NS
Pos				***	NS			NS
Daged	***	***	0.06	***	***	NS	***	***
Hang*Pos				*	NS			NS
Hang*daged	NS	NS	NS	NS	**	NS	NS	NS
t@pH6	NS	NS	NS	NS	NS	NS	NS	NS
GM	*	NS	*	**	***	*	NS	**

Tables 5 a) and b) showed the significance of treatment effects in the reduced model. Predicted means for the GM effects were shown in Table 6. In all cases those carcasses with the low MVP for shear force showed higher tenderness scores than carcasses with high MVP for shear force. The magnitude of the differences between muscles ranged from 0.8 MQ4 scores to a maximum of 6.9 MQ4 units in the STR045.

Table 6 Predicted means for MQ4 scores for individual muscles with low and high gene marker status

Cook	Cut	GM Tender (low MVP)	GM tough (high MVP)	Difference	(SE)
GRL	CUB045	61.2	58.2	1.9	(1.7)
GRL	CUB081	78.6	73.7	4.9	(1.6)
GRL	EYE075	45.8	41.9	3.9	(1.2)
GRL	KNU066	58.8	55.2	3.6	(1.4)
GRL	KNU099	47.2	46.4	0.8	(1.5)
GRL	OUT005	45.9	42.1	3.8	(1.2)
GRL	OYS036	72.4	69.7	2.7	(1.4)
GRL	RMP005	68.9	66.9	2.0	(1.8)
YAK	RMP087	55.3	51.1	4.2	(1.4)
GRL	RMP131	61.0	58.8	2.2	(1.7)
GRL	RMP231	67.0	61.3	5.7	(1.8)
GRL	STR045	60.3	53.4	6.9	(1.7)
GRL	TDR062	74.7	68.6	6.1	(1.1)
YAK	TOP001	65.0	61.6	3.4	(1.2)
YAK	TOP033	65.0	62.6	2.4	(1.3)
GRL	TOP073	44.6	40.5	4.1	(1.1)

Although not shown when t1, t2, t3 and t4 were substituted in place of the treatment groups based on MVP estimates the difference due to gene markers were largely associated with calpastatin (t1), calpain (t2 and t3 variants 1316 and 14751). This was supported by Thompson (2011) using the samples in the BLUE data base where the majority of the effects due to the calpastatin marker and the 1316 variant of the calpain marker. In this study T4 (calpain 3) played a minimal role and when significant the differences were not always as expected.

Conclusion

This experiment showed that gene marker effects were apparent for a number of muscles in the carcass. What was of interest was that the effect of gene markers did not interact with days aged or rigor temperature. The lack of an interaction with days aged was surprising as the markers work via the ageing enzymes. The lack of an interaction with rigor temperature was supported by the results from the Beef CRC where the same magnitude of the GM effect was evident in normally processed and heat shortened carcasses.

It appears that the effects due to rigor temperature were not important in this experiment. This was surprising given the range in rigor temperature achieved experimentally (see Figure 1). As a check the data was reanalyzed using the exponential estimate of rigor temperature but as expected from the high correlation between the estimates this had little impact on the outcomes. The lack of any effect probably means that the importance of rigor temperature in the MSA scheme may need to be reviewed at some stage soon.

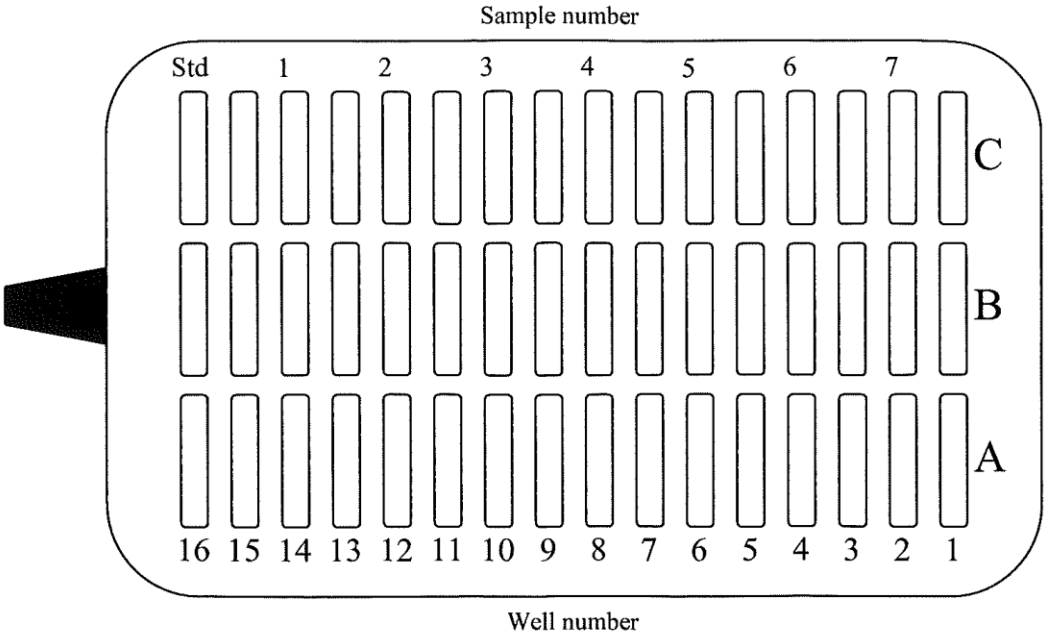
Appendix 2: Protocol for quantification of titin degradation products in soluble muscle extracts using slot-blotting (Fisher Biotech CSL-S48 – Slot Blotter, 48 Sample).

Preparation of the soluble muscle fraction

- Homogenise 1 g of muscle tissue in 5 mL of ice-cold extraction buffer (50 mM Tris/HCl, 10 mM EDTA, pH 8.3)using a polytron on high speed.
- Centrifuge the samples at 2,000 x g_{max} for 5 min. and collect the supernatant.
- Dilute the samples using Tris Buffered Saline (TBS) according to the schedule below:
    - 1:4 = 750µL TBS : 250µL sample
    - 1:16 = 750µL TBS : 250µL 1:4 dilution
    - 1:32 = 500 µL TBS: 500µL 1:16 dilution
    - 1:64 = 500 µL TBS: 500µL 1:32 dilution
- Keep the samples and buffers on ice during processing. Diluted samples can be kept at -20°C until later use.

Loading the slot blot

- Prepare blotting paper and membranes and place them in the manifold as described in the instruction manual.
- Load 10µL of sample in duplicate for the 1:16, 1:32 and 1:64 dilutions (see figure below for an example)
- Attach the pump to the manifold and run the pump for 10 minutes to absorb the proteins onto the membrane.
- Remove the membranes and process them according to the standard Western blotting protocol using the anti-titin antibody 9D10 (1:250 dilution) as a primary antibody.

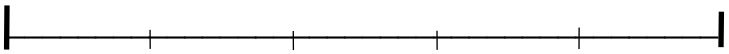


Appendix 3: Form used for the evaluation of sensory quality traits of MAP- and skin-packed- samples.

Please tick one of the following to rate the degree of doneness of the beef sample you have just been served.

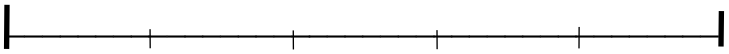
Rare ( )
Medium Rare ( )
Medium ( )
Medium Well ( )
Well Done ( )

Tenderness



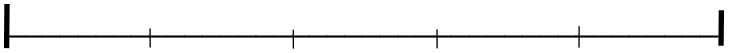
Not Tender Very Tender

Juiciness



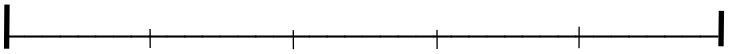
Not Juicy Very Juicy

Liking of flavour



Dislike Extremely Like Extremely

Overall Liking



Dislike Extremely Like Extremely

Please tick one of the following to rate the quality of the beef sample you have just eaten.

Unsatisfactory ( )
Good everyday quality ( )
Better than everyday quality ( )
Premium quality ( )