







final report

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Investigation of the interaction of selected value added processes on selected cuts of varied quality – FINAL INDUSTRY REPORT

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Executive summary

This project has confirmed substantial commercial opportunities for the beef industry to capitalise on value adding technology to deliver consistent high quality meals and meal components from raw material that currently fails consumer expectations as defined by MSA grade results.

This project investigated 5 alternative enhancement and enhancement plus mechanical tenderisation processes across striploin, rump and oyster blade primal cuts collected from a diverse carcase quality range of grass and grain fed product, defined by MSA grading and cooked by grill, roast and re-heat after industrial cooking methods. All product was evaluated by untrained consumers in conjunction with untreated controls utilising MSA consumer testing protocols.

In all cases the treated product was rated significantly higher than the control, typically by 20 points on a 100 point eating quality scale. A critical finding however was that the cut-off scores that delineate product into quality categories are 5 to 6 MQ4 points higher than those for non-enhanced product. The MQ4 score improvement was similar for tenderness and flavour ratings, but typically slightly less for juiciness contrary to expectation. No further improvement was found with mechanical tenderising after the injection process. Variation in the percent of weight added through the injector had little if any effect on the outcome, indicating that existing injection equipment is adequate. The product was not massaged.

Results were similar for grass and grain fed product indicating that raw material of common MSA predicted quality from either can be mixed for processing. Differences in initial raw material quality, defined by MQ4 (Meat Standards Australia eating quality points) remained after treatment although this difference reduced as raw material quality increased.

As a consequence, a combination of raw material selection based on MSA cut MQ4 estimates and treatment applied may be utilised to deliver high and consistent quality consumer product within desired quality and pricing bands.

There are also strong indications that improved flavour scores are the result of precursors influencing muscle biochemical reactions related to taste compounds such as sugars rather than through the expected flavour volatiles, however additional study is required to define the mechanisms involved.

Analysis of the data highlights that value added products as assessed by consumer sensory testing show different MQ4 value cut-offs and weightings to untreated fresh beef products. This is an important finding as it would appear that a separate model may be appropriate for value added products.

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1 Background

1.1 Project purpose

The Australian beef processing industry currently uses available commercial value adding processes to transform source beef cuts to value added product lines to meet demand in key retail and foodservice market channels. Consistent consumer related product performance is a vital requirement and it is critical to better understand the link between initial raw material quality and the value adding process treatments. A detailed knowledge of such interactions will enable consistent quality product to be produced, either by control and procurement of raw material specification or by adaption of the value adding process to best create and capture value.

1.2 Project background

A growing portion of the Australian beef market is in branded fresh beef products with new branding strategies based on predicted eating quality ranges for individual cuts. Carcases can be graded using Meat Standards Australia (MSA) procedures, eating quality (MQ4) scores calculated for major carcase muscles and cuts with these then used to assign the carcase to a boning run and also to determine the brand level for the individual cuts.

As carcase quality decreases some cuts fail to meet the minimum eating quality standards established from consumer testing and used as brand minimums. These cuts, which include large volumes of striploins and rumps in addition to traditional secondary cuts, are currently downgraded and sold as unbranded commodity product.

It is postulated that if such cuts were subjected to appropriate processing they could be raised to acceptable or higher eating quality and marketed at a higher price reflecting their performance thereby improving overall carcass yield and delivering more product consistency. Successful marketing however is dependent on delivering a consistent product quality with minimal variation to create a clear value proposition. To achieve a consistent outcome value adding procedures must be tightly controlled as must raw material specification. Traditionally raw material for value adding has been described by AUS-MEAT cipher, a description now known to be inadequate and largely unrelated to eating quality.

The potential for value adding has been established in some preliminary MSA studies, initially at Texas Tech University (TTU) with Australian striploins and rumps consumer tested utilising a commercial phosphate based solution that indicated substantially higher consumer scores (Garmyn et al. 2012). This study was followed by limited opportunistic Australian consumer based studies of striploins from carcase competition cattle and striploins and outside flats from a long distance transport trial (McGilchrist et al. 2013). The show cattle striploins were utilised to compare phosphate from the same TTU batch with a kiwifruit extract and needling without injection against untreated controls.

Australian consumer results aligned well with the TTU study with the kiwifruit extract performing similar to the phosphate. The transport trial cuts utilised the kiwifruit extract but expanded comparison to include grilled and roasted product with a further comparison of freezing after

injection, as applied in the previous trials, versus ageing for 21 days post injection (McGilchrist et al. 2013). Further variations were conducted with roasting variations of slice thickness and hot versus cold servings. All product was evaluated by untrained Australian consumers utilising MSA consumer protocols (Anon 2008) with additions to specify procedures for cold serving and 2mm rather than 10mm slice thickness.

Results from this study were mixed and indicated no improvement with ageing of enhanced product suggesting that an optimum result could be to market commercial product in frozen form. The control product aged as expected leaving the mechanism for the lack of response in the enhanced product open to question. Was ageing inhibited by enhancement or was ageing "normal" but flavour scores reduced an offsetting amount due to enhancement by time interactions?

A further MLA funded study by Geesink (2015) investigated the potential to use alternative plant extracts with a mushroom derived product producing the greatest improvement in Instron shear values. This product was then consumer tested with the result an effective nil result due to dramatic and highly significant flavour score reduction fully offsetting the tenderness improvement.

Consequently while enhancement was found to have a very positive effect in some instances there were attendant problems in further studies. The early studies also had very limited structured control of the raw material quality with each study reflecting opportunistic use of cuts from projects designed for different purposes.

The current project was designed to source selected high priority cuts from a comprehensive range of MQ4 score bands and apply further alternative value adding treatments to all with the product evaluated by formal consumer testing. An objective was to quantify the interaction between raw material MQ4 for selected muscles and the value adding processes. This knowledge may then be applied to either adapt the raw material and process specification to produce a uniform article from diverse raw material or to underpin multiple product quality ranges at alternative value points.

As an adjunct to the project data at carcase grading was collected from a range of technologies being evaluated for objective carcase measurement. The cuts utilised for value adding were drawn from a larger number of instrumentally assessed carcasses to enable sensory outcomes to be correlated with prospective objective inputs in addition to creating data for instrument comparison.

1.2.1 Fundamental questions

- Can eating quality be improved with value adding treatments & by how much?
- Are effects consistent?
- Can we predict consumer response?
- What is the relationship between initial raw material quality and specific value adding treatments?
- Is there a basis for a value added grading model?

1.2.2 Prediction of value added eating quality

The MSA grading prediction model is built from statistical evaluation of untrained consumer sensory scoring under rigid protocols in association with a wide range of grade inputs reflecting genetic, on

farm, processing and post processing ageing and cooking factors. As such the model assigns an MQ4 point score for each muscle for defined cooking methods. The test protocols used to date assume common domestic cooking appliances and do not include raw material value adding treatments or commercial cooking alternatives.

This research project was designed to evaluate the need and potential to develop a similar approach to model value adding processes and predict a consumer response. Potential interaction of initial raw material MQ4 and subsequent value adding processes, together with the consistency of any outcome, is central to this proposition.

1.2.3 How will raw material & process interact?

Possible outcomes for 2 beef samples of different initial quality are represented in Figure 1.

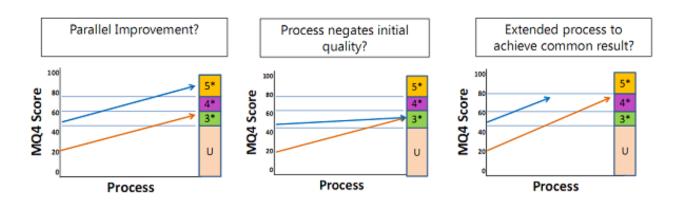


Fig. 1: Alternative sensory outcomes (MQ4) from value adding processes.

The project was designed to provide some preliminary indication of raw material by process response for the muscles and processes evaluated. It was anticipated that results would indicate whether a value adding prediction model development may be feasible and the degree to which linking to the MSA model outcome could improve outcomes through categorising raw material.

1.2.4 Value of the research

This trial was designed to inform raw material purchasing and processing decisions based on:

- A rigorous, evidence based assessment of eating quality from specific value adding processes.
- A protocol to clearly determine whether trends in value adding are worthy of investment.
- The opportunity and basis for developing a value added prediction model to produce an estimated consumer response from inputs including raw material and process interactions.

2 Project objectives

The study encompassed the following objectives:

To evaluate the interaction of raw material eating quality and alternative value adding

- processes as measured by consumer sensory appraisal of the final product to determine if product consistency can be created from various combinations.
- To review the latest trends in ingredient functionality for value adding red meat (including enzymes/phosphate tenderisers and enhancers) and process variations (including massaging and maceration/ needling) relative to alternative solutions on primary muscles from the striploin and rump as viable intervention for value add product design.
- To evaluate alternative sous-vide cooked products employing several processes across oyster blades sourced from 5 base eating quality ranges.
- To accumulate the collected data in a database that can be expanded and used as a base for development of a value added prediction model to produce an estimated consumer response from inputs including raw material and process interactions.
- To examine flavour chemistry changes resulting from process and raw material interactions including precursors.
- To investigate the possibility of process modification to positively impact flavour modification.
- To collect data at MSA grading from multiple potential objective measurement tools
 (Hyperspectral camera, Kuchida camera, HunterLab and NIX) that have potential as grading or yield assessment inputs.

3 Methodology

3.1 Carcase selection

Based on previous analysis of raw material quality as assessed by the MSA grading system prospective carcases were categorised into 5 grades. The 1st grade required high MSA MQ4 values across all cuts. The 3rd grade represented base MSA graded scores with the 2nd grade being intermediate. The 4th grade carcasses were those where some carcase muscles achieved the 3rd grade settings but others fell below MSA thresholds, including commercially important ones such as striploin, whereas those assigned to the 5th grade were of low eating quality across the carcase with a minimum number of muscles achieving an MSA level. Cuts from the 4th and 5th grades were identified as priority value adding targets with the objective being to raise them to a consumer acceptable standard. Both grass and grain fed carcasses were included in the design for 1st grade to 4th grade categories to determine if segregation was needed.

Carcasses were selected during MSA grading at a major processor to provide a range of product for trial selection. The 5 grade categories were then used to select carcasses for measurement with the Kuchida camera system and a subset of 59 carcasses were selected for cut collection, subsequent value adding treatment and consumer evaluation. No 1st grade grainfed carcasses were available within the kill resulting in a grass fed range from 1st to 5th grade and a grainfed range from 2nd to 4th grade. Each of the 59 carcasses were assessed by a Carometec hyperspectral camera, a HunterLab spectrophotometer and NIX tristimulus colorimeter prior to marshalling for boning.







Fig. 2: Carcase selection grading inputs

3.2 Processing

3.2.1 Process selection

It was agreed that initial work should focus on high value cuts and on processing that could be achieved with existing equipment. It was agreed that the primary commercial target was for steak cuts from the rump muscles and that grill and roast were of interest for the striploin. Oyster blade is extensively utilised in RTH (ready to heat) pre-cooked products so it was elected to test these samples, with identical prior treatment, in a pre-cooked and then re-heated form.

Existing processing equipment provided basic processes to mechanically tenderise, to inject, to massage and to cook industrially. After discussion, it was agreed that combinations of injection and tenderising were expected to deliver a greater impact than massaging leading to this process being left out of the initial trial. Five proprietary treatments were selected for evaluation plus a non-treated control. In this report the treatments will be referred to as T1, T2, T3, T4 and T5.

3.2.2 Processing

All collected cuts were prepared for value adding with control files created through MSA software. Treatments were allocated to positions within cuts and across right and left sides. In total 8 samples were designated for striploin (STR045) from one carcase, 2 for rump cap (RMP005), 2 for the $1/3^{rd}$ portions of the rostbiff (RMP231) and 4 portions from the larger 2/3rds portion of rostbiff (RMP131) and 4 from the oyster blades (OYS036). Treatment allocation was rotated to balance side and position within cut.

During preparation for treatment cuts were individually removed from their vacuum packaging and the laminated primal label retained with the cut to ensure continuous identification. Each cut was then fabricated in preparation for value added treatment. The control rump and striploin samples were firstly fabricated into MSA grill or roast consumer samples following MSA protocols (Anon, 2008). The control oyster blade portions were firstly weighed and then divided along the tissue seam to produce two sub portions.





Fig. 3: Fabrication of portions into consumer samples

All portions to be treated were firstly weighed. The value adding processes were then applied with each portion weighed and then further fabricated to consumer and flavour steaks as described above for the control samples.

All cuts were 22 days aged when frozen post processing or, for the oyster blade, not frozen but cooked within a period from 22 days post slaughter to their use by date.

3.3 Consumer allocation and preparation

The samples were sorted into 'picks', a pick being the 42 samples to be tested by 60 consumers. MSA sensory software was utilised to allocate the samples and produce related 'posting sheets', labels for plates and questionnaires and files for sensory result checking, serving time control etc. The sensory files were emailed to TastePoint in Melbourne to facilitate consumer recruitment and testing.

Under MSA protocols the 42 samples in a pick are arranged as 7 groups/products, each with 6 samples. The products are ideally relatively uniform within each and diverse in expected quality across the products. The pick design used assumed that the largest score difference was likely to be across the 5 grades, this being based on a 30 point range for untreated muscles as estimated by the MSA grading model. It was assumed that the value added treatments would each impact the score but that the difference between them would be less, as would the difference between the striploin and rump muscles. Accordingly, a general pick design which transitioned from Manufacturing grade in product 2 to high grade in product 7 with the 6 samples within each product including a control and each of the 5 value added treatments, rotated in order across picks, was adopted.

To test all trial product 18 grill, 5 roast and 7 reheat picks were required. Each pick comprised 42 grill samples, each a thermoform pack of 6 small steaks. Each grill pick then required 'posting', which arranged the samples for cooking to ensure each of the 5 component steaks were served in a different order and to different consumers as designated. The 6th steak was removed, labelled and packed as a flavour sample and stored frozen until needed for flavour volatile analysis.

3.4 Consumer testing

Consumers met the screening criteria of being aged between 18 and 70, eating beef at least once

per fortnight and preferring it cooked medium. Testing was conducted within the Australian Market and Social Research Society code of professional behaviour. No information was retained that could identify individual consumers. The origin of all samples and treatments was not made available, ensuring a blind test regime. Standard MSA protocols were designated for the roast and grill sensory testing and this roast protocol was adapted to test the re-heated (RHT) oyster blade derived products.

For all protocols, each consumer was served 7 samples of beef, the first (product 1) an assumed mid quality common 'link', in these picks an untreated portion of the posterior striploin, followed by one sample from each of the 6 test products. The order of product serving was dictated by a 6 x 6 Latin square which ensured each product was served an equal number of times in each serving order (2nd to 7th), and equally before and after each other product. Further detail is provided by Anon, (2008).



Fig. 4: Consumer testing booth, 6 x 6 Latin square and plate ID label example.

Each sample was assessed by 10 consumers in turn comprised of 5 paired people. The 5 pairs were distributed across the 60 with one pair drawn from each subset of 12; ie 2 people from consumers 1 to 12, 2 people from 13 to 24 and 2 people from 49 to 60. This sample allocation was identical for all cooking protocols. For the grill picks the 60 consumers were seated and served in three sittings of 20 whereas all 60 were seated in a single session for the roast and re-heat picks. Figure 4 provides an example of the sensory booth layout, Latin square employed to control serving order for the six products and an example plate ID label. On receipt by Birkenwood, MSA error checking routines were utilised to verify that each sample was served in the pre-allocated order to the nominated consumer and the final file combined with the original carcase grading and cut treatment data in a master database.

4 Results

4.1 Treatment weight analysis.

The mean weight of individual raw muscle portions after primal fabrication and immediately prior to treatment or further fabrication to consumer or flavour samples is summarised in Table 1 and shown visually in Figure 5. While variation in carcase and initial primal weight created a range of portion

weights the mean weights and distribution of portions allocated to each treatment were numerically similar with no statistically significant difference between the means (P=0.907).

Table 1: Mean raw and processed weight (Kg), mean weight added (Kg) and mean percent weight added by treatment.

	T1	T2	Т3	T4	T5	Sig	p-value
Raw wt	0.787	0.786	0.778	0.768	0.777	NS	0.907
Processed wt	0.882	0.855	0.891	0.845	0.928	S	0.011
Wt added	0.095	0.07	0.113	0.077	0.151	S	<0.001
%Wt added	11.9%	8.8%	14.7%	10.5%	19.0%	S	<0.001
.	0.00/	45.00/	0.20/	27.20/	1.00/		
Min	0.0%	-15.0%	-0.3%	-27.2%	1.9%		
Q1	9.8%	7.0%	9.8%	7.3%	12.0%		
Med	11.9%	8.8%	14.1%	9.2%	14.8%		
Q3	14.3%	10.4%	16.8%	11.3%	18.6%		
Max	35.9%	18.4%	83.4%	87.9%	79.0%		

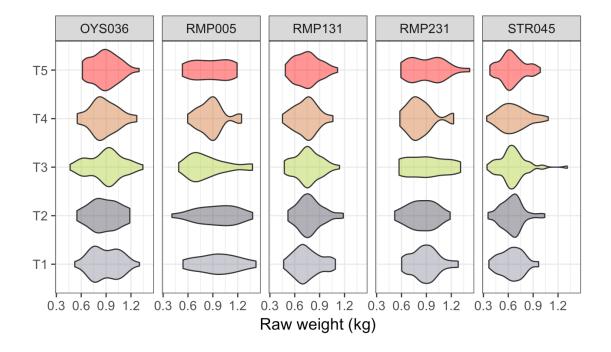


Fig. 5: Distribution of raw weights (Kg) by muscle and treatment method.

In contrast, there were significant differences between the mean percentage weight added between treatments (P<0.001). All treatment pairwise mean differences were significant (P<0.05) other than T4-T2 and T4-T1 as illustrated by Figure 6. T5 had the greatest mean % increase followed by T3, with the tenderised treatments lowest. While it was assumed that the mean % weight added related to moisture binding, and possible losses through the needle tenderisation process, a batch injection difference cannot be ruled out.

95% family-wise confidence level

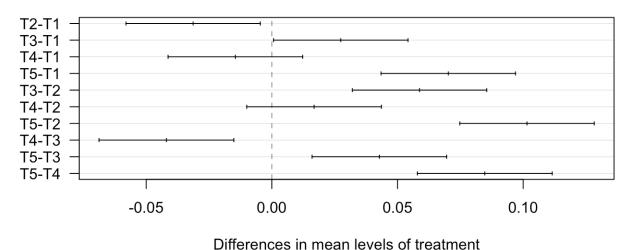


Fig. 6: 95% family-wise confidence interval between pairwise treatment mean differences

The individual % weight added was calculated for each treated portion and varied widely within each muscle as shown in Figure 7. From Table 1 some extreme outlier values are observed but the middle 50% of samples achieved a percent weight added between 8.3 and 14.8%. As illustrated by Figure 8 there was no relationship across all cuts between raw treatment weight and percent weight added.

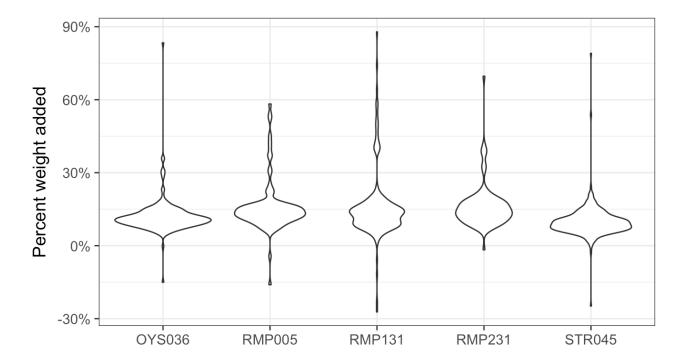


Fig. 7: % weight added by muscle.

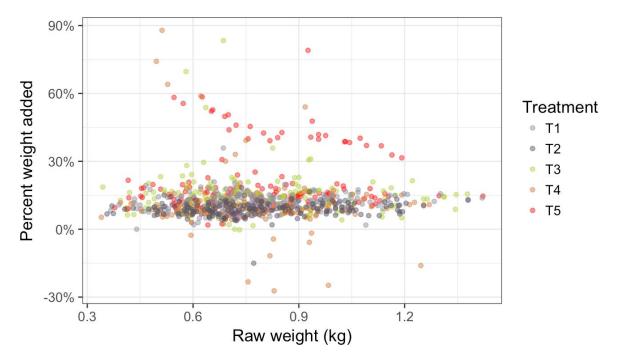


Fig. 8: Percent of weight added by treatment relative to raw sample weight.

The boxplots in Figure 9 show that there is a reasonably consistent percent weight added increase across treatments. However, within the rump muscles, T5 is higher on average with a larger variability than the other treatment methods. Predicted means and confidence intervals are displayed in Figure 10.

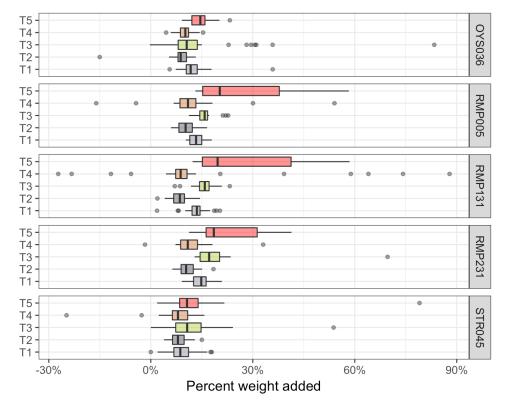


Fig. 9: Distribution of % weight added by muscle and treatment.

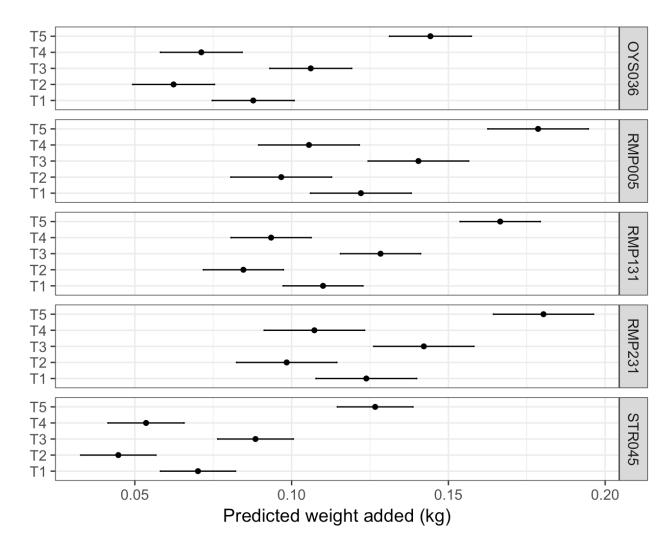


Fig. 10: Predicted means and 95% confidence intervals for weight added by treatment and cut.

4.2 Sensory analysis.

Raw sensory values (MQ4) and standard deviations for each muscle by treatment class are displayed in Table 2. All treatments resulted in highly significant (P<0.001) increases in MQ4 values relative to control samples for each cooking method and muscle as shown in Figure 11. Mean values for individual sensory traits for all treatments are shown in Table 3.

It can be seen that the observed sensory raw mean (MQ4) score difference between control and treated samples within each cut by cook combination ranged from 16.2 to 28 points. The standard deviation was also typically reduced within the treated samples. There were no significant differences between treatments when controlling for cut and cook (P<0.294).

Table 2: Raw mean sensory (MQ4) scores and standard deviation for muscle, cook and treatment.

			MQ4		Diff to	
Muscle	Cook	Treatment	mean	MQ4 sd	Control	n
RMP005	GRL	Control	59.9	11.1		19
RMP005	GRL	T1	76.5	9.5	16.6	20
RMP005	GRL	T2	78.9	7.4	19.0	20
RMP005	GRL	T3	79.6	4.6	19.7	20
RMP005	GRL	T4	78.2	7.9	18.3	19
RMP005	GRL	T5	80.7	7.6	20.8	20
RMP131	GRL	Control	44.5	9.7		39
RMP131	GRL	T1	66.3	9.7	21.8	39
RMP131	GRL	T2	67.7	9.8	23.2	40
RMP131	GRL	T3	70.8	7.6	26.3	39
RMP131	GRL	T4	66.6	10.3	22.1	40
RMP131	GRL	T5	72.5	7.0	28.0	39
RMP231	GRL	Control	47.2	9.6		20
RMP231	GRL	T1	70.4	8.9	23.2	19
RMP231	GRL	T2	71.6	8.4	24.4	20
RMP231	GRL	T3	73.5	12.1	26.3	20
RMP231	GRL	T4	69.5	8.9	22.3	19
RMP231	GRL	T5	73.3	7.1	26.1	20
STR045	GRL	Control	47.0	11.1		30
STR045	GRL	T1	72.5	7.9	25.5	30
STR045	GRL	T2	71.0	8.6	24.0	30
STR045	GRL	T3	69.2	10.2	22.2	30
STR045	GRL	T4	69.0	11.0	22.0	30
STR045	GRL	T5	72.8	8.6	25.8	30
OYS036	RHT	Control	58.6	11.8		39
OYS036	RHT	T1	76.6	9.5	18.0	40
OYS036	RHT	T2	74.8	9.9	16.2	40
OYS036	RHT	T3	74.3	8.7	15.7	39
OYS036	RHT	T4	75.2	8.1	16.6	39
OYS036	RHT	T5	77.5	8.4	18.9	39
STR045	RST	Control	37.6	13.6		32
STR045	RST	T1	61.1	10.4	23.5	25
STR045	RST	T2	59.3	12.8	21.7	28
STR045	RST	T3	63.0	13.1	25.4	26
STR045	RST	T4	58.2	14.0	20.6	26
STR045	RST	T5	61.0	11.2	23.4	27

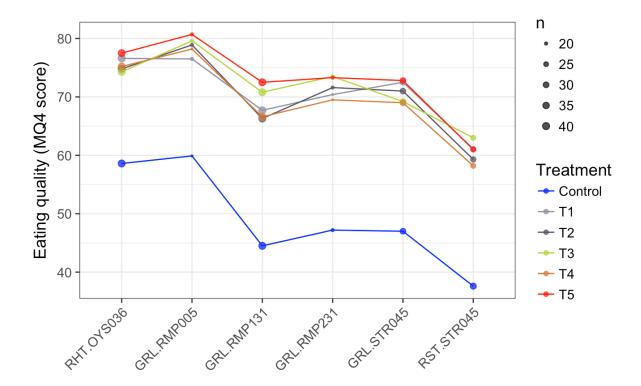


Fig. 11: Mean eating quality (MQ4) values for control and treated samples by muscle, cooking method and treatment.

It was also observed that the large MQ4 value increases were mirrored by the individual MQ4 components with tenderness and flavour each increasing in tandem as shown in Figure 12.

Somewhat surprisingly, given the moisture addition associated with treatments, juiciness was found to be less responsive. The juiciness values are noticeably low relative to other sensory traits for both control and treated samples in the roasts indicating that they were overcooked.

While standard MSA roast protocols were observed (dry heat of 160°C until reaching an internal 65°C followed by 10 minutes rest) it is hypothesised that the relatively small sample size and associated short cook time resulted in over cooking.

The pattern of juiciness score increases being slightly less than those for tenderness and flavour is further illustrated for striploin in both grills and roasts in Figure 13. While not reaching significance T5 was typically slightly higher for grill and reheat cooking methods while T3 outperformed in the roasts. The tenderised treatments are lower in each method.

Further work would be required to establish if these trends are reproducible.

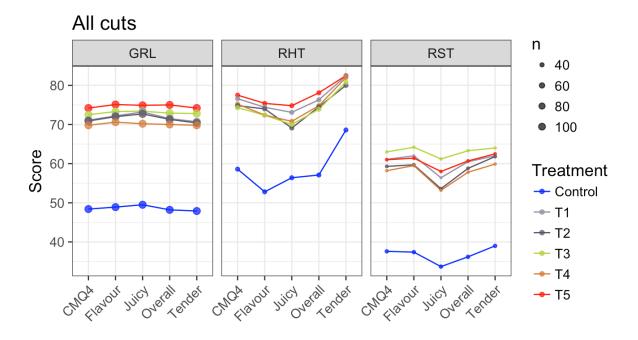


Fig. 12: Mean MQ4 eating quality component values for control and treated product within cooking method.

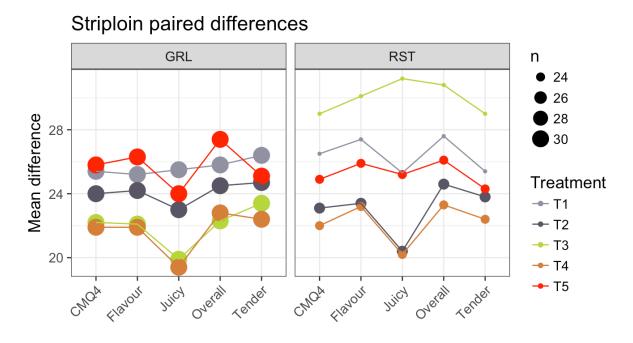


Fig. 13: Mean striploin consumer score differences between control and treatment by sensory trait.

Table 3: Mean sensory trait values for control and treated samples within cooking method.

Cut	Cook	Treatment	MQ4	Tender	Juicy	Flavour	Overall	n
STR045	GRL	Control	47	47.4	48.3	47.3	46.4	30
STR045	GRL	T1	72.5	73.8	73.8	72.5	72.3	30
STR045	GRL	T2	71	72.1	71.3	71.5	70.9	30
STR045	GRL	T3	69.2	70.8	68.2	69.4	68.8	30
STR045	GRL	T4	69	69.8	67.7	69.2	69.3	30
STR045	GRL	T5	72.8	72.5	72.4	73.6	73.9	30
STR045	RST	Control	37.6	39	33.7	37.4	36.2	32
STR045	RST	T1	61.1	62	56.4	62	60.5	25
STR045	RST	T2	59.3	61.8	53.6	59.7	58.8	28
STR045	RST	T3	63	64	61.2	64.2	63.3	26
STR045	RST	T4	58.2	59.9	53.2	59.5	57.8	26
STR045	RST	T5	61	62.5	58	61.4	60.7	27
OYS036	RHT	Control	58.6	68.6	56.4	52.8	57.1	39
OYS036	RHT	T1	76.6	82.4	73.1	74.4	76.3	40
OYS036	RHT	T2	74.8	80	69.1	74	74.5	40
OYS036	RHT	T3	74.3	80.8	70	72.4	73.9	39
OYS036	RHT	T4	75.2	82	70.8	72.4	74.8	39
OYS036	RHT	T5	77.5	82.5	74.8	75.4	78.1	39
RMP005	GRL	Control	59.9	59.5	62.8	59.7	60.4	19
RMP005	GRL	T1	76.5	79.2	79.4	75.7	76.6	20
RMP005	GRL	T2	78.9	79.2	81.4	79.2	79.9	20
RMP005	GRL	T3	79.6	82.8	82	79.3	79.5	20
RMP005	GRL	T4	78.2	79.1	79.1	78.3	78.5	19
RMP005	GRL	T5	80.7	82.7	82.8	79.9	81.5	20
RMP131	GRL	Control	44.5	43.4	46.2	45.3	44.1	39
RMP131	GRL	T1	67.7	64.6	70	70.3	69	40
RMP131	GRL	T2	66.3	64.3	69.5	68.7	66.9	39
RMP131	GRL	T3	70.8	69.2	72.6	72.1	72	39
RMP131	GRL	T4	66.6	65.3	68.1	68.3	66.8	40
RMP131	GRL	T5	72.5	71.7	73.7	74.4	72.9	39
RMP231	GRL	Control	47.2	46.3	44.9	48.2	47.1	20
RMP231	GRL	T1	70.4	69.7	72.3	71.9	70.4	19
RMP231	GRL	T2	71.6	71.3	72.2	71.9	72	20
RMP231	GRL	T3	73.5	73	74	75.3	74.1	20
RMP231	GRL	T4	69.5	69.9	69.8	70.1	69.7	19
RMP231	GRL	T5	73.3	73.3	73.4	73.9	74.2	20

Given the variation in weight added within cut and treatment within cut the potential for an interaction with eating quality was examined and found to be significant within the striploin but not in the rumps or oyster blades. In the striploins, the results suggested that there may be as much as a 1 MQ4 point increase for every 1% increase in weight added, with similar results across the different treatments. The possibility that this may be driven by a larger effect at low pump rates and decrease subsequently was tested using a curvilinear function, but there was no evidence of this over the

limited range of observed values. Restricting attention to samples with a pump rate of 7% or more resulted in no significant relationship between percentage weight added and eating quality. This suggests that there is a minimum threshold above which there is no further improvement. In this study, the majority of the percentage weight added values were clustered around 10% with only a few observations found in the extremes. To confirm any relationship between pump rate and eating quality, further study is required with samples over a more diverse range of percentage weight added.

Figures 14 (oyster blade) and 15 (striploin) shown below illustrate the observed relationships.

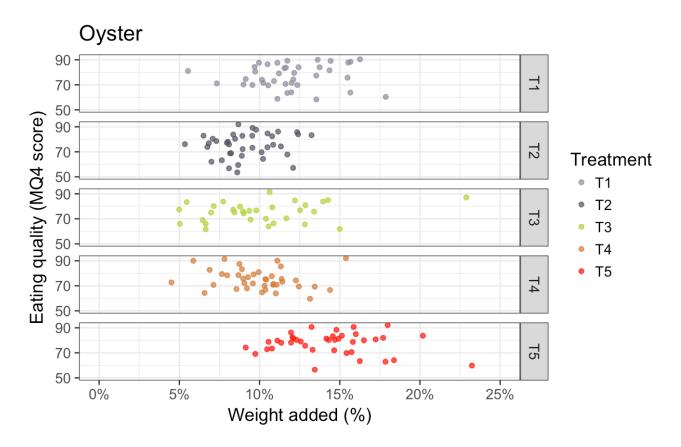


Fig. 14: Relationship of eating quality (MQ4) and weight addition for oyster blade by treatment.

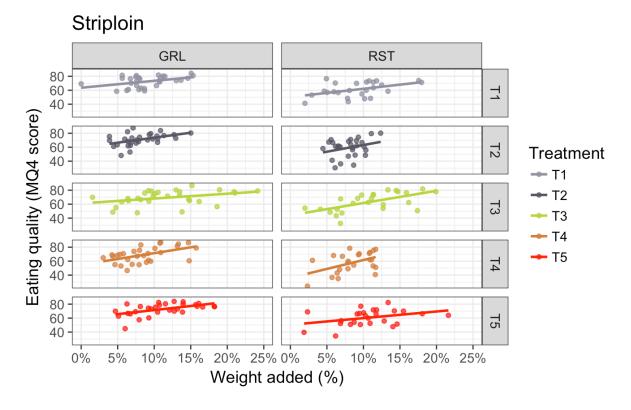


Fig. 15: Relationship of eating quality (MQ4) and weight addition for striploin by treatment.

Analysis found no difference in treatment effect between cuts from grain and grass fed groups with similar improvement in the order of 20 MQ4 points. As noted there were no 1st grade grain fed carcasses to compare but the similarity in treatment effect at progressive control MQ4 bands is illustrated in Figure 16.

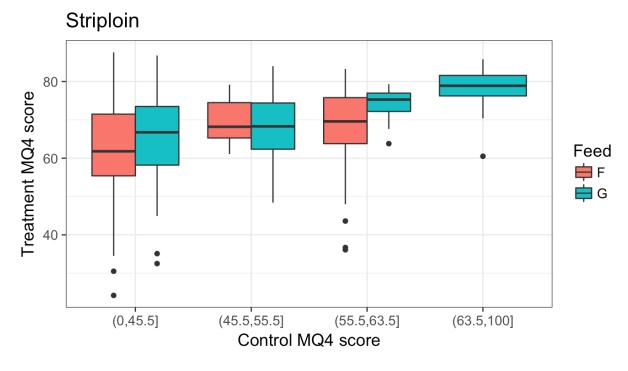


Fig. 16: Comparison of control and treated sensory differences for grain and grass fed carcasses.

Figure 17 demonstrates that grass and grain fed relationships are similar when control and treated means are compared within sensory traits with the higher grass fed values related to the inclusion of 1st grade carcasses.

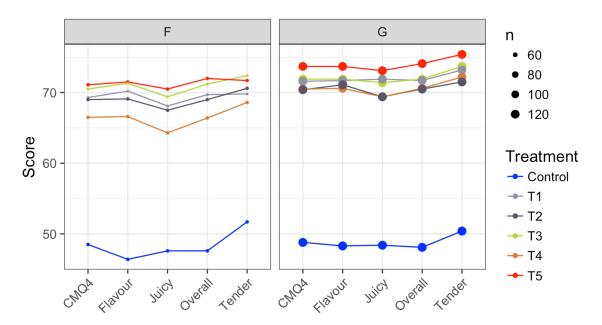


Fig. 17: Sensory scale comparison between grain and grass fed cuts.

Figure 16 also demonstrates that the treated eating quality (MQ4) score is positively related to the initial raw control scores indicating that raw material eating quality impacts the final treated result. This is further illustrated in Figure 18 for striploins with the shaded area around the trend line illustrating the 95% confidence interval for predictions from the linear model. (Any horizontal line that remains within the shaded area represents a non significant difference)

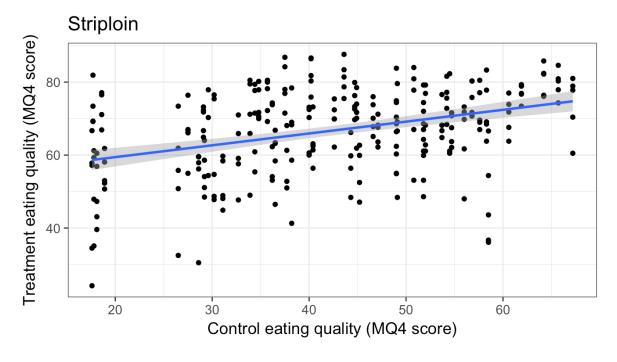


Fig. 18: Relationship of control and treated sensory scores (MQ4).

The positive trend between raw material eating quality and eating quality after treatment was also found across the carcase grade categories as shown by the fitted lines for control and treated samples in Figure 19. Statistical modelling of the relationship between eating quality for the control and treatment samples suggested that for each additional MQ4 point in the raw material, on average, the treated sample would improve by 0.25 MQ4 points. This result was consistent across both striploins and oyster blades (the muscles with substantial control/treatment pairings). As found previously, there were no significant differences between the treatments. To put this in context,

a product with control MQ4 score of 30 => predicted treated MQ4 score of 67.5,

a product with control MQ4 score of 40 => predicted treated MQ4 of score 70,

a product with control MQ4 score of 50 => predicted treated MQ4 of score 72.5.

These results suggest that the there is a decreasing return to increased raw eating quality. Higher scoring product will improve, but proportionally less than lower scoring product. In the scenario above, the product with an MQ4 score of 30 more than doubled its eating quality when treated. In contrast, the product with a score of 50 increased its eating quality by less than 50%.

These results also show how the treatment process can be used to yield a more consistent consumer experience. Low scoring products tend to result in highly variable consumer eating experiences, however, the treatment process boosts scores across the scale so that the final product has a much tighter range of eating quality. In the above example, the three initial samples had a range of 20 MQ4 points, but when treated the range became 5 MQ4 points.

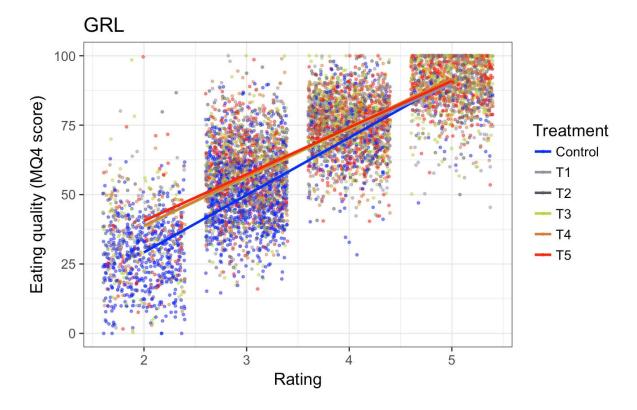


Fig. 19: Relationship between source carcase grade and treated eating quality score (Grill).

4.3 Willingness to pay data

Willingness to pay (WTP) data was collected from all consumers. The WTP question was presented after all 7 sensory samples had been evaluated to avoid price influencing sensory response. Results were recorded in \$ per kg on line scales for each category choice (unsatisfactory, good everyday, better than everyday and premium) with the scale \$0 to \$80 per kg. A copy of the sheet is in the appendix. It should be noted that the question asked was "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". The objective was to collect data based on "beef that they would assign to each category" rather than to actual samples presented as consumers may not accurately recall "the second sample" etc.

The responses to the WTP question were typical of other MSA tested Australian consumers with ratios relative to 3* of around 50% for unsatisfactory, 150% for 4* and 200% for 5*.

The category chosen for each sample by each consumer (10 consumer scores per sample) was then related to the price elected for that category by that consumer and used to produce a WTP for the control and treated products. The result is shown in Figure 20 with supporting data in Table 4.

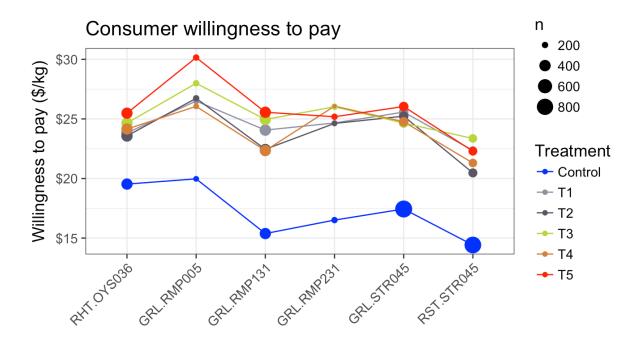


Fig. 20: Willingness to pay for control and treated products.

Table 4: Willingness to pay values by cut and category for control and treated samples.

Tuestment	Cook	Mussle	Average	Av	verage		
Treatment	Cook	Muscle	Rating	\$	S/Kg	n	
Control	GRL	RMP005	3.31	\$	19.97	190	
Control	GRL	RMP131	2.78	\$	15.38	390	
Control	GRL	RMP231	2.89	\$	16.51	200	
Control	GRL	STR045	2.99	\$	17.44	870	
Control	RHT	OYS036	3.21	\$	19.53	390	
Control	RST	STR045	2.58	\$	14.42	840	
T1	GRL	RMP005	4.05	\$	26.50	200	
T1	GRL	RMP131	3.64	\$	24.06	400	
T1	GRL	RMP231	3.69	\$	24.66	190	
T1	GRL	STR045	3.81	\$	25.57	300	
T1	RHT	OYS036	3.8	\$	23.83	400	
T1	RST	STR045	3.35	\$	22.36	250	
T2	GRL	RMP005	4.05	\$	26.73	200	
T2	GRL	RMP131	3.56	\$	22.45	370	
T2	GRL	RMP231	3.76	\$	24.62	190	
T2	GRL	STR045	3.74	\$	25.23	300	
T2	RHT	OYS036	3.73	\$	23.57	400	
T2	RST	STR045	3.27	\$	20.47	280	
Т3	GRL	RMP005	4.08	\$	27.99	200	
Т3	GRL	RMP131	3.75	\$	24.95	390	
Т3	GRL	RMP231	3.91	\$	26.02	200	
Т3	GRL	STR045	3.69	\$	24.65	300	
Т3	RHT	OYS036	3.72	\$	24.65	390	
Т3	RST	STR045	3.47	\$	23.36	260	
T4	GRL	RMP005	3.95	\$	26.05	190	
T4	GRL	RMP131	3.52	\$	22.34	390	
T4	GRL	RMP231	3.71	\$	26.07	180	
T4	GRL	STR045	3.7	\$	24.76	300	
T4	RHT	OYS036	3.73	\$	24.15	390	
T4	RST	STR045	3.33	\$	21.30	260	
T5	GRL	RMP005	4.18	\$	30.15	200	
T5	GRL	RMP131	3.77	\$	25.56	390	
T5	GRL	RMP231	3.83	\$	25.18	200	
T5	GRL	STR045	3.89	\$	26.04	300	
T5	RHT	0YS036	3.85	\$	25.48	390	
T5	RST	STR045	3.43	\$	22.29	270	

These results indicated a \$5.00 to \$10.00 /kg premium for an enhanced product due to the increased eating quality delivered and provide strong support for the economic case to pursue development and marketing of a value added product.

However, given that the typical enhanced improvement was found to be 20 MQ4 points or more, virtually all enhanced product would be expected to be a full MSA grade equivalent above controls which would equate to a 50% to 100% variation from the "good everyday" 3* value. As the actual difference appeared slightly less than this the MQ4 weightings and grade cut-off values were further investigated.

Table 5 displays the results of this analysis.

Table 5: MQ4 sensory component weightings and category cut-off scores.

	Gr:	i11	Reh	eat	Roast	
No of observations	1650	5390	390	1970	840	1320
Treatment	Control	Enhanced	Control	Enhanced	Control	Enhanced
Tender	36	26	8	7	9	15
Juicy	8	8	7	12	31	18
Flavour	27	34	51	48	39	38
Overall	29	32	34	33	22	29
2*/3* cut-off	40	46	45	50	35	40
3*/4* cut-off	62	67	65	71	60	64
4*/5* cut-off	78	82	80	85	75	82
Accuracy 4 variable	68.3	66.2	57.9	57.9	74	68.3
Std MQ4 accuracy	67.6	64.9	54.9	57.1	73.9	67.6

This analysis provided valuable insight into apparent differences in consumer assessment of enhanced versus control product that need to be considered in developing standards and a potential value adding model. The relative MQ4 cut-off values for enhanced relative to control products are around 5 points higher within each cooking method. The higher cut-offs can be readily applied in future modelling and it is recommended that they be utilised in electing new brand standards.

Further insights were gained on examination of the weightings for sensory traits used to generate the MQ4 although the data is minimal, and perhaps inadequate, to make a definitive judgement. The numbers against each trait (tender, juicy, flavour overall) reflect the optimal proportion of each trait to combine in an MQ4 score for these data. Due to a generally strong correlation between the traits these weightings can safely be rounded to the nearest round number divisible by 10, reflected in the current MSA standards of 30:10:30:30. It can be seen in Table 5 that the control grill weightings of 36(T), 8(J), 27(F) and 29(O) are entirely normal and fully align with standard MSA practice. The enhanced grill values also fit the general model, but less convincingly with signs of a trade-off from tenderness to flavour.

The re-heat weightings were quite different and massively weighted to flavour for both the control and treated samples. As all product was oyster blade and essentially 3* and 4* for the control it is possible that the low tenderness weighting reflects that tenderness was not an issue but this would need replication to make any firm conclusion. The re-heat protocol was developed within the project as pre-cooked and re-heated product had not been tested by MSA previously. Given the high consumer satisfaction generated and the apparent weighting on flavour more work on this product category is recommended after examination of flavour chemistry results from AFBI.

The roast results as noted earlier were abnormally low relative to the grilled striploin pairs leading to a tentative conclusion that the samples may have been overcooked due to their small size despite adherence to MSA temperature standards and protocol. Again the weighting to flavour is high relative to tenderness and similar for control and treated product. Juiciness also increased considerably for the roast weightings and more so for the control which would appear to support the assumption that the small size and associated short cooking time (at the 160°C protocol) may have resulted in a dry overcooked product, with the added moisture in the enhanced samples reflected in the lower juiciness weighting. Further work post flavour analysis is recommended to examine the issue further.

The "Accuracy 4 variable" values represent the % of consumer responses allocated correctly to the grades using an optimum weighting for this specific set of data whereas the "Std MQ4 accuracy" reflects the equivalent accuracy for the standard MSA 30:10:30:30 weightings. It should be noted that the % is a reflection of consumer variance and not related to model accuracy; rather it is a measure of how well a "perfect" model could perform given inherent consumer variation. The 4 variable and standard weightings are seen to perform similarly despite the unusual weightings. This consumer variance measure was similar to other MSA based fresh untreated beef data with the overall scale contributing to the standard MQ4 performance.

From prior MSA experience the overall scale reflects "something else" rather than the net effect of tenderness, juiciness and flavour with the addition of overall improving the precision of the MQ4 statistic rather than being an equivalent rating. In this case, the overall weighting may be reflecting a consumer view that while the enhanced product is highly acceptable it differs in some way from typical fresh untreated beef. The differences in cut off values for enhanced product hint at a difference in consumer appraisal relative to fresh untreated beef; despite rating enhanced samples higher for tenderness and flavour the consumer is also demanding a higher score before granting a higher rating relative to untreated product scored much lower by the same consumer groups in the same sessions.

This deserves further attention in conjunction with flavour chemistry to ensure the observation is understood and applied but would suggest at this point that enhanced product should perhaps be marketed as a separate category to the fresh beef offer. The product scores highly, significantly above controls, and the WTP values are higher providing a real opportunity to build a successful proposition if positioned as a standalone product rather than as another form of fresh beef that is perhaps "NQR" (not quite right) or more correctly not quite the same.

4.4 Flavour chemistry

Samples for flavour chemistry analysis were fabricated in conjunction with those for consumer testing. There was a considerable delay in transporting the flavour samples to AFBI in Belfast due to the need to obtain special export and import clearance. This became necessary due to the Hemmant TAFS facility and UNE being domestic registered with samples required to leave EU registered premises to access the enhancement equipment. John Langbridge consulted with AQIS and finally obtained the necessary approvals to export given they were purely laboratory samples and not for human consumption. The analyses included volatile analysis using Solid Phase Micro-extraction (SPME) and analysis of reducing sugars by ion exchange liquid chromatography.

The difficulties encountered and desire to reduce the need for special arrangements in subsequent projects has resulted in plans to build at least base chemistry (with analysis of data at some later point) capacity at both Charles Sturt University (CSU) in Wagga and at Texas Tech University (TTU) in Lubbock, Texas. Both Universities have strong chemistry departments with CSU specialising in wine and TTU more meat oriented. Unlike Warner Bratzler tenderness evaluation, where a higher value equates to greater toughness, flavour chemistry is less easy to analyse due to the importance of myriad compounds from Mass Spectrometry being essentially unrelated to their peak area volume. Consequently considerable experience is needed to provide reliable analysis. Further issues relate to quality assessment of data on a daily basis with GCMS with the use of internal standards and intensive data checks needed.

Dr Linda Farmer at AFBI is a global expert in this field and it is planned to have her lead a flavour group including TTU and CSU. In the initial stage this should enable the samples to be processed on GCMS at CSU for Australian samples or TTU for USA. The data files can then be reviewed by AFBI and the necessary experience built at CSU and TTU. A PhD student with University College Cork, Ms Irene Chong, has been employed in the AFBI laboratory to utilise these samples and associated analysis within her thesis. Irene has previously conducted consumer testing to MSA protocols in the UK and Ireland so she has an excellent background in closely related work during her Masters studies. She is currently conducting further work on the samples to enable some of the new issues raised to be investigated.

The balance of the flavour chemistry reported is largely drawn from the AFBI report.

4.4.1 Volatile analyses

The beef samples were stored at -80°C at AFBI prior to cooking according to MSA cooking protocol for medium grilled beef. The following modifications were conducted to ensure that the cooking process was comparable to consumer panels: scrap meat was grilled before cooking actual samples and standard portions of striploin steaks were used to surround the actual samples during the cooking process. The volatile aroma compounds were collected using the SPME technique and analysed by GC-MS (Agilent 5973 MSD and HP6890 GC) using a Zebron-5MS, 30m length, 0.32m diam., 0.50µm column, following the procedure developed by Hagan, Legako and Farmer (Farmer, 2010).

An Agilent integration method was employed to quantify the data based on one quantification ion and three target ions. The resulting data was exported to an Excel spreadsheet using an AFBI "inhouse" macro. Extensive quality assurance was conducted by comparing 10% of the results from automatic quantification with manual integration, as well as investigating any results that appeared unusual. Mass spectra and linear retention time were checked against published values or authentic standards.

Bromobenzene, n-alkanes and an external standard to ensure the performance and reproducibility of GC-MS were analysed at least once daily.

189 samples were analysed as detailed in Table 6.

Table 6 Samples analysed for volatile compounds

Treatment		Exp A						
116	atment	RMP131	STR045	RMP131	STR045	RMP005*	RMP231*	Total
T1	Control	5	5	5	5	6	6	32
T2	F+T	5	6	6	5	6	6	34
T3	F10	5	6	6	5	6	6	34
T4	K+T	4	3	7	8	6	6	34
T5	K10	5	5	6	5	6	6	33
Т6	P10	5	5	5	5	6	6	32
	Total	29	30	35	33	36	36	189

^{*} Some results had to be omitted from statistical analysis due to instrument problems.

4.4.2 Sugar analyses

Sugars and sugar phosphates were extracted from duplicate samples (2g of lean meat) which was homogenised at 13,000 rpm with perchloric acid solution, deionised water and internal standard (rhamnose), until a smooth homogenate was obtained without heat generation. The tubes were then centrifuged, the supernatant was removed and its pH adjusted to pH 6.5. The potassium perchlorate formed was removed by centrifugation. The final "aqueous meat extract" was stored at -80°C until required for analysis. One aliquot of the sample was treated with a mixture of 1:1 (w/w) Dowex SOWX4-400 and WGR-2 resin, which was then removed by centrifugation. To obtain the concentration of phosphate sugars by difference, a second aliquot was treated with alkaline phosphatase prior to mixing with the resin.

Analysis was conducted on a Dionex ion chromatograph with electrochemical detection.

4.4.3 Statistical analysis

Flavour chemistry statistical analysis has been conducted by AFBI statisticians. For the results of volatile analyses, REML variance components analysis was conducted on peak areas which were converted to log10 values using GenStat version 18.1, in order to achieve a normal distribution. Experiments A and B / C were analysed separately.

For the sugar analyses, REML analysis was conducted on the quantitative data, without the need for logarithmic treatment.

4.4.4 Quality assurance and validation of the method

The identities of the volatile compounds were confirmed by comparison of the mass spectra and retention indices with those of the authentic compounds wherever possible. Where an authentic sample was unavailable, identification was by comparison with published literature data for the mass spectrum and retention index. A list of compounds identified is provided in Table 7.

The data was checked for the effect of individual fibres used and date of analysis. The use of individual fibres was distributed across treatments to avoid bias. There was no evidence of any consistent effect of the fibre used on the results.

Table 7. Volatile compounds included in analysis

Compounds	Mean RT	Mean LRI	Literature LRI	Ident. Method**	Formation
Short chain ketones					
2,3-Butanedione	1.35	<700	596	lri+ms	From Maillard reaction
2-butanone,3-hydroxy	3.09	705	718	lri+ms	Maillard sugar and amino acids.
2-butanone	1.44	<700	572	lri+ms	From Maillard reaction
Strecker aldehydes					
2-Methyl butanal	2.25	<700	652	lri+ms	Strecker aldehyde. Marker for Maillard reaction.
3-Methyl butanal	2.15	<700	646	lri+ms	Strecker aldehyde. Marker for Maillard reaction.
2-Methyl propanal	1.28	<700	637	lri+ms	Strecker aldehyde. Marker compound for the Maillard reaction
Benzaldehyde	11.34	957	996	LRI+MS	Strecker aldehyde. Marker for Maillard reaction.
Other Maillard					
Dimethyl trisulphide	11.58	965	984	lri+ms	From Maillard reaction
Dimethyl disulphide	4.04	731	785	LRI+MS	From Maillard reaction
2,5-dimethyl pyrazine	10.11	911	892-913	LRI+MS	Maillard - sugars and amino acids.
3-Ethyl-2,5-	14.22	1072	1093	lri+ms	Maillard - sugars and amino
dimethylpyrazine					acids.
n-Aldehydes					
Pentanal	2.65	<700	697	LRI+MS	From thermal breakdown of lipids.
Hexanal	6.00	785	798-802	lri+ms	From thermal breakdown of lipids.
Heptanal	9.57	893	892-908	lri+ms	From thermal breakdown of lipids.
Octanal	12.51	1000	1002-1005	LRI+MS	From thermal breakdown of lipids.
Nonanal	14.93	1102	1107	LRI+MS	From thermal breakdown of lipids.
Decanal	16.87	1204	1209	lri+ms	From thermal breakdown
Alkanes and ketone					
Heptane	2.73	<700	700	lri+ms	
Octane	6.04	786	800	lri+ms	
Nonane	9.38	893	900	LRI+MS	
2-Heptanone	9.42	888	898	LRI+MS	Lipid oxidation
Acids					
Nonanoic Acid	17.98	1260	1275	lri+ms	
Hexadecanoic Acid	28.94	1958	2010	lri+ms	
Octadecanoic Acid	30.73	2161	2200	LRI+MS	
Pentadecanoic Acid	27.65	1855	1820, 1851	lri+ms	
Miscellaneous					
Toluene	4.60	747	774	LRI+MS	
1-Octene	5.347	777		lri+ms	
Alpha pinene	10.55	927	939	lri+ms	*Olive oil spray?
* I.R.I = linear retention in	11.00	944	953	lri+ms	*Olive oil spray?

^{*} LRI = linear retention index.

^{**} Identification method: MS or LRI = mass spectrum or linear retention index compared with authentic compound; ms or lri = mass spectrum or linear retention index compared with literature values.

There was evidence of an effect of date of analysis with deterioration in the analyses of some later eluting compounds in the most recently analysed samples. These data were omitted from the statistical analysis. In addition, there were some differences in the first batch of samples analysed (Experiment A, August 2017) and those from late 2017/2018 (Experiments B and C). Therefore, statistical analysis of samples from Experiment A was conducted separately from those in Experiment B/C.

There was considerable variability between analyses of samples from the same treatments. Some variability is usual for analysis by manual SPME, but this was greater than previously observed. This method was selected for the work during previous consultations, due to its wide availability in many labs. However, given the developments in technologies for SPME and related analyses, consideration will be given to adjusting the method to improve reproducibility.

Further discussion on this aspect has centred on potential reasons for variability and related implications for both further analysis and commercial application. The variability in actual weight increase (pump rate) has been shown in Table 1 and Figure 5 with discussion as follows:

"Given the variation in weight added within cut and treatment within cut the potential for an interaction with eating quality was examined and found to be significant within the striploin but not in the rumps or oyster blades. In the striploins, the results suggested that there may be as much as a 1 MQ4 point increase for every 1% increase in weight added, with similar results across the different treatments. The possibility that this may be driven by a larger effect at low pump rates and decrease subsequently was tested using a curvilinear function, but there was no evidence of this over the limited range of observed values. Restricting attention to samples with a pump rate of 7% or more resulted in no significant relationship between percentage weight added and eating quality. This suggests that there is a minimum threshold above which there is no further improvement. In this study, the majority of the percentage weight added values were clustered around 10% with only a few observations found in the extremes. To confirm any relationship between pump rate and eating quality, further study is required with samples over a more diverse range of percentage weight added".

A more definitive understanding of this issue is required as a need for greater control could create challenges for consistent product utilising current equipment. A further possibility is that the flavour sampling process of taking 3 cores from each cooked sample could inadvertently sample directly on an injection site or alternatively not be close which in turn could create variation. Massaging could reduce this issue in commercial application and taking a full face of the flavour steak surface and grinding might reduce variation in flavour chemistry variance.

4.4.5 Flavour chemistry results

The flavour chemistry results are intriguing being both different than expected and providing some previously unreported characteristics compared to that reported in non-enhanced beef. Further work is planned as a result, particularly in regard to flavour precursors including ribonucleotides to be analysed by HPLC, some short chain peptides and amino acids to be analysed by chemometric measures. Irene Chong will continue this work within her PhD study.

In general while muscle effects were consistent with previous work and continuing to indicate clear flavour chemistry differences the enhancement treatments had a lower than presumed impact on flavour volatiles in contrast to the very large impact on consumer flavour ratings. The RMP131 muscle typically was higher in lipid oxidation products such as n-aldehydes than the STR045. This may reflect the quantities of polyunsaturated fatty acids in these muscles. However, as advised by Dr Farmer this tends to be fixed by the proportion of cell membranes, where these fatty acids are mainly found. It may be, therefore, that the differences between muscles reflect the propensity of the fatty acids to oxidise, i.e. the balance of antioxidants and pro-oxidants in the muscle.

Similarly the Strecker aldehydes which had previously been found to correlate with flavour liking were higher in the RMP131 but at levels that were thought unlikely to significantly impact consumer scoring. A number of short chain ketones that occur during sugar breakdown reactions (part of the Maillard reaction) tended to be higher in the RMP31 samples with the variability however reducing significance to one compound.

Further discussion has ensued regarding potential analyses that may correct for pump rate to remove potential impact on variability and to investigate the potential to utilise a compound(s) not present in untreated controls to standardise for addition rate. In general, enhancement had less effect on the volatile compounds than expected, partly due to the wide variation in the quantities of some volatiles detected. It is possible that enhancement may have multiple effects including:

- Dilution of meat-based flavour precursors.
- Addition of fruit-based precursors.
- Increased water holding will inhibit the Maillard reaction.
- Inhibition of certain oxidation reactions.

However, as the application of the enhancement materials is inevitably inhomogeneous, this may result in wider variations from sample to sample than have been observed previously in untreated beef and as discussed above.

4.4.6 Analyses for sugars

More than 90 samples from six treatments and four muscles were analysed for sugars. In contrast to the aldehydes and volatiles highly significant differences were reported relating to the sugars and sugar phosphates.

Four sugars and four sugar phosphates were quantified together with an additional unidentified sugar, detected in some runs. A corresponding sugar phosphate was not detected. This "unknown sugar" was detected only in the enhanced samples. Figures 21-24 compare typical chromatographic runs from control and treated samples.

The control run (Figure 21) shows the expected peaks for glucose, mannose, fructose and ribose together with rhamnose (internal standard). In contrast an additional peak between mannose and fructose is seen in the enhanced samples.

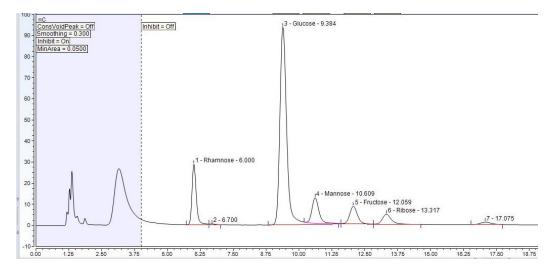


Figure 21. Chromatogram for control beef - S2W7

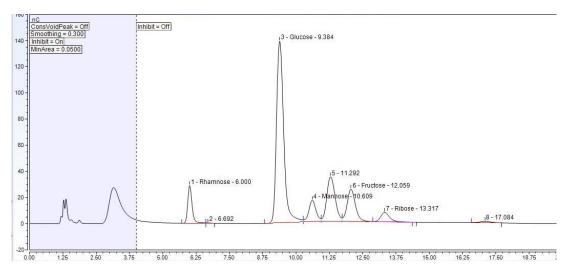


Figure 22. Chromatogram for F10 beef – W4V2

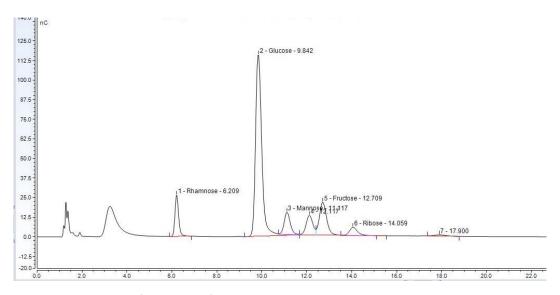


Figure 23. Chromatogram for K10 beef - A6T1

The P10 treatment (Figure 24) also shows the same peak which is unexpected if this treatment contains *only* phosphate. This result would suggest that there is some additional sugar in this treatment or (even more interesting) that phosphate releases sugar from some source within the meat.

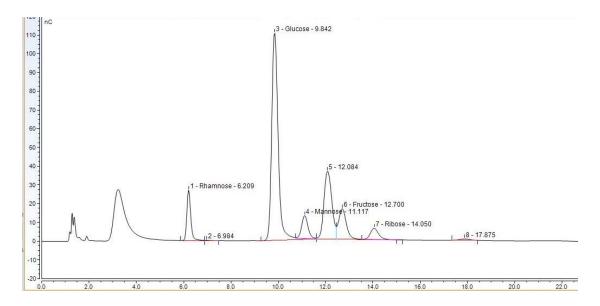


Figure 24. Chromatogram for P10 beef – G2F1

Enhancement treatments produced highly significant effects for most sugars. The treated samples were significantly different from the controls and, except for glucose, there is a significant difference between those treatments giving higher and lower sugar concentrations. Mannose did not show significant effects and did not follow the same trend. Ribose-5-phosphate and fructose-6-phosphate did not show significant effects, but followed a similar trend to the other sugar phosphates, suggesting higher variability within treatments.

Interestingly, most sugars were increased by all the enhancement treatments as shown in Figures 25 and 26. This suggests either that there were considerable concentrations of sugars in all these samples (including the phosphate treatment, P10) or that the conditions caused by these treatments were conducive to the formation of additional sugars. This latter idea is supported by the fact that sugar phosphates were reduced by enhancement treatments. However, these reductions were not as great as the increases in sugars, so additional mechanisms of formation must also occur.

As indicated previously, the unknown sugar was present only in the treated samples and not in the control meat. The role of sugars and further consideration of whether their increase is due to sugar inclusion in the enhancement additive or to enhancement influencing sugar formation within muscle biochemistry is an important issue with considerable potential impact on the possibility of manipulating cooked product flavour through manipulation of precursors.

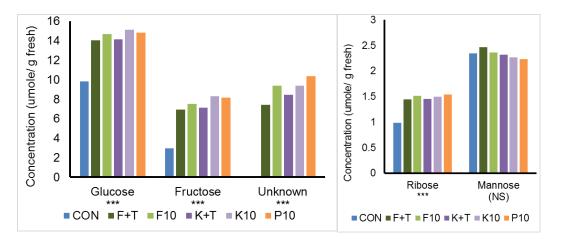


Figure 25: Effect of enhancement on the concentrations of sugars in raw meat.

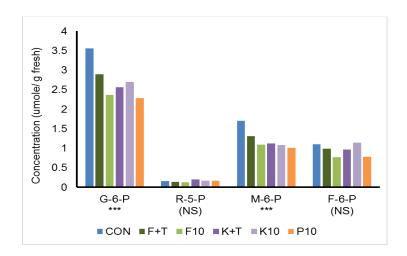


Figure 26. Effect of enhancement on the concentrations of sugar phosphates in raw meat.

These results suggest that either there were considerable concentrations of sugars in all these samples (including the phosphate treatment, P10) or that the conditions caused by these treatments were conducive to the biochemical formation of additional sugars.

Enhancement has shown a substantial increase in consumer scores, including the scores for flavour liking which raises the question: "Why?" It is possible that there is an effect on volatiles but it is hidden by the aforementioned causes of variation. However, there is no evidence of a large effect. However, flavour is a combination of taste and aroma and it is possible that taste is responsible for this flavour change. An addition of salt (NaCl) would have this effect, as could an increase in certain amino acids and peptides from the enzymic activity of the kiwi and ficus. The planned analyses of amino acids may help explain this effect.

If the significant differences in sugars, or indeed the volatile compounds, can be linked to the consumer scores for flavour, this suggests a promising route for influencing flavour with new and modified enhancement treatments.

5 Discussion

5.1 Inferences and insights from the data relative to previous research

The project aligns with previous MSA and published studies that report improved consumer sensory ratings with enhanced product. It builds on these studies to compare 5 alternative treatments and reports very similar sensory outcomes for each. The results obtained without massaging may surprise some and it is clear that further mechanical tenderising after injection provided no benefit. With the exception of striploin with less than 7% weight addition, product results were relatively stable across muscles and little affected by a broad range of weight addition. This and the lack of response to tenderising indicate that a relatively simple production process utilising existing equipment is viable.

The study adds useful knowledge in regard to individual muscle effects and raises some issues demanding further work to clarify some differences with alternative cooking methods. An important finding is that while raw material eating quality differences may be reduced with treatment they remain important so that a raw material MQ4 needs to be matched to appropriate process to generate a consistent consumer outcome. This suggests that a range of quality and price points may be created within a value added range to provide value choices. An independent economic assessment by Green et al. (2017) supported this assumption. The opportunity to utilise alternative muscle x raw MQ4 x process combinations to produce common consumer eating quality outcomes appears promising but requires additional work to define the interactions and to extend these to a form of prediction model.

This study differs from the majority reported in that outcomes were measured utilising untrained consumers and MSA cooking protocols. Further evaluation of the consumer data has identified a clear difference in consumer sensory rating of enhanced products relative to untreated controls. While the enhanced products are rated highly and beyond the controls consumers clearly identify some difference to untreated fresh product and elect higher minimum MQ4 scores in relation to grade cut-offs. These findings deserve further study and replication but suggest that successful branding and marketing strategies may favour creation of a separate meat and meal category sold under a final meal outcome description rather than marketing as a fresh beef extension. Willingness to pay data confirms that consumers place a higher value on the enhanced products creating an opportunity for increased industry revenue.

The flavour chemistry provided some unexpected outcomes in that enhancement had a relatively small effect on flavour volatiles including n-aldehydes and Strecker aldehydes despite the considerable increase in consumer flavour scores. In contrast all enhancement treatments resulted in highly significant changes in sugars, including the observation of an "unknown" sugar not found in the control. It is as yet unclear whether this sugar reflects an addition within the enhancing mix or the biological triggering of sugar production. Further evaluation together with evaluation of amino acids and other potential precursors will address these issues.

5.2 Practical implications for industry

This project has confirmed that commercial opportunities exist to capitalise on value adding technology to deliver consistent, high-quality meals and meal components from a large supply of raw material that currently fails consumer expectations. Results were similar for grass and grain fed product indicating that raw material of common MSA predicted quality from either can be mixed for processing.

A combination of raw material selection based on MSA cut MQ4 estimates and treatment applied may be utilised to deliver high and consistent quality consumer product within desired quality and pricing bands.

Analysis of the data highlights that value added products as assessed by consumer sensory testing show different MQ4 values and weightings to untreated fresh beef products. This is an important finding as it would appear that a separate model may be appropriate for value added products.

The flavour chemistry outcomes indicate a strong potential for precursor adjustment in the raw meat to trigger further flavour enhancing outcomes in the final cooked product. Initial findings illustrate a highly significant addition to muscle sugars with amino acid and other precursors being further evaluated. Increased understanding of these mechanisms combined with documented muscle differences could provide a powerful commercial tool to influence targeted cooked product flavour through the enhancement process.

5.3 Unanswered questions / additional research recommended

The research has raised a number of issues that deserve further attention with the following considered of substance:

- 1. Further analysis of consumer sensory response to enhanced versus untreated product. The improved eating quality is not in doubt but differences in cut-off scores and sensory weightings need to be explored further.
- Additional flavour chemistry by AFBI will provide further understanding of the consumer response. Identification of further precursor interactions and interpretation of the increased sugar levels in enhanced product provide opportunities to modify flavour precursors through ingredient selection and inclusion in value adding formulations.
- 3. Further analysis may identify easy practical marker compounds associated with desirable volatiles and open the possibility of in plant detection with an electronic nose.
- 4. A study to confirm sensory and consumer visual response to enhanced product held chilled post treatment. Limited prior MSA work found no improvement and possible degradation with an 18 day post treatment delay. More detailed study would confirm relationships and be central to electing a fresh, frozen or pre-cooked meal approach.
- 5. Additional study to confirm acceptable weight addition % ranges with particular attention to possible flavour interactions.

- 6. Measurement of purge from vacuum packed treated primal and cooking loss relative to untreated controls to provide a baseline for costing and customer advice.
- 7. Extension to further cuts.

6 Conclusions/recommendations

6.1 Conclusions

Principal conclusions from the research were:

- 1. Enhancement can deliver significantly improved consumer outcomes from cuts of differing quality.
- 2. The 5 treatments tested each delivered a similar positive result allowing a choice of product and ability to substitute between them.
- 3. The results were achieved without massaging and were not improved by tenderising post injection.
- 4. Consumer response was not sensitive to a range of injection rates with the possible exception of striploin injected at less than 7%.
- 5. Treated consumer score ratings were significantly above control for all cooking methods.
- 6. Consumers nominated higher willingness to pay values for enhanced versus control product.
- 7. While assigning higher values for all sensory traits consumer data indicates that enhanced product is assessed differently to fresh untreated product with higher grade cut-off values and changes in sensory scale weightings. This is an important finding as it would appear that a separate model may be appropriate for value added products.
- 8. All sensory scales are increased by the treatments but appear greater for flavour and tenderness rather than an anticipated increase in juiciness values. The overall scale may be reflecting further nuances in consumer reaction to a desirable product that is in some way "different" to untreated beef.
- 9. While final definition of the flavour improvement mechanism is still required the results indicate that the enhancement benefit may be less than anticipated through change in volatiles and more related to taste compounds such as additional sugars.
- 10. There is now strong evidence for a potential to achieve significant flavour change through manipulation of flavour precursors.
- 11. There is considerable potential for additional revenue through marketing of value added product with this revenue resulting from delivery of superior consumer value.

6.2 Next steps

Further flavour chemistry will examine amino acid and ribonucleotide reactions to further quantify the mechanisms influencing precursor interactions.

Follow on activities that flow from the project results include selection of additional cuts for further testing, consideration of further value adding processes and refinement of these to sufficiently understand their interaction and enable development of a value adding prediction model.

One factor to be considered is linkage to MSA certification or endorsement. The research indicates that an MSA assessment of the raw material is a useful component in delivering consistent value added outcomes. Currently value added product does not qualify for MSA grade endorsement. Industry must develop a policy position on use of the existing or an alternative MSA certification and further the degree to which value adding technologies are supported by industry funds and where further processes remain proprietary.

Whatever the outcome the current project has demonstrated the value of utilising consumer testing protocols to define process outcomes.

7 Key messages

- This project confirmed substantial commercial opportunities to capitalise on value adding technology to deliver consistent high-quality meals and meal components from a large supply of raw material that currently fails consumer expectations.
- This is in contrast to the traditional view that such raw material can only be sold at a discount regardless of processing. The value adding treatments tested in this project produce a premium product rather than a discounted product as shown by the willingness to pay data collected at consumer testing.
- Diversion from a bulk commodity to consumer products offers increased revenue potential.
- This project investigated 5 alternative value adding processes across striploin, rump and oyster blade primal cuts collected from a diverse carcase quality range of grass and grain fed product, defined by MSA grading and cooked by grill, roast and re-heat after industrial cooking methods.
- In all cases the treated product was rated significantly higher than the control.
- Results were similar for grass and grain fed product indicating that raw material of common MSA predicted quality from either can be mixed for processing.
- A combination of raw material selection based on MSA cut MQ4 estimates and treatment applied may be utilised to deliver high and consistent quality consumer product within desired quality and pricing bands.
- Analysis of the data highlights that value added products as assessed by consumer sensory testing show different grade cut-off values and scale weightings to untreated fresh beef products. This is an important finding as it would appear that a separate model may be appropriate for value added products.

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9 Appendix

9.1 Annexure A – Reheat protocol

Preparation, heating and serving of pre-cooked beef products for MSA consumer testing. Addendum to MSA Roast Cooking Protocol.

Authors: R Polkinghorne, J Philpott and M Porter.

Requirements:

- A fan forced oven of sufficient size to hold and heat 42 Gastonorm1/9 x 100mm deep bain-marie steamer pans on 5 GN 1/1 trays. (10 tray oven minimum due to pan height).
- 5 bain-maries capable of maintaining 50°C when loaded with 9 x Gastronorm 1/9 steamer pans of 100mm depth together with power boards and leads for power connection.
- 42 x Gastronorm1/9 x 100mm deep bain-marie steamer pans and lids. (Note: the 1/9 pans require a rectangular rather than 180° curved bottom ends to accommodate the keeper).
- Bucket to facilitate filling and draining of bain-maries.
- Sufficient (minimum 5) full gastronome trays to suit the oven racks. (25 or 50mm deep recommended for safety during transport to and from the oven).
- 42 MSA specified stainless steel roast keepers complete with inbuilt cutting boards.
- Cutting board and large sharpened knives suitable for sizing product.
- A 65mm x 65mm x 110mm template or equivalent markings under a clear cutting board.
- 42 x 50mm stainless steel trussing pins.
- A cork pin board.
- A roll of aluminium foil and 42 pre-cut sections to cover and seal individual bain-marie pans.
- Food grade gloves and a protective cut proof glove for use in sample preparation.
- Printed pick sheet with sample ID's.
- 42 x individual oven proof labels pre-printed with relevant EQSRef numbers.
- 42 x individual EQSRef ID labels printed on Avery 7160, 21 up label stock or equivalent.
- 5 x printed serving schedules.
- 5 x small cutting boards and filleting knives (as specified for MSA roast serving).
- Two pairs of elbow length well insulated oven gloves.
- Minimum of two calibrated probe thermometers suitable for internal temp checking of meat in the keepers and of water in the bain-maries.
- 5 x count-up timers of at least 60 minutes duration.
- Chux on a roll or equivalent towelling.
- Cleaning materials suitable for cleaning of the oven, trays, preparation equipment and bench areas.
- Suitable washing facilities to clean all equipment.
- Rubbish bin and liners.
- Fly swat.
- Highlighter pens
- Sharp scissors

Procedure:

- A pick sheet listing the EQSRef and Sequence codes for the 42 samples within the pick should be received prior to the test day. The 42 EQSRef codes should be replicated on individual oven proof labels by printing the 2 label file sheets, laminating and cutting out each EQSRef code. To maintain alphanumeric order pierce each code with a 50mm stainless steel trussing pin and pin in order on to a cork board.
- 2. Delivery of samples.
 - Chilled samples are to be delivered to the test location in sealed packaging with temperature not to exceed 4°C. A count should be made to confirm that 42 samples are present and their individual ID checked against the pick sheet.
 - (If there are any roast samples to be cooked in conjunction with the re-heats they should be identified and separated out as they need to be prepared first.)
- 3. Check that the bain-maries, pans and oven are clean and re-clean if needed. Fit bars to the bain-maries. Check that all needed equipment and consumables are on hand.
- 4. The 5 x bain-maries should be located in five positions suitable for serving, filled with warm water to the recommended level, connected to power and turned on. If possible spread the bain-maries across multiple electrical circuits to prevent overloading. Each should be covered with either a full gastronorm tray or foil to reduce heat loss. Alternatively, if the bain-marie construction and bar attachment allows for the lids to be securely held without the pans, the lids can be used during heating. The final temperature must be a stable 50°C. If necessary a higher temperature may be set to reduce the initial heating period but care must be taken to ensure 50°C is not exceeded. A temperature check should be made at 15 minute intervals to ensure effective operation and to ensure that power has not been lost.
- 5. Select a suitable meat preparation area, ideally on a drained bench and adjacent to a sink. If space allows and there is sufficient staff set up three adjacent work stations. The first for sample presentation with a few clean trays, scissors and a large lined bin. The second station for sample preparation with knife, keepers, sizing template and the cork board of oven proof tags and skewers. The third station has the empty bain-marie pans, 5 x gastronorm trays (GN1/1: 530mm x 325mm) each allocated to a bain-marie numbered 0 4 and pre-cut foil pieces to cover each bain-marie pan. If space is restricted these work areas can be reduced to suit but will increase preparation time.
- 6. Two hours prior to the session commencing and one hour prior to heating turn oven on and set to 190°C dry heat and fan forced. A temperature check should be made at 15 minute intervals to ensure effective operation.
- 7. If roast samples are to be served within the same session for comparison a second oven is required with cooking of roast samples in accordance with the roast protocol commencing prior to loading the re-heat oven.
- 8. After re-heat oven is turned on (two hours prior to session commencement) remove one sample at a time from its packaging taking care to maintain individual ID. IT IS RECOMMENDED THAT ONLY ONE SAMPLE BE OPENED AT ANY TIME TO AVOID ID CONFUSION. If more than one work station is in use place opened sample with its original ID (cut out from bag) onto a tray. On opening, the sample should be checked visually and by odour to verify it is of food grade standard. Each tray should only have a single sample and ID label on it at any time.

- 9. At station one open the sample bag and place sample on a tray. Cut the label off the bag and place beside the sample. Discard the bag and juices. If an identifying disc has been used within the sample bag it should be retrieved at this point. Pass tray on for sample preparation and then start on the next sample. NOTE: EACH SAMPLE MUST HAVE ITS OWN TRAY.
- 10. At the second station each sample is cut to size using the template to achieve a notional dimension of 65mm x 65mm x 110mm to fit into an MSA roast stainless steel keeper. After sizing fit the sample into the keeper and place to ensure the stainless steel spike holds it in position. If the sample is irregular in size place the least suitable end on the spike. The spike should be to the left end of the keeper with the curved edge toward the operator. Select the ovenproof sample identification tag (pinned to the corkboard) which matches the sample EQSRef ID on the original bag. Attach it to the top rear (at the spike end) of the sample using a 50mm stainless steel trussing pin. Pass tray on to the next stage.
- 11. Place the filled keeper into a 400mm deep 1/9th bain-marie steamer pan (GN1/9: 108mm x 176mm x 100mm). Verify that the original ID and that on the trussing pin agree. Place on the gastronorm tray designated on the ID label. The bain-marie allocation number for each sample is printed to the right of the EQSRef label. Cover the pan with a pre-cut foil section. Wipe tray clean ready for re-use.
- 12. If preparation is scheduled to take more than one hour or room temperatures are excessive the 5 loaded trays should be held under refrigeration.
- 13. The procedure above is to be repeated for all of the 42 samples. When completed the trays are ready for heating.
- 14. The 5 loaded gastronorm trays should be transferred to the oven 1 hour prior to the scheduled commencement of the sensory session. Oven temperature should be verified prior to tray placement. A timer should be set to 40 minutes immediately the last tray is placed and door closed.
- 15. While the samples are heating lay the 1/9th steamer pan lids out in a 3 x 3 configuration adjacent to each of the 5 bain-maries and attach the appropriate EQSRef label. The labels are printed on 2 x 21-Up, A4 Avery sheets in alpha-numeric order working top to bottom and left to right. Remove labels for each bain-marie starting from the top left and working down the columns of both sheets. This will maintain alpha numeric order for each bain-marie. There will be 9 labels for bain-maries 1 to 4 and 6 for the bain-marie (0) holding the links.
- 16. Once completed leave the lids in this configuration to indicate where each 1/9th steamer pan should be placed within the bain-marie after heating is completed.
- 17. At 40 minutes a temperature check should be made on representative samples in the oven with a probe thermometer. If the temperature is below 65°C the oven temperature should be checked and heating continued until 65°C is reached.
- 18. After validating the temperature, the trays should be removed from the oven and transferred to the allocated bain-marie (0 4,). Remove the foil cover from each steamer pan, match the oven proof EQSRef label pinned to the sample with its corresponding lid and place under the lid. When all 9 samples are lidded transfer to the bain-marie maintaining alpha numeric order. Once completed each pan will be positioned in alphanumeric sequence within the bain-marie. A final check should be made to ensure the ovenproof tag and lid ID align.
- 19. Serving procedures are to be identical to the MSA roast protocol from this point.

9.2 Annexure B – Cost benefit analysis

Greenleaf Pty Ltd has conducted an independent cost-benefit analysis of pre-grading and treating raw material to enhance value added product eating quality. Their report provides insight into future commercialisation opportunities beyond the trial. It will be submitted independently to Teys.

9.3 Annexure C – TastePoint Consumer Questionnaire Sheets

Prior to being served consumers were briefed on the scoring procedure and completed a basic demographic survey. In all sensory sessions for all cooking methods consumers scored the 7 samples served in a standard manner. Four 100mm line scales were marked to record the consumer rating for tenderness, juiciness, flavour and overall satisfaction. The scales were anchored with the words not tender/very tender, not juicy/very juicy and dislike extremely/like extremely for the flavour and overall scales. In addition, one of four category boxes was marked to assign a description of unsatisfactory, good everyday, better than everyday or premium quality to each sample. After evaluating the last sample consumers completed a willingness to pay sheet that comprised 4 line scales. The line scale descriptions matched the 4 category box category descriptions and had price increments from \$0 to \$80 per kg. Consumers were asked to mark the line for each category at the price they considered appropriate value for samples they would rate at that quality. In addition, they were asked to record whether they were the primary meat shopper in their household.

The questionnaire sheets utilised may be viewed in the following four pages.

TPB TPB

Thank v	you for	vour p	articii	oation	todav	with	our	meat	tastino
	,	,							

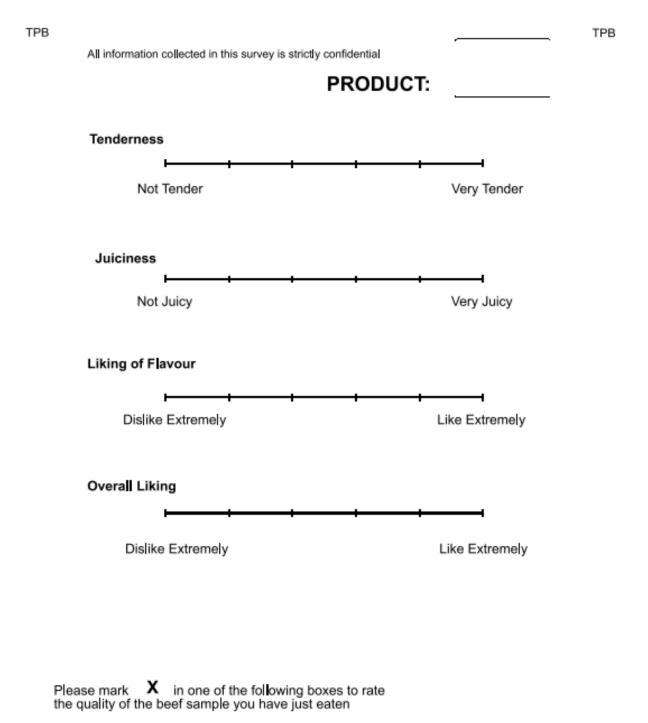
\odot	Our team is here to help you during your session and make this easy for you.
\sim	

Please use a black pen to fill in the form and where asked:

Before you start please listen to the instructions on how to use the scales contained in this questionnaire

	write crosses in box	es like	this		
	mark on the line sca	le like		Very Juicy	
- 1	n between each sample please cleanse y	our p	1.50		
	* first taking a s		•		
	* then chew a		,		
	· ·		ner sip of diluted apple juice		
We	are after YOUR opinion and theref	ore a	sk that you do not talk to anyone els esearch session.	e in	
Now	just a few questions about your	self (All this information is strictly confider	ntial)	
Date				_	
Your	Group's Name		<u> </u>		
1. Ple	ease write in the boxes the postcode y	ou no	rmally live in		
2. Ag	e Group: (Use X in one box only) 18,19 20–25 26–30	3	1-39 40-60 61-70		
3. Ge	nder: (Use X in one box only)				
	Male Female _				
4. WI	nat is the occupation of the main incor (Use X in one box only)	ne ea	rner in your household?:		
\Box	Manager (Ose X III One box Only)	\Box	Professionals (includes health professional etc.)		
\sqcup	Technicians and Trade Workers		Community and Personal Services Workers		
Γ	Clerical and Administrative workers		Sales Workers (includes retail sales etc.)		
L	Machinery operators and Drivers		Labourers		
Γ	Home Duties		Student		
Γ TPB	Other			TPB	

TPB Please use a black pen to fill in the form and w	rite 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	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 Medium / Well done Well done
6.1. How many adults (18 and over) normally live in your household? (Use X in one box only) 1 Adult 1 2 Adults 1 3 Adults 4 Adults 5 Adults 6 Adults 7 Adults 7 Adults 8 and over adults 6.2 How many children under 18 years normally live in your household??	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
(Use X in one box only) O Children I 1 Child 2 children 3 Children 4 Children 5 Children 6 Children 7 and over children	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
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	11. What is your cultural heritage? (Use X in one box only) Australian British descent European descent Asian descent Other Prefer not to say



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

Unsatisfactory

Good everyday quality

Better than everyday quality

Premium quality

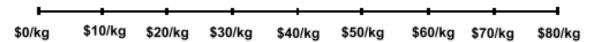
TPB TPB

TPB TPB

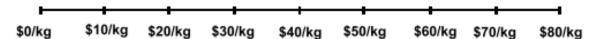
Based on the beef you have just consumed:

Please mark the line at the price per Kg you believe best reflects the value for each category.

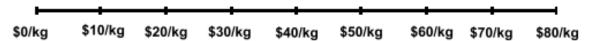
Unsatisfactory Quality



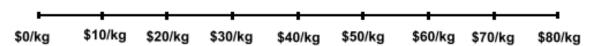
Good Everyday Quality



Better Than Everyday Quality



Premuim Quality



Are you the regular purchaser for your family?

(Use X in one box only)

Yes

Νo

TPB TPB