final report

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Super-Tenderisation processes

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Executive summary

Trials evaluating potential super tenderisation waveforms were carried out in beef at Auckland Meat Processors. Two approaches have shown some promise during past trials:

1). Effect of varying pulse widths, and
2). A frequency modulated waveform.

While these results did not suggest that pulse width modifications alone are likely to generate consistent super-tenderisation effects, understanding the effects of pulse width will help refine the waveforms, particularly those that have recently been identified that use an amplitude and frequency modulated waveform (AFM & FM).

Therefore, this report consists of two parts; the first was to build on the preliminary pulse width data that was reported in milestone 2; and the second was to start screening the AFM waveform options.

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Background

The objective of this project is to identify and commercialise electrical stimulation waveforms that are able to accelerate the tenderisation process through mechanisms independent of accelerated pH decline. The conventional approach to stimulation (for example the AC&A process) is based on producing a rapid pH decline and earlier onset of rigor mortis, which stimulates initial tenderisation but can have some meat quality drawbacks: the ultimate level of tenderness can be compromised, as can other commercially important quality attributes such as colour, colour stability and purge losses. There is therefore a clear commercial benefit in accelerating tenderisation without generating an excessively rapid pH decline, the key objective of this project. The results so far demonstrated that unconventional electrical stimulation waveforms are able to accelerate tenderisation compared with conventional stimulation methods, recent trial work has shown that using specialised waveforms the shear force at 26 hours can be reduced to less than 7Kgf. This effect was independent of the typical stimulation induced pH drop. However, at this stage, the effect is not sufficiently consistent to become a commercial system.

A likely explanation is that this lack of consistency reflects differences in the way carcasses respond to the stimulation waveform. This application therefore proposes to directly measure the muscle responses to the potential tenderisation waveforms, in order to characterise the different responses and use this information to tailor the new stimulation waveforms to produce a more consistent effect.

In addition, this work so far has been carried out on lamb carcases, mostly because they are an easier model to screen a wide range of stimulation waveforms. We propose now to compare the effects identified so far in lamb carcasses to beef carcasses, in combination with the measurement of muscle response characteristics.

We propose also to expand the scope of the research to include measurement of other quality characteristics besides tenderness. These will include meat colour, colour stability and purge losses.

Project Outline

The following are the milestones:

Milestones
1. Screen a range of low frequency waveforms and stimulation conditions in extended muscles to establish an optimum stimulation protocol to accelerate tenderness
2. Using the muscle pressure technique, establish the response characteristics of post mortem muscle to a range of hybrid frequency waveform characteristics.
3. Evaluate the meat quality consequences of using hybrid frequencies on meat quality outcomes, including pH decline, colour, water binding and tenderness.
4. Using the most effective waveforms, evaluate the effects of these on tenderness using the calpain and susceptibility assays; colour in terms of oxygen consumption rate and muscle reductive capacity and waterbinding capacity in terms of myofibrillar density.
5. Using data generated in the above milestones, provide a document outlining the key findings from the above work and identify the potential applications of these in commercial situations.

Project Objectives

The objectives of this project proposal are:

1. Using muscle contraction measurements, identify the nature of the different responses between sheep carcasses in response to the range of accelerated tenderization waveforms and develop procedures to tailor the waveform to produce more consistent tenderness responses.
Commercial outcome and expected date: Electrical stimulation waveform able to accelerate tenderization without affecting pH decline – 2008 (Sheep)

2. Evaluate the tenderization waveforms in beef carcasses, in combination with muscle contraction measurements
Commercial outcome and expected date: Electrical stimulation waveform(s) able to accelerate tenderization without affecting pH decline – 2008 (Beef)

3. Evaluate the super-tenderisation waveforms for effects on meat colour, colour stability and purge losses
Commercial outcome and expected date: Objective data on the effect of the super-tenderisation waveform(s) on purge during storage, colour and colour stability during retail display – 2008 (Beef & Sheep)

Experimental work

The following methods will be used:

1. Muscle responses to tenderisation waveforms: Muscle contraction measurements will be carried out using our established muscle pressure technique. In addition, subject to funding and successful validation under the smart stimulation objective, sonomyography will also be used when it is available. Using these methodologies, the most promising waveforms identified during this year's work will be assessed using a minimum of 20 carcasses for each to identify any differences in the response characteristics. For this purpose, the muscle contraction model will be used where possible to provide a possible physiological explanation for the differences. Response characteristics will be correlated to 24 hour shear force values.

Once differences in response characteristics have been identified, these will be used to develop modified waveforms designed to overcome the sources of variable responses. Two possible strategies present themselves, depending on the results of this stage: an improved waveform will be defined which can accommodate the range of response characteristics. Alternatively, the characteristics of a specific carcass will be defined using a preliminary assessment of its response characteristics (using muscle pressure measurements); then, a predefined waveform appropriate to the behaviour of that carcass will be delivered. The potential commercialisation pathway for this approach will then be based on the Smart Stimulation platform, which has the capability of identifying and responding to the unique responses of individual carcasses.

2. Effects of super-tenderisation waveforms in beef. A similar format as described in 1 will be applied to the super-tenderisation of beef carcasses.

3. As promising waveforms are identified in 1. and 2. above, a full assessment of a range of meat quality attributes will be conducted. In addition to the rate of post mortem pH decline and tenderness and colour measurements at 24 hour measurements, samples will be aged for different periods up to a maximum of 6 weeks to define the ultimate tenderness, colour and colour stability (shelf life) and purge loss during storage.

Pulse width trials: The pulse width treatments were applied on dressed ½ carcasses via the smart stimulation rail (20-25 minutes post slaughter) using the mid voltage electronics (1 Amp at 300V) with the controller programmed to deliver the 3 pulse width treatments (0.1, 0.5, 1.0 msec) at a frequency of 15Hz and the control group using 10 msec pulses at 15Hz.

AFM Waveforms: The AFM waveforms were applied in the bleeding area (hide-on, uneviscerated carcasses). The current controlled system could not be used with multiple carcasses in the smart stimulation tunnel, so a constant voltage waveform that modulated both pulse frequency and pulse width was used instead (FM modulation). The AFM waveforms were modulated with a maximum current of 2 Amps while the FM waveforms generated 1 Amp at 300V.

The controllers for both the experimental stimulation unit that was used in the bleeding area and the smart stimulation unit were programmed with the various waveforms prior to the start of the trial. Depending upon stock availability, either 2 or 3 waveforms were tested in any one day and compared to a control group, the standard 300V, 15Hz stimulation applied using the smart stim rail. The pH fall following stimulation was measured at 1.5 (pH1) and 2.5 hours (pH2) post mortem while the carcasses were in the chillers.

The following day, a sample of striploin was removed during the usual boning operations, and returned to the Carne Technologies laboratory. On arrival, the ultimate pH of each sample was measured and it was then cut into two equal pieces, one was allocated to bloomed colour evaluation and the other for shear force evaluation. A further small sample (less than 5g) was removed from the shear force samples, this was used to measure the water binding capacity.

The colour samples were placed on a polystyrene tray and overwrapped with a standard oxygen permeable retail overwrapping film. The samples were allowed to ‘bloom’ for 3 hours at 2 deg C and then the colour was measured using a Minolta colour meter (CR 400). The lightness, (L*), redness (a*) and yellowness (b*) values were collected and these were also converted into hue angle (3 dimensional colour) and chroma (colour intensity).

The water-binding capacity was determined by calculating the meat area and the liquid area after pressing 500 mg of sample on a filter paper sandwiched between two Perspex plates and pressed at a standard pressure for 1 minute. Photos were taken and the areas were measured using digitising software.

The shear force samples were cooked in accordance with the protocols for shear force evaluation. After overnight cooling of the cooked samples, the shear force was measured using the prototype G2 tenderometer developed under contract to MWNZ and MLA.

The data were analysed using a general linear model and differences between treatments were derived from one-way ANOVA (Minitab 14 – 2006). As with previous trials, the so called 'super tender' effect was defined as samples with a shear force equivalent to or less than 8 kgf. However, previous trials have used the original pneumatic tenderometer where the shear force scales tend to be higher. In this trial, all samples were evaluated using the prototype G2 tenderometer and therefore the super tender cut off was re-defined using the previously derived conversion equation which allows G1 shear force values to be converted to G2. This effectively generated a G2 super tender cut off of equivalent to or less than 5.7 kgf.

Results & Discussion

Pulse width trials

An earlier milestone identified some unexpected results using a range of different pulse widths delivered at the traditional 15Hz stimulation frequency. This work was repeated using between 60-80 carcasses per treatment to evaluate the effect of varying pulse widths on the pH at various times post mortem.

The results were broadly similar to those reported previously; the pH fall following a 15Hz stimulation using 0.1 msec pulse widths was significantly less compared to pulse widths of 0.5, 1 and 10 msec but there were no differences between these last three (Table 1).

Loin samples from a total of 60 samples per treatment were selected during the course of these trials and the shear force was measured at 24 to 26 hours post mortem. The results in Table 1 show that there was no significant effect of pulse width on the average shear force at 24 hours post mortem although the highest mean shear force values were from the 10 msec control and 0.1 msec treatment groups (Table 1). Looking at these data for evidence of the supertender effect found that, consistent with previous reports, the 1 msec pulse duration produced the greatest proportion of super tender samples (27%). By comparison, the control group, using a 10 msec pulse duration, generated only 9% of samples in this category.

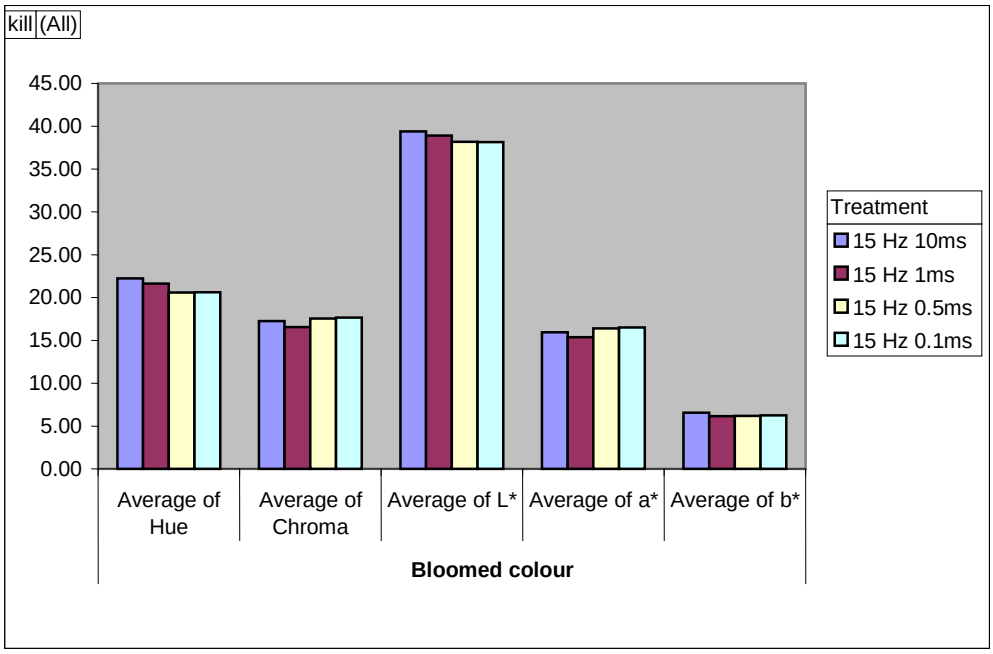
Table 1. Effect of stimulation applied to dressed carcasses (via smart stim rail) using varying pulse widths at 15Hz

Treatment	pH1	pH2	Shear force (1 day post mortem	% in the 'super tender' category
15 Hz/0.1 msec	6.17[a] (0.01)	5.91[a] (0.02)	6.51 (0.29)	20.0
15 Hz/0.5 msec	6.10[b](0.01)	5.84[b] (0.02)	6.42 (0.20)	15.0
15 Hz/1.0 msec	6.11 [b] (0.01)	5.84[b] (0.02)	6.44 (0.23)	27.1
*15 Hz/10.0msec	6.10 [b] (0.01)	5.84 (0.01)[b]	6.72 (0.14)	9.1
Significance	P<0.01	P<0.01	NS	N/A

* control group

The effect of pulse width on bloomed colour is shown in Figure 1. To summarise these data, the 0.1 and 0.5 msec pulse width treatments resulted in a slightly better bloomed colour; the samples were a stronger, slightly darker red with less of a brown component when compared to both the 1.0 and 10 msec pulse width treatments. Despite these differences, all treatments generated a bloomed colour that would be regarded as acceptable if assessed on retail display by consumers.

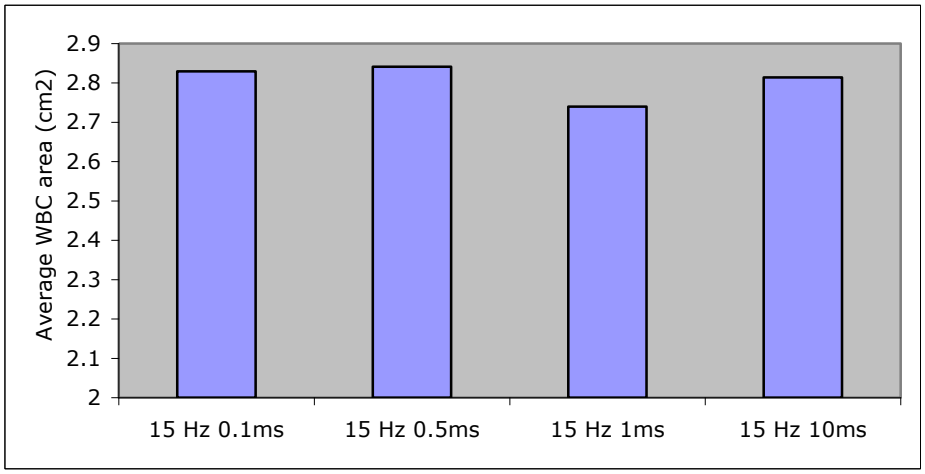
Figure 1. The effect of varying stimulation pulse widths on bloomed colour



There were no effects of pulse width on the amount of expressible water (water binding capacity) (Figure 2) although the average value for the 1 msec pulse width treatment was slightly less than the other three treatments.

Typically, the water binding capacity of meat declines with faster rates of rigor onset; the more the myofibrillar proteins are denatured during the pre-rigor period, the less they are able to maintain intramuscular fluid during post-rigor storage and thus the higher is their expressible fluid. For these data, the higher water binding capacity is produced by the 1.0 msec treatment although these differences are not significant (Figure 2). This is in contrast to the pre-rigor pH falls, where the slowest rate of decline was measured in the 0.1 msec samples. However, the differences in both pH decline and subsequent WBC between treatments are relatively small and therefore do not have any commercial value.

Figure 2. The effect of varying pulse widths on water binding capacity



Modulated waveforms

Three separate trials of each modulated waveform, involving 15-20 carcasses per treatment, were carried out. The AFM waveforms, delivered during bleeding, were based on a waveform described in the previous milestone. This waveform was generated at 3 different frequencies, described using arbitrarily units as 9.766, 78.125 and 156.25 and which represent a 1/8 and 1/16 change in the baseline frequency.

Two separate FM modulated waveforms were assessed. Waveform one involved only a modulated pulse width, which varied between 0.2 and 20 msec in 5 decreasing and 5 increasing steps, at a continuous 15 Hz over a time base of 2.6 seconds. The second waveform varied both pulse width and pulse frequency, varying from 20 msec pulses at 11.5 Hz to 0.2 msec at 909 Hz, over a period of 4 seconds.

Table 2 shows the results of the different modulated waveform treatments. The AFM waveform produced a consistently lower number of super-tenderised carcasses relative to controls. While this is obviously of little commercial benefit, it still demonstrates that tenderness can be influenced by stimulation waveforms through a mechanism dissociated with the rate of pH decline.

This conclusion is reinforced by the effects of the FM waveforms, which both showed an increase in the number of super-tenderised carcasses.

Table 2. Effect of stimulation using AFM waveforms with different characteristics applied either at bleeding or on dressed ½ carcasses.

Treatment	pH1	pH2	Shear force (1 day post mortem	% in the 'super tender' category
AFM 78.125	6.25 (0.03)^{a}	6.02 (0.04)^{a}	8.94 ^{a} (0.40)	3.3
AFM 9.766	6.18 (0.03)^{bc}	5.98 (0.04)^{ab}	7.83 ^{bc} (0.26)	12.9
AFM 156.25	6.31 (0.04)^{ab}	6.14 (0.05)^{b}	7.17 ^{bc} (0.73)	11.1
FM Waveform 1	6.12 (0.02)^{c}	5.92 (0.03)^{ac}	6.27 ^{c} (0.29)	45.0

FM Waveform 2	6.23 (0.02)[ab]	6.09 (0.03)[ac]	6.67 [c] (0.32)	45.0
Control	6.12 (0.02)[c]	5.87 (0.03)[c]	7.85 [bc] (0.29)	16.3
Significance	P<0.001	P<0.001	P<0.001	N/A

Project Outcomes

Screen potential waveforms based on the amplitude frequency modulated waveform approach in beef and evaluate on shear force outcomes.

Conclusion

The results reported here are based on three replicates of each treatment and each showed a similar pattern of response to the different treatment. This suggests a reliable response, even though the effects are probably not sufficiently consistent at this stage to provide a clear commercial advantage.

The benefit of a 1 msec pulse width relative to 10 msec is particularly surprising, as was the particularly high incidence of super-tenderised carcasses with the FM waveform. These results provide encouraging new avenues for exploring super-tenderisation waveforms and will be incorporated into the new trials under MQST 07/08.

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