Final Report

AUSTRALIAN MEAT PROCESSOR CORPORATION

Meat Industry Services

Ultrasonics to improve meat texture

Project code:	A.MIS.1003
Prepared by:	Anita Sikes, Raymond Mawson, Janet Stark CSIRO Animal, Food and Health Sciences
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Meat Industry Services – Ultrasonics to improve meat texture

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Executive summary

A strategic priority for the red meat industry is to improve the tenderness and colour of beef and sheep meat. Conventional applications of ultrasound such as medical imaging, cleaning and measuring fat depth of live animals are well established. In the last few years, the frequencies that can be delivered through ultrasound have changed significantly and have more potential for application to meat for improved quality.

Ultrasound applied to pre-rigor meat is postulated to transiently affect membranes, releasing proteases (cathepsins) and calcium (activator of calpains), thus influencing metabolism as well as proteolytic activity. The acceleration of metabolism pre-rigor could potentially be used, particularly alongside hot-boning, to improve meat colour at grading and to enhance tenderness through proteolysis and reduced cold-toughening. The application of ultrasound to post-rigor meat has the potential to alter muscle structure by physical disruption of the muscle tissue, hence improving tenderness. This proof of concept study was conducted to demonstrate whether ultrasound can be used to accelerate post-mortem tenderisation and control rigor mortis onset, thus enabling delivery of a consistent quality product to the consumer.

Low frequency ultrasound (600 kHz at 40% and 100% power) was applied to post-rigor beef striploin (*M. longissimus dorsi*) steaks to examine the effects on meat texture and colour. Texture was evaluated on cooked steaks using a shear force measurement (Tenderometer) and colour evaluated after ultrasound treatment and ageing using a HunterLab Miniscan EZ. Aged samples were held at 4°C for 7 days. High frequency ultrasound (2 MHz at 60% power) was applied to pre-rigor beef neck muscle (*M. sternomandibularis*) and quality measurements were assessed (texture and colour). Metabolism was measured by muscle pH and tensile testing (measurements from force deformation curves can be used as an indicator of ATP turnover). Texture was evaluated on cooked muscle after treatment and ageing using a Tenderometer and colour assessed using a using a Minolta chromameter. Samples for ageing were stored at 0°C for 3 weeks.

There was no significant effect of the application of low frequency ultrasound (600 kHz) at either power level (40% or 100%) on the texture of post-rigor beef striploin steaks. On ageing of the steaks, there was a significant tenderisation of the meat, however there was no added benefit of ultrasound treatment above that of the normal ageing process. Ultrasound treatment resulted in significant darkening of fresh steaks. H owever on ageing for 7 days, the colour of the ultrasound treated steaks was lighter then the aged, untreated muscle. As measured by differential scanning calorimetry (DSC), there was no indication of denaturation of muscle proteins due to ultrasound treatment of post-rigor beef muscle.

High frequency ultrasound (2 MHz, 60% power) had no significant effect on the pH of pre-rigor beef neck muscle, which is one way to measure metabolism of the muscle. The calculated exhaustion factor, an indication of the ATP turnover and actin-myosin interaction, showed that ultrasound treatment had effectively 'relaxed' the pre-rigor muscle, suggesting some effect on metabolism and actin-myosin interaction. However, the resultant texture of cooked, treated muscle was lower in tenderness compared to the control sample, as indicated by a higher peak shear force value. After ageing at 0°C for 3 weeks, the ultrasound-treated samples had the same peak force value as the control, aged muscle (7.7 kgF). This indicates that the initial toughening of the muscle by the ultrasound treatment was nullified by ageing. High frequency ultrasound had no significant effect on the colour parameters (L*, a*, b*, ΔE) of pre-rigor beef neck muscle. Although the treatment produced toughening, the result is of interest as it does show that high frequency ultrasound had an effect on ATP turnover and the actin-myosin interaction. Although we expected to increase metabolic rate, the far harder thing to achieve is slowing down metabolic rate in pre-rigor

muscle. Thus the application of high frequency ultrasound shows potential for modifying the metabolism of pre-rigor beef muscle.

Recommendations

Although the application of low frequency ultrasound had no effect on the texture of post-rigor beef muscle, it did influence the colour of the muscle. The effect on the colour stability after ageing of the muscle would be worthwhile investigating in more detail. The application of lower frequencies to that used in this study for affecting the texture of post-rigor meat also has merit.

The application of high frequency ultrasound to pre-rigor beef muscle and the potential modification of muscle metabolism (ATP turnover and actin-myosin interaction) warrant further investigation. The application of different frequencies and power to that used in this study for affecting the metabolism and relaxation of pre-rigor meat has merit.

Table of contents

Background

Improving the tenderness and colour of beef and sheep meat is a strategic priority of the meat industry. Conventional applications of ultrasound such as medical imaging, cleaning and measuring fat depth of live animals are well established. The sonochemical and sonomechanical effects of sound waves (20 kHz -100 kHz) at a sufficiently high power range (100 W-10 kW*)* radiated through food (aqueous or semisolids) can alter the intrinsic property of the foods. The application of ultrasound to meat to increase tenderness is proposed to have its effects either through physical disruption of the muscle tissue (Jayasooriya *et al.,* 2004), or through damage to membranes and release of cathepsins and calcium (Roncales 1993). Calcium is a potent activator of the muscle proteases calpains and these proteases, as well as cathepsins, are involved in muscle breakdown during ageing. The application of ultrasound to pre-rigor meat is postulated to transiently affect membranes, releasing cathepsins and also causing calcium release, which can activate glycolysis as well as stimulate calpain activity; thus potentially influencing metabolism as well as proteolytic activity. The acceleration of metabolism pre-rigor could potentially be used, particularly alongside hot-boning, to improve meat colour at grading and to enhance tenderness through proteolysis and reduced cold-toughening. In the review conducted by (Kentish *et al.* 2009) for MLA, they identified the variable results obtained for improving the tenderness of meat through application of ultrasonics. In the last two years, the frequencies that can be delivered through ultrasound have changed significantly (Kai Knoerzer, *pers. comm.*) and have more potential for application to meat.

The project will undertake proof of concept R&D to investigate whether ultrasound can be used to control muscle metabolism pre-rigor and alter muscle structure post-rigor.

Project objectives

To investigate whether ultrasound can be used to accelerate the post-mortem tenderisation process

To investigate whether the application of ultrasound to pre-rigor meat has any effect on the muscle metabolism, with potential for improving meat colour at grading

Application of low frequency ultrasound to post-rigor beef

Aim

To demonstrate that the application of ultrasound at a frequency optimal for penetration of acoustic energy into post-rigor meat can stimulate the intrinsic proteolytic enzymes to enhance the rate of tenderisation or alter muscle structure.

Methods

M. longissimus dorsi (striploin) muscles were removed from 10 Halal-slaughtered beef carcasses, 24 hours post-mortem. The muscle from each carcase was cut into 25 mm thick steak-sized portions from the same end of the striploins and randomly allocated to 6 treatments, as outlined below:

- Control, fresh
- Control, aged
- Ultrasound 40%, fresh
- Ultrasound 40%, aged
- Ultrasound 100%, fresh
- Ultrasound 100%, aged

Measurements of pH and colour were taken prior to, and after treatment. Weights were also recorded. pH was measured with a digital pH Meter (TPS W80) and intermediate junction pH electrode (TPS IJ44, spear tip). Triplicate colour measurements (L*, a*, b* values) were conducted using a Hunterlab Miniscan EZ (illuminant A, observer angle 10°, aperture size 5 cm) at 5°C after blooming for 20 minutes. The steaks were vacuum sealed into polypropylene plastic bags and stored in a 4ºC chiller overnight. Fresh samples were treated on the day after collection (day 1); samples for ageing were treated on the day after collection (day 2).

The sample was immersed in a waterbath (Julabo F38-ME, Germany) at approximately 10˚C. The 600 KHz ultrasound transducer was placed vertically in the tank (Figure 1).

Figure 1: Set-up for ultrasonic trials of post-rigor meat. Left: SONOSYS® generator, Middle: water bath controller, Right: sample immersed in tank, showing position of transducer.

The samples were exposed to ultrasound at 40% or 100% power for 10 minutes. Control samples were not treated with ultrasound and were stored at 5°C until further analysis. The temperature of the water was recorded before and after ultrasound treatment.

Following treatment and measurements (pH, colour, weights), subsamples were taken for differential scanning calorimetry (DSC) and myofibrillar fragmentation index (MFI).

The samples were weighed and suspended in plastic bags in a 75˚C circulating water bath for 30 minutes until an internal temperature of 72ºC was reached, cooled in an ice slurry for 10 minutes, dried and reweighed to determine cook loss. Samples were stored overnight at 4˚C to set before cutting.

The samples were sectioned into 10 mm x 10 mm x variable length slices with the fibres parallel to the long axis. The samples were placed into a G2 Tenderometer cutting chamber for measurement (Figure 2). The G2 Tenderometer gives a shear force value (Kgf) and 7 to 10 replicates were measured on each sample and the means determined.

Figure 2: Sample preparation for texture measurement using the G2 Tenderometer. Left, samples placed into texture chamber. Middle, G2 Tenderometer. Right, sample chamber is brought up to the cutting blade.

Samples treated on day 1 were cooked the same day; on day 2, samples for ageing were vacuum-packed and stored at 4°C for 7 days prior to pH and colour measurement, subsampling, cooking and texture analysis.

Results

From the analysis of the texture measurements, there was no significant (P>0.05) effect of ultrasound at either power level (40% or 100%) on the peak force value of beef striploin steaks (Figure 3). There was a significant effect (P<0.001) of ageing at 4°C for 7 days on the peak force value, however there was no effect (P>0.05) of the interaction between treatment and storage (Figure 3). As there was no significant effect of ultrasound on the texture of LD steaks, the myofibrillar fragmentation method was not performed on these samples.

The L* (lightness), a* (redness) and b* (yellowness) colour values of control and treated, fresh and aged samples were determined using a Hunterlab Miniscan. Three determinations were carried out on the fresh surface of the muscle samples. The lightness (L^* value) of striploin steaks after ultrasound treatment was significantly decreased (P=0.008) compared to untreated control samples (Figure 4). There was also a significant effect of ageing (P<0.001), with steaks becoming lighter after storage at 4°C for 7 days. The control samples did not increase in lightness with ageing whereas the ultrasound samples (40% and 100% power) significantly increased in lightness with ageing (treatment x storage interaction, P=0.004). A significant effect (P<0.001) was found with ageing on the b^* value, indicating a more yellow colour.

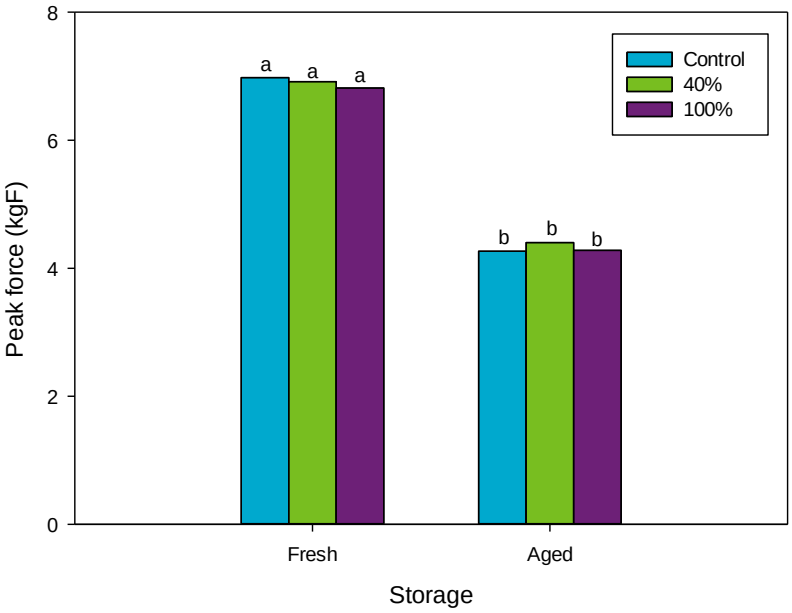

Figure 3: The effect of low frequency (600 kHz) ultrasound (40% or 100% power) on the texture of beef striploin steaks (n=10), as measured using a Tenderometer. Texture was assessed on fresh steaks (no ageing) and aged (7 days at 4°C) steaks.

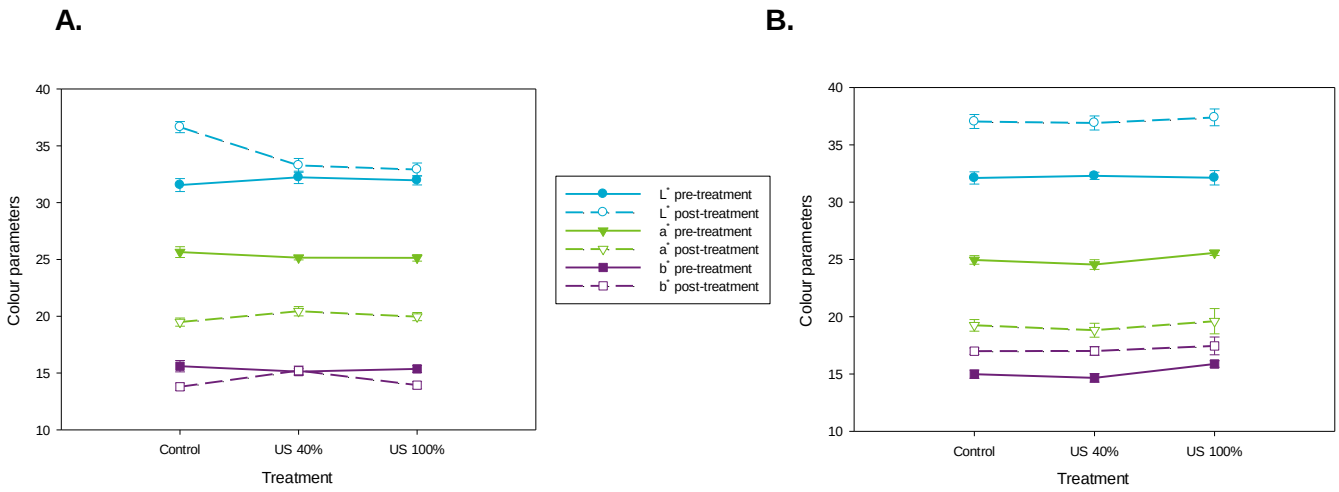

Figure 4: The effect of low frequency (600 kHz) ultrasound (40% or 100% power, US 40% and US 100%) on the Minolta colour parameters (L^*, a^*, b^*) of beef striploin steaks. Mean ± SE (n=10).
A, colour measurements of unaged (fresh) steaks; B, colour measurements after ageing for 7 days at 4°C.

Averaged colour values (L*, a*, b*) were used to calculate the total colour difference (ΔE), with the untreated control sample used as a reference, according to the equation, $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$. Ultrasound treatment at both 40% and 100% power significantly (*P*=0.002) decreased the total colour difference of fresh beef striploin steaks (Table 1). This was due largely to a decrease in the L* (lightness) value, indicating that muscle was darker (less light) after ultrasound treatment. However, after ageing, the colour of ultrasound-treated muscle was similar to untreated, aged samples (Figure 4).

Table 1: Effect of low frequency ultrasound (600 kHz) at 40% and 100% power on the total colour difference (ΔE) of fresh and aged beef striploin steaks (n=10).

Treatment	Control		US 40%		US 100%		*P*-value (S.E.D.)		
Storage	Fresh	Aged	Fresh	Aged	Fresh	Aged	T[a]	S[b]	T*S
	8.72	8.03	5.40	8.07	5.83	9.03	0.002	<0.001	<0.001
							(0.443)	(0.361)	(0.626)

[a] T – treatment effect (ultrasound)

[b] S – storage effect (unaged or aged)

Measurements of the thermal behaviour of proteins in the muscle were performed using differential scanning calorimetry (DSC). Initially, 2 samples from each treatment were analysed to assess differences in thermal stability of muscle proteins between treatments. The transition temperature (T_m) was recorded and the transition enthalpy (ΔH) for individual peaks was calculated from the peak area. The total enthalpy of denaturation (ΔH_T) was calculated by constructing a baseline from 35 – 80°C. From the results in Table 2, it can be seen that there are no differences in the transition temperatures or enthalpy values for individual peaks or the total enthalpy when ultrasound was applied. There was also no effect of ageing of control or ultrasound-treated beef muscle. It can be concluded that the application of ultrasound under these conditions did not denature the muscle proteins. Therefore, no further DSC analysis of samples was undertaken.

Table 2: Thermal behaviour parameters (T_m, transition temperature, °C; ΔH, transition enthalpy, J/g; ΔH_T, total enthalpy from 35-80°C, J/g) of untreated (control) and ultrasound-treated (600 kHz at 40% or 100% power, US40 or US100) beef muscle determined by differential scanning calorimetry (DSC). Peak 1 represents denaturation of myosin, Peak 2 includes sarcoplasmic proteins, myosin and connective tissue, and Peak 3 represents actin denaturation. Fresh samples were unaged; aged samples were stored at 4°C for 7 days), n=2.

Treatment	Storage	Peak 1		Peak 2		Peak 3		Total enthalpy
		T_m	ΔH	T_m	ΔH	T_m	ΔH	ΔH_T
Control	Fresh	52.2	0.87	62.4	0.57	75.0	0.64	5.06
	Aged	53.2	1.21	61.6	0.50	75.4	0.67	5.63
US40	Fresh	52.2	0.71	62.5	0.51	75.3	0.64	4.92
	Aged	53.1	1.01	62.7	0.49	75.6	0.65	5.47
US100	Fresh	52.7	0.93	62.1	0.54	75.1	0.62	5.30
	Aged	53.3	1.07	62.6	0.50	75.4	0.72	5.44

Application of high frequency ultrasound to pre-rigor beef

Aim

To investigate the use of ultrasound at 2 MHz to control muscle metabolism and improve quality of pre-rigor beef muscle.

Methods

M. sternomandibularis muscle (neck muscle / tongue root fillet) was dissected from freshly Halal slaughtered beef neck tissue. Samples were collected four times daily at 2 hourly intervals, for use in the trials. Muscle was cut longitudinally into approximately 40 mm x 70 mm portions, with four samples taken from the one animal (Figure 5).

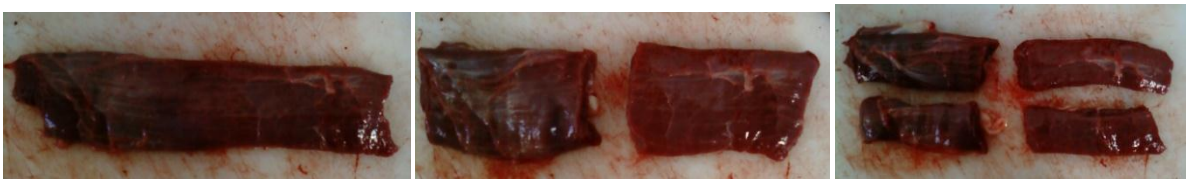

Figure 5: Cutting of tongue root fillet into 4 portions.

The muscle pieces were measured for pH immediately prior to treatment with a pH meter (TPS W80), fitted with an intermediate junction pH sensor. Triplicate colour measurements (L*, a*, b* values) were also measured at this time with a Minolta CR300 chromameter (illuminant D65, aperture 10mm).

The strips were clamped onto holders and attached to a Lloyd LRX Universal testing machine with a 2.5 KN load cell attached. The sample was immersed in a phosphate-buffered saline solution (PBS, pH 7) at approximately 30˚C in a perspex tank fitted with a 2 MHz transducer (vertical alignment) and opposing reflector plate, on the Lloyd platform (Figure 6). The time of treatment was recorded to ascertain time post slaughter. The temperature of the saline solution was recorded before and after treatment.

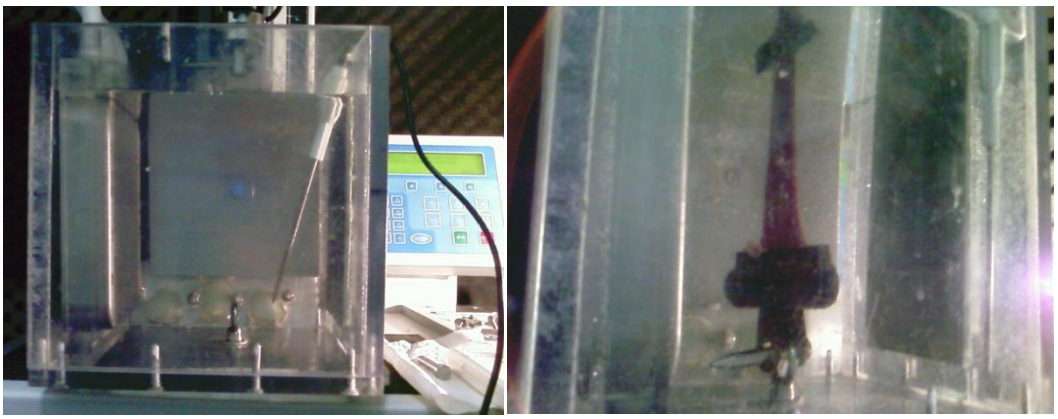

Figure 6: Left, tank and ultrasound setup on Lloyd Universal testing machine platform. Right, sample placement in tank.

The sample had a preload of 2 N applied before the extension was set to 15 mm. The samples were kept at this extension for 2.5 minutes to allow the force to stabilise before being exposed to ultrasound at 60% power for 5 minutes. The samples were then subjected to the ultrasound treatment a second time under the same conditions. After treatment, the pH was recorded and colour measurements were recorded. Control samples were treated to the same conditions but no ultrasound was applied.

The samples for fresh analysis (unaged) were wrapped individually in a plastic bag and stored over night at 4ºC. Ultimate pH was measured prior to cooking to ensure that the samples had gone into rigor (pH ≤ 5.7). The samples for ageing were vacuum sealed in polypropylene plastic bags and stored at 0ºC for 3 weeks, before being processed the same as the unaged samples.

The samples were weighed and suspended in plastic bags in a 74˚C water bath until an internal temperature of 72ºC was achieved (20 – 23 minutes), cooled in ice slurry for 10 minutes, dried and reweighed to determine cook loss. Samples were stored at 4˚C to set before cutting. Day 1 to 3 samples were stored overnight and day 4 samples were stored for 2 hours.

The samples were sectioned into 10 mm x 10 mm x variable length slices with the fibres parallel to the long axis. The samples were placed into the G2 Tenderometer cutting chamber for measurement. The G2 Tenderometer gives a shear force value Kgf and 3 to 6 replicates were measured on each sample and the means determined.

Results

High frequency ultrasound (2 MHz, 60% power) applied to pre-rigor beef neck muscle resulted in an increased peak force value of fresh muscle, indicating toughening of the muscle, however this was not significant ($P>0.05$) (Figure 7). After ageing at 0°C for 3 weeks, the ultrasound-treated samples had the same peak force value (7.7 kgF) as the control, aged muscle; ageing of muscle, irrespective of treatment, resulted in a significant increase in tenderness ($P<0.001$) (Figure 7). This indicates that the initial toughening of the muscle by the ultrasound treatment was nullified by ageing and suggests that ultrasound applied pre-rigor does not provide any additional benefits to texture compared to the normal ageing process under these conditions.

The application of ultrasound to pre-rigor neck muscle had no effect on the total colour difference (ΔE) ($P>0.05$) (Figure 8). Overall, there was a significant effect ($P=0.043$) of ageing of the muscle, resulting in a decrease of the ΔE value. On ageing, the ΔE value of the control sample decreased by two-thirds, whereas the total colour difference for the ultrasound-treated sample was similar to the fresh (unaged) ultrasound-treated sample. The application of ultrasound or ageing also had no significant effect ($P>0.05$) on the individual colour parameters (L*, a*, b* values) compared to the untreated control samples (Table 3).

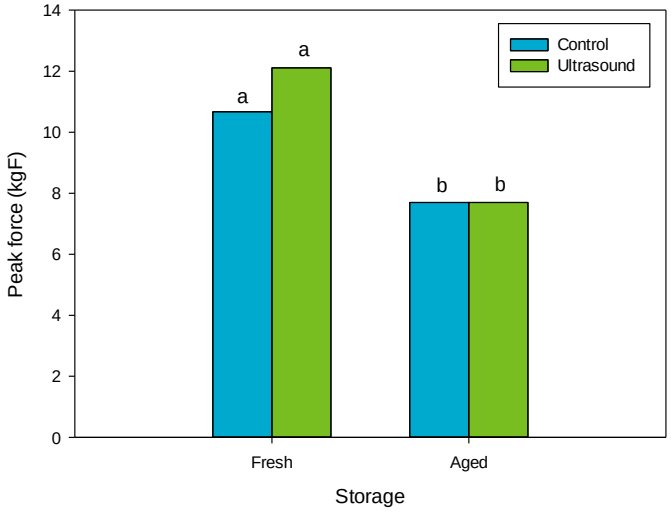

Figure 7: The effect of high power ultrasound (2 MHz, 60% power) and ageing (3 weeks at 0°C) on the texture of beef neck muscle.

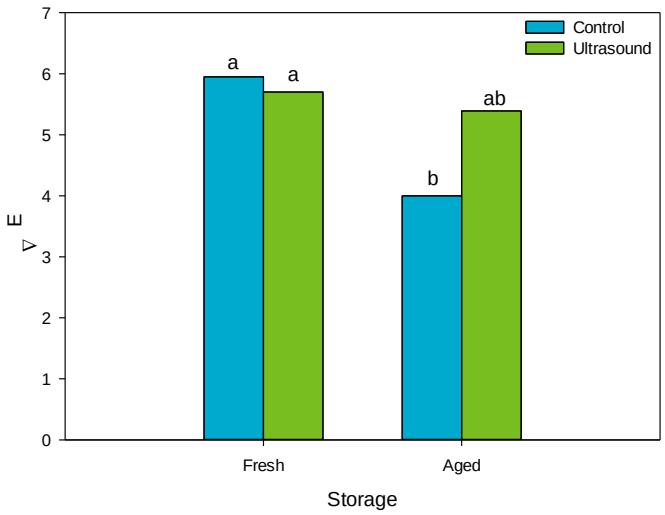

Figure 8: Changes in meat colour (total colour difference, ΔE) of ultrasound-treated pre-rigor bovine neck muscle. Mean ± SE, n=14.

Table 3: Meat colour changes (L*, a*, b* values) of ultrasound-treated (2 MHz, 60% power) bovine neck muscle. Mean ± SE, n=7.

Treatment	Pre-treatment			Post-treatment			Aged		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control	28.51 ± 0.721	16.58 ± 0.464	-1.40 ± 0.276	30.84 ± 0.726	14.72 ± 0.560	-2.57 ± 0.255	33.26 ± 0.757	20.42 ± 0.452	-1.73 ± 0.381
Ultrasound	27.84 ± 0.423	17.12 ± 0.571	-1.25 ± 0.271	31.14 ± 0.692	13.97 ± 0.388	-2.71 ± 0.236	33.03 ± 0.716	20.77 ± 0.333	-1.30 ± 0.302

In this study, high frequency ultrasound was applied to pre-rigor muscle, essentially with the intention of changing metabolism/glycolysis. The initial pH of pre-rigor beef neck muscles ranged from 6.62 to 6.76 which was within the expected pH values for pre-rigor muscle. The application of ultrasound had no significant effect (P>0.05) on pH (Table 4).

Table 4: The effect of high frequency ultrasound (2 MHz, 60% power) on the pH of pre-rigor beef neck muscle. Mean ± SE, n=28.

Treatment	Pre-treatment pH	Post-treatment pH
Control	6.65 ± 0.044	6.54 ± 0.052
Ultrasound	6.69 ± 0.039	6.58 ± 0.042

An example of the output from the forces recorded during ultrasound treatment is shown in Figure 9. The data for peak stress, peak strain, area under the curve and work done were used to calculate the exhaustion factor (%); a parameter which describes the metabolic state (glycolytic rate) of the muscle without the rigour of doing extensive biochemical analyses.

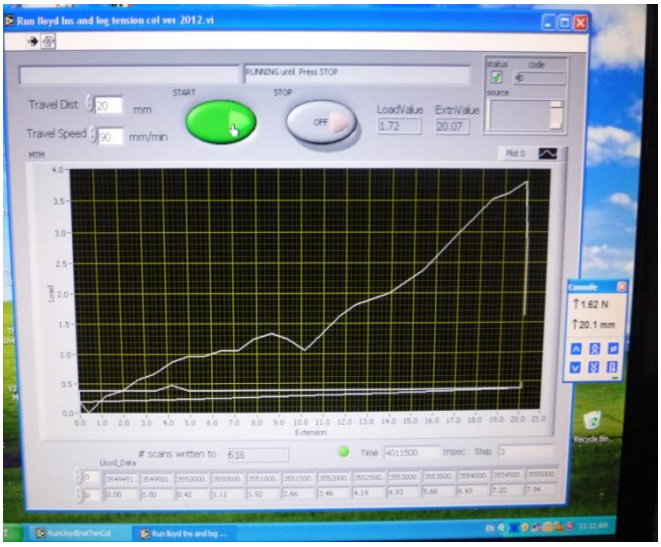

Figure 9: An example of the force deformation curve during ultrasound treatment of pre-rigor beef neck muscle.

The exhaustion factor tended to be higher in the ultrasound-treated sample (60.31%) compared to the control sample (56.36%) (*P*=0.073, SED 2.168). This would suggest however, that an increase in the ATP turnover or glycolytic rate had occurred due to ultrasound treatment. It is postulated that the resultant texture (peak force) of the ultrasound-treated sample may have been more tender than the control sample as contraction of the ultrasound-treated sample would have been avoided. However, the opposite was observed (Figure 7). Thus this requires further investigation as a potential technology to change pre-rigor metabolism. Although we expected to increase metabolic rate, the far harder thing to achieve is slowing down metabolic rate in pre-rigor muscle. Thus this is actually an interesting finding and warrants further investigation, using a wider range of power and frequency ultrasound.

Conclusions

- The application of low frequency ultrasound (600 kHz, 40% and 100% power, 10 min) to post-rigor beef LD muscle did not alter muscle structure or stimulate proteolytic enzymes to enhance the rate of tenderisation. This was evidenced by no significant effect on the objective texture measurements of cooked steaks or denaturation of muscle proteins (analysed by DSC). Ultrasound using these conditions resulted in darkening of fresh steaks, however on ageing for 7 days, the colour of the ultrasound treated steaks was lighter then the aged, untreated muscle.

- The application of high frequency ultrasound (2 MHz, 60% power) showed potential for modifying the metabolism of pre-rigor beef muscle. This relaxation of the muscle did not result in improvement in the quality (texture, colour) of pre-rigor beef muscle but did change pre-rigor metabolism. Although we expected to increase metabolic rate, the far harder thing to achieve is slowing down metabolic rate in pre-rigor muscle. For example, by slowing down metabolic rate, we could avoid heat-toughening and ensure muscles/carcasses enter the correct part of the MSA pH-temperature window. Thus this is actually an interesting finding and warrants further investigation, using a wider range of power and frequency ultrasound.

Recommendations

Although the application of low frequency ultrasound had no effect on the texture of post-rigor beef muscle, it did influence the colour of the muscle. The effect on the colour stability after ageing of the muscle would be worthwhile investigating in more detail. The application of lower frequencies to that used in this study for affecting the texture of post-rigor meat also has merit.

The application of high frequency ultrasound to pre-rigor beef muscle and the potential modification of metabolism (ATP turnover and actin-myosin interaction) warrant further investigation.

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