MEAT & LIVESTOCK AUSTRALIA

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Microwave surface inactivation of microorganisms : Preliminary trials on red meat

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Abstract

Inactivating (or killing) contaminating microorganisms on the surface of meat is a problem that is not adequately solved through taking care not to contaminate the carcase during hide removal and dressing, by washing, application of hot water, steam or various chemicals. All of these potential solutions leave bacteria on the surface of the carcase, and make the carcase unsuitable for certain uses or markets. A microwave technology (gyrotron) that is capable of heating the surface of the meat to a very high temperature in a very short time without causing a change in the appearance of the carcase may be a technology that will be successful in decontaminating the carcase or other piece of meat. A trial of the technology was conducted using a laboratory-scale gyrotron and meat pieces inoculated with *Escherichia coli* and *Enterococcus faecium*. In the case of both organisms, on both fat and lean surfaces, suspended in broth or in the presence of feces, greater than a 5 $\log_{10}$ reduction in bacterial count was observed. Changes in the appearance of meat surface was minimal, if at all noticeable. This technology is of great potential benefit to the industry for decontamination of meat.

Executive summary

Inactivating (or killing) contaminating microorganisms on the surface of meat is a problem that is not adequately solved through taking care not to contaminate the carcase during hide removal and dressing, by washing, application of hot water, steam or various chemicals. All of these potential solutions leave bacteria on the surface of the carcase, and make the carcase unsuitable for certain uses or markets. A microwave technology (gyrotron) that is capable of heating the surface of the meat to a very high temperature in a very short time without causing a change in the appearance of the carcase may be a technology that will be successful in decontaminating the carcase or other piece of meat. A trial of the technology was conducted using a laboratory-scale gyrotron and meat pieces inoculated with *Escherichia coli* and *Enterococcus faecium*. In the case of both organisms, on both fat and lean surfaces, suspended in broth or in the presence of feces, greater than a 5 $\log_{10}$ reduction in bacterial count was observed. Changes in the appearance of meat surface was minimal, if at all noticeable. This technology is of great potential benefit to the industry for decontamination of meat.

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1 Background

Microwaves with appropriate characteristics are capable of heating a surface rather than heating deep within a mass. Heat can be generated at the surface very rapidly and so there is a possibility that microorganisms can be deactivated without having a significant effect on meat characteristics.

Some earlier work, performed through an MLA Donor Company project (P.PSH.0624 - Microwave E-coli eradication process intervention – Stage 1), and by Gyrotron Technologies Inc. (GTI) have suggested that bacterial deactivation can occur without affecting meat colour, but trials have not been in a well-controlled gyrotron, with careful microbiological validation or instrumental measurement of meat colour.

2 Project objectives

Determine a level of microwave treatment that will inactivate meatborne pathogens without affecting meat colour.

3 Methodology

3.1 Overview

Meat pieces, both fat and lean, were inoculated with two species of bacteria at high levels in broth and also a faecal slurry, and subjected to microwave at various doses. The reduction in numbers of bacteria and the change in meat colour were measured to determine a level of microwave treatment that would result in significant reduction in bacteria without affecting meat colour.

3.2 Microwave treatment

3.2.1 Gyrotron

A Gyrotron is a high powered vacuum tube which generates millimeter-wave electromagnetic waves by bunching electrons with cyclotron motion in a strong magnetic field. Output frequencies range from about 20 to 250 GHz, covering wavelengths from microwave to the edge of the terahertz gap. Typical output powers range from tens of kilowatts to 1-2 megawatts.

The Gyrotron Beam is a new, efficient source of energy. Its high frequency and high energy concentration combined with the microwave nature of this novel product results in an energy source, different from any other. Unique properties include:

- ultra-rapid volumetric heating of non-metallic materials with heating rate of hundreds and thousands of degrees in one second
- selective heating of target regions inside an exposed material
- volumetric heating of small and large objects uniformly through varying thicknesses
- Ability to take any form, including: circular with Ø from 3mm (0.12”), strip with length up to 2m (6’), square and ellipse up to 60 sq ft. The beam can also be split to support two production lines or heat two sides of a product being processed simultaneously
- Ability to provide unique heating uniformity – up to 1%

3.2.2 Treatments

The microwave treatment process requires a layer of water on the surface of the meat. This may be applied along with the inoculum, but may be better applied with an additional spray of gelatin solution (3% gelatin).

Four treatments were applied:

- A high power short time treatment (T1)
- A high power, short time treatment, with an additional overlay of gelatin solution (T2)
- A low power, long time treatment (T3)
- A low power, long time treatment, with an additional overlay of gelatin solution (T4)

3.3 Microbiological validation

Samples of beef carcass pieces (fat and lean) will be surface inoculated with cultures of generic *E. coli* and *Enterococcus faecium* (as surrogates for pathogenic *E. coli* and *Salmonella* spp.). Post-inoculation, samples will be exposed to varying antibacterial treatments. The amount of each challenge organism remaining on the surface of the samples after each treatment regimen were compared to the amount present on a set of untreated controls to determine the effect of the process on each challenge organism.

3.3.1 Challenge organisms

The following challenge organisms will be prepared for this study as surrogates for pathogenic *E. coli* and *Salmonella* spp.:

- *Enterococcus faecium* (ATCC #8459)
- *Escherichia coli* (ATCC #BAA-1427)

The cultures will be prepared from a fresh lyophilized preparation (KWIK-STIK™, Microbiologics, St. Cloud, MN) according to manufacturer's instructions. The cultures will be transferred onto Tryptic Soy Broth (TSB, Neogen, Lansing, MI) and incubated 18-24 hours at 35 ± 2°C. These stocks will then be plated onto Tryptic Soy Agar (TSA, Neogen) at appropriate dilutions to determine the actual final concentration (targeted at 7.0-8.0 log10 cfu/mL).

3.3.2 Sample matrices and preliminary treatment

Beef samples will be collected from a processing facility from either Thin Flank Meat or Navel End Brisket. Pieces will include approximately 20mm x 40mm of external surface and be approximately 10mm thick. Pieces will include surface samples containing both fat and naturally lean tissue (approximately half of each). Samples will be maintained refrigerated, as close to 0°C as practical. Samples will be placed on a sanitized stainless steel tray and exposed to varying levels of each portion of the antibacterial treatment.

3.3.3 Inoculation of matrix

Beef samples will be inoculated with both surrogate cultures both as a broth preparation and in the context of a fecal slurry preparation. Cattle fecal material will be collected from a meat processing facility and formed into a slurry. The material will be strained through a cheesecloth filter to remove

the larger particles. The remaining liquid material will be combined 1:1 with a cocktail of the stock cultures.
Both this combination and the broth preparations will be used to surface inoculate beef samples. A 0.1mL volume of a preparation will be spread over the surface of the upper surface sample, avoiding the edges of the sample to prevent inoculum from dripping over the sides. An additional set of 8 pieces of each type of product (16 total) will remain uninoculated.

3.3.4 Microbiological testing

Product samples will be mixed with a volume of sterile Butterfield's Phosphate Buffer (prepared according to FDA-BAM guidelines) at a 1:10 dilution to the product. Samples will be rinsed by hand (shaken 25 times in a 30cm arc over 7 seconds) before plating at appropriate dilutions.

Samples will be plated onto MacConkey Agar (MAC, Neogen) as well as KF Streptococcus Agar (KFS, Neogen) to recover the *E. coli* and *E. faecium* cultures. MAC and KFS plates will be incubated at 35 ± 2°C for 24-48 hours.
After incubation, samples will be enumerated by hand using a Quebec colony counter (Model #3325, Reichert Technologies, Depew, NY). Uninoculated control samples will be also be evaluated for the background presence of each organism.

3.3.5 Data processing

Bacterial counts were converted to $\log_{10}$ before calculating average counts or subtracting one count from another.

3.4 Meat Colour

3.4.1 Equipment

Minolta Color Meter reading results according to the Hunter L*a*b* scale

3.4.2 Samples

Meat samples were maintained under refrigeration and were transported in a cooler with ice bricks until analysis. Meat samples (lean surface) treated by microwave treatment following spraying with the gelatin solution were compared to control (untreated in any way) samples.

Six samples of control, and 3 samples of meat from each treatment were tested. Three sites on each meat piece was tested

3.4.3 Sub heading

Measurements were made and differences to controls were calculated:
ΔL* (L* sample minus L* standard) = difference in lightness and darkness (+ = lighter, - = darker)
Δa* (a* sample minus a* standard) = difference in red and green (+ = redder, - = greener)
Δb* (b* sample minus b* standard) = difference in yellow and blue (+ = yellower, - = bluer)

4 Results

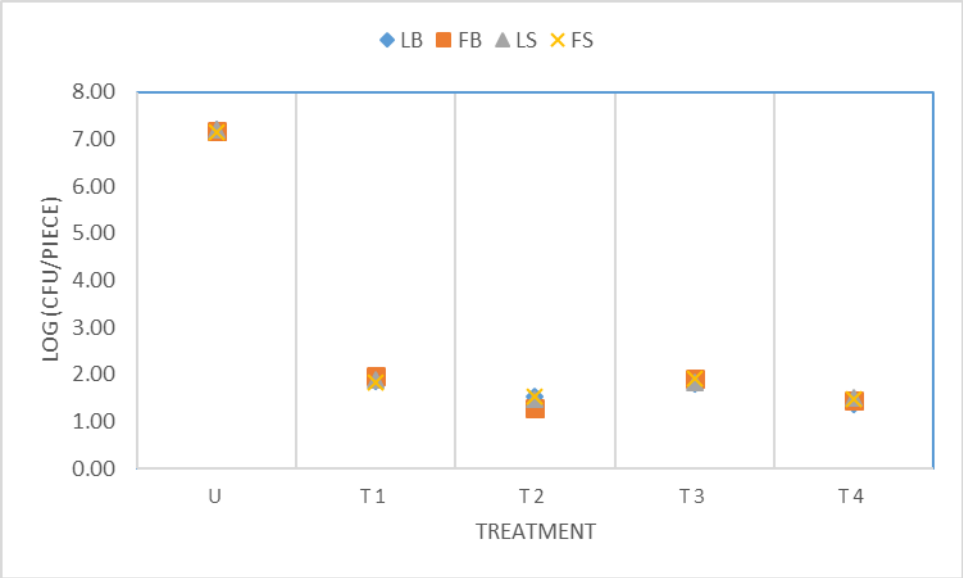
4.1 Microbiology

4.1.1 E. coli

E. coli was applied to both lean (L) and fat (F) surfaces of meat, suspended in broth (B) or a fecal slurry (S). The 0.1ml inoculum contained over 10^7 cfu (7 $\log_{10}$).

The inoculum was consistent across all pieces, and the reduction in counts were highly uniform, so only the mean results are presented in the figure 1 (all results in Appendi 8.1)

Figure 1: Effect of microwave treatments on *E. coli*



Treatments: Untreated, and treatments T1-T4 as defined in method
Inoculum and meat surface defined above

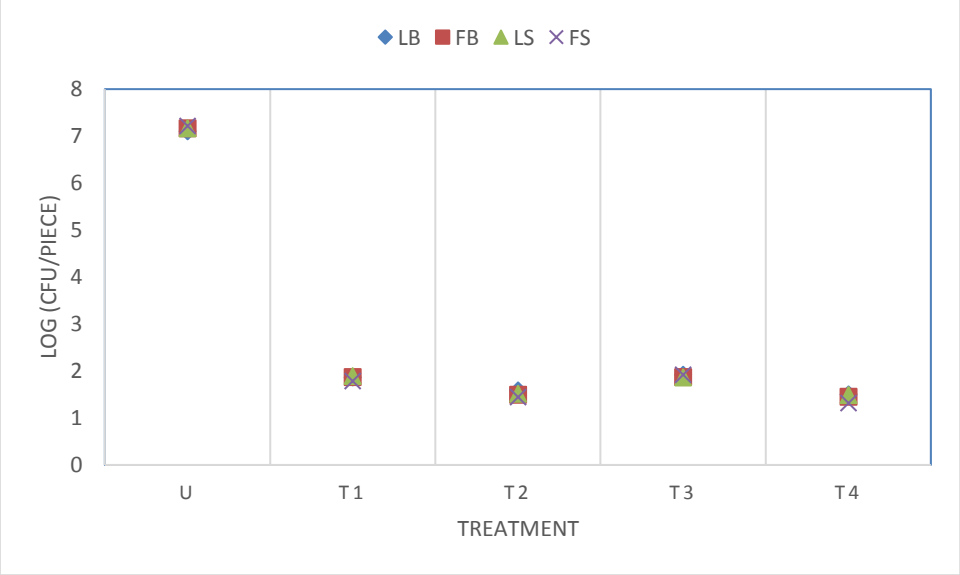
On average, the simple treatments resulted in a 5.3 log reduction in *E. coli*, and those with the sprayed overlay resulted in a 5.73 log reduction.

4.1.2 E. faecium

E. faecium was applied to both lean (L) and fat (F) surfaces of meat, suspended in broth (B) or a fecal slurry (S). The 0.1ml inoculum contained over 10^7 cfu (7 $\log_{10}$).

The inoculum was consistent across all pieces, and the reduction in counts were highly uniform, so only the mean results are presented in figure 2 (all results in Appendix 8.1)

Figure 2: Effect of microwave treatments on *E. faecium*



Treatments: Untreated, and treatments T1-T4 as defined in method
Inoculum and meat surface defined above

On average, the simple treatments resulted in a 5.36 log reduction in *E. coli*, and those with the sprayed overlay resulted in a 5.83 log reduction.

4.2 Colour

In general, the differences in colour between pieces of meat within each treatment group was greater than the difference in colour between treatment group. This feature could have been controlled by better matching of appearance of meat pieces prior to treatment.

A visual assessment of the pieces (appendix 8.2) suggest that there is no significant difference in the appearance of treated and untreated pieces. Instrumental analysis was inconclusive (Table 1)

Table 1: Colour measurements of meat – treated pieces and controls

Treatment	measurement	L*	a*	b*
untreated	mean	36.0	17.6	7.3
	range	4.6	6.2	2.7
High power – short time	mean	41.4	15.0	5.8
	range	14.7	6.1	5.0
	treated-untreated	5.4	-2.6	-1.5
Low power – long time	mean	36.9	16.4	6.4
	range	1.6	1.8	0.7
	treated-untreated	0.9	-1.2	-1.9

5 Discussion

5.1 Points for future attention

5.1.1 Experimental design

The bacterial inoculum in fecal slurry was not particularly thick with feces. The effect of having bacteria within materials such as feces or ingesta have not been fully assessed.

The meat pieces were not of uniform color, so that the instrumental analysis could not reliably detect any differences that may have been due to the microwave treatment.

5.1.2 Microwave technology

Reducing the power of the treatment, and modifying the requirement for the liquid overlay would provide options for optimising the process.

6 Conclusions/recommendations

The gyrotron treatment, as applied, was capable of delivering bacterial inactivation in excess of 5 logs without having an appreciable impact on lean meat appearance.

7 References

8 Appendix

8.1 Microbiological results

8.1.1 Lean meat inoculated with broth

	E. coli								E. faecium					
replicate	uninoc	untreated	T1	T2	T3	T4	replicate	uninoc	untreated	T1	T2	T3	T4
1	50	13,900,000	80	20	60	40	1	10	11,600,000	80	50	60	30
2	40	16,700,000	60	50	50	20	2	10	15,600,000	50	40	100	30
3	50	16,800,000	90	50	90	20	3	<10	10,700,000	100	50	100	40
4		15,800,000	60	40	70	10	4		13,300,000	60	30	100	30
5		17,600,000	80	20	60	40	5		14,000,000	90	30	70	30
n	3	5	5	5	5	5	n	2	5	5	5	5	5
log 1	1.70	7.14	1.90	1.30	1.78	1.60	log 1	1.00	7.06	1.90	1.70	1.78	1.48
log 2	1.60	7.22	1.78	1.70	1.70	1.30	log 2	1.00	7.19	1.70	1.60	2.00	1.48
log 3	1.70	7.23	1.95	1.70	1.95	1.30	log 3	n/a	7.03	2.00	1.70	2.00	1.60
log 4		7.20	1.78	1.60	1.85	1.00	log 4		7.12	1.78	1.48	2.00	1.48
log 5		7.25	1.90	1.30	1.78	1.60	log 5		7.15	1.95	1.48	1.85	1.48
min	1.60	7.14	1.78	1.30	1.70	1.00	min	1.00	7.03	1.70	1.48	1.78	1.48
mean	1.67	7.21	1.86	1.52	1.81	1.36	mean	1.00	7.11	1.87	1.59	1.92	1.50
max	1.70	7.25	1.95	1.70	1.95	1.60	max	1.00	7.19	2.00	1.70	2.00	1.60
Reduction			5.34	5.69	5.40	5.85	Reduction			5.24	5.52	5.19	5.61

8.1.2 Fat meat inoculated with broth

	E. coli								E. faecium					
replicate	uninoc	untreated	T1	T2	T3	T4	replicate	uninoc	untreated	T1	T2	T3	T4
1	20	17,600,000	70	10	70	10	1	10	19,700,000	50	30	70	10
2	70	20,000,000	100	30	80	40	2	10	15,300,000	90	50	70	40
3	10	11,300,000	100	30	90	30	3	10	12,900,000	80	10	100	30
4		15,200,000	90	30	70	50	4		10,400,000	80	40	70	40
5		12,200,000	100	10	90	30	5		17,400,000	80	50	70	40
n	3	5	5	5	5	5	n	3	5	5	5	5	5
log 1	1.30	7.25	1.85	1.00	1.85	1.00	log 1	1.00	7.29	1.70	1.48	1.85	1.00
log 2	1.85	7.30	2.00	1.48	1.90	1.60	log 2	1.00	7.18	1.95	1.70	1.85	1.60
log 3	1.00	7.05	2.00	1.48	1.95	1.48	log 3	1.00	7.11	1.90	1.00	2.00	1.48
log 4		7.18	1.95	1.48	1.85	1.70	log 4		7.02	1.90	1.60	1.85	1.60
log 5		7.09	2.00	1.00	1.95	1.48	log 5		7.24	1.90	1.70	1.85	1.60
min	1.00	7.05	1.85	1.00	1.85	1.00	min	1.00	7.02	1.70	1.00	1.85	1.00
mean	1.38	7.17	1.96	1.29	1.90	1.45	mean	1.00	7.17	1.87	1.50	1.88	1.46
max	1.85	7.30	2.00	1.48	1.95	1.70	max	1.00	7.29	1.95	1.70	2.00	1.60
Reduction			5.21	5.89	5.27	5.72	Reduction			5.30	5.67	5.29	5.71

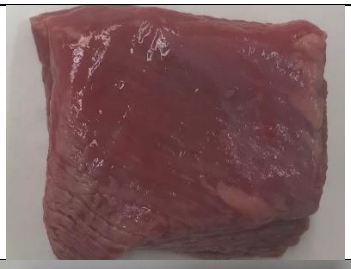
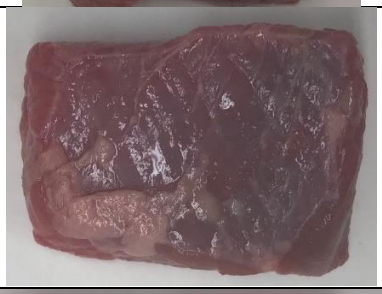
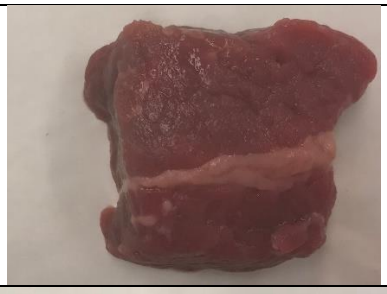
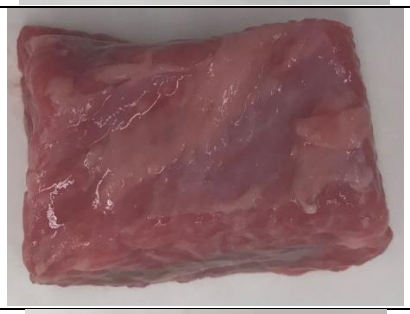
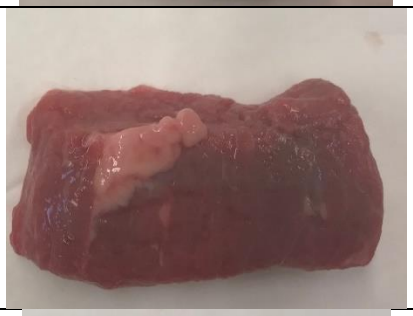
8.1.3 Lean meat inoculated with fecal slurry

	E. coli								E. faecium					
replicate	uninoc	untreated	T1	T2	T3	T4		replicate	uninoc	untreated	T1	T2	T3	T4
1	20	13,000,000	50	50	90	30		1	<10	12,100,000	90	50	100	30
2	50	15,100,000	90	20	80	20		2	<10	17,100,000	80	50	60	30
3	10	19,400,000	90	50	60	40		3	10	17,600,000	80	20	100	20
4		16,300,000	80	10	60	50		4		15,400,000	100	40	60	40
5		16,000,000	80	50	60	30		5		13,300,000	50	20	60	40
n	3	5	5	5	5	5		n	1	5	5	5	5	5
log 1	1.30	7.11	1.70	1.70	1.95	1.48		log 1	n/a	7.08	1.95	1.70	2.00	1.48
log 2	1.70	7.18	1.95	1.30	1.90	1.30		log 2	n/a	7.23	1.90	1.70	1.78	1.48
log 3	1.00	7.29	1.95	1.70	1.78	1.60		log 3	1.00	7.25	1.90	1.30	2.00	1.30
log 4		7.21	1.90	1.00	1.78	1.70		log 4		7.19	2.00	1.60	1.78	1.60
log 5		7.20	1.90	1.70	1.78	1.48		log 5		7.12	1.70	1.30	1.78	1.60
min	1.00	7.11	1.70	1.00	1.78	1.30		min	1.00	7.08	1.70	1.30	1.78	1.30
mean	1.33	7.20	1.88	1.48	1.84	1.51		mean	1.00	7.17	1.89	1.52	1.87	1.49
max	1.70	7.29	1.95	1.70	1.95	1.70		max	1.00	7.25	2.00	1.70	2.00	1.60
Reduction			5.32	5.72	5.36	5.69		Reduction			5.30	5.67	5.29	5.71

8.1.4 Fat meat inoculated with fecal slurry

	E. coli								E. faecium					
replicate	uninoc	untreated	T1	T2	T3	T4		replicate	uninoc	untreated	T1	T2	T3	T4
1	50	12,400,000	50	40	100	50		1	<10	18,800,000	50	10	50	20
2	10	19,000,000	80	50	70	50		2	<10	15,900,000	70	40	100	20
3	80	16,600,000	70	40	80	10		3	<10	18,300,000	60	30	90	50
4		10,600,000	70	50	60	40		4		16,100,000	60	50	90	10
5		12,400,000	80	10	100	20		5		14,000,000	70	30	100	20
n	3	5	5	5	5	5		n	0	5	5	5	5	5
log 1	1.70	7.09	1.70	1.60	2.00	1.70		log 1	n/a	7.27	1.70	1.00	1.70	1.30
log 2	1.00	7.28	1.90	1.70	1.85	1.70		log 2	n/a	7.20	1.85	1.60	2.00	1.30
log 3	1.90	7.22	1.85	1.60	1.90	1.00		log 3	n/a	7.26	1.78	1.48	1.95	1.70
log 4		7.03	1.85	1.70	1.78	1.60		log 4		7.21	1.78	1.70	1.95	1.00
log 5		7.09	1.90	1.00	2.00	1.30		log 5		7.15	1.85	1.48	2.00	1.30
min	1.00	7.03	1.70	1.00	1.78	1.00		min	n/a	7.15	1.70	1.00	1.70	1.00
mean	1.53	7.14	1.84	1.52	1.91	1.46		mean	n/a	7.22	1.79	1.45	1.92	1.32
max	1.90	7.28	1.90	1.70	2.00	1.70		max	n/a	7.27	1.85	1.70	2.00	1.70
Reduction			5.30	5.62	5.24	5.68		Reduction			5.43	5.77	5.30	5.90

8.2 Meat appearance

	Treated	Control
High power – short time		
		
		
Low power – long time