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Prepared by: Dr A. Small, Ms R. Goulter and Dr G. A. Dykes
Food Science Australia

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The effect of different water temperatures on knife decontamination

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EXECUTIVE SUMMARY

In Australia and internationally knives used during the slaughter and dressing of carcasses are required to be sanitized in water at 82^{o}C. Regulations allow science-based equivalent alternative procedures to 82^{o}C dipping to be used. Recent studies have established the potential for alternative practices to achieve the same or better reduction in bacterial numbers than the typical 82^{o}C immersion. There is, however, limited sufficiently replicated time-temperature combination data available on the response of bacteria to hot water treatment on knives. The current study was undertaken to determine the effect of time:temperature combinations ranging from 1 to 60s and 60 to 82^{o}C on the disinfection of knives artificially contaminated with *Escherichia coli* and *Listeria monocytogenes*. In addition the effect of a pre-rinse at 40^{o}C on the disinfection of artificially contaminated knives treated under the same controlled conditions as above was established.

Triplicate experiments on one strain of each of the above bacterial species on knives in a meat matrix at each of 42 time:temperature combinations, with and without the pre-rinse, were performed in a laboratory water bath. Bacterial reductions were established by plate counts from the knife blade before and after emersion. Data was converted to mean log reductions, subjected to statistical analysis and basic models generated from the results.

The results of the study demonstrated that dipping knives in water for shorter times at higher temperatures or longer times at lower temperatures can produce equivalent reductions of bacteria. Pre-rinsing knives at 40^{o}C increases the performance of the subsequent dipping step. The reductions in bacterial numbers reported here are consistent with those in previous laboratory and processing plant-based studies. Models produced from the data in this study can be used to predict suitable time/temperature combinations to achieve a desired bacterial reduction. To achieve a reliable >3 log reduction performance criteria with current industry practice or small variations of these is not easily achievable.

It is recommended that models developed in this study should be used to establish alternative time/temperature combinations which will result in equivalent reductions to current industry practice where appropriate. The feasibility of a >3 log reduction criteria needs to be revaluated in the light of the findings of this study. Work validating models and assessing the role of microbial transference from knives to caresses in the hygiene of meat production would be useful additions to this study.

1.0 INTRODUCTION

In Australia and internationally knives used during the slaughter and dressing of carcasses are sanitized in water at 82^{o}C (Eustace et al. 2007; Taormina and Dorsa 2007). In Australian slaughter facilities knives are generally rinsed before sanitation in tepid water (~40^{o}C) (Eustace et al. 2007), while in facilities in the USA the substitution of hot water with sanitizers is permitted but not widely implemented (Taormina and Dorsa 2007). In Australia regulations allow science-based equivalent alternative procedures to 82^{o}C dipping to be used (AS4696 2007). The scientific basis for the use of the 82^{o}C temperature is not clear and appears to be based on convention established from previous regulatory practices (USDA 2004) rather than from empirical data. A number of studies have indicated some issues with the use of hot water as a sanitizer and in particular the role organic matter on knives can have in reducing the effectiveness of this practice (Peel and Simmons 1976; Snijders et al. 1985). Furthermore, it has been suggested that meat residue denatured in hot water may act to protect bacteria from subsequent sanitation (Peel and Simmons 1978).

More recent studies have examined the potential for alternative practices to achieve the same or better reduction in bacterial numbers than the typical 82^{o}C immersion (Midgely and Eustace 2003; Eustace et al. 2007; Taormina and Dorsa 2007). The effectiveness of brief immersion at 82^{o}C in significantly reducing bacterial numbers on knives with meat residue on them was questioned by all three of these studies with a <1 log CFU/cm^{2} typically observed. A 15s immersion time in hot water at 82^{o}C or warm water (40^{o}C) containing quaternary ammonium compounds, however, were suggested as being effective in reducing bacterial numbers by ~3 log CFU/cm^{2} (Taormina and Dorsa 2007). In a separate study Midgely and Eustace (2003) demonstrated that immersion of knives in 72^{o}C water for 15s after a rinse in hand-wash water was as effective as rapid dipping in 82^{o}C water in the laboratory. Eustace et al. (2007) went on to demonstrate that the use of a two-knife system with rinsing in hand-wash water then immersing in 60^{o}C between uses was as effective as the typical 82^{o}C system in small slaughter facilities.

In all the above studies limited time-temperature combinations were investigated and an overall indication of the response of bacteria to hot water treatment on knives with meat residue on them was not apparent. In addition, a lack of sufficient replication in some of the studies did not allow clear statistically valid

conclusions to be drawn. This study was undertaken to overcome these gaps in knowledge by establishing the response of an *Escherichia coli* and a *Listeria monocytogenes* strain on meat-soiled knives to time-temperature combinations ranging from 1 to 60s and 60 to 82^{o}C.

2.0 OBJECTIVES

The objectives of this study were to:

- Establish the effect of time:temperature combinations ranging from 1 to 60s and 60 to 82^{o}C on the disinfection of knives artificially contaminated with *Escherichia coli* and *Listeria monocytogenes* under controlled laboratory.
- Establish the effect of a pre-rinse at 40^{o}C on the disinfection of artificially contaminated knives treated under the same controlled conditions as above.

3.0 METHODS

3.1 Bacterial strains and growth conditions

Single *E. coli* (ATCC 25922) and *L. monocytogenes* (ATCC 7644) strains were used in this study. Bacterial strains were revived from stock cultures stored at -80^{o}C on Protect Bacterial Preservers (Technical Service Consultants, UK) and checked for purity after growth at 37^{o}C for 18h on Tryptone Soy Agar (TSA) plates (Oxoid, UK). For all experiments bacterial strains were grown for 18h at 37^{o}C in Tryptone Soy Broth (TSB; Oxoid, UK) before being inoculated on knives as described below.

3.2 Inoculation and heating experiments

Knife blades were scanned and imaging software (ImageJ, U. S. National Institutes of Health) was used to determine the surface area of the blades. This was established to be 28cm^{2}. A 2mL aliquot of each of the 18h cultures prepared as described above was added to 150g of ground meat in a stomacher bag and mixed by kneading the bag by hand. Both sides of a knife blade were covered with the meat/culture mixture, ensuring an even coating of meat product on both sides of the knife blade. A 28mL aliquot of 0.85% saline was added to a sterile Speci-Sponge (Nasco, USA) in a Whirl-pak® bag (Nasco) which was then squeezed to remove excess liquid. A volume of 28mL was used so that subsequently 1mL of liquid

represented 1cm^2 of knife blade area. One side of the knife was swabbed with the sponge and the sponge returned to the Whirl-pak bag. The knife was then immersed in a waterbath at a set temperature for a specific period of time. The times and temperatures used in the treatments were combinations of 1, 5, 10, 20, 30, 45, 60s and 60, 65, 70, 75, 80, 82^{o}C, respectively. After the treatment was applied, another Speci-Sponge prepared in the same way was used to swab the other side of the knife. All combinations were tested in triplicate, and all combinations were then repeated with a 1s 40^{o}C pre-rinse prior to immersion of the knife blade. To investigate the changes in temperature at the knife blade surface, a thermocouple was placed on the blade of a typical knife, closely apposed to the metal of the blade. This was held in place using a rubber band. A second thermocouple was attached to the handle of the knife, and held in place using Araldite™ plastics adhesive. The knife was allowed to stabilise at room temperature (20°C) before being immersed in the water bath used for the investigation. The temperatures detected by the two thermocouples were logged at 1 s intervals for 16 seconds using a Squirrel Logger 1250 series (Grant Instruments, UK). Temperature change measurements were taken in triplicate for each of the water bath temperatures used in the investigation.

3.3 Microbiological analysis

The Speci-Sponge were homogenised in their Whirl-pak bags in a stomacher (Interscience, Canada) for 30s. A decimal dilution series was prepared in 0.85% saline, using 0.5mL of liquid from the Whirl-pak bags. Volumes of 1mL of each dilution were plated onto Petrifilm™ *E.coli*/Coliform Count Plates (3M, USA) and Petrifilm™ Environmental Listeria Plates (3M) and were incubated at 37^{o}C for 18h and 48h, respectively before counting.

3.4 Statistical analysis

Counts were reported per cm^2 of knife area and mean log reductions were calculated from these counts. The results were analysed using Tukey's familywise comparison using Minitab® 14 (Minitab Inc., USA) and second-order polynomial models were fitted to the data using Response Surface Methodology (Minitab).

4.0 RESULTS

4.1 Effect of various time/temperature combinations on Escherichia coli populations on knives immersed without prior rinsing

Extending the immersion time of the knife blade increased the performance of this method as a decontamination intervention with respect to *E. coli* (Table 1, Figure 1). A temperature of 60°C performed poorly at the immersion times investigated with less than a 2 log reduction achieved in all cases. Similarly, a 1s immersion time also performed poorly in all cases with no significant differences between reductions in numbers of *E. coli* at 60, 65, 70 and 75°C (mean log reduction of -0.02 to 0.68).

Table 1: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	$0.47^{a,d}$ ±0.07	$0.68^{a,d}$ ±0.25	-0.02^{a} ±0.19	$0.36^{a,d}$ ±0.31	2.05^{c} ±0.59	$1.17^{c,d}$ ±0.30
5	1.40^{e} ±0.08	1.71^{e} ±0.31	1.83^{e} ±0.58	1.97^{e} ±0.78	3.78^{f} ±0.82	4.72^{f} ±0.16
10	1.71^{g} ±0.19	1.88^{g} ±1.00	3.81^{h} ±0.14	4.64^{h} ±0.13	4.72^{h} ±0.37	4.81^{h} ±0.08
20	1.60^{i} ±0.26	2.57^{j} ±0.33	2.58^{j} ±0.51	4.70^{k} ±0.40	5.29^{k} ±0.06	4.65^{k} ±0.20
30	1.73^{l} ±0.23	$2.45^{l,m}$ ±0.10	$3.48^{l,n}$ ±0.26	5.08^{n} ±0.06	$3.85^{m,n}$ ±1.67	4.65^{n} ±0.11
45	1.85^{p} ±0.32	3.23^{q} ±0.82	4.77^{r} ±0.18	5.11^{r} ±0.33	4.85^{r} ±0.06	5.02^{r} ±0.01
60	1.86^{s} ±0.20	3.75^{t} ±0.50	4.62^{t} ±0.03	$4.58^{t,u}$ ±0.78	5.33^{u} ±0.08	4.93^{u} ±0.10

Values across rows bearing different superscripts differ significantly at P<0.001

At 1s temperatures of 80°C and 82°C performed slightly better (mean log reductions 1.17 to 2.05) although there was no significant difference between these latter two temperatures. At 5s immersion, there were again no significant differences between performances of 60, 65, 70 and 75°C (1.40 to 1.97 log reduction), but both 80°C (3.78 log reduction) and 82°C (4.72 log reduction) performed significantly better ($P<0.001$) than the lower temperatures. When the knives were immersed for 10s, there was no significant difference between the performances of 70, 75, 80 and 82°C (3.81 to 4.81 log reduction), but each of these temperatures performed significantly better ($P<0.001$) than either 60°C (1.71 log reduction) or 65°C (1.88 log reduction).

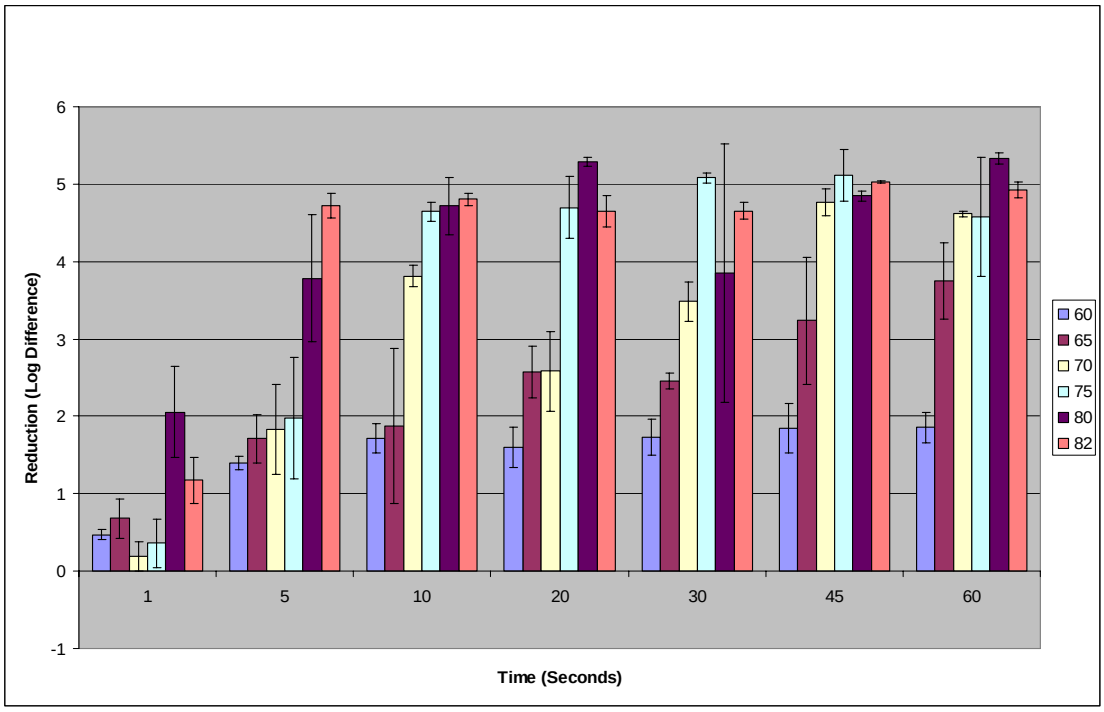


Figure 1: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures (°C)

Immersion for 20s at 60°C gave only a 1.60 log reduction, significantly lower ($P<0.001$) than either 65 or 70°C (2.57 and 2.58 log reduction respectively), and these in turn were significantly ($P<0.001$) poorer performers than 75, 80 and 82°C (4.70, 5.29 and 4.65 log reduction respectively). At 30s immersion, 60°C gave the lowest reduction, and 75, 80 and 82°C greatest, with 65 and 70°C forming an intermediate grouping. There was no significant difference between the performances of 60, 65 and 70°C with 30s immersion (1.73, 2.45 and 3.48 log reduction). 80°C performed at a similar level to 65 and 70°C (3.85±1.67 versus

2.45±0.10 and 3.48±0.26 log reduction), but better than 60°C. Overall, there was no statistically significant difference between the performances of 70, 75, 80 and 82°C (3.48, 5.08, 3.85 and 4.65 log reduction respectively). At 45s immersion, 65°C performed significantly better than 60°C (3.23 versus 1.85 log reduction, P<0.001), while each of the 70, 75, 80 and 82°C temperature performed significantly (P<0.001) better again. There was no significant difference between the performances of each of these four temperatures (4.77, 5.11, 4.85 and 5.02 log reduction, respectively). If knives were immersed for 60s, three groups of performance, between which there were statistically significant differences (P<0.001), could be discerned: Poor performance (60°C; 1.86 log reduction), Good performance (65, 70 and 75°C; 3.75 to 4.62 log reduction) and High performance (75, 80 and 82°C; 4.58 to 5.33 log reduction).

With respect to percentage reduction in *E. coli* populations (Table 2), a reduction of 99.9% was achieved by immersion for 5s or more at 80 and 82°C, 10s or more at 75°C, 30s or more at 70°C (10s immersion achieved <99.9% reduction, but 20s only achieved 99.6%), and 45s or more at 65°C. Immersion at 60°C did not achieve more than a 98.5% reduction.

When the outlying variable of 1s immersion was ignored, there was a good positive correlation between immersion time and mean log reduction at temperatures between 60 and 70°C (P<0.05). At 75°C and above, the reductions had reached a plateau after 10s immersion, so there was poor linear correlation.

Table 2: Percentage reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	66.1	76.7	<1	49.4	98.6	92.2
5	96.0	97.8	97.8	97.2	99.96	>99.99
10	98.0	96.5	99.98	>99.99	>99.99	>99.99
20	97.2	99.7	99.6	>99.99	>99.99	>99.99
30	97.2	99.6	99.96	>99.99	99.6	>99.99
45	98.3	99.9	>99.99	>99.99	>99.99	>99.99
60	98.5	99.98	>99.99	>99.99	>99.99	>99.99

4.2 Effect of various time/temperature combinations on *Escherichia coli* populations on knives immersed following rinsing at 40°C

In general, there tended to be a greater overall reduction in *E. coli* counts on the knife blades when the knife was rinsed in 40°C water prior to immersion than when knives were not pre-rinsed (Table 3, Figure 2). A 1s immersion gave a mean reduction of 1.92 to 3.44 log, and there were no statistically significant differences between the reductions achieved at each of the immersion temperatures trialled. At 5s immersion, temperatures of 70°C or above achieved a mean reduction greater than 3.46 log, significantly greater ($P<0.001$) than at temperatures of 60 and 65°C (1.96 and 1.24 log respectively). This pattern, temperatures of 70°C and above giving significantly greater mean log reductions than 60 or 65°C, was maintained throughout the immersion times of 10s and above. The mean log reduction increased in all cases with longer immersion times, except for 60°C, where the 60s immersion only gave a mean reduction of 1.53 log, compared with 3.69 log at 45s immersion. There were few differences between the mean log reductions achieved by 70, 75, 80 and 82°C with immersion times of 5s or more. Where differences were noted (20 and 60s), the performance of 70, 75 or 80°C were often slightly better than the performance of 82°C.

Table 3: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures after a pre-rinse at 40°C

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	2.07^{a} ±0.69	1.92^{a} ±0.12	1.85^{a} ±0.21	2.70^{a} ±0.39	3.41^{a} ±1.20	3.44^{a} ±0.34
5	$1.96^{b,e}$ ±0.45	$1.24^{b,d}$ ±0.22	$3.46^{c,e}$ ±0.47	$3.75^{c,e}$ ±1.16	$3.63^{c,e}$ ±0.98	4.56^{c} ±0.07
10	1.90^{f} ±0.32	$1.85^{f,g}$ ±0.23	3.51^{h} ±0.84	4.97^{i} ±0.09	4.95^{i} ±0.18	$4.47^{h,i}$ ±0.38
20	2.71^{j} ±0.48	$3.50^{j,k}$ ±1.11	$4.75^{k,l}$ ±0.17	5.11^{l} ±0.06	4.91^{l} ±0.06	$4.74^{k,l}$ ±0.21
30	2.62^{m} ±0.53	$2.86^{m,n}$ ±0.18	5.29^{p} ±0.18	4.98^{p} ±0.13	4.93^{p} ±0.30	4.83^{p} ±0.10
45	$3.69^{q,r}$ ±0.17	$3.31^{q,s}$ ±0.40	5.15^{t} ±0.22	4.91^{t} ±0.19	5.01^{t} ±0.30	$4.46^{r,t}$ ±0.50
60	1.53^{u} ±0.44	4.30^{w} ±0.24	5.23^{x} ±0.10	$4.90^{w,x}$ ±0.32	4.44^{w} ±0.06	$4.78^{w,x}$ ±0.10

Values across rows bearing different superscripts differ significantly at $P<0.001$

In terms of percentage reduction in *E. coli* populations (Table 4), when knives were pre-rinsed a reduction of 99.9% was achieved by immersion for 1s or more at 82°C, 5s or more at 70°C or above, and 45s or more at 65°C. Immersion at 60°C did achieve 99.98% reduction at 45s, but only a 96.1% reduction at 60s.

When the outlying variable of 1s immersion was ignored, there was a good positive correlation between immersion time and mean log reduction at all temperatures except 60 and 82°C ($P<0.05$). At 82°C, the reductions had reached a plateau after 10s immersion, so there was poor linear correlation, while at 60°C there

were outliers at 45 and 60s immersion that prevented development of a good time/temperature relationship.

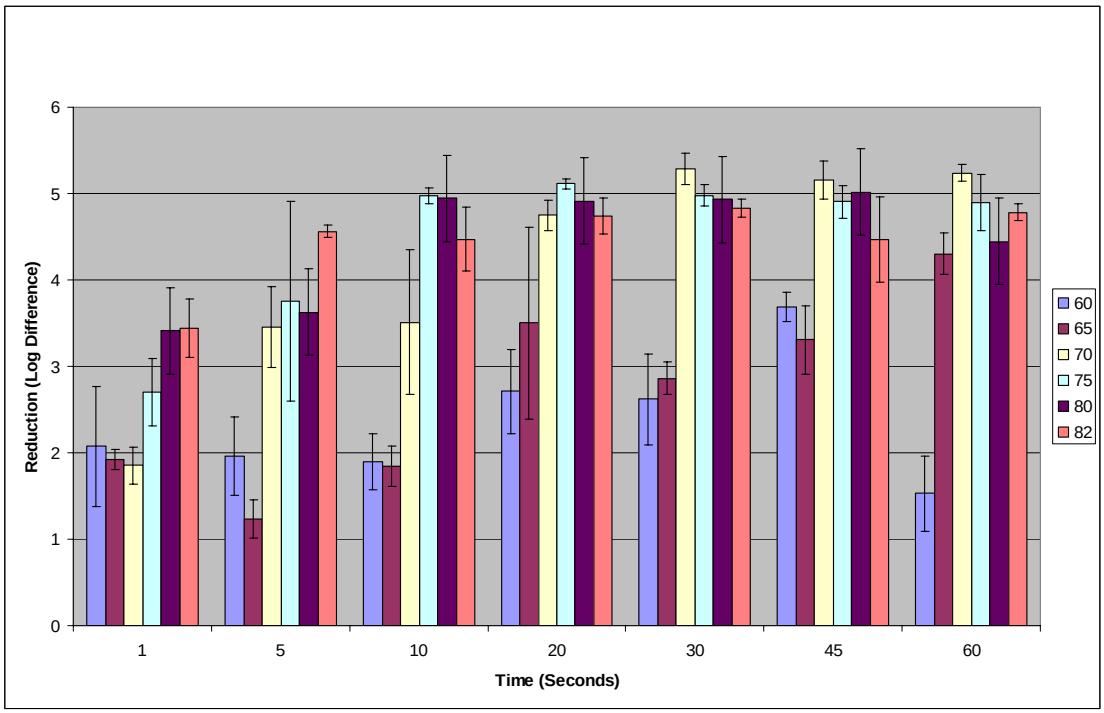


Figure 2: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures (°C) after a pre-rinse at 40°C

Table 4: Percentage reduction in *Escherichia coli* populations on knife blades following immersion for different periods of time in water at different temperatures after a pre-rinse at 40°C

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	98.5	98.8	98.5	99.7	99.7	99.95
5	98.4	93.6	99.95	99.93	99.94	>99.99
10	98.5	98.4	99.93	>99.99	>99.99	>99.99
20	99.7	99.8	>99.99	>99.99	>99.99	>99.99
30	99.6	99.89	>99.99	>99.99	>99.99	>99.99
45	99.98	99.93	>99.99	>99.99	>99.99	>99.99
60	96.1	99.99	>99.99	>99.99	>99.99	>99.99

4.3 Effect of various time/temperature combinations on *Listeria monocytogenes* populations on knives immersed without prior rinsing

Immersion for 1s at any temperature was not particularly effective in reducing *L. monocytogenes* (Table 5, Figure 3). There were few differences between the performances of each temperature evaluated, but 80°C gave a greater log reduction (1.09) than 60, 75 and 82°C (0.54, 0.48 and 0.35 log respectively). These temperatures in turn gave greater log reduction than 65 and 70°C (0.10 and 0.15 log). At 5s immersion, 80°C gave the best performance (2.99 log reduction), followed by 82°C (2.68 log reduction), but this difference was not statistically significant. The reduction achieved at 82°C for 5s was in turn not significantly different from that achieved at 70 and 75°C (1.64 and 2.14 log respectively). The reduction achieved at 70°C was not significantly different from the reduction achieved by 65°C (0.64 log), which in turn was not significantly different to that achieved ay 60°C (0.29 log). When the knife blades were immersed for 10s, there were two distinct groups. Temperatures of 70°C and above performed significantly better ($P<0.001$) than those below 70°C (3.35 to 4.84 log reduction compared with 0.68 to 1.82 log). Within each of these two groups, the performance of individual temperature points did not differ significantly.

Table 5: Mean log reduction in *Listeria monocytogenes* populations on knife blades following immersion for different periods of time in water at different temperatures

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	$0.54^{a,b}$ ±0.33	0.10^{a} ±0.10	0.15^{a} ±0.04	$0.48^{a,b}$ ±0.25	1.09^{b} ±0.34	$0.35^{a,b}$ ±0.17
5	0.29^{c} ±0.15	$0.64^{c,d}$ ±0.21	$1.64^{d,e}$ ±0.38	2.14^{e} ±0.85	2.99^{f} ±0.25	$2.68^{e,f}$ ±0.51
10	0.68^{g} ±0.30	1.82^{g} ±0.48	3.35^{h} ±0.40	4.29^{h} ±0.28	3.60^{h} ±1.05	4.84^{h} ±0.46
20	0.88^{i} ±0.08	1.17^{i} ±0.36	2.55^{k} ±0.59	3.05^{k} ±0.38	5.56^{m} ±0.01	5.48^{m} ±0.26
30	1.29^{n} ±0.63	$1.86^{n,p}$ ±1.04	$3.76^{p,q}$ ±0.29	4.98^{q} ±0.96	4.82^{q} ±1.42	5.36^{q} ±0.10
45	0.96^{r} ±0.19	2.44^{s} ±0.15	4.67^{t} ±0.05	5.50^{t} ±0.75	5.57^{t} ±0.02	5.19^{t} ±0.28
60	1.07^{u} ±0.21	2.94^{w} ±0.44	4.58^{x} ±0.01	5.46^{x} ±0.48	5.51^{x} ±0.27	4.70^{x} ±0.48

Values across rows bearing different superscripts differ significantly at P<0.001

At 20s immersion, 80 and 82°C gave greater (P<0.001) log reductions (5.56 and 5.48 log) than 70 and 75°C (2.55 and 3.05 log), which in turn gave greater (P<0.001) reductions than 60 and 65°C (0.88 and 1.17 log). An immersion time of 30s showed a return to there being no significant differences between the reductions achieved by temperatures of 70°C and above (3.76 to 5.36 log). The reduction achieved by 60°C was significantly less (1.29 log, P<0.001) than those at 70°C and above.

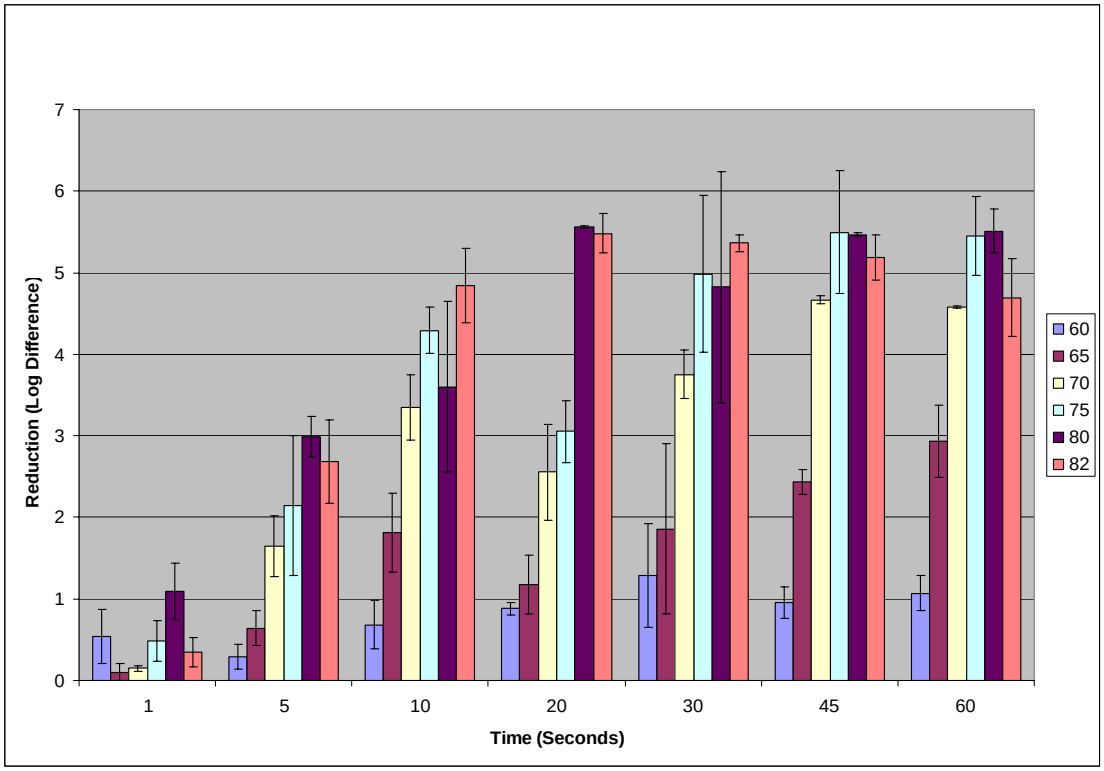


Figure 3: Mean log reduction in *Listeria monocytogenes* populations on knife blades following immersion for different periods of time in water at different temperatures (°C)

On the other hand the reduction at 65°C was intermediate (1.86 log) but not significantly different from 60°C or 70°C. When the knives were immersed for 4s or 60s there were three distinct groups of reduction. Temperatures of 70°C and above performed significantly better ($P<0.001$) than 65°C, which in turn performed significantly better than 60°C. At both 45 and 60s immersion, temperatures of 70°C and above achieved mean log reductions of greater than 4.5 log, while 65°C achieved 2.44 to 2.94 log reduction while 60°C achieved only 0.96 to 1.07 log reduction.

In terms of percentage reduction in *L. monocytogenes* population (Table 6), a reduction of 99.9% was achieved by immersion for 10s or more at 82°C, 5s or more at 80°C or above, and 30s or more at 70°C or above. Immersion at 60 and 65°C achieved a maximum of 91.7 and 99.8% respectively. At 60°C this maximum was achieved at 30s.

When the outlying variable of 1s immersion was ignored, there was a good positive correlation between immersion time and mean log reduction at temperatures of 65 and 70°C ($P<0.05$). At 75°C and above, the reductions had reached a plateau

after 10s immersion, so there was poor linear correlation. At 60°C there was little difference between the reductions in population achieved by any immersion period.

Table 6: Percentage reduction in *Listeria monocytogenes* populations on knife blades following immersion for different periods of time in water at different temperatures

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	60.5	19.8	29.0	63.0	90.4	52.4
5	46.1	75.5	97.0	98.1	99.9	99.7
10	76.1	97.9	99.9	99.99	99.9	>99.99
20	86.8	91.4	99.5	99.9	>99.99	>99.99
30	91.7	93.9	99.97	>99.99	99.98	>99.99
45	88.1	99.6	>99.99	>99.99	>99.99	>99.99
60	90.8	99.8	>99.99	>99.99	>99.99	>99.99

4.4 Effect of various time/temperature combinations on *Listeria monocytogenes* populations on knives immersed following rinsing at 40°C

Overall, including a pre-rinse in 40°C water increased the log reduction achieved at each time-temperature point when compared to immersion without pre-rinse. Once again 60°C did not perform particularly well, achieving no more than a 1.86 log reduction even with an extended immersion time of 60s (Table 7, Figure 4). In fact, there was no significant difference between the reductions achieved with any immersion time of 5s or above. At all temperatures, 1s immersion gave poor reductions in counts with only 80 and 82°C achieving greater than a 2 log reduction (2.86 and 2.17 log respectively). This reduction was significantly greater ($P<0.001$) than that achieved at 60, 65 and 70°C (0.71, 0.25 and 0.72 log). On the other hand 75°C gave an intermediate reduction of 1.89 log, which was neither significantly different from the reductions at 80 and 82°C, nor from the reductions at 60 and 70°C. At 5s immersion there were no significant differences between the reductions achieved at 70, 75, 80 and 82°C (range 2.46 to 3.40 log). A temperature of 65°C performed poorly giving only a 0.27 log, while a temperature of 60°C gave a 1.18 log reduction, which was neither significantly different from that achieved by 65°C nor from that achieved by 75°C (2.46 log). Once immersion times exceeded 10s or more, there was a clear division between the performances of 70°C and above as

compared to 60 and 65°C (P<0.001). At 10s, 30s and 45s immersion, there were no significant differences between the reductions achieved by each of the temperatures of 70°C and above, which collectively were significantly greater (P<0.001) than the reductions achieved at 60 and 65°C. At 20s immersion, three groupings could be identified in which the reductions seen were not significantly different, although overlaps between groupings were evident at 70 and 80°C. There was no statistically significant difference between the performances of 60, 65 and 70°C (1.69, 1.95 and 3.02 log reduction), neither was there significant difference between the performances of 70, 75 and 80°C (3.02, 3.57 and 4.11 log reduction), nor were there significant differences between the performances of 80 and 82°C (4.11 and 5.18 log reduction). With 60s immersion, three groups were also evident, but with no overlaps. Immersion at 60°C gave significantly lower (P<0.001) reductions in count than 65°C (0.076 and 2.87 log respectively), which in turn gave significantly lower reductions than immersion at 70°C or above. There were no significant differences between the reductions achieved at 70, 75, 80 or 82°C, all of which were greater than 5 log.

Table 7: Mean log reduction in *Listeria monocytogenes* population on knife blades following immersion for different periods of time in water at different temperatures, after a pre-rinse at 40°C

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	$0.71^{a,b}$ ±0.08	0.25^{a} ±0.10	$0.72^{a,b}$ ±0.15	$1.89^{b,c}$ ±0.76	2.86^{c} ±0.48	2.17^{c} ±0.65
5	$1.18^{d,e}$ ±0.53	0.27^{d} ±0.31	2.91^{f} ±0.37	$2.46^{e,f}$ ±0.13	3.40^{f} ±0.93	3.40^{f} ±0.82
10	1.22^{g} ±0.41	1.53^{g} ±0.52	2.82^{h} ±0.29	2.72^{h} ±0.15	3.60^{h} ±0.36	3.56^{h} ±0.59
20	1.69^{i} ±0.01	1.95^{i} ±0.26	$3.02^{i,j}$ ±0.42	3.57^{j} ±0.69	$4.11^{j,k}$ ±0.89	5.18^{k} ±0.26
30	1.64^{l} ±0.21	2.45^{l} ±0.74	4.69^{m} ±0.46	4.76^{m} ±0.37	5.47^{m} ±0.27	5.31^{m} ±0.09
45	2.48^{n} ±0.62	3.13^{n} ±0.49	5.20^{p} ±0.31	5.18^{p} ±0.30	5.28^{p} ±0.17	5.21^{p} ±0.17
60	0.76^{q} ±0.23	2.87^{r} ±0.64	5.34^{s} ±0.08	5.40^{s} ±0.41	5.27^{s} ±0.03	5.31^{s} ±0.13

Values across rows bearing different superscripts differ significantly at $P<0.001$

In terms of percentage reduction in *L. monocytogenes* population (Table 8), when knives were pre-rinsed, a reduction of 99.9% was achieved by immersion for 5s or more at 80°C or above, 20s or more at 70°C or above, and 45s or more at 65°C. Immersion at 60°C did achieve 99.5% reduction at 45s immersion, but only 80.9% reduction with 60s immersion.

When the outlying variable of 1s immersion was ignored, there was a good positive correlation between immersion time and mean log reduction at temperatures of 65°C and above ($P<0.05$).

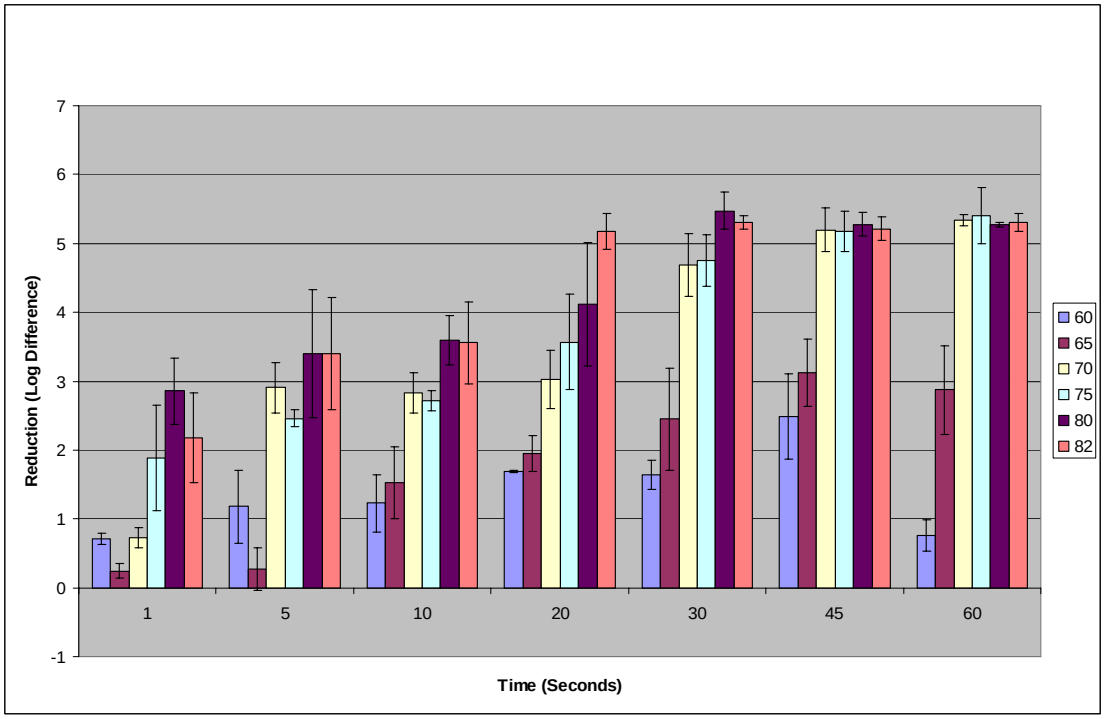


Figure 4: Mean log reduction in *Listeria monocytogenes* population on knife blades following immersion for different periods of time in water at different temperatures (°C) after a pre-rinse at 40°C

Table 8: Percentage reduction in *Listeria monocytogenes* population on knife blades following immersion for different periods of time in water at different temperatures after a pre-rinse at 40°C

Immersion time (sec)	Temperature (°C)					
	60	65	70	75	80	82
1	80.1	42.1	80.4	96.7	99.8	98.8
5	89.8	37.9	99.8	99.6	99.9	99.9
10	92.2	95.8	99.8	99.8	99.96	99.94
20	98.0	98.7	99.9	99.93	99.98	>99.99
30	97.5	99.2	>99.99	>99.99	>99.99	>99.99
45	99.5	99.9	>99.99	>99.99	>99.99	>99.99
60	80.9	99.8	>99.99	>99.99	>99.99	>99.99

4.5 Analysis of time:temperature curves

When the mean log reductions are plotted against time, there is an initial rapid increase in log reduction as time increases, and this begins to level off to a plateau (Figures 5 to8). The plateau stage occurs earlier at low temperatures (60°C) and high temperatures (80 and 82°C) than at intermediate temperatures (65-75°C), although the height of the plateau is markedly different. Logarithmic lines of best fit

were applied to these plots (Table 9), and used to predict the time in seconds required to achieve log reductions of 1 to 5 in *E. coli* (Table 10) and *L. monocytogenes* (Table 11) populations. In Tables 10 and 11, predicted immersion times of less than 1s are highlighted in yellow, and the times required for a 3 log reduction in count highlighted in blue.

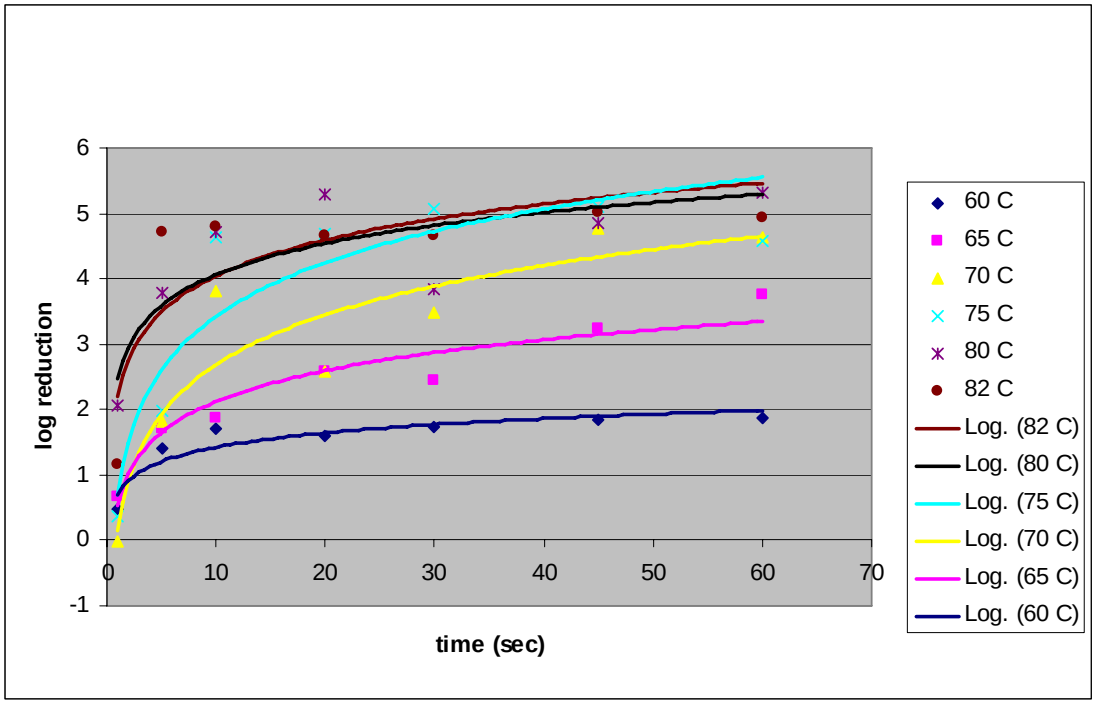


Figure 5: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperatures

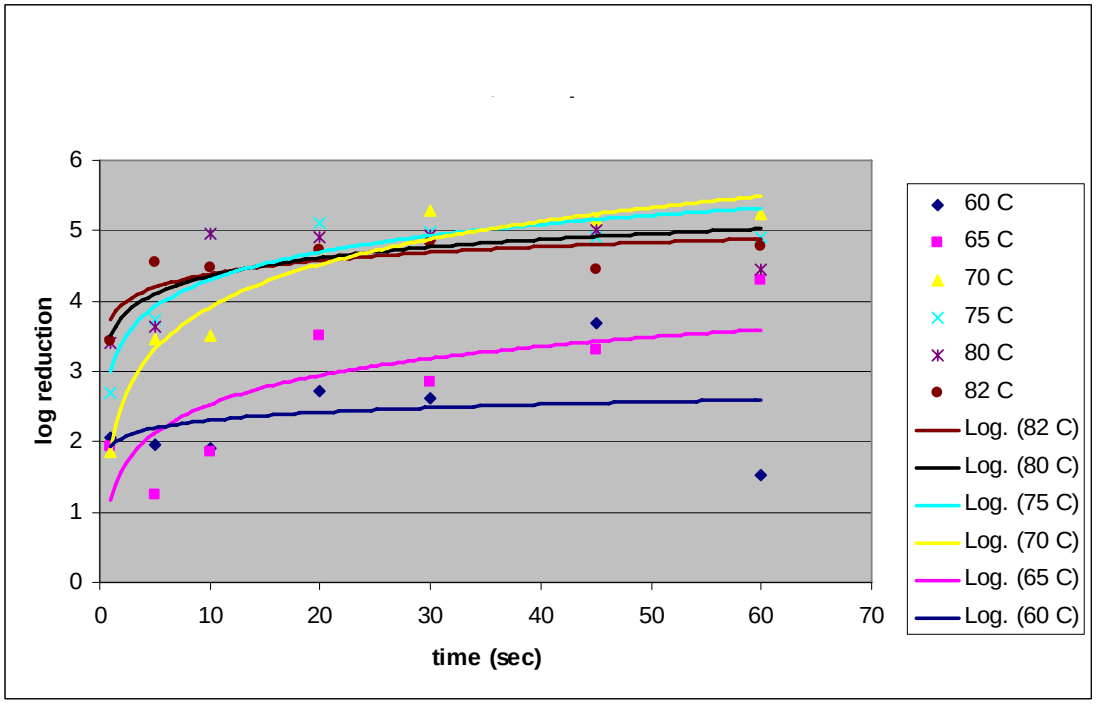


Figure 6: Mean log reduction in *Escherichia coli* population on knife blades following immersion for different periods of time in water at different temperature after a pre-rinse at 40°C

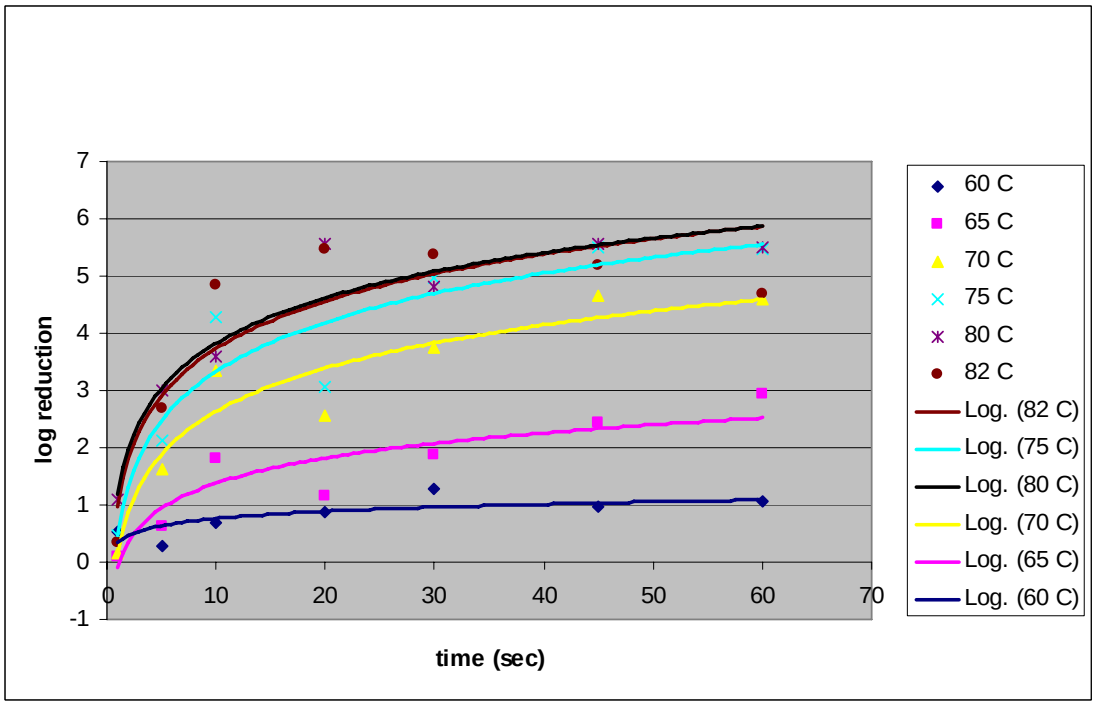


Figure 7: Mean log reduction in *Listeria monocytogenes* population on knife blades following immersion for different periods of time in water at different temperatures

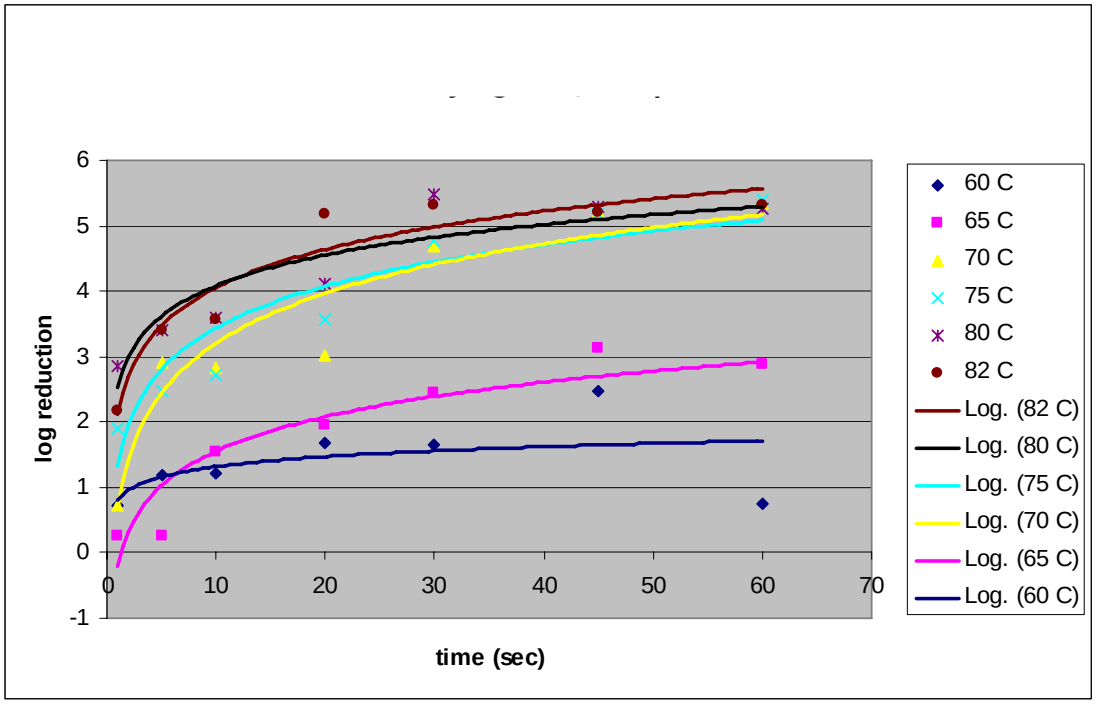


Figure 8: Mean log reduction in *Listeria monocytogenes* population on knife blades following immersion for different periods of time in water at different temperatures after a pre-rinse at 40°C

Table 9: Equations of lines of best fit, log reduction plotted against time

	Equation(x = time; y = log reduction)	
Treatment	**E. coli**	**L. monocytogenes**
60°C	y = 0.3165Ln(x) + 0.6938	y = 0.1817Ln(x) + 0.3432
65°C	y = 0.685Ln(x) + 0.5424	y = 0.6365Ln(x) - 0.0887
70°C	y = 1.0997Ln(x) + 0.1493	y = 1.0928Ln(x) + 0.1143
75°C	y = 1.1882Ln(x) + 0.6862	y = 1.2431Ln(x) + 0.4661
80°C	y = 0.688Ln(x) + 2.4773	y = 1.1455Ln(x) + 1.183
82°C	y = 956Ln(x) + 2.2089	y = 1.1938Ln(x) + 0.9802
Rinse + 60°C	y = 0.1622Ln(x) + 1.9322	y = 0.2208Ln(x) + 0.8085
Rinse + 65°C	y = 0.5926Ln(x) + 1.1699	y = 0.764Ln(x) - 0.2088
Rinse + 70°C	y = 0.8788Ln(x) + 1.8909	y = 1.1024Ln(x) + 0.6607
Rinse + 75°C	y = 0.5685Ln(x) + 2.9955	y = 0.9214Ln(x) + 1.3144
Rinse + 80°C	y = 0.373Ln(x) + 3.4982	y = 0.6769Ln(x) + 2.5233
Rinse + 82°C	y = 0.2789Ln(x) + 3.743	y = 0.8455Ln(x) + 2.1062

4.6 Surface-response modelling

Polynomial regression of the log reduction data gave the equations shown in Table 12 and the fitted plots shown in Figures 9 to 12. In all cases the 1 second data point was an outlier, so the regression was repeated omitting these data points. This resulted in the equations and plots illustrated in Table 13 and Figures 13 to 26. When these equations are used to predict log reductions, the equations derived from the entire data set (Table 12) predict a slightly lower log reduction (between 0.1 and 0.7 log) than the equivalent equations derived omitting the 1 second data points (Table 13). With regard to *E. coli*, equations including the rinse step predict 0.5-0.9 log greater reductions than those without the rinse step whereas for *L. monocytogenes*, only a 0.3 log difference in reduction was seen, and only in the equations derived from the complete data set. Where knives were not rinsed, equations for *L. monocytogenes* predicted 0.2 log less reduction than equations for *E. coli*. Where knives were rinsed, there was a greater impact on *E. coli* numbers than on *L. monocytogenes* numbers. With pre-rinse, reductions in *E. coli* counts were predicted to be 0.9 log greater than reductions in *L. monocytogenes* counts.

Table 10: Prediction of time (seconds) of immersion required to achieve a particular log reduction in *E. coli* population (based on log regression)

E. coli		**Immersion Temperature**											
required log reduction		60	65	70	75	80	82	rinse + 60	rinse + 65	rinse + 70	rinse + 75	rinse + 80	rinse + 82
	0	0.11	0.45	0.87	0.56	0.03	0.06	0.00	0.14	0.12	0.01	0.00	0.00
	0.5	0.54	0.94	1.38	0.85	0.06	0.12	0.00	0.32	0.21	0.01	0.00	0.00
	1	2.63	1.95	2.17	1.30	0.12	0.22	0.00	0.75	0.36	0.03	0.00	0.00
	1.5	12.77	4.05	3.42	1.98	0.24	0.41	0.07	1.75	0.64	0.07	0.00	0.00
	2	61.99	8.40	5.38	3.02	0.50	0.77	1.52	4.06	1.13	0.17	0.02	0.00
	2.5	300.90	17.42	8.48	4.60	1.03	1.44	33.14	9.44	2.00	0.42	0.07	0.01
	3	1460.56	36.15	13.36	7.01	2.14	2.70	722.87	21.94	3.53	1.01	0.26	0.07
	3.5	7089.36	75.00	21.05	10.68	4.42	5.07	15769.68	51.01	6.24	2.43	1.00	0.42
	4	34410.93	155.62	33.17	16.26	9.15	9.50	344021.24	118.60	11.02	5.85	3.84	2.51
	4.5	167026.60	322.91	52.26	24.77	18.92	17.81	7504945.41	275.74	19.47	14.10	14.67	15.09
	5	810727.44	670.01	82.35	37.73	39.12	33.39	163723044.70	641.12	34.39	33.99	56.05	90.65

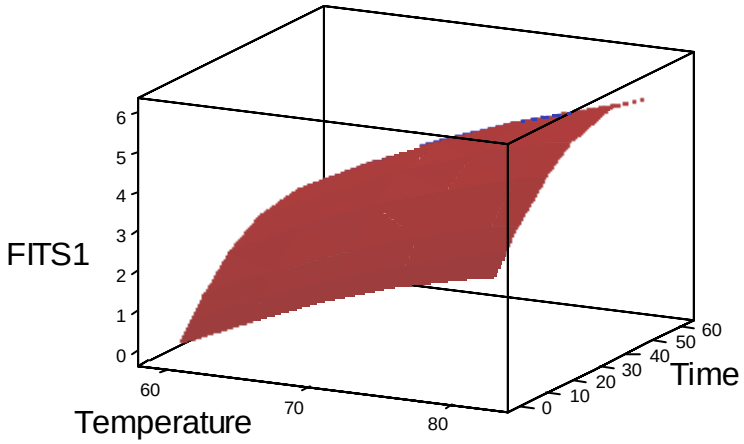
Table 11: Prediction of time (seconds) of immersion required to achieve a particular log reduction in *L. monocytogenes* population (based on log regression)

L. monocytogenes		**Immersion Temperature**											
required reduction	log	60	65	70	75	80	82	rinse + 60	rinse + 65	rinse + 70	rinse + 75	rinse + 80	rinse + 82
	0	0.15	1.15	0.90	0.69	0.36	0.44	0.03	1.31	0.55	0.24	0.02	0.08
	0.5	2.37	2.52	1.42	1.03	0.55	0.67	0.25	2.53	0.86	0.41	0.05	0.15
	1	37.14	5.53	2.25	1.54	0.85	1.02	2.38	4.87	1.36	0.71	0.11	0.27
	1.5	582.04	12.13	3.55	2.30	1.32	1.55	22.92	9.36	2.14	1.22	0.22	0.49
	2	9120.93	26.62	5.62	3.43	2.04	2.35	220.59	18.01	3.37	2.10	0.46	0.88
	2.5	142930.76	58.39	8.87	5.14	3.16	3.57	2123.41	34.66	5.30	3.62	0.97	1.59
	3	2239815.91	128.08	14.02	7.68	4.89	5.43	20440.46	66.69	8.35	6.23	2.02	2.88
	3.5	35099340.55	280.95	22.16	11.48	7.56	8.25	196764.82	128.31	13.14	10.72	4.23	5.20
	4	550029000.52	616.29	35.01	17.16	11.70	12.55	1894105.49	246.88	20.68	18.44	8.86	9.39
	4.5	8619304428.76	1351.90	55.33	25.66	18.10	19.08	18233114.92	475.02	32.55	31.73	18.55	16.97
	5	135069984974.58	2965.54	87.43	38.37	28.00	29.00	175516349.07	913.97	51.23	54.60	38.82	30.65

Table 12: Surface response models of each knife treatment

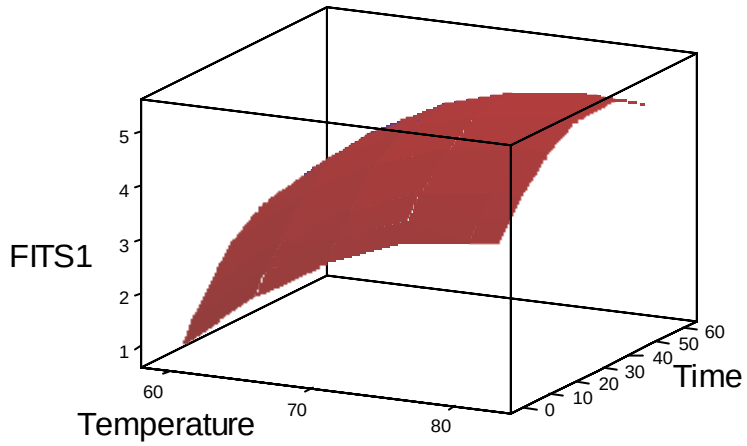
Organism	Treatment	Polynomial Regression Equation	Fitted plot
E. coli	No pre-rinse	$\boldsymbol{Z}$ = -19.2+0.0935$\boldsymbol{x}$+0.465$\boldsymbol{y}$-0.00140$\boldsymbol{x}^2$-0.00243$\boldsymbol{y}^2$+0.000427$\boldsymbol{xy}$	Figure 14
E. coli	With pre-rinse	$\boldsymbol{Z}$ = -34.6+0.127$\boldsymbol{x}$+0.939$\boldsymbol{y}$-0.00117$\boldsymbol{x}^2$-0.00579$\boldsymbol{y}^2$-0.000441xy	Figure 15
L. monocytogenes	No pre-rinse	$\boldsymbol{Z}$ = -31.5+0.0187$\boldsymbol{x}$+0.802$\boldsymbol{y}$-0.00167$\boldsymbol{x}^2$-0.00483$\boldsymbol{y}^2$+0.00182$\boldsymbol{xy}$	Figure 16
L. monocytogenes	With pre-rinse	$\boldsymbol{Z}$ = -27.2+0.0406$\boldsymbol{x}$+0.696$\boldsymbol{y}$-0.0013$\boldsymbol{x}^2$-0.00409$\boldsymbol{y}^2$+0.00116$\boldsymbol{xy}$	Figure 17

$\boldsymbol{Z}$: Log reduction; $\boldsymbol{x}$: time (sec); $\boldsymbol{y}$: temperature (°C)



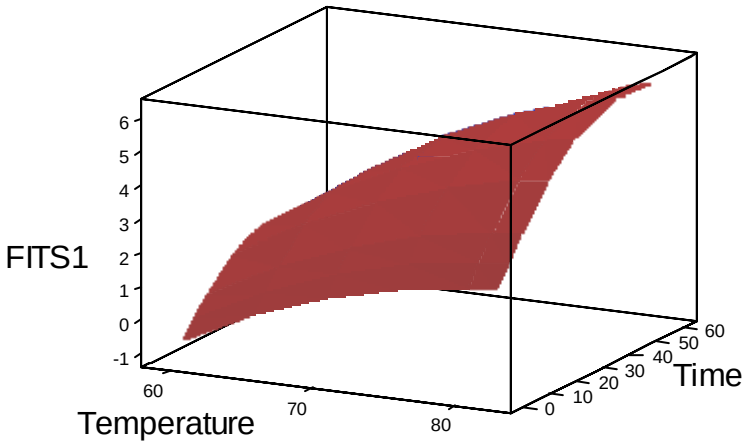
*FITS1: Fitted log reductions

Figure 9: Fitted plot of *E. coli* log reductions without pre-rinse at 40°C



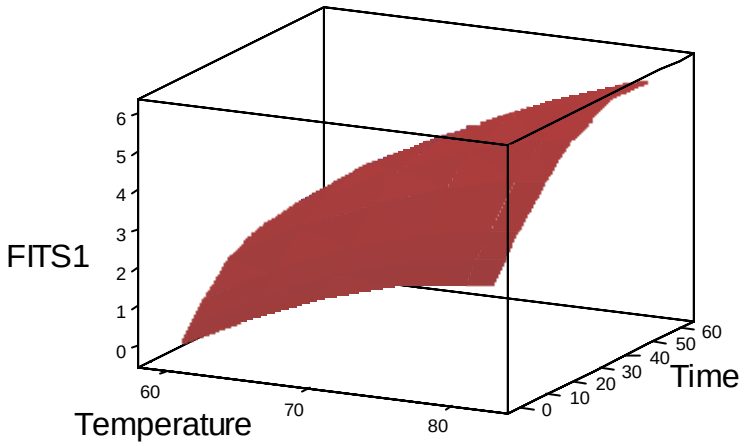
*FITS1: Fitted log reductions

Figure 10: Fitted plot of *E. coli* log reductions with pre-rinse at 40°C



*FITS1: Fitted log reductions

Figure 11: Fitted plot of *L. monocytogenes* log reductions without pre-rinse at 40°C



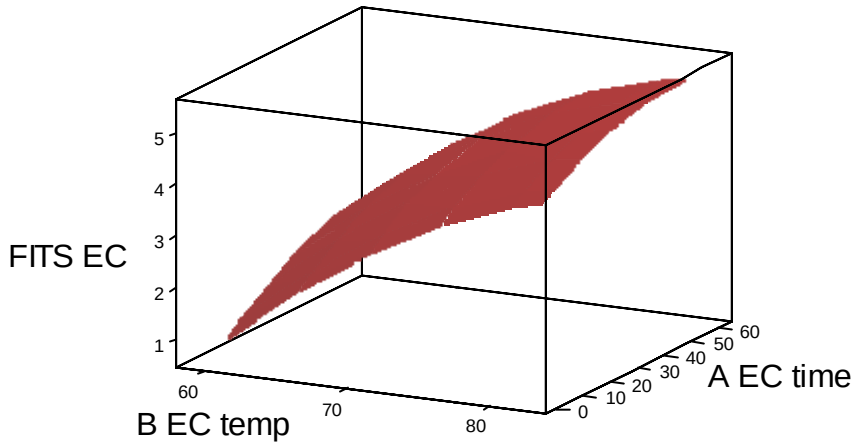
*FITS1: Fitted log reductions

Figure 12: Fitted plot of *L. monocytogenes* log reductions with pre-rinse at 40°C

Table 13: Surface response models of each knife treatment omitting 1s data points

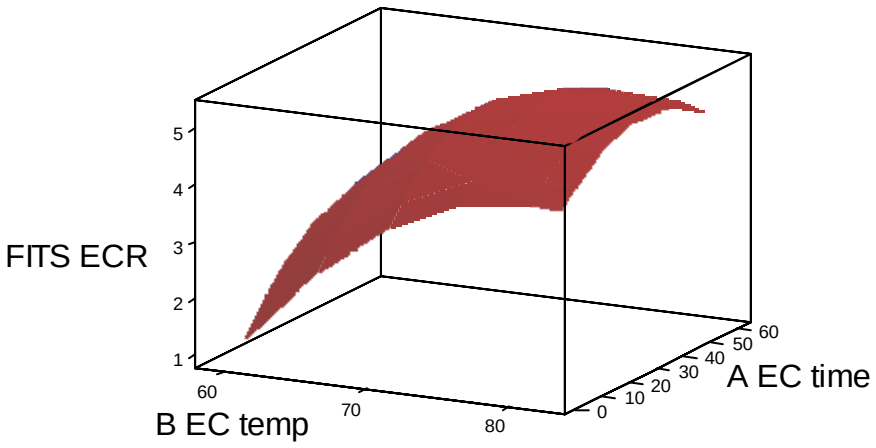
Organism	Treatment	Polynomial Regression Equation	Fitted plot
E. coli	No pre-rinse	$Z = -28.1+0.0791x+0.713y-0.000415x^2-0.00392y^2-0.000407xy$	Figure 18
E. coli	With pre-rinse	$Z = -45.4+0.148x+1.23y-0.000998x^2-0.00770y^2-0.000914xy$	Figure 19
L. monocytogenes	No pre-rinse	$Z = -40.6+0.0568x+1.03y-0.00102x^2-0.00605y^2+0.000608xy$	Figure 20
L. monocytogenes	With pre-rinse	$Z = -34.5+0.0376x+0.903y-0.00116x^2-0.00551y^2+0.00104xy$	Figure 21

Z: Log reduction; ***x***: time (sec); ***y***: temperature (°C)



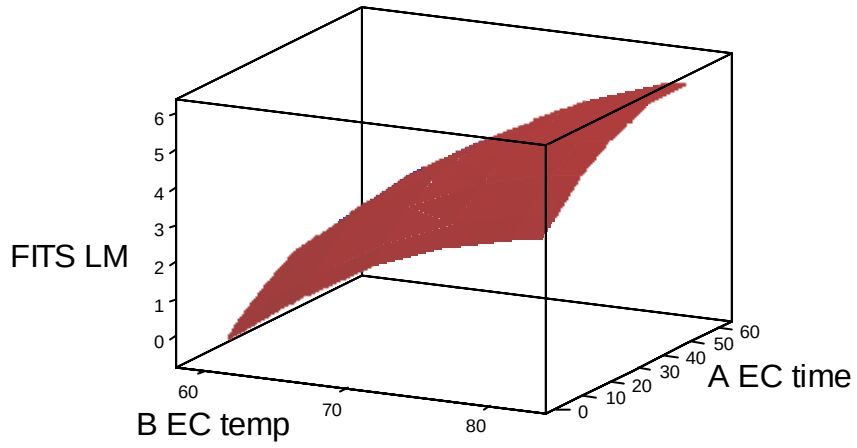
*FITSEC: Fitted log reductions; AEC time: time (s); BEC temp: temperature (°C)

Figure 13: Fitted plot of *E. coli* log reductions without pre-rinse at 40°C omitting 1s data points



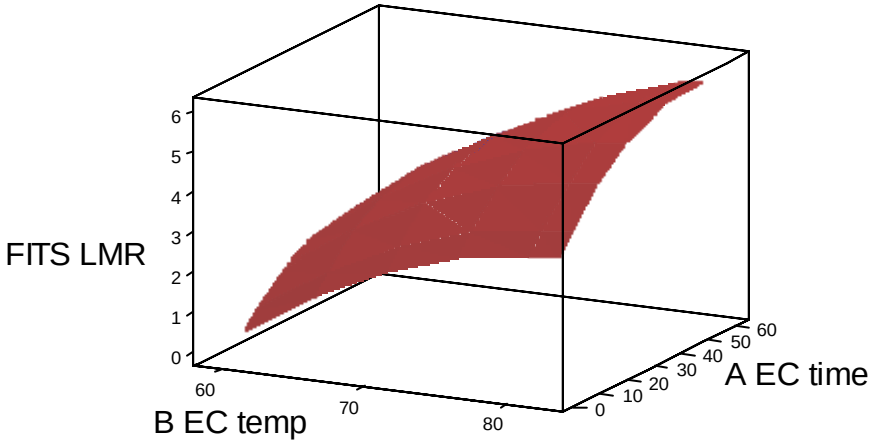
*FITSECR: Fitted log reductions; AEC time: time (s); BEC temp: temperature (°C)

Figure 14: Fitted plot of *E. coli* log reductions with pre-rinse at 40°C omitting 1s data points



*FITSLM: Fitted log reductions; AEC time: time (s); BEC temp: temperature (°C)

Figure 15: Fitted plot of *l. monocytogenes* log reductions with pre-rinse at 40°C omitting 1s data points



*FITSEC: Fitted log reductions; AEC time: time (s); BEC temp: temperature (°C)

Figure 16: Fitted plot of *l. monocytogenes* log reductions with pre-rinse at 40°C omitting 1s data points

4.7 Thermal effects at knife blade

At all temperatures, there was an initial rapid rise in blade temperature (Figure 17). Within the first second the blade temperature rose to a level of 72% of the final blade temperature. The temperature rise then progressively slowed. In all cases, the maximum blade temperature was reached after 8s, and this temperature was approximately 0.5°C cooler than the water temperature. The temperature of the knife handle rose more slowly, and reached its plateau, which was again 0.5°C lower than that of the blade, after 20s.

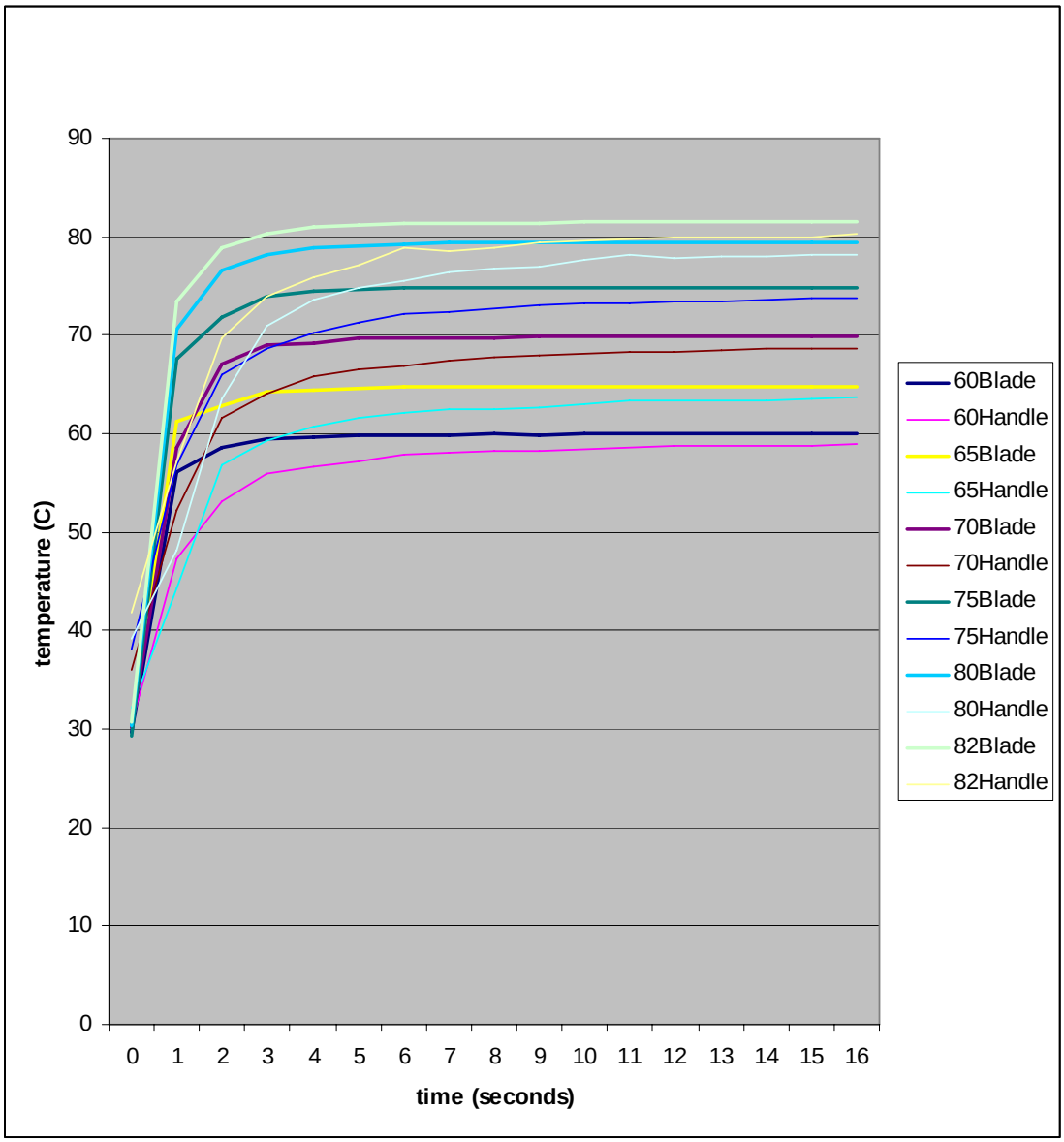


Figure 17: Rise in temperature of knife blade and handle following immersion in water at different temperatures

4.8 Summary of results

- Immersion of a knife for 1s at any temperature in the range 60 to 82°C, without a pre-rinse phase, is an ineffective intervention for microbial reduction.
- Without pre-rinse, 80 and 82°C can achieve a 3 log reduction in *E. coli* population or a 2 log reduction in *L. monocytogenes* population when knives are immersed for 5s.
- Without pre-rinse, 75°C will achieve a 3 log reduction in *E. coli* and *L. monocytogenes* population when 10s immersion is used, 70°C will achieve this after 20s immersion.
- Without pre-rinse, 60°C does not achieve more than a 2 log reduction even if the immersion time is extended to 60s. A temperature of 65°C achieves a 2 log reduction in *E. coli* after 20s, and in *L. monocytogenes* after 45s.
- When a pre-rinse is applied, 80 and 82°C can achieve a 3 log reduction in *E. coli* population or a 2 log reduction in *L. monocytogenes* population with a 1s immersion.
- With a pre-rinse, immersion at temperatures of 70°C or above will achieve a 3 log reduction in *E. coli* population after 5s immersion, and in *L. monocytogenes* after 20s.
- Even with pre-rinse, 60°C does not achieve a 3 log reduction in *L. monocytogenes* population, even with extended immersion times of 60s, although a 3 log reduction in *E. coli* was evident at 45s, but not at 60s. This was probably an artefact of the study, caused by very good performance of the pre-rinse rather than the immersion.
- With a pre-rinse, 65°C can achieve a 3 log reduction in *E. coli* population after 45s. A 3 log reduction in *E. coli* and *L. monocytogenes* was also seen at 20s and 45s, respectively, at 65°C. These again are probably artefacts of the study.

5.0 DISCUSSION

The results of this study are consistent with those of previous studies both in Australia and in the USA (Midgely and Eustace 2003; Eustace et al. 2007; Taormina and Dorsa 2007). Specifically, the finding that a 1s “brief dip treatment of contaminated knives has limited efficacy” (Taormina and Dorsa 2007) is confirmed by

absence of consistent and effective decreases at any temperature, with or without a pre-rinse treatment. Reductions in numbers of bacteria at these immersion times are frequently less than 1 log. This finding is important from a practical perspective since it is well established that immersion times in processing facilities are frequently 1s or less, and the contamination on knives frequently exceeds 1 log per unit area (Midgely and Eustace 2003; Eustace et al. 2007). Data form the present study also indicated that longer immersion times at lower temperatures can achieve equivalent reductions in numbers to momentary immersion at higher temperatures. This finding is not surprising and is the basis of the alternative procedures suggested in previous studies (Midgely and Eustace 2003; Eustace et al. 2007; Taormina and Dorsa 2007). What is clear though, is that the time: temperature combination of this treatment is critical and an equivalent reduction is only achievable by adhering to a given set of criteria. Under practical conditions in a processing facility such criteria may be difficult to enforce and chain speed may make some options unviable.

To achieve an effective performance criterion of greater than 3 log reduction on knives as suggested by Midgley and Eustace (2003) is more difficult still, in practical terms, than achieving the equivalent reduction discussed above. Specifically, the two knife system suggested by Midgley and Eustace (2003) with immersion times at 60^{o}C for up to 30s would be ineffective for this purpose even with a pre-rinse. A 3 log reduction is possible at higher temperatures and immersion times, but these time:temperature combinations would need to be carefully monitored to consistently result in the required performance. It was apparent from this aspect of the study that the pre-rinse step is a useful one and significantly reduces the stringency of the subsequent time:temperatures required to produce a effective decrease. As such a pre-rinse should be implemented where possible to increase the general hygienic status of knives in slaughter facilities.

In the present study only one strain of two different bacterial species grown under one set of conditions were used. As with other studies of this type the limited use of strains and growth conditions results in some issues which should be considered. It is not clear, for example, that all strains of *E. coli* have the same resistance to heat as the strains used here (Benito et al. 1999). Similarly, strains that are grown to stationary phase may not have the same heat resistance as strains in the logarithmic phase, a feature which has been known for some time (Elliker and Frazier 1983). Overall, however, the patterns observed and the similarity of the

results to previous studies with naturally contaminated knives (Eustace et al. 2007) indicates that the results are likely to be representative.

It was apparent from this study that *E. coli* and *L. monocytogenes* have different resistances to heat. This too is an expected result as *L. monocytogenes* was included in this study as a more resistant bacterial indicator of heat sensitivity than *E. coli* (Doyle et al. 2001), and not because it is an issue in primary meat processing. It is apparent that the use of *E. coli* alone is not representative of the behaviour of all bacteria of concern as a difference of 1-2 log reduction was observed between the two species at some time:temperature combinations. The use of aerobic plate counts under natural conditions (Eustace et al. 2007) or a range of bacteria including spore formers (Taormina and Dorsa 2007) may be a better option.

The simple models developed in this study may be useful to establish suitable and practical time:temperature combinations for use in individual processing plants. The models were not thoroughly investigated or validated in the present study as their development was not officially part of the project. They were included as an addendum to the work to further analyse the data and present it in a way that may be more useful to industry. Examination of the data and the models overall indicate that this study fulfilled its aim of providing statistically valid information on the effect of a wide range of time:temperature combinations on bacterial survival on knives.

6.0 CONCLUSIONS

- Dipping knives in water for shorter times at higher temperatures or longer times at lower temperatures can produce equivalent reductions of bacteria.
- Pre-rinsing knives at 40^{o}C increases the performance of the subsequent heat treatment step.
- The reductions in bacterial numbers reported here are consistent with those in previous laboratory and processing plant-based studies.
- Models produced from the data in this study can be used to predict suitable time/temperature combinations to achieve a desired bacterial reduction.
- With current industry practice, it would not be easy to achieve a reliable >3 log reduction performance criterion.

7.0 RECOMMENDATIONS

- Models developed in this study should be used to establish alternative time/temperature combinations which will result in equivalent reductions to current industry practice where appropriate.
- The feasibility of a >3 log reduction criteria needs to be revaluated in the light of the findings of this study.

8.0 FURTHER WORK

- A number of models have been developed from the data and presented in this study. If they are to be applied in the industry an appropriate model/s needs to be selected. While some are clear candidates, models selected will need to be validated under laboratory and possibly processing facility conditions before they could be used.
- As indicated above, this and other studies have demonstrated that short time/ high temperature or long time/low temperature dipping in water can produce equivalent reductions of bacteria on knife blades to each other. However, under practical conditions in slaughter facilities the time/temperature combinations required to produce an effective 3 log or greater reduction are unlikely to consistently occur. How well bacteria transfer onto knives from carcasses and vice versa is unknown. The question therefore remains as to the significance of knives in the contamination of carcasses? Specifically, whether the application of strict time/temperature dipping regimes impact on carcass hygiene is not clear. Further work investigating the role of transfer of bacteria to and from carcasses and knives on hygiene could clarify this issue.

9.0 REFERENCES

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