final report

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Cold Pasteurisation using Ultrasonics

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30th May 2004

PROJECT CLOSE OUT SUMMARY DOCUMENT[1]
PRTEC.003 – Cold Pasteurisation using Ultrasonics

Introduction to Project

High power ultrasound was investigated for its ability to effect reductions in numbers of bacteria in intact packs. Investigations were undertaken in a small system that was designed to treat small sealed sachets containing either broth culture of *E. coli* or small pieces of fat tissue painted with the culture.

Project Objectives

High power ultrasound (**HPU**), a recent innovation in ultrasound technology, offers an innovative, acceptable alternative to ozone, ultraviolet, high pressure and heat pasteurisation for the treatment of food products. HPU can treat liquids such as food products, which contain high levels of particulate whilst ozone and ultraviolet can only operate effectively in filtered liquids.

HPU processes are currently being developed in a variety of industrial applications including the food industry. Applications in the food industry have focused particularly on using HPU as a technology for emulsification, crystallisation, extraction and activation/enhancement of cell growth.

A process using HPU has not been commercially developed for the cold pasteurisation of meat products. It is believed that HPU processes may enhance food safety in meat processing, which will result to *E.coli* free meat, which in turn, will lead to increased sales/exports of meat products and premium price for pathogen free meat, among other benefits.

The pasteurisation process is chemical-free; the HPU equipment is modular, easy to install and operate, and is suitable for both small and large operators and is relatively less expensive to operate compared with alternative equipment. However, the capital cost of the HPU equipment is higher than e.g. ultraviolet equipment. The operating cost in terms of both power consumption and maintenance costs of HPU is considerably less.

These studies were aimed to determine:

- whether the ultrasound treatments given to the packs have measurable effects on the meat quality parameters pH, meat colour, and tenderness; and
- whether ultrasound treatments applied by the Consultant reduce the numbers of test organisms with which portions of beef have been before they are vacuum-packaged and treated;

Conclusions

High power ultrasound is a processing technology that should be considered for applications in vacuum-packed and processed meat. It offers an alternative to heat pasteurisation, high pressure, and irradiation for the treatment of intact packs of meat and other food products. A process using high power ultrasound has not been commercially developed for the cold pasteurisation of meat products

[1] Majority of text extracted from Project reports

Results

Experiments using broth cultures of *E. coli* achieved reductions of 2.8 log10 *E. coli* ±0.8 cfu/mL in sachets held at 60°C for 30 s in zone B of the treatment vessel – a region where the ultrasonic energy was intentionally focused.

The effect was not restricted to that zone however; there were also significant reductions at positions outside zone B.

The orientation of the sample in zone B appeared not to influence the reductions observed. Further investigations using small sachets of vacuum-packed fat and lean tissue held at 75°C and sonicated for 15-30 seconds, achieved microbial reductions of up to 3 logs, though reductions were not always consistent. In order to identify potential treatment parameters for a commercial application, a better understanding of the reasons for the inconsistent reductions is necessary.

This study demonstrated that the lethal effect on microorganisms is a synergistic effect and is not due to heating alone. A scaled-up treatment vessel must employ heat and sonication simultaneously.

Where to from here?

A three way project meeting held at the conclusion of the project between Valley Beef (Mike Jackson), Food Science Australia (Ian Eustace) and Meat and Livestock Australia (Ian Jenson and Sean Starling), concluded that the project had been successful.

The next step for the evaluation of the technology would be to run an extended product trial that utilised up to 100 cuts of treated meat and 100 control cuts of meat. The treated meat would be analysed for shelf life, microbial quality, general appearance and eating quality over 4 months tested every 15 days.

However, prior to any further attempts at evaluating the technology it was concluded that the following needed to be understood on a basic level to ensure that if the technology functioned as required it would be commercially viable. Items included:

- capital costs including installation costs and required footprint (it was concluded that $1 million would be too much and 20 metres would be too long);
- how technology may integrate with a process;
- occupational health and safety issues;
- operating costs;
- any competing technologies that may be equivalent; and
- expected uptake if successful

Food Science Australia is responding to MLA with a proposal. In addition MLA will commence discussions with potential commercialisers of the technology to determined potential capital and operating costs.

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PROJECT PRTEC.003B(V1)

Pilot plant for cold pasteurisation of beef – equipment and technical expertise

Prepared for
Meat & Livestock Australia

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March 2004

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SUMMARY

High power ultrasound was investigated for its ability to effect reductions in numbers of bacteria in intact packs. This report provides the results of a series of investigations that was undertaken in a small system that was designed to treat small sealed sachets containing either broth culture of *E. coli* or small pieces of fat tissue painted with the culture. It also identifies a possible system design and treatment conditions for a pilot scale system for treating vacuum-packed meat primals.

The system used for the investigations was designed to focus the ultrasound energy from a 4 kw radially-emitting sonotrode into a specific treatment zone. These investigations were done after ultrasonication treatments, using one or two 4 kw sonotrodes in a much larger vessel of cold (10°C) water, proved ineffective. A revision of the ultrasound parameters using laboratory-scale ultrasonic equipment at the Food Science Australia Werribee facility, indicated that the original treatment vessel needed to be reconfigured to increase the power and should also operate at a higher temperature.

Experiments using broth cultures of *E. coli* achieved reductions of 2.8 $\log_{10}$ *E. coli* ±0.8 cfu/mL in sachets held at 60°C for 30 s in zone B of the treatment vessel – a region where the ultrasonic energy was intentionally focused. The effect was not restricted to that zone however; there were also significant reductions at positions outside zone B. The orientation of the sample in zone B appeared not to influence the reductions observed.

Further investigations using small sachets of vacuum-packed fat and lean tissue held at 75°C and sonicated for 15-30 seconds, achieved microbial reductions of up to 3 logs, though reductions were not always consistent. In order to identify potential treatment parameters for a commercial application, a better understanding of the reasons for the inconsistent reductions is necessary.

This study demonstrated that the lethal effect on microorganisms is a synergistic effect and is not due to heating alone. A scaled-up treatment vessel must employ heat and sonication simultaneously.

High power ultrasound is a processing technology that should be considered for applications in vacuum-packed and processed meat. It offers an alternative to heat pasteurisation, high pressure, and irradiation for the treatment of intact packs of meat and other food products. A process using high power ultrasound has not been commercially developed for the cold pasteurisation of meat products.

INTRODUCTION

This project, initiated by Valley Beef and funded by AMPC and MLA, was commenced at Food Science Australia Cannon Hill by Innovative Ultrasonics in 2001. During 2002 and 2003, work focused on a physical method - aluminium foil erosion – for characterising the performance of the system. The investigation was undertaken using either one or two sonotrodes operating in a large cylindrical tank, volume approximately 450 L. Figure 1 shows one of the sonotrodes supported in the tank.

Figure 1. Sonotrode supported in original cylindrical tank.

It proved impossible to achieve useful reductions in numbers of *E. coli* in the system however. A critical review of possible reasons for the system's ineffectiveness identified two likely reasons. Firstly, the power to volume ratio, or energy intensity of ultrasound in the treatment vessel was too low - probably less than 0.1 W cm^{-3} - even using small parabolic reflectors. Secondly the temperature at which the investigation was done – 10°C – was too low. It was decided that the system needed to be reconfigured to increase the intensity of sonication, and that there should be provision for operating the system at elevated temperature. It was also decided that the position of maximum sonication needed to be mapped using bacteria in broth rather than gravimetrically by aluminium foil erosion.

Food Science Australia assumed responsibility for the project in 2003. Since that time, research has involved an exploratory investigation at Food Science Australia Werribee with a small (400 W) flow-through cell unit, and a more detailed

investigation at Cannon Hill. This report provides the results of the recent investigation at Cannon Hill.

The objective of this investigation was to generate performance information necessary for the design of a pilot scale system for treating vacuum-packed primals.

The aims of the investigation were to:

1. Identify a possible ultrasonic design that demonstrates an effective treatment zone for vacuum-packed meat, including the position, depth and orientation.

2. Identify conditions (temperature of water, duration of sonication) for inactivation of bacteria sealed in small vacuum packs and gain better understanding of what influences the microbiological effectiveness of ultrasound.

SONICATION TRIALS

Preparation of samples

The *E. coli* culture was a mixture of strains that have no known virulence markers for pathogenic *E. coli*. Broth cultures of each of five *E. coli* strains (EC1604, EC1605, EC1606, EC1607, EC1608) were grown overnight at 37°C in tryptose soya broth (TSB).

Small (~5 cm x 5 cm) sachets were made from standard vacuum packaging film (Barrier Bag™, Cryovac Australia) and filled with either the diluted broth (broth sachets) or fat to which diluted culture had been applied (fat sachets) or aluminium foil sheets (aluminium foil sachets), as described below.

Broth sachets

Equal volumes (1 mL) of the five cultures were added to 300 mL of sterile TSB to give a cell density of around 10 million *E. coli* per mL. The broth cultures were stored in an ice/water mixture until added to the sachets. A small volume (5 mL) of the broth was added to each sachet with a pipette. Each sachet was then sealed. The sealed sachets were kept at 4-10°C until treatment. They were treated within 2 hours of preparation.

Fat sachets

Subcutaneous beef carcass fat trim (from rumps, striploins etc) was chosen for treatment because for most primal cuts, it is the main surface of contamination, not lean tissue. Chilled fat trim was collected from a local boning room and samples ~5 x 5 x 1 cm were prepared. A broth culture of *E. coli* was painted onto the outer surface (ie. that which is on the surface of the carcass) of the samples using a sterile, 30 mm paintbrush and allowed to air-dry for 15 min. The fat samples were then inserted individually into the sachets, vacuum-packed under a light vacuum, and kept at 4-10°C until treatment. The sachets were treated within 2 hours of preparation.

Aluminium foil sachets

Aluminium foil sheets were cut into 5 cm x 5 cm squares, which were weighed and individually inserted into sachets. 5 mL of water was added to each and the sachets were sealed.

Configuration of ultrasound equipment

A vessel, capacity 38 L and ellipsoidal in cross-section, was constructed from stainless steel (Figure 2). It was designed to focus the energy from the 4 kW sonotrode fitted to it, into a useable zone. Although the intensity of ultrasound energy in the system could not be measured, the intensity is likely to be at least 1 W/cm^3 (and possibly up to 10 W/cm^3) in Zone B (Figure 3).

Figure 2. Ellipsoidal vessel and sonotrode supported in cylindrical tank

Figure 3 shows, in plan view, the positions in the vessel where the test sachets were held during treatment. The positions are marked as zones A, B and C. The sachets were held at depths of 150, 300 or 450 mm, and in the 'X' or 'Y' orientation.

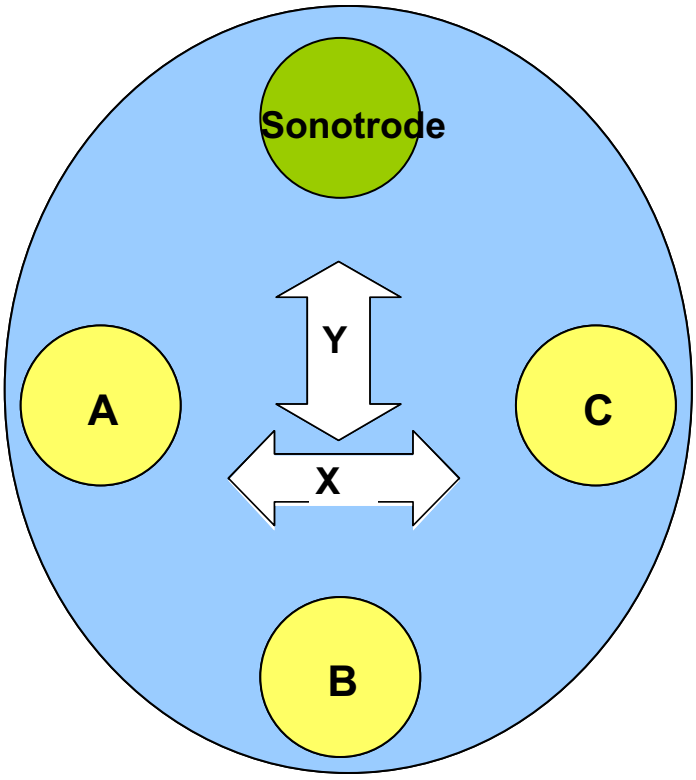


Figure 3. Cross-section of ellipsoidal treatment vessel showing the relative position of the sonotrode and positions in which sachets were held for treatment (A, B and C). The orientations in which sachets were held are depicted by X and Y.

The power supply to the transducer and sonotrode was located in a room adjacent to the vessel so that the operator could be behind a closed door during each sonication run. This was a requirement of the OHS&E Committee for the Cannon Hill facility.

For operation, the vessel was filled with water to within 50 mm of the top of the sonotrode. The water temperature for the trials ranged from 55 to 75°C. The water temperature was maintained at the treatment temperatures by recirculating water from a temperature-controlled bath. The temperature during each run was monitored and recorded.

Ultrasound treatment procedure

Broth sachets

A single sachet of *E. coli* culture was located in the vessel, at a position specified at the commencement of each trial. The operator moved to the adjacent room where the power supply was located, closed the door and commenced sonication. Upon completion of sonication for the designated time, the door was opened and the sachet was retrieved from the vessel. The need to follow this procedure meant that total immersion times were around 10 s longer than the corresponding sonication times. Sachets were sonicated individually at full power (100% amplitude) at a temperature of 55, 57.5, 60, or 62.5°C for times up to 120 s. After retrieval, the contents of the sachets were transferred to sterile tubes, which were kept, on ice until their transfer to the laboratory. Each broth was serially diluted and plated in duplicate on to tryptone

soya yeast + glucose (TSYG) agar. The plates were incubated overnight at 37°C, and colonies were counted.

Fat sachets

A modification to the treatment procedure outlined above was made before the fat sachets were treated in order to avoid the immersion time having to be longer than the sonication time. A pulley system was devised to allow the (weighted) sachets to be lowered into the treatment vessel after the sonication had been started from the adjacent room. The fat samples were treated at full power (100% amplitude), water temperatures of 60°C and 75°C and for times up to 60 s. After sonication treatment, sachets were placed in a water bath at 10°C until being transported to the laboratory. The fat samples were transferred to sterile stomacher bags, 100 mL of sterile saline was added and the contents were stomached for 1 min. Samples were then serially diluted and plated in duplicate on to TSYG agar. The plates were incubated overnight at 37°C, and colonies were counted.

Foil sachets

Sachets were placed in zone A, B or C (Figure 3) and treated under the same conditions as the broth sachets. After treatment, the aluminium foil was dried and weighed again; weight loss of foil due to erosion was recorded.

Statistical analysis

Means and standard deviations were calculated from $\log_{10}$ counts of *E. coli*. One-way analysis of variance and of means was performed using the Minitab statistical package.

RESULTS

Sonication efficacy in broth

Initial experiments investigated the effects of ultrasound on *E. coli* in sachets immersed in hot water (55-62.5°C) for periods up to 130 s. Sonication times were for periods up to 120 s.

The beneficial effect of immersing the sachets in water at temperatures higher than 55°C is very evident in the results presented in Table 1. At temperatures greater than 55°C, exposure of the *E. coli* culture to ultrasound had a dramatic effect over and above that of temperature alone. Based on these results, it was decided to focus further trials on microbial inactivation at 57.5°C and 60°C.

Table 1. Effect of temperature, duration of immersion, and sonication time on reductions in numbers of *E. coli* in broth sachets.

Water temperature (°C)	Time of immersion (s)	Time of sonication (s)	Depth (mm)	Position	Reduction in *E. coli** (cfu/mL)
55	120	0	150	B	0.2 ±0.04
55	130	120	150	B	1.1 ±0.4
57.5	120	0	150	B	0.6 ± 0.06
57.5	130	120	150	B	2.8 ± 1.0
60	60	0	150	B	0.6 ± 0.05
60	70	60	150	B	4.0 ± 1.1
62.5	60	0	150	B	2.1 ± 0.07
62.5	70	60	150	B	>6.8

* from initial inoculum of 7.4 $\log_{10}$ cfu/mL.

Sonication at 57.5°C or 60°C was assessed at 15 s intervals from 0 up to 120 seconds (up to 130 s immersion). Heat alone at these temperatures was also assessed at two time points - 45 and 90 s (Figure 4). Sonication in combination with heat had a very significant inactivation effect over and above heat alone. This is particularly so at 60°C where the effect is clearly evident after 30 seconds; the average reduction in *E. coli* was 2.82 $\log_{10}$ ±0.77 cfu/mL.

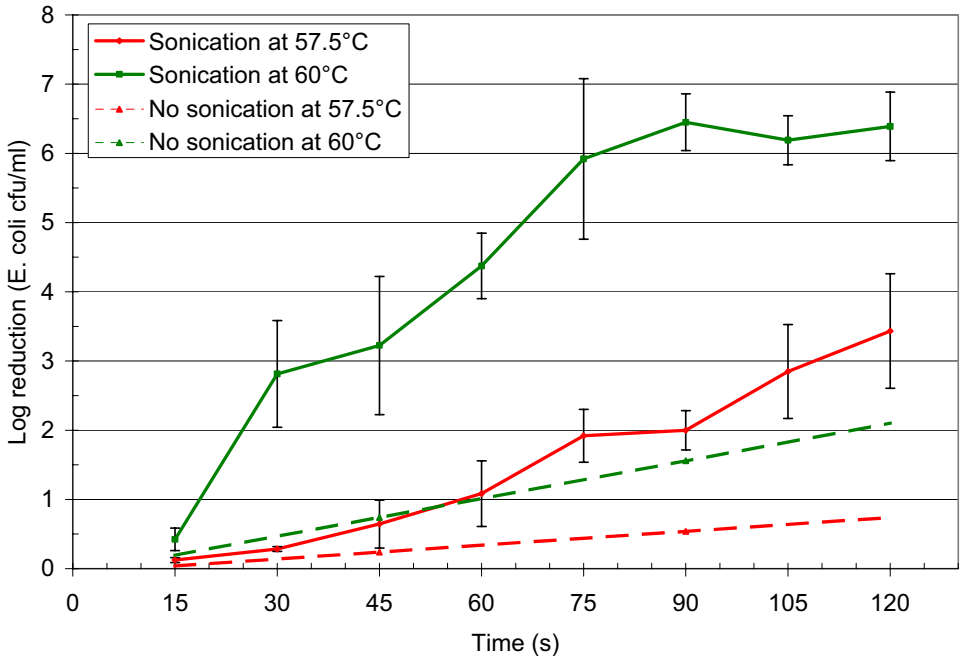


Figure 4. The effect of temperature and sonication time on the reduction in numbers of *E. coli* in broth sachets. Sachets were held in zone B in the X orientation and at a depth of 150 mm.

As well, some sachets were subjected to sonication at 10°C for 30 s, either before or after they were subjected to hot water (60°C) for 30 s. Neither of these sequential treatments resulted in any reduction in the numbers of *E. coli*.

In addition to plating treated sachets onto TSYG agar, some sachets were also serially diluted and plated in duplicate on to eosine methylene blue (EMB) agar and/or *E. coli* Petrifilm plates. The microbial reductions on EMB were sometimes greater than on TSYG, but generally they were similar.

Investigation of the effective sonication zone

Results of the trials to obtain evidence that ultrasound energy from the sonotrode was being reflected from the wall of the ellipsoidal vessel and focused in Zone B are summarised in Table 2.

Table 2. Effect of sonicating broth sachets and aluminium foil sachets at specified depths and positions in water at 60°C for 60 s.

Position	Depth (mm)	Orientation	Log reduction in *E. coli** (cfu/mL)		Aluminium foil weight reduction (mg)
A	150	X	1.20	2.05	0.03
A	150	Y	1.50	1.98	0.01
A	300	X	2.62	1.98	0.03
A	300	Y	1.70	2.00	0.03
A	450	X	1.78	1.32	0.03
A	450	Y	2.00	2.15	0.03
B	150	X	5.47	3.74	0.01
B	150	Y	2.71	0.13	0.01
B	300	X	4.65	4.08	0.01
B	300	Y	5.58	6.73	0.02
B	450	X	2.24	2.54	0.01
B	450	Y	3.51	4.89	0.01
C	150	X	1.43	1.24	0.02
C	150	Y	1.35	1.33	0.01
C	300	X	2.27	4.03	0.03
C	300	Y	1.95	2.28	0.02
C	450	X	1.60	6.25	0.02
C	450	Y	2.09	2.26	0.01

* from initial inoculum of 7.3 $\log_{10}$ cfu/mL.

The average *E. coli* reductions for zones A, B and C respectively, were 1.86 $\log_{10}$ ±0.39 cfu/mL, 3.86 $\log_{10}$ ±1.79 cfu/mL and 2.34 $\log_{10}$ ±1.44 cfu/mL. Overall, the $\log_{10}$ reductions of *E. coli* in zone B were significantly different ($p<0.05$) to those in zones A and C, although the effect of the ultrasound is clearly not limited to zone B. There was no significant difference ($p>0.05$) between sachets held at different depths, nor in different orientations. These microbiological results support the theory that sonication apparatus can be designed to focus the ultrasound energy into an active zone.

One data point in zone B (0.13 $\log_{10}$ cfu/mL) appeared to be an outlier. However; statistical tests (box plot and Grubb's test) indicated that though it is furthest from the other data points, it was not a significant outlier ($p>0.05$).

The weight losses from the aluminium foil due to erosion by ultrasound did not show any significant trends in any of the positions tested. There was no relationship between erosion of the foil and reduction in numbers of *E. coli*.

Sonication efficacy on fat surfaces

Sonication treatments at 60°C were repeated using small samples of fat in the sachets instead of broth. However, using fat, the average $\log_{10}$ reduction in *E. coli* on 12 samples was only 0.46 ±0.27 cfu/cm^2 after 30 s.

During some of the runs, the temperature of the fat surfaces in the sachets was logged using fast response sensors. A sensor was placed on either side of the tissue; one sensor on the 'fat' side and one sensor on the 'lean' side. The samples were vacuum-packed and sonicated in a 60°C water bath for 60 s. Figure 5 shows that at a water temperature of 60°C, the temperature of the fat surface within the sachet failed to rise above 50°C after 30 s.

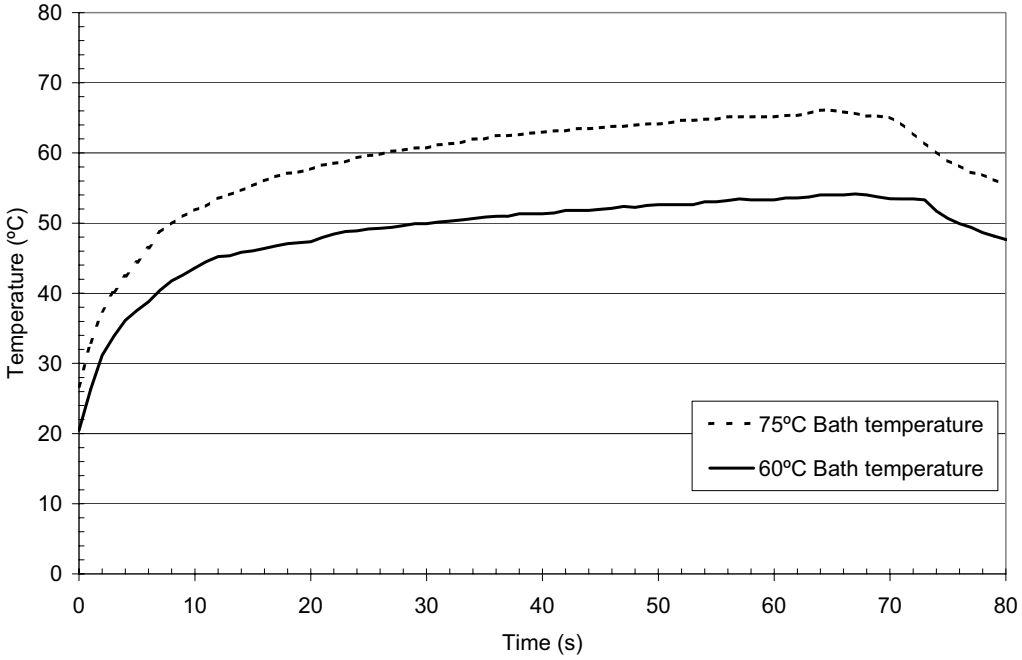


Figure 5. Surface temperatures of fat samples sonicated in a water bath at 60°C and 75°C respectively for 60s.

Further trials indicated that in order for the temperature at the fat surface to reach 60°C after 30 s, the sachets had to be held in water at 75°C.

Results of further tests with fat to which *E. coli* had been applied indicated that after 30 seconds sonication, reductions up to 2.6 $\log_{10}$ units in *E. coli* could be achieved at a water temperature of 75°C (Figure 6). After longer exposure, heat appeared to be the greater factor influencing microbial inactivation as indicated by the 'no sonication' line. The reductions were variable however.

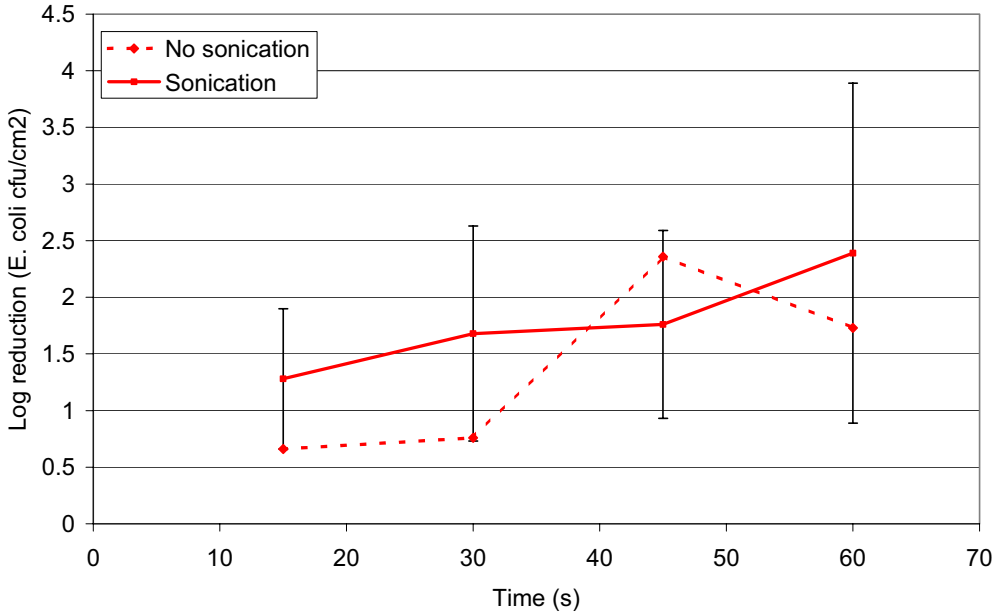


Figure 6. Log reduction over time of *E. coli* on fat surfaces, with or without ultrasound at 75°C (Zone B, X orientation, depth 150 mm).

In total, 28 fat sachets were treated using sonication for 15 or 30 s in water at 75°C (Figure 7). Reductions were variable, ranging from 0.5 to up to 3.0 $\log_{10}$ units. The average reduction after 15 s treatment was 1.19 $\log_{10}$ *E. coli* ± 0.6 cfu/ cm^2 and after 30 s it was 1.55 $\log_{10}$ *E. coli* ± 0.93 cfu/ cm^2

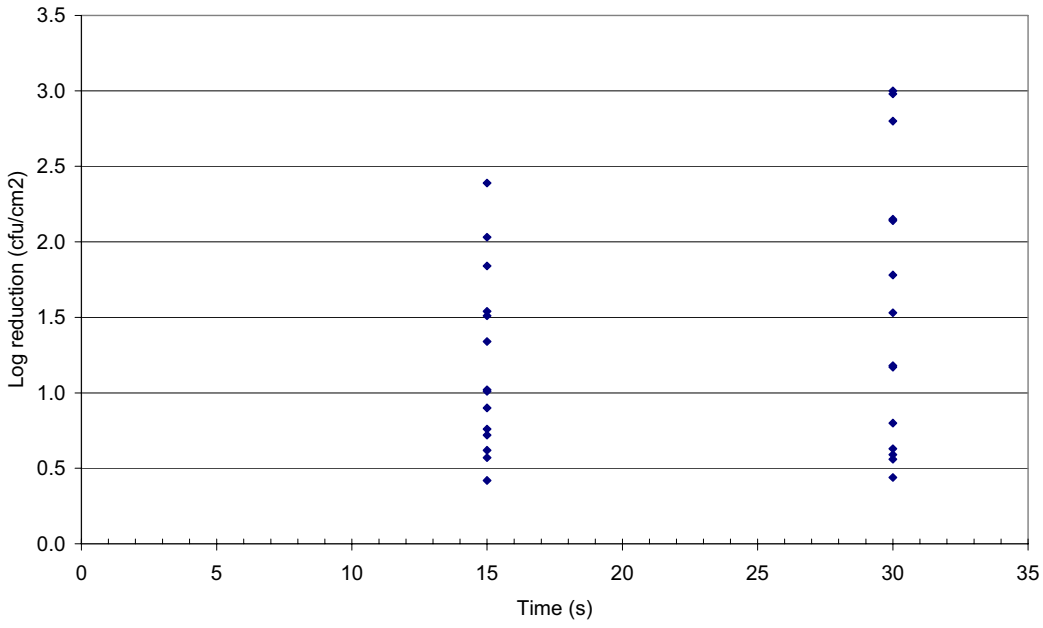


Figure 7. The reduction in numbers of *E. coli* on fat surfaces of 14 samples sonicated at 75°C for 15 or 30 s.

DISCUSSION

Results in this report indicate that microbial reductions of up to 3 $\log_{10}$ units of *E. coli* were achieved in broth treated at 60°C for 30 s. In fat, held in sachets in water at 75°C and sonicated for 15 to 30 seconds, microbial reductions of up to 3 logs were achieved, but not reliably.

A possible reason for the inconsistent reductions in fat sachets is because the surface of the fat did not consistently reach the temperature of that of the water bath. Temperature logs of the fat surface demonstrated that at 60°C and after 30 s, the fat surface was 10°C cooler than the water temperature. Another possible reason may be incomplete exhaustion of air from the sachets. Air pockets have been reported to reduce the effectiveness of sonication. They would also retard the transfer of heat to the fat surface. However in this investigation, sachets with visible air bubbles after sealing were discarded, so air is not a likely reason for the ineffectiveness. Another possible reason is that for some samples, overlying fat tissue may have protected a small quantity of the bacterial culture. In order to identify potential treatment parameters for a commercial application, a better understanding of the reasons for the inconsistent reductions is necessary.

The results indicated that in the small sachets used for this investigation, the orientation of the surface for treatment, and the depth of immersion did not influence the reductions in numbers of *E. coli* significantly. However, reductions were significantly greater if the sample was placed in zone B of the treatment vessel (Figure 3) – the region where energy from the sonotrode was intentionally focused. This is an important finding because it provides a basis for the design of a system that can be scaled-up sufficiently to treat vacuum-packed primals.

The current study has demonstrated that the lethal effect on microorganisms is a synergistic effect and was not due to heating alone or sonication at lower temperature. In addition, our results suggest that sequential treatments - sonication in water at boning room temperature (10°C) either prior to, or following, hot water treatment - are also not effective. Therefore, it appears that an effective system must employ heat and sonication simultaneously.

Vacuum packs of meat are routinely heat-shrunk by immersion in, or spray treated with very hot water at 85-90°C. Our limited measurements suggest that the temperature at the surface of meat within a vacuum pack will briefly reach 60-70°C from heat shrinking. It appears that there is an opportunity to incorporate the ultrasound treatment into heat-shrink systems. Sonication time and temperature conditions of 15-30 s at 75°C were shown to be effective, albeit variably so. Higher temperatures (>75°C) for shorter times (5-8 seconds) as used for heat shrinking may also be effective. In order to investigate these conditions and verify that useful reductions in numbers of *E. coli* can be obtained, certain modifications would need to be made to the current system configuration to overcome the current OHS&E concerns.

Initial work using *E. coli* cells grown in buffered peptone water (BPW), suggested that these cells were more susceptible to the heat inactivation treatments than those grown in TSB (Appendix, Table A). For trials done subsequently, the *E. coli* was

grown in TSB. However, because the physiological state of cells may influence their susceptibility, further investigations should be conducted to allay the possibility that different growth media and conditions in abattoirs and boning rooms, are more likely than TSB to encourage the *E. coli* to 'switch on' mechanisms to make them more resistant to temperature and sonication conditions. In addition, there may also be differences in physiological influence on cells that are in different phases of growth, eg. differences between lag, log or stationary phase cells. Most microorganisms that contaminate a carcass will be unable to grow for a time (that is, they will enter lag phase), particularly under the conditions of good manufacturing practice.

In some trials, reductions of *E. coli* were also counted on EMB agar or *E. coli* Petrifilm plates (data shown in Appendix, Table B and C). EMB and Petrifilm are more inhibitory, selective media than TSYG, which is a non-selective and non-inhibitory medium. Although the reductions were sometimes greater than on TSYG, they were generally similar. This suggests that in general the damage done to cells exposed to the treatment was such that they were unable to recover.

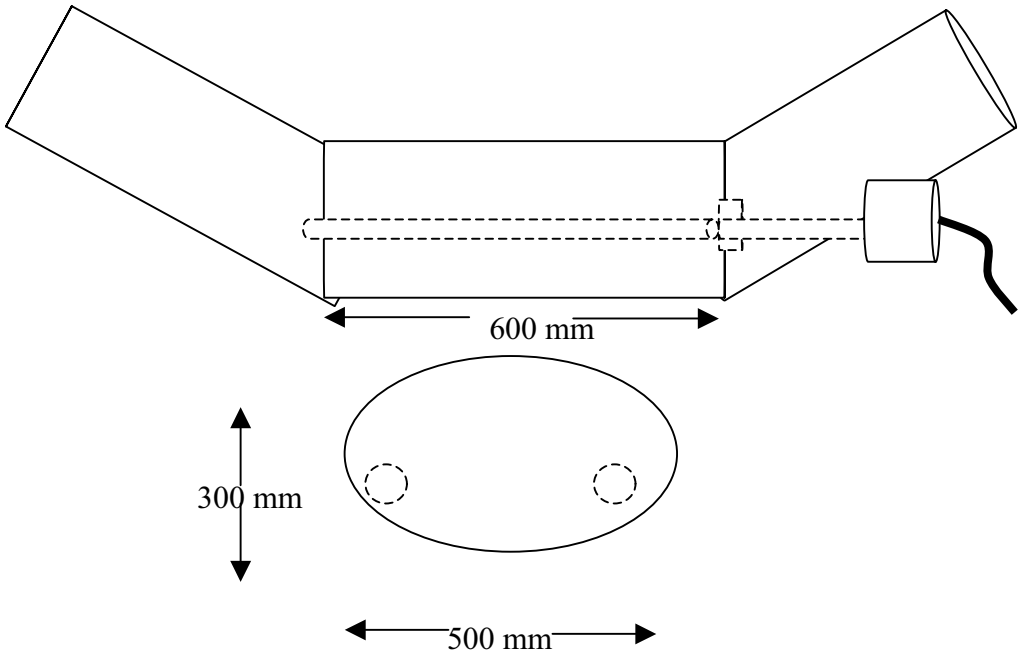
CONCLUSIONS AND RECOMMENDATIONS

This study has demonstrated that high power ultrasound is a processing technology that should be considered as a bacterial intervention for intact vacuum of fresh and processed primals and sub-primals. It is likely that only the surface of the primal will be contaminated and need to be treated, in which case ultrasound will be effective if applied appropriately.

In terms of bacterial inactivation, it appears that heat and ultrasound have a synergistic effect. Vacuum-packaging systems in most boning rooms already incorporate a hot water, heat-shrinking procedure. Whether this process can be successfully scaled up to be economically viable is a question that industry needs to consider. The ellipsoidal design of the vessel used in this investigation appears to focus the energy into a target zone as intended, however; areas outside this zone also showed good microbial reductions in broth sachets. Only one sonotrode was used. A modified design that includes two sonotrodes has the potential to provide a treatment zone that is large enough to effectively treat vacuum-packaged full primals.

Minor modifications to the current unit shown in Figure 2 will make it suitable for obtaining further information on the behaviour of the system. However its small size will limit the mass of test vacuum packs of meat that can be treated. Packs much larger than 100 to 200 g may affect the ability of the unit to reflect energy and focus it into an active zone.

In order to be able to investigate the characteristics of a system where packs pass through a zone of elevated energy intensity between two sonotrodes (the concept originally proposed to Valley Beef and MLA by Innovative Ultrasonics), a larger unit should be designed and fabricated. This larger unit should be based on a modified ellipsoidal design that incorporates the two sonotrodes that are currently available. Unlike the current vertical unit, it should be horizontal in order to demonstrate – in principle at least – that vacuum packs can be conveyed through water between two sonotrodes and receive treatment that is uniform over the entire surface. A possible configuration is shown below.


600 mm
300 mm
500 mm

APPENDIX

Table A. Broth cultures of *E. coli* grown in tryptone soya broth (TSB) or buffered peptone water (BPW) and treated at 55°C without sonication for 2 or 5 min.

Broth type	Time in 55°C water bath (min)	Trial 1 (*E. coli* cfu/mL)		Trial 2 (*E. coli* cfu/mL)	
BPW	2	0.46	0.55	0.99	0.92
BPW	5	1.16	1.09	1.39	1.23
TSB	2	0.13	0.12	0.1	0.24
TSB	5	0.49	0.57	0.43	0.38

Table B. Log reduction of *E. coli* in broth sachets treated at various temperatures and EMB.

Agar	Treatment	Log reduction in *E. coli* (cfu/mL)	
		Trial 1	Trial 2
TYSG	Control 55°C	0.27	0.2
TYSG	Control 55°C	0.27	0.2
EMB	Control 55°C	0.35	0.4
EMB	Control 55°C	0.32	0.3
TYSG	55°C	1.09	1.3
TYSG	55°C	1.14	0.9
EMB	55°C	1.46	2.3
EMB	55°C	-0.25	1.6
TYSG	Control 57.5°C	0.65	0.6
TYSG	Control 57.5°C	0.51	0.6
EMB	Control 57.5°C	0.96	1.0
EMB	Control 57.5°C	0.88	0.9
TYSG	57.5°C	1.96	2.1
TYSG	57.5°C	2.92	4.1
EMB	57.5°C	3.4	3.2
EMB	57.5°C	4.78	6.5
TYSG	Control 60°C	0.57	0.6
TYSG	Control 60°C	0.62	0.7
EMB	Control 60°C	0.89	0.9
EMB	Control 60°C	0.72	1.2
TYSG	60°C	2.53	4.0
TYSG	60°C	5.09	4.5
EMB	60°C	4.33	5.2
EMB	60°C	6.63	5.6
TYSG	Control 62.5°C	2.1	2.0
TYSG	Control 62.5°C	2.12	2.1
EMB	Control 62.5°C	3.38	3.5
EMB	Control 62.5°C	3.27	3.2
TYSG	62.5°C	6.63	6.8
TYSG	62.5°C	6.63	6.8
EMB	62.5°C	6.63	6.8
EMB	62.5°C	6.63	6.8

Table C. Log reduction of *E. coli* in fat sachets treated at 60°C for 60 s.

Sonic time (sec)	Heat (sec)	*E. coli* reduction on TYSG agar (Log_{10} cfu/mL)		*E. coli* reduction on Petrifilm (Log_{10} cfu/mL)	
		Trial 1	Trial 2	Trial 1	Trial 2
0	70	0.10	0.66	0.04	-0.09
60	60	0.63	1.25	0.99	1.61
60	60	0.17	0.54	0.55	1.17
60	60	0.35	0.86	0.56	1.07
60	60	0.10	0.81	0.87	1.43
60	60	0.26	0.56	0.34	0.79
60	60	0.25	0.49	0.50	1.00
0	70	0.34	0.59	0.01	0.04

Ultrasonics for in-pack pasteurisation

- **Aims - FSA**
  - **Review scientific literature and databases**
  - **Acquire suitable pilot equipment for trials**
  - **Conduct microbiological studies on vacuum packs**
  - **NEW Define operating parameters for consistent intervention process**

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Ultrasonics for in-pack pasteurisation

- **Outputs**
  - **Validation trials with test bacteria**
  - **Information on system configuration to be used to configure industrial unit**
  - **NEW Information to optimise pilot scale system**

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Ultrasonics for in-pack pasteurisation

- **Gravimetric – erosion of alfoil**
  - **Energy in the treatment vessel increased with power output.**
  - **As the exposure time of alfoil to the ultrasound energy increases, wt loss increases.**
  - **At 10°C, approx 10% energy lost through vacuum-pack film.**
  - **Energy greater at 5 or 10°C than when warmer.**
  - **Energy declines as radial distance from sonotrode increases.**
  - **At a given radial distance from sonotrode, energy varied with depth in a way that couldn't be explained.**
  - **Were other inconsistencies that needed to be explored further.**

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Ultrasonics for in-pack pasteurisation

- **Microbiological**
  - **Vacuum packs** of meat spiked with *E. coli* and *Salmonella* subjected to 4 kW for one or 3 min.

    Only small decreases in numbers of the test bacteria.
  - **Broth culture** of *E. coli* was sealed in small pouches and treated at 4 kW for one or three minutes.

    After treatment for one min only small reductions in *E. coli*.

    After three min, reductions of around 6 log units in *E. coli* in a few but not all pouches.

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Ultrasonics for in-pack pasteurisation

- **Erosion of alfoil**
  - **Response surface mapping approach to gain improved understanding of energy distribution in vessel**

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What's been done so far…

- Mapped the 'power' intensity of the round tank

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Response Surface I

Factor	Treatment	
	Low	High
Power (W)	4000	6000
Depth (mm)	150	300
Distance (mm)	50	150
Time (seconds)	60	180

Centre Points for each factor were 5000 W, 225 mm, 100 mm and 120 seconds, respectively.

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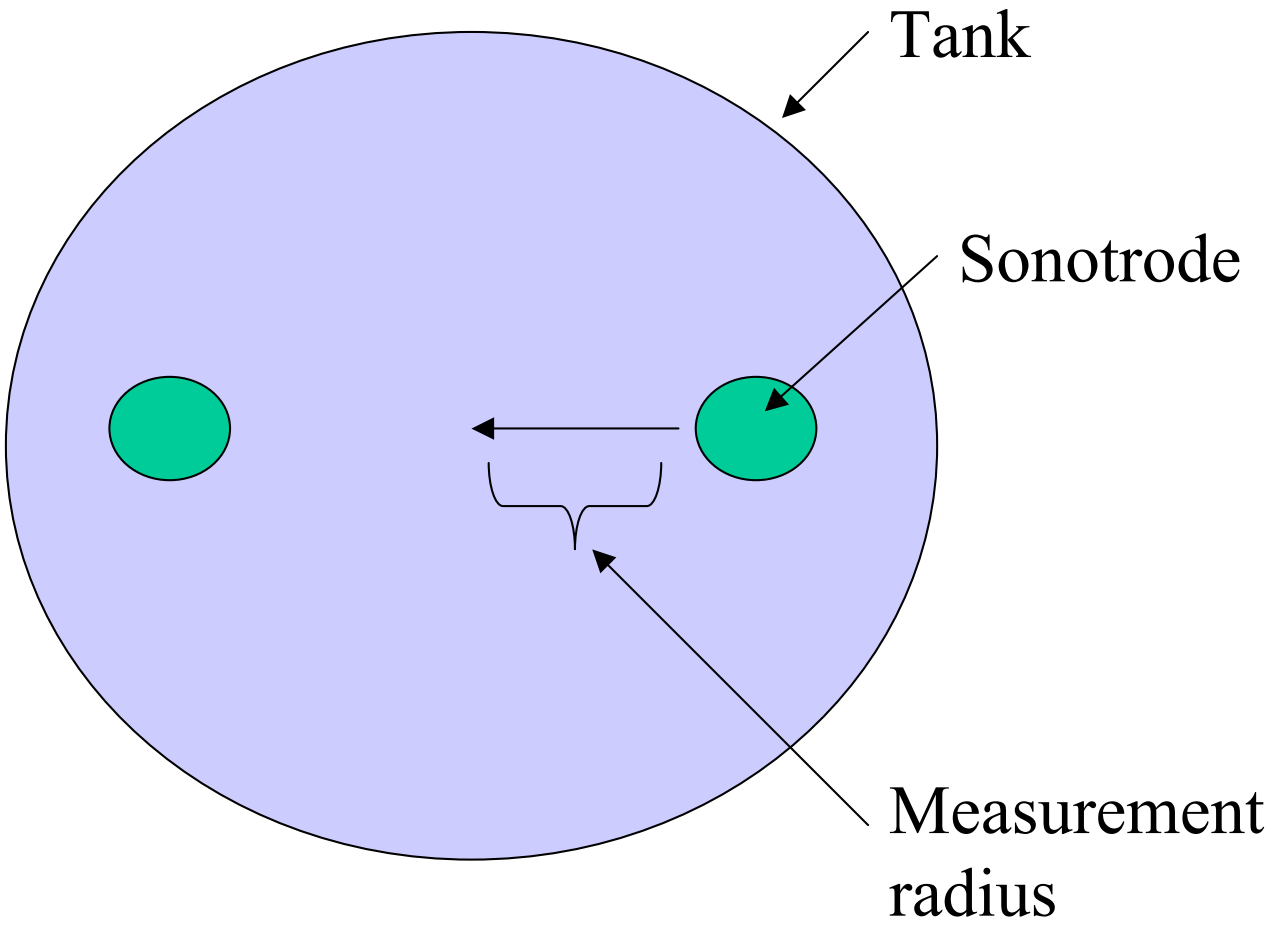
Response surface I continued



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Results I

Foil erosion = 22.33 – 0.0233(power) +
1.455(depth) – 2.696(radius) + 2.83(time)

Tells us that:

1. power had little effect
2. as depth increased, erosion increased
3. as the radius increased, erosion decreased
4. foil erosion increased dramatically with time.

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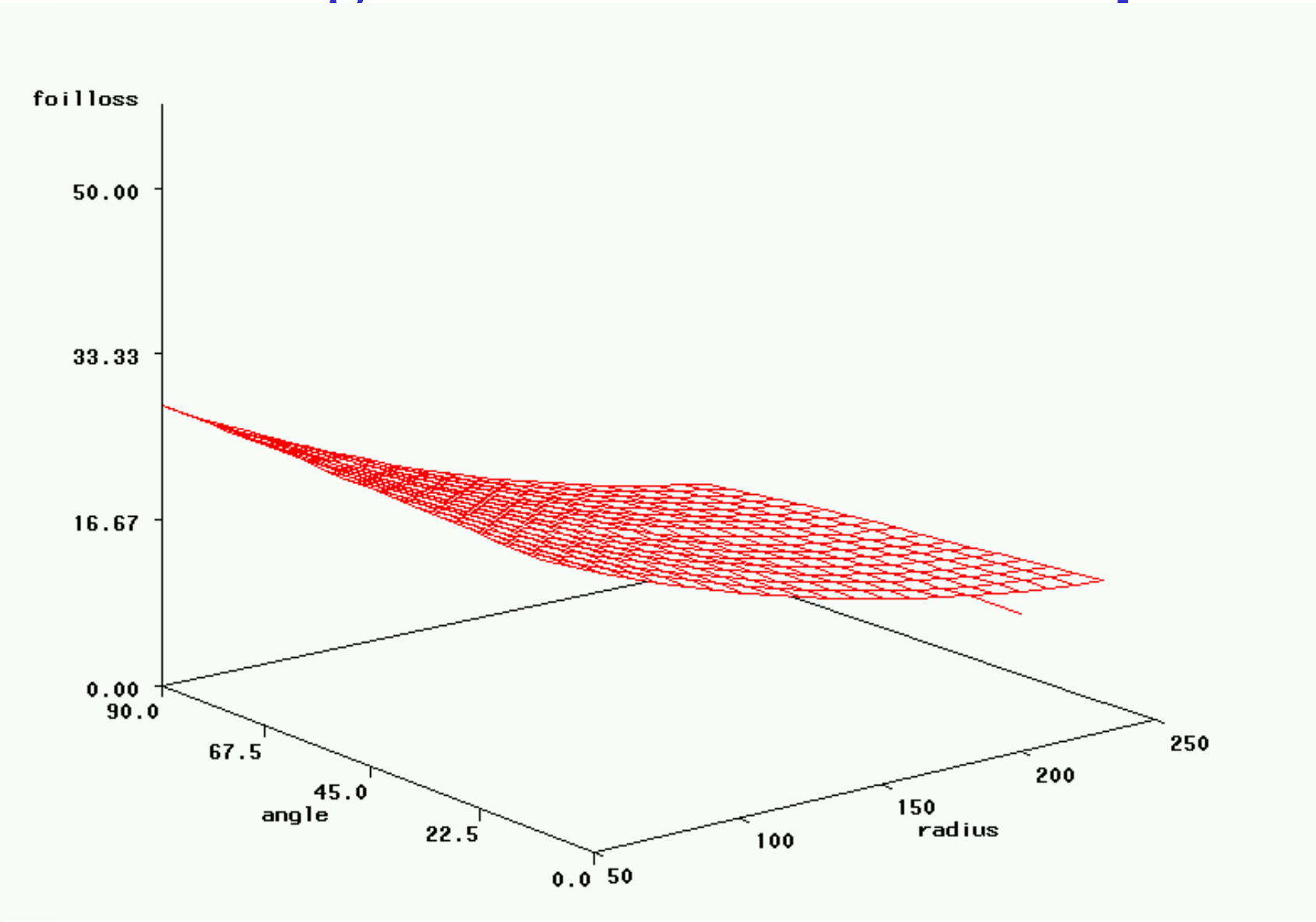
Modelling erosion at 100 mm deep



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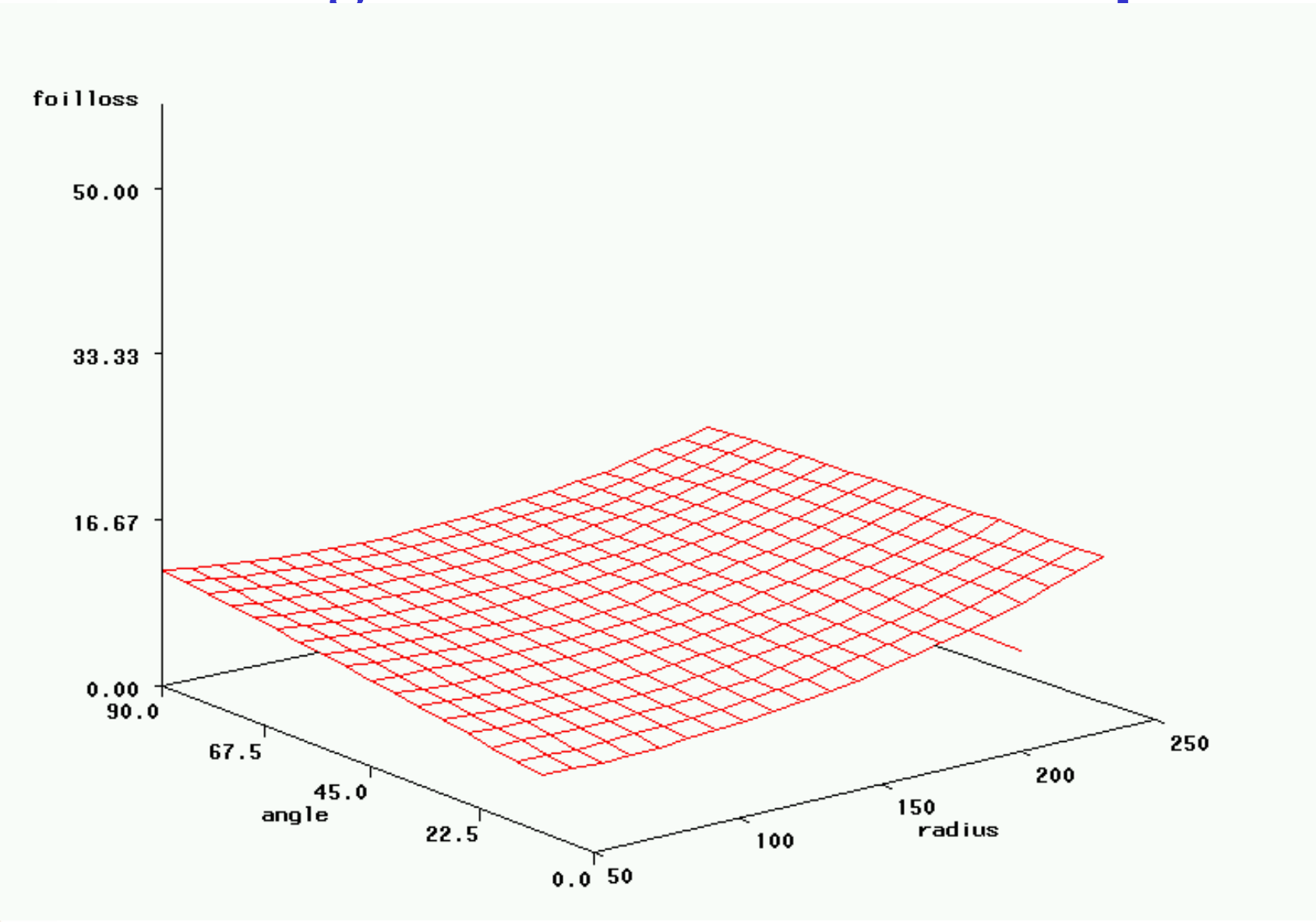
Modelling erosion at 400 mm deep



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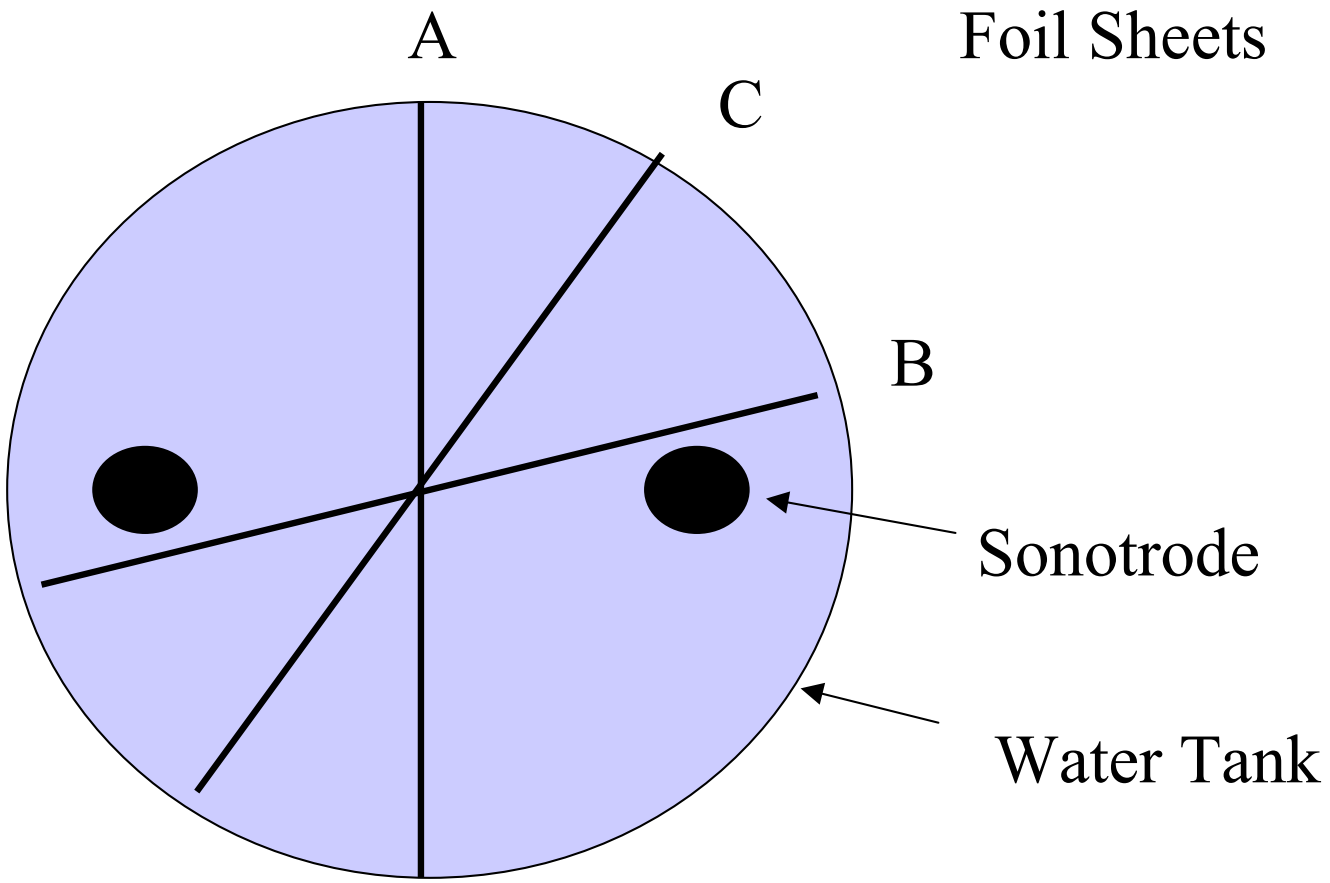
Experimentation with foil sheets



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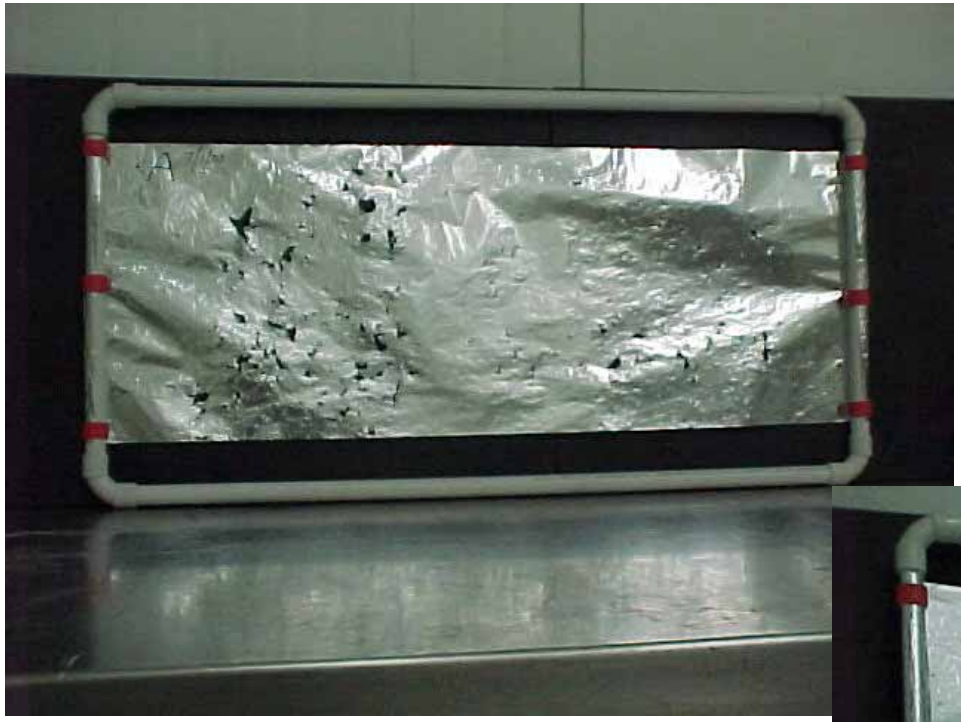
Sheet A



Top half ←

Bottom half →

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Sheet B

Top half

Bottom half

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Sheet C

Top half

Bottom half

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Sheet A – half diameter, top to bottom

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Ultrasonics for in-pack pasteurisation

- **Microbiological**
  - **Broth culture** of *E. coli* in small pouches treated at 8 kW for one or three minutes**.**

    Negligible decreases in numbers
  - **Relevance of alfoil approach to energy mapping?**

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