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Prepared by: Scott Technology Australia Pty Ltd

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Microwave E. coli Eradication Process Intervention – Stage 1

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Executive Summary

Introduction

Escherichia coli (E. coli) are harmless gram-negative, rod-shaped bacteria that form part of the normal human gut flora. Shiga-toxin producing *E. coli* (STEC) may be especially harmful to humans with *E. coli* O157:H7 being the most common. Ruminants, particularly cattle and sheep, are reservoirs for STEC especially during processing where it is possible for meat to be exposed to faecal matter.

Current methods target on-farm practices, pre-slaughter holding practices, within slaughter processing practices, strict chilling regimes, hygienic boning room and finished goods handling procedures, and control points mechanisms such as carcase wash systems. However, current methods do not guarantee eradication of bacteria from meat products.

The project aims to ascertain whether principles behind the Gyrotron can be applied to the meat processing sector to eradicate microorganisms, with the initial focus on *E. coli*. The Gyrotron delivers microwaves to the surface of meat in a non-contact method. The project determined whether the technology can eradicate *E. coli* without deteriorating meat quality.

Method

The trials were carried out at the Plasma Physic Institute (IFP), which had access to a 28GHz, 15kW Gyrotron, and *E. coli* inoculation and cell count verification was provided by the Institute of Sciences of Food Production (ISPA). Both institutes are part of the National Research Council of Italy.

A combination of meat samples and inoculated agar plates were exposed to 149 various settings. Meat samples which showed *E. coli* reduction without surface damage, were further analysed by the Department of Food, Environmental and Nutritional Sciences (DeFENS) for any effects the Gyrotron may have had on meat colour and tenderness.

Results

Initially, there were three settings that could reduce *E.coli* cell numbers without deteriorating meat quality (refer to Table 1). However, further analysis by DeFENS found a significant difference in colour for Sample A compared to the control. In saying that, Sample A managed a 4.7 log reduction (refer to Table 2).

Table 1. Treatment cycles of three settings using the Gyrotron (GYCOM 28GHZ/15kW/cw) that deactivated *E. coli* without damaging meat surface (prior to DeFENS analysis)

Sample	A	B	C
Power (kW)	10	9	8
Pulse length (sec)	0.03	0.02	0.03
Number of pulses	10	10	13
Wait time between pulses (sec)	5	5	5
Total treatment cycle (sec)	46	46	61

Table 2. Log reduction of the three samples which had reduced *E. coli* cell number without visual meat impact (prior to DeFENS analysis)

Sample	CFU[A]/g	CFU[A]/plate	Log Reduction
Control	$4.5x10^5$	$1.9x10^6$	-
A	<10	<36	4.7
B	$9.5x10^3$	$4.7x10^4$	1.6
C	$1.4x10^4$	$6.0x10^4$	1.5

[A]Colony Forming Units

Although results were promising, there was uncertainty around progressing with the 28GHz Gyrotron. Continued discussions between Scott Technology and IFP led to a simulation report, which used the results from the microwave trials to determine whether higher frequency microwaves could deactivate *E. coli* in 20 seconds or less.

On a 100GHz Gyrotron, one setting was found to deactivate 90% of *E. coli* cells within 20 seconds. Similarly, one setting on the 28GHz Gyrotron could achieve the same level of deactivation within 20 seconds. Theoretically, the 100GHz Gyrotron would be more ideal as it delivers higher frequency microwaves and can be adjusted to deliver higher amounts of energy to the meat surface unlike the 28GHz. This is because a higher frequency source will not penetrate beyond the surface of meat and will not cause any damage to meat surfaces.

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1.0 Background

Current methods in the meat processing sector revolve around preventing microbiological contamination. These methods target on-farm practices, pre-slaughter holding practices, within slaughter processing practices, strict chilling regimes, hygienic boning room and finished goods handling procedures, and control points mechanisms such as carcase wash systems. Although these practices are effective, there is not one approach that can guarantee complete bacterial eradication.

The project aims to ascertain whether principles behind the use of a high power microwave source (namely Gyrotron) can be applied to the meat processing sector to eradicate microorganisms, with the initial focus on *Escherichia Coli (E. coli)*. The Gyrotron emits microwaves with wavelengths from 1mm to 100mm. A range of settings with varying power, pulse length and pulse time were emitted on the surface of meat. The study will determine whether microwave technology can eradicate *E. coli* without deteriorating meat quality.

This report will conclude Stage 1 of microwave *E. coli* eradication process intervention. Microwave energy, which was delivered by the Gyrotron (GYCOM 28GHZ/15kW/cw), could rapidly heat surfaces of meat samples and deactivate *E. coli* on agar plates. Specifically, there were three settings that could deactivate *E. coli* without damaging meat surface using the 28GHz unit.

Additionally, microwave trials determined no significant difference in colour and tenderness for Samples B and C compared to its controls. A significant difference in colour for Sample A was observed, however, tenderness was not effected. A log reduction of 4.7 was achieved for Sample A and preliminary tests on *Listeria innocua (L. innocua)* demonstrated that deactivation was possible for other heat-sensitive microbes.

The report will determine whether microwave technology can be applied to the meat processing sector to inactivate E. coli without visual meat degradation. If successful, microwave technology can improve product integrity and ensure quality assurance for processors and consumers.

2.0 Projective Objectives

The objectives of this project were to:

- Ascertain if the application of microwave technology has the potential to deactivate microorganisms on meat pieces. Specifically:

    Does the system inactivate *E. coli*?

    Does the system have any visual detrimental effects on the raw meat surface?

    What are the ideal settings and configuration of the system?

3.0 Methodology

3.1 Gyrotron (microwave source)

The Plasma Physic Institute (IFP), an Institute of Italian National Research Council, had access to a 28GHz, 15kW Gyrotron. Prior to conducting the experiments, IFP had added modifications to the Gyrotron to deliver microwaves onto the surface of lamb and beef samples.

Figure 1. Side view of the 28GHz, 15kW Gyrotron at IFP without modifications

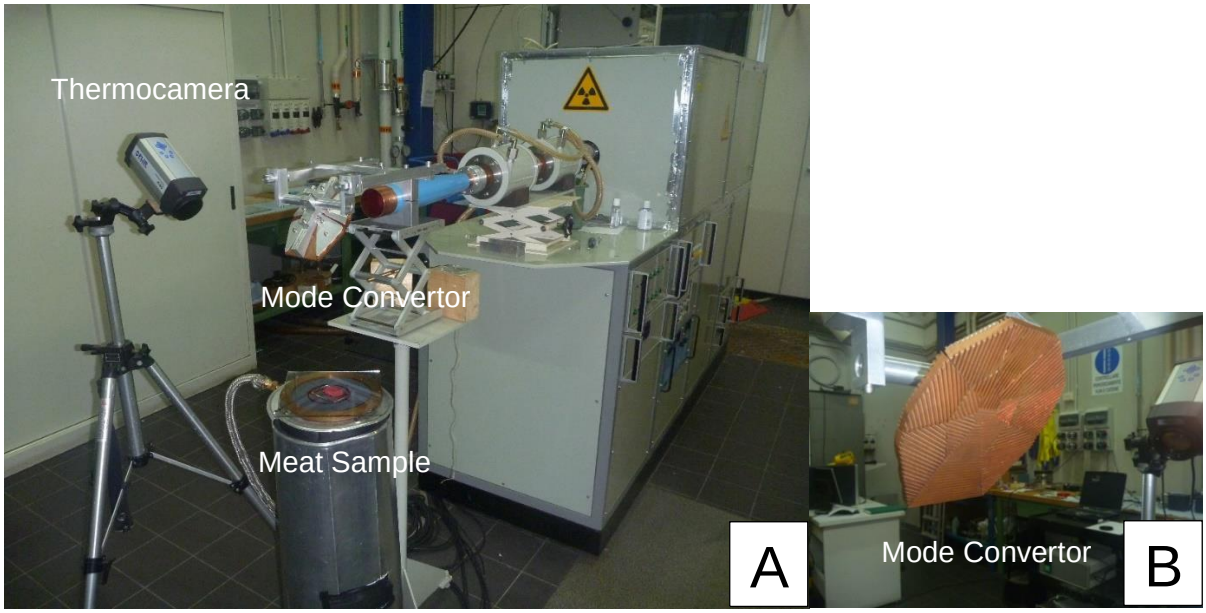

Figure 2. (A) Front view of the Gyrotron with modifications to deliver microwaves onto the surface of meat at IFP; (B) Mode convertor

The 28GHz, 15kW Gyrotron had a range of parameters that could be modified and controlled by the operator including power, number of pulses and pulse length.

A combination of meat samples and inoculated agar plates were exposed to 149 various microwave settings on the 28GHz Gyrotron. The meat samples were sent to the Department of Food, Environmental and Nutritional Sciences (DeFENS) for image, colour and tenderness acquisition and inoculated agar plates were delivered to the Institute of Sciences of Food Production (ISPA) for microbiological analyses.

3.2 Bacterial strains and enumeration of *E. coli*

E. coli inoculation and cell count verification was managed by the Institute of Sciences of Food Production (ISPA), an Institute of National Research Council.

E. coli ATCC 8739 were sub-cultured aerobically at 35ºC each night. Microbiological analyses of the *E. coli* agar plates involved weighing each BHI agar plate in a sterile Stomacher bag, where an amount of sterile buffered peptone water (Oxoid Ltd., Basingstoke, England) was subsequently added into the stomacher bag to make a 1 in 10 dilution. After homogenisation, serial dilutions in sterile Ringer (Scharlau Microbiology, Barcelona, Spain) solution were prepared and the appropriate dilutions were plated onto *E. coli* / Coliform Count Plates Petrifilm (3M, St. Paul, MN, USA) and incubated at 35ºC for 24 hours. Enumeration of *E. coli* Petrifilms occurred after 24 hours.

During the last few microwave trials, *L. innocua* CV46 were sub-cultured aerobically at 35ºC. *L. innocua* in Agar Listeria acc. Ottaviani & Agosti (ALOA) (Biolife Italiana, Milan, Italy) were used with ALOA medium instead of BHI and the plates were incubated at 37ºC for 48 hours. *L. innocua* plates were subjected to the same microwave power settings as Sample A, B and C. This determined effect of microwaves on other heat-sensitive bacteria apart from *E. coli.*

3.3 Image, colour and tenderness acquisition

Department of Food, Environmental and Nutritional Sciences (DeFENS) assisted in determining the sensory qualities of beef treated with microwaves. Specifically, colour analysis and tenderness was tested. In addition, image acquisition of test samples and control samples were taken for comparison.

Lean beef was cut to 1cm thick slices with an electric slicer. From each slice, two 50x50mm samples were obtained. Samples were identified as A, B and C and corresponding controls were A(C), B(C) and C(C).

3.3.1 Image acquisition

For each sample, the image of the surface was obtained by using a flatbed scanner (Epson Expression 8000, Seiko Epson Corporation, Japan). The images were acquired at a resolution of 600 dpi and a colour depth of 24 bits.

3.3.2 Colour

Colour analyses were performed on the surface of the samples. International Commission on Illumination (CIE) L*a*b* indexes were established using a reflectance colorimeter Chroma Meter CR 210 (Minolta Camera Co. Ltd, Japan), with standard illuminant C.

Illuminant C is a daylight stimulator representing average day light. L* is an index of brightness, a* is the green-red component, and b* is the blue-yellow component. For each sample, three different points of the lean part were analysed.

3.3.3 Tenderness

Tenderness was measured at room temperature with a 3365 Instron Universal Testing Machine (Instron Division of ITW Test and Measurement Italia S.r.l, Trezzano sul Naviglio, Italy) with shear-cutting (Warner Bratzler blade). The Universal Testing Machine was equipped with a 100 N load cell and a 200mm/min crosshead speed was used during the test.

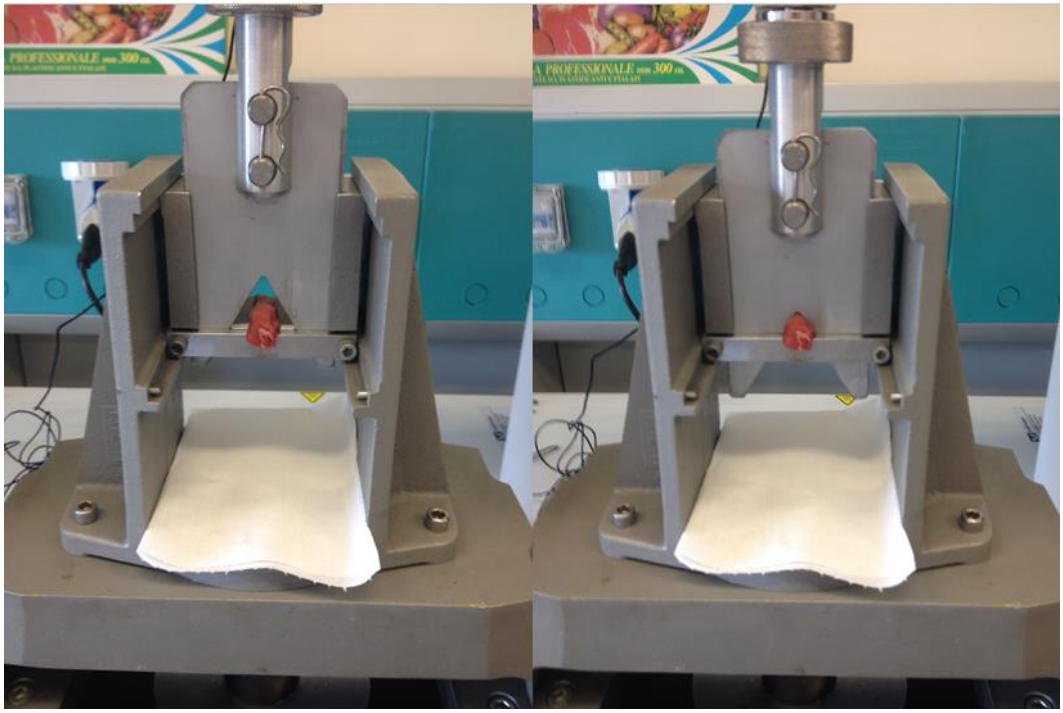

Figure 3. 3365 Instron Universal Testing Machine with a shear-cutting blade (Warner Bratzler blade) to identify shear force value at DeFENS

3.4 Statistical analysis

3.4.1 Image, colour and tenderness acquisition

Results obtained from the samples of each microwave power setting were compared to those obtained from the corresponding control by one-way analysis of variance (ANOVA; Statgraphics Plus 5.1, Statistical Graphics Corp., Herndon, VA, USA).

3.5 Further research

Although results were promising, there was uncertainty around using the 28GHz Gyrotron. Currently, the 28GHz Gyrotron takes around 40 seconds to 1 minute to deactivate *E. coli.* Theoretically, a higher frequency Gyrotron (80GHz or more) could deliver higher amounts of microwaves to the meat surface without penetrating and causing damage. Thus a shorter deactivation time. Continued discussions between Scott Technology and IFP led to a simulation report. This simulation report used the results from the microwave trials to

determine whether there is an optimum treatment cycle on the 28GHz to deactivate *E. coli* within 20 seconds. Similarly, the simulation report determined an optimum treatment cycle for a 100GHz unit to deactivate *E. coli* without causing meat damage.

4.0 Results

4.1 Gyrotron (microwave source)

Results proved that microwaves delivered through a 28GHz, 15kW Gyrotron were effective in heating surfaces of meat samples. At certain settings, microwaves caused damage to meat surfaces. Likewise, there were three settings that could heat the surface without causing damage (refer to Table 3). Inoculated agar plates were used for settings that did not cause damage to meat samples. These were taken to ISPA for further analysis.

Table 3. Treatment cycles of three settings using the Gyrotron (GYCOM 28GHZ/15kW/cw) that could deactivate E. coli without damaging meat surface

Sample	A	B	C
Power (kW)	10	9	8
Pulse length (sec)	0.03	0.02	0.03
Number of pulses	10	10	13
Wait time between pulses (sec)	5	5	5
Total treatment cycle (sec)	46	46	61

4.2 Bacterial strains and enumeration of *E. coli*

Analysis on the *E. coli* inoculated agar plates by ISPA determined the largest log reduction of 4.7 for the setting at Sample A. Log reductions for Sample A, B and C are shown in Table 4.

Table 4. Log reductions of *E. coli* on Brain Heart Infusion agar plates at microwave power settings for Sample A, B and C

Sample	CFU[A]/g	CFU[A]/plate	Log Reduction
Control	$4.5x10^5$	$1.9x10^6$	-
A	<10	<36	4.7
B	$9.5x10^3$	$4.7x10^4$	1.6
C	$1.4x10^4$	$6.0x10^4$	1.5

[A]Colony Forming Units

The same microwave power settings for Sample A, B and C were applied to *L. innocua* ALOA agar plates. Log reductions for *L. innocua* are shown in Table 5.

Table 5. Log reduction of *L. innocua* on Agar Listeria acc. Ottaviani & Agosti at microwave power settings that were performed on *E. coli* agar plates

Sample	CFU[A] / g	CFU[A] / plate	Log Reduction
Control	$3.0x10^4$	$1.8x10^5$	-
A	$1.0x10^2$	$6.7x10^2$	2.4
B	$6.8x10^3$	$4.5x10^4$	0.6
C	$2.6x10^3$	$2.1x10^4$	0.9

[A]Colony Forming Units

4.3 Image, colour and tenderness acquisition

4.3.1 Image acquisition

Images of the test samples and control samples from the flatbed scanner are shown in Fig. 4-6 below. Sample A (Fig. 4) displayed a brown spot in the centre of the meat surface, corresponding to the treated area.

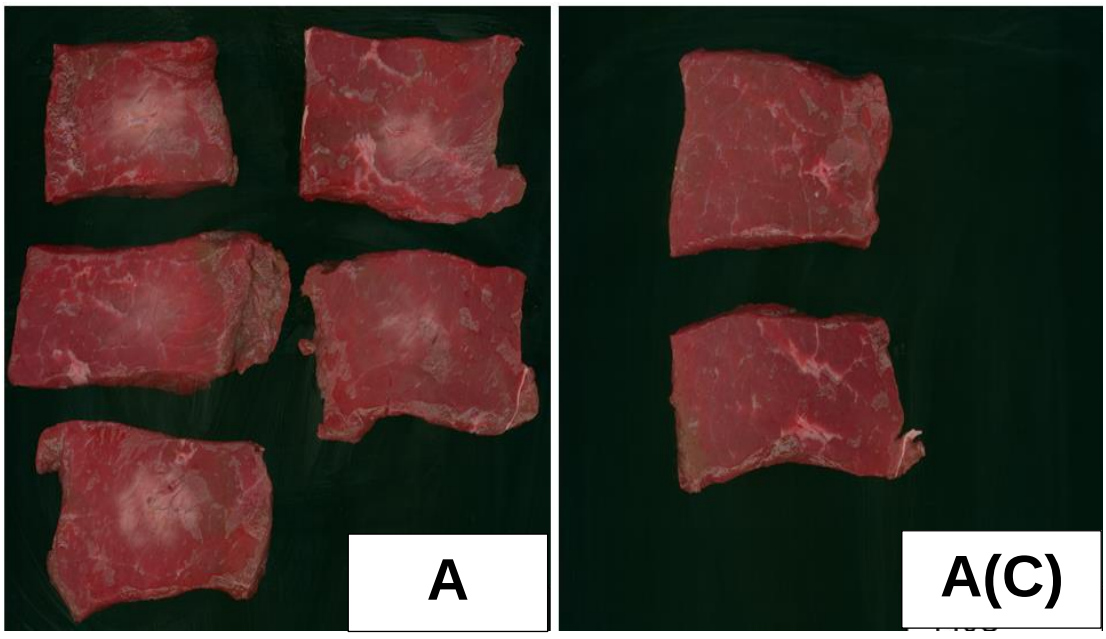

Figure 4. Image acquisition of Sample A and control sample A(C) from the flatbed scanner at DeFENS

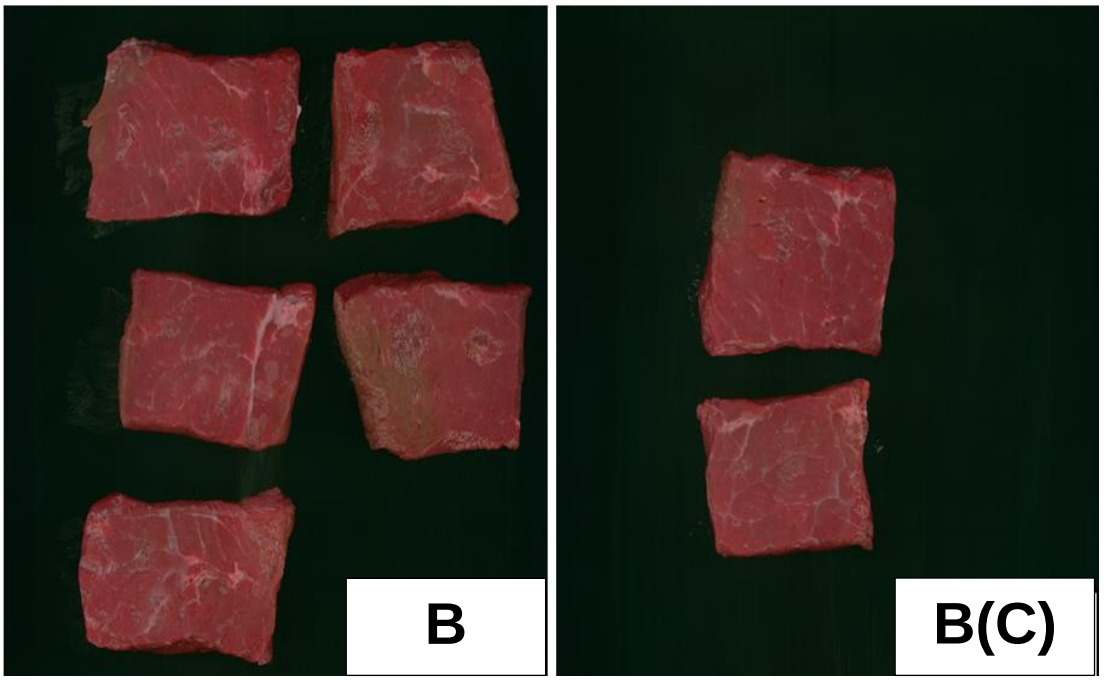

Figure 5. Image acquisition of Sample B and control sample B(C) from the flatbed scanner at DeFENS

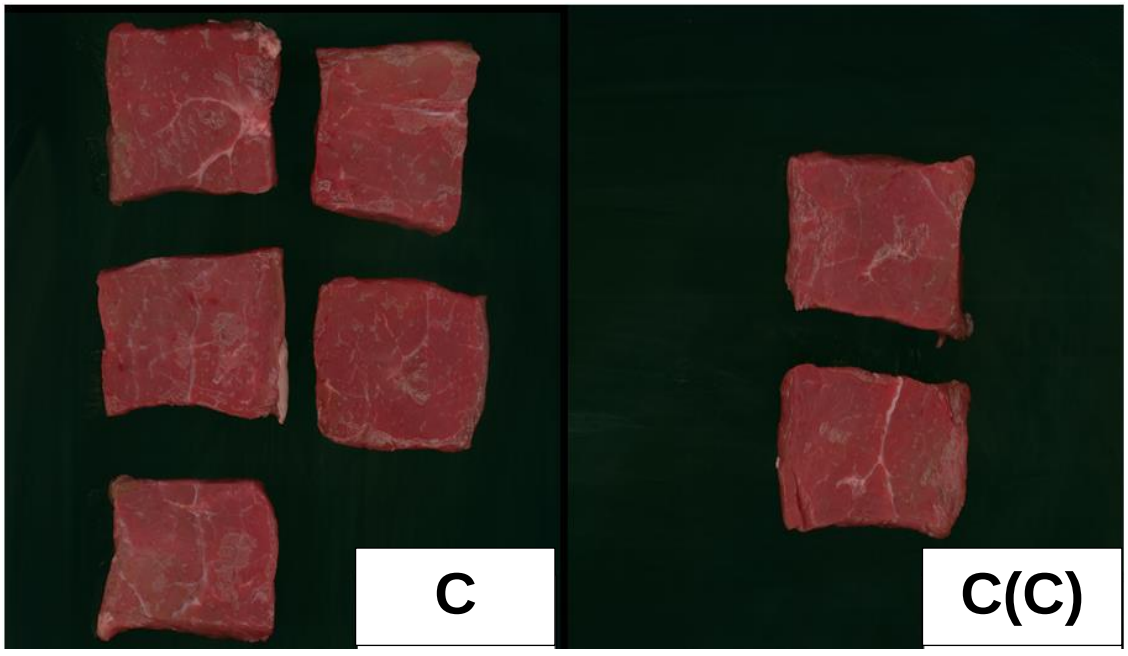

Figure 6. Image acquisition of Sample C and control sample C(C) from the flatbed scanner at DeFENS

4.3.2 Colour and tenderness

Results for tenderness using the Warner-Bratzler shear force test and colour analyses are reported in Table 6. For Sample A, the microwaves delivered to the meat surface caused a darkened central portion to result, where it didn't occur in previous meat samples under the same setting. This was separately tested and labelled A2. When significant differences between treated samples and corresponding controls were identified by ANOVA ($p<0.01$), superscript letters were used.

Table 6. Results (mean ± standard deviation values) of Warner-Bratzler shear force and colour analyses

Sample	**Shear Force (N)**	**Colour**		
		L[A]	**a[B]**	**b[C]**
A	38.0±8.4	42.9±5.5	19.3±1.9	7.5±1.1
A2	40.9±9.8	50.0±2.8[b]	17.8±1.7[a]	6.7±1.0[a]
A(C)	30.1±2.1	41.5±2.2[a]	20.2±1.1[b]	8.2±0.9[b]
B	39.3±9.8	41.2±2.2	20.6±1.2	8.0±0.9
B(C)	29.3±2.1	41.1±1.6	20.9±0.7	7.3±0.7
C	33.3±13.6	39.5±1.4	19.1±0.9	7.3±0.7
C(C)	37.0±12.6	39.9±0.7	19.6±0.8	7.1±0.8

[A]L scale represents light vs. dark where a low number (0-50) is dark and a higher number (51-100) is lighter.

[B]a scale represents red vs. green where a positive number indicates red and a negative number indicates green.

[C]b scale represents yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

4.4 Simulation Report

4.4.1 Simulation of Sample A

The following time-temperature simulation of Sample A was determined.

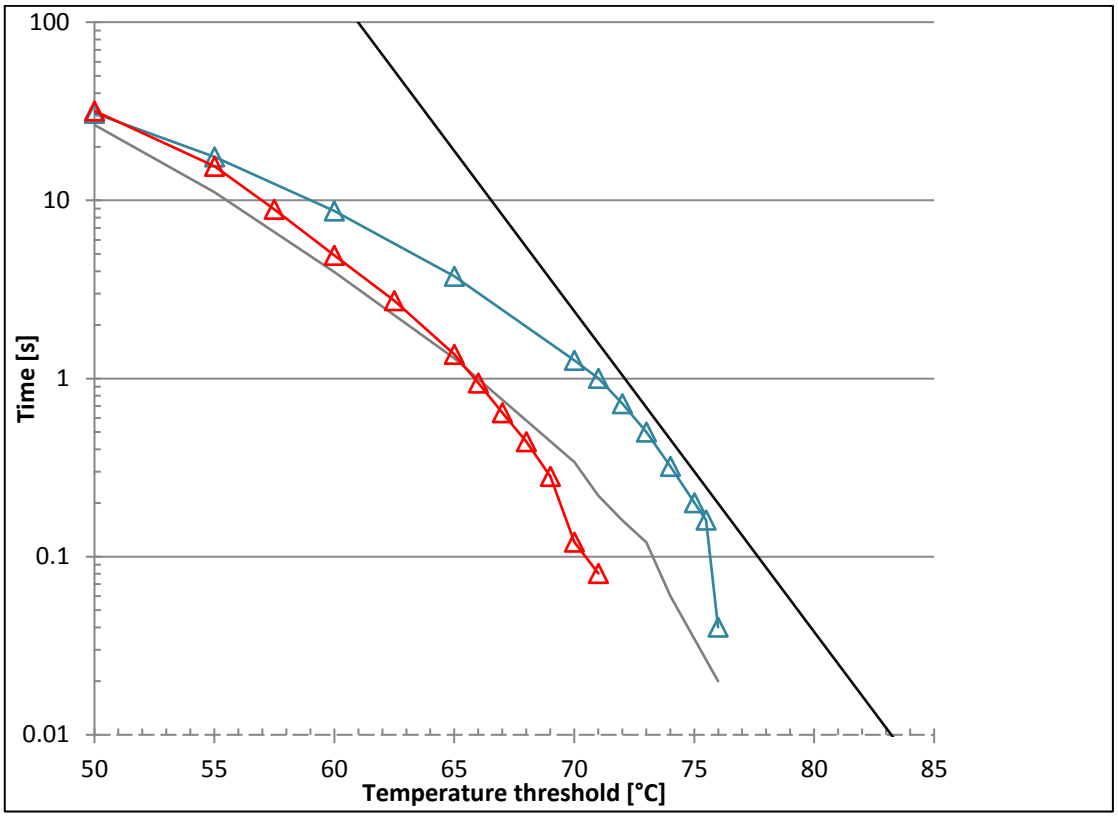

Figure 7. Sample A time-temperature curve for meat (blue) and agar (red) measured by infra-red camera compared to the simulation (grey) results. The reference curve (black) depicts the level required to reach 90% deactivation of *E. coli* cells.

Using Fig. 8, the variation between meat and agar can be better explained. At each pulse delivered to Sample A, the temperature increase is larger on meat then on agar. That is, there is less reflection of microwaves off of the meat surface when compared to agar. It is hypothesised that meat will require lower power to reach the same temperature as agar to achieve *E. coli* reduction. This would indicate that the operational window could be larger than expected.

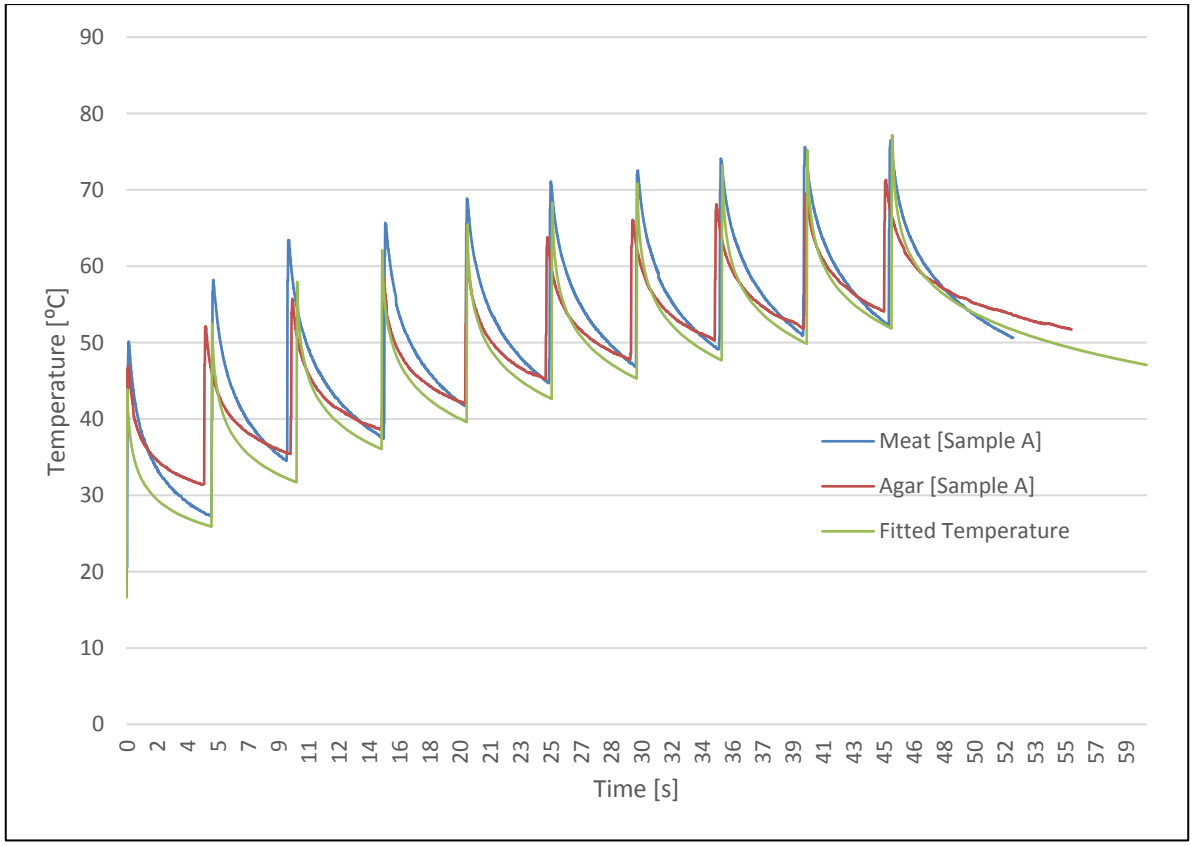

Figure 8. Temperature evolution for Sample A temperatures on meat (blue) and agar (red) as measured by the infrared camera compared to the simulation equation results for Sample A (grey)

4.4.2 Simulation of the 28GHz Gyrotron to reduce cycle time from 50 seconds to 20 seconds

Fig. 9 represents the optimal treatment cycle predicted to reduce up to 90% of *E. coli* cells without damaging meat surface on a 28GHz Gyrotron unit. In addition, Fig. 10 shows temperature evolution of the optimised treatment cycle.

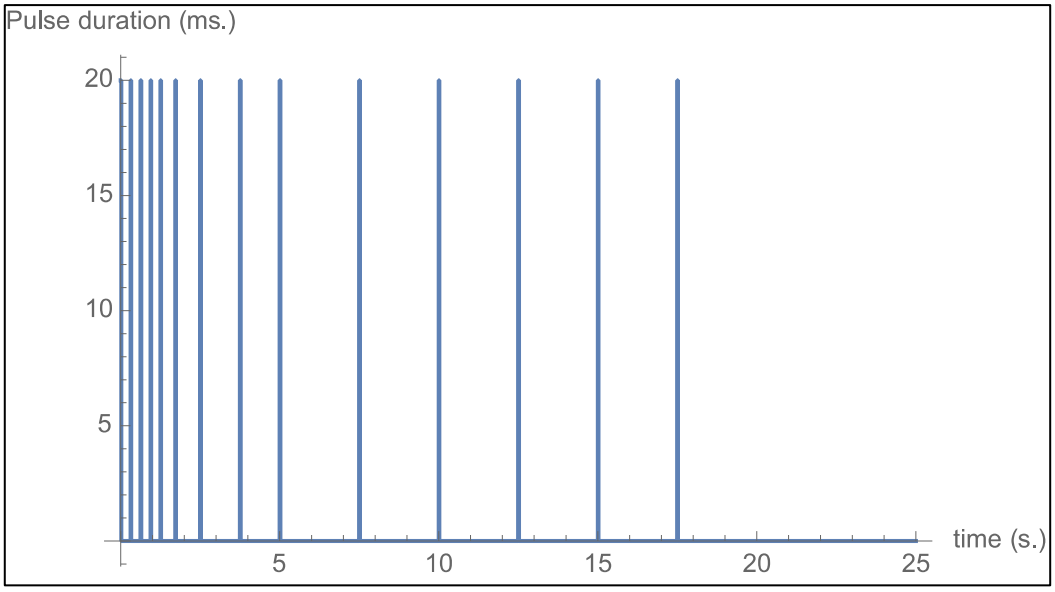

Figure 9. Pulse duration and total time required to reduce 90% of *E. coli* cells without causing meat damage on the 28GHz Gyrotron

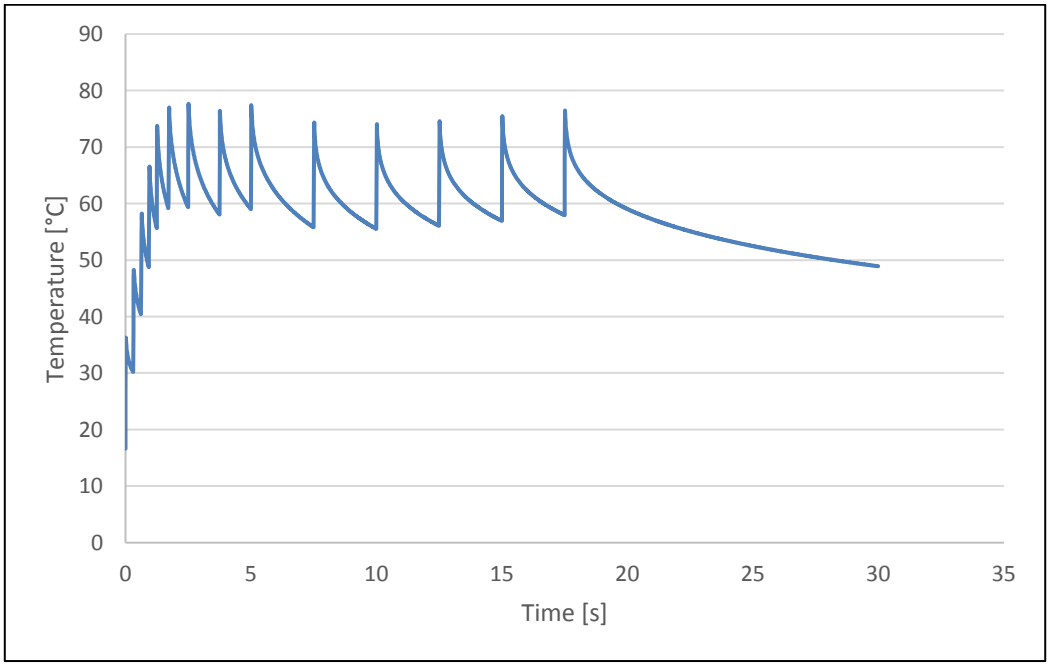

Figure 10. Temperature evolution using the optimal parameters on meat surface for the 28GHz Gyrotron

4.4.3 Simulation of a 100GHz Gyrotron to reduce *E. coli* without effecting meat surface in 20 seconds or less

Compared to the 28GHz Gyrotron, temperature rise of every pulse using the 100GHz Gyrotron is much higher. In order to avoid over-heating the meat surface, a sequence of faster but shorter pulses is recommended. Fig. 11 focused on the first 10 seconds of the optimised treatment cycle, where pulse length and time was dynamic. The last 6 seconds was not depicted as repletion rate after 10 seconds was constant. Temperature evolution of meat is shown in Fig. 12.

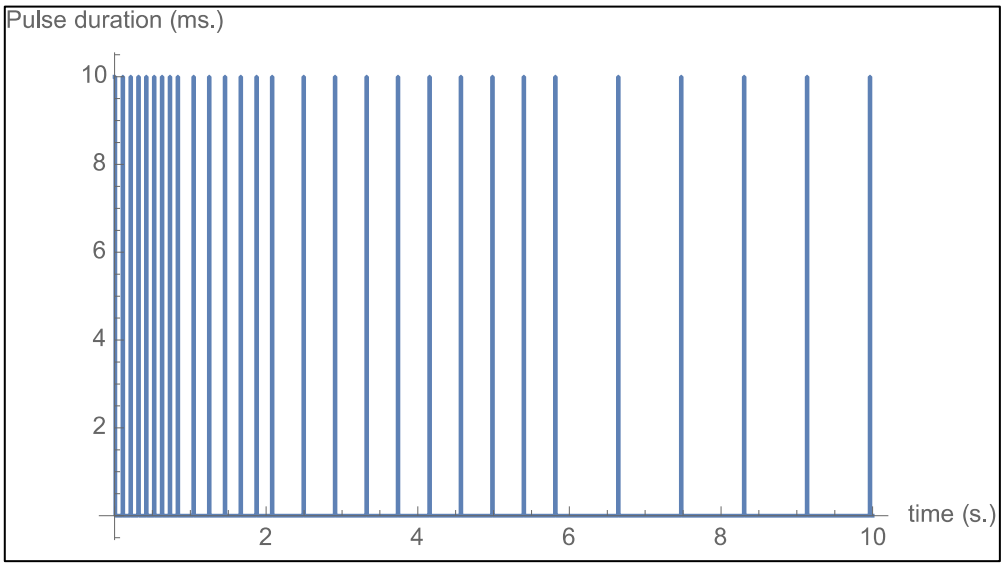

Figure 11. Pulse duration and total time required to reduce 90% of *E. coli* cells without causing meat damage on the 100GHz Gyrotron

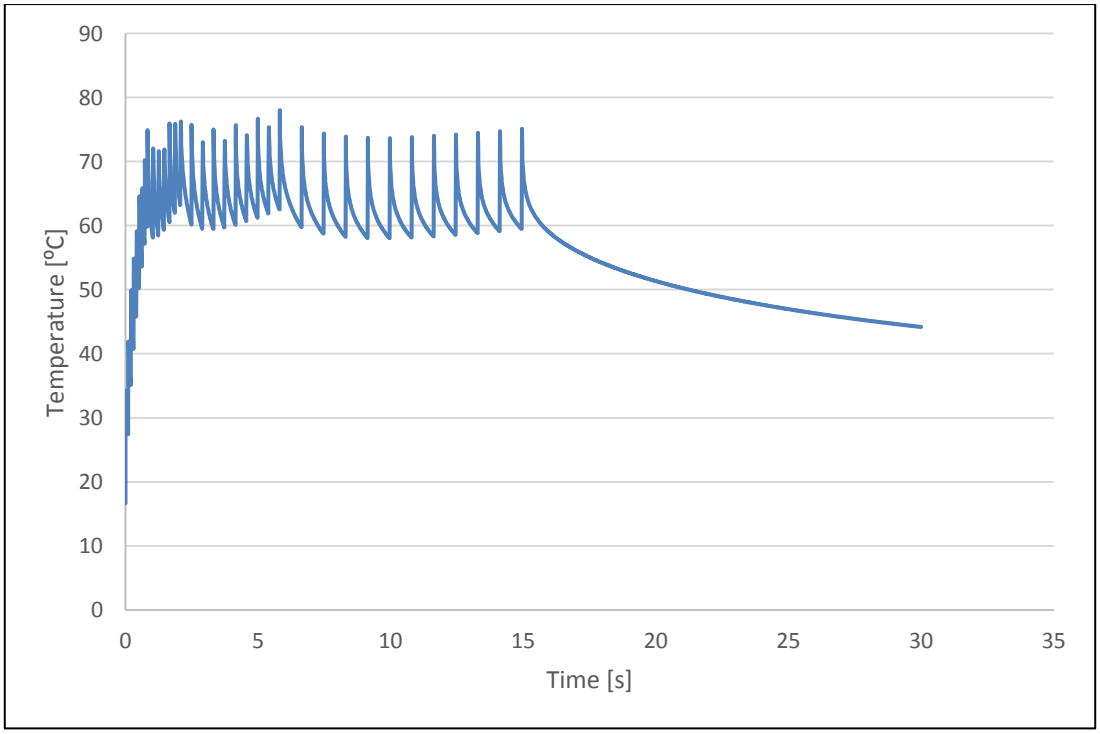

Figure 12. Temperature evolution using the optimal parameters on meat surface for the 100GHz Gyrotron

5.0 Discussion

Microwave energy eradicates *E. coli* without deteriorating meat quality. The results of the microwave trials revealed that it was possible to deactivate *E. coli* in a non-contact method without causing any associated meat damage. However, with the 28GHz Gyrotron, there were limitations that could be revoked with a Gyrotron that can deliver higher frequency microwaves.

In summary, the *E. coli* trials at IFP established that microwave energy can rapidly heat the surface of meat. Likewise, over-heating was possible and there was damage to the surface as a result. The advantage of the 28GHz Gyrotron as a microwave source is the range of settings that were achieved through controlling power, pulse time and pulse length. A log reduction of 4.7 for Sample A demonstrated that *E. coli* could be deactivated by microwaves. Likewise, microwaves were effective in deactivating *L. innocua*, however, the level of reduction was lower for the same setting (2.4 compared to 4.7). The results on *L. innocua* identified opportunities for microwave energy to deactivate other heat-sensitive microbes.

DeFENS confirmed there was no significant difference between treated samples and non-treated samples for Sample B and C in terms of colour and Sample A, B and C for firmness. A significant difference was observed for the central portion of Sample A in comparison with the control sample. However, the same setting on previous meat samples did not result in a darkened region. Initial surface temperature is a factor that most likely effected this result and it was not stringently controlled during the microwave trials. Temperature fluctuation depended on the transit time between ISPA and IFP as meat samples and agar plates were

prepared at ISPA before being delivered to IFP every day. Future research will need to consider a more controlled environment. There was a variation coefficient within samples ranging from 15 to 34% for the Warner-Bratzler shear force, which is common for meat due to the complex structure of meat and to the different location of the samples in the muscular mass.

In a meat processing plant, the total treatment cycle time for Sample A, B and C (46, 46 and 61 seconds, respectively) are unacceptable. With the simulation report prepared by IFP, the idea was to predict whether this time could be reduced to 20 seconds on the 28GHz Gyrotron while deactivating 90% of *E. coli* cells. Similarly, the simulation was applied to a 100GHz Gyrotron to predict whether a higher frequency Gyrotron could deactivate 90% of *E. coli* cells in 20 seconds or less. According to the results of the simulation, it was possible for both the 28GHz and 100GHz to deactivate *E. coli* within 20 seconds. It is believed that the higher frequency unit will absorb less on the surface of meat, thus a higher amount of microwaves and heat can be delivered to the surface without penetrating as deep as a lower frequency unit (28GHz). An advantage is that the surface will receive enough microwaves and heat to reduce *E. coli* without causing meat damage. Hence microwaves of higher frequency is preferred. Compared to the 28GHz unit, temperature rise of every pulse in the 100GHz unit is much higher. In order to avoid over-heating the meat surface, a sequence of faster but shorter pulses was recommended.

A disadvantage of the experimental set-up at IFP was the uneven distribution of heat across the meat surface as a result of the mode convertor (refer to Fig. 2). Assuming all samples were 30x30mm, there was a coupling efficiency of 33%. In other words, for every Watt of microwave delivered by the Gyrotron, only 0.33 Watts heated the sample. In addition, the actual power density on the meat samples varied by 30% from the centre to the edge. This variation may be larger depending on the amount of energy that is reflected off the surface. Moreover, the meat surface was irregular causing different degrees of cooking on the same sample at one treatment cycle, the degree of cooking on meat was influenced by initial sample temperature which was not controlled during the microwave trials on the 28GHz Gyrotron, and the infrared camera was not sensitive enough to capture the correct temperature reached. It constantly gave an underestimation of the results.

At each pulse delivered to the meat samples, the temperature increase was larger on meat then on agar. That is, there is less reflection of microwaves off of the meat surface when compared to agar. It is hypothesised that meat will require lower power to reach the same temperature as agar to achieve *E. coli* reduction. This would indicate that the operational window could be larger than expected. Future research will need to consider this difference and possibly eliminate the use of agar plates. Agar plates were used for these microwave trials as the *E. coli* was seeping through the meat fibres.

6.0 Conclusions/Recommendations

Meat & Livestock Australia and Scott Technology would like to ascertain whether microwave technology can deactivate microorganisms on meat surfaces. In particular, to confirm (1) whether microwave technology can deactivate *E. coli*, (2) whether microwaves cause visual damage on meat surfaces, and (3) to determine the ideal settings and configuration for the Gyrotron to deactivate *E. coli* without causing damage on meat surfaces.

It was established that microwave technology delivered through the 28GHz Gyrotron can successfully deactivate *E. coli* without causing damage on meat surfaces. A 4.7 log reduction was achieved and there was no significant texture and colour effects on meat. However, the size of deactivation was small and a tight control of the settings between Sample A and C was needed. Currently, there are still uncertainties around whether the settings for Sample A, B and C are the ideal settings particularly when exposure time was above 45 seconds.

The simulation report produced by IFP from the microwave trials confirmed that an ideal setting exists for the 28GHz Gyrotron to deactivate 90% of *E. coli* cells without damaging meat surface in 20 seconds or less. Similarly, the same level of deactivation was possible on a 100GHz Gyrotron. A Gyrotron with higher frequency then the 28GHz Gyrotron will deliver a higher amount of heat to the surface of meat and deactivate *E. coli* without penetrating and causing damage to meat.