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food and drink innovation

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Report on:

Assessment of the Microbiological Efficacy of Decontamination Technologies for Treating Frozen Raspberries Using Aqualution Technology

Work performed by Campden BRI (Chipping Campden) Limited
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1. INTRODUCTION

This report describes the use of a decontamination treatment for inactivation of bacteria and viruses on fresh raspberries. The Technology that was assessed in this trial is a hypochlorous acid dry misting system produced by Aqualution Systems Ltd. The project was done to provide evidence for Marks and Spencer plc, the dry misting approach would be a suitable intervention strategy to minimise microbial risks on raw fruit used as an ingredient in their chilled deserts.

Marks and Spencer plc. chose raspberries as the target for this trial as a "worst case scenario" for decontamination, due to their large irregular surface and plug. If the decontamination approaches worked with raspberries, the method would be moved to other berry types.

The raspberries currently used by Marks and Spencer plc are grown in Poland or Chile and due to quality reasons are frozen directly after harvest without the application of any decontamination strategy. The frozen raspberries are then used as an ingredient in desserts without further treatment. The study tested the application of the Aqualution dry mist to the raspberries whilst in a frozen state to evaluate the effect of treatment immediately before use in the final product. In addition, the application of the Aqualution dry mist to fresh raspberries before freezing was also tested.

This protocol was designed to assess the efficacy of Aqualution dry mist on *E. coli*, *Salmonella* and *Listeria monocytogenes* as bacterial pathogenic organisms, and against MS2 bacteriophage which is a well known surrogate for human viruses such as Norovirus and Hepatitis.

This protocol represents the current best practice in the evaluation of produce decontamination technologies. It follows the protocols developed over many years at Campden BRI (R&D report 209: A study to establish a standard produce decontamination protocol and evaluation of a selection of wash agents) and incorporates the principles of the Marks and Spencer plc's Code of Practice for the Decontamination of Prepared Produce.

The study was executed according to TES-MB-196 for challenge testing.

2. METHODS

2.1 Products

The products tested are shown below along with the sample code.

Table 1: Products

Clients product	Sample code	Sample receipt
Fresh raspberries BB 13/9/13	MB/130524/1-3 virus	12/9/13
Fresh raspberries	MB/130524/1-3 bacterial	16/9/13
Frozen British raspberries M&S BB end 12/2014	MB/130524/0 min, 1 min, 3 min, 5 min	17/9/13

The samples were in satisfactory condition.

All samples were labelled with appropriate sample code.

Samples were stored chilled and frozen conditions prior to testing.

Testing was carried out between 17/09/13 and 24/09/13.

2.2 Organisms

The following microbial strains were used in this trial

Organism	Culture code
Escherichia coli O157 non toxigenic	CRA 16039 CRA 16040 CRA 16244
MS2 *E.coli* bacteriophage *Escherichia coli* K-12	ATCC 15597-B1 ATCC 12435
Listeria monocytogenes	CRA 1177 CRA 1101 CRA 1102
Salmonella Typhimurium	CRA 5452 CRA 1092 CRA 16305

The *Listeria* was grown in Tryptone Soya Broth (TSB) at 30^{o}C overnight (up to 24h) and the *Salmonella* and *E. coli* O157 were grown in Nutrient Broth (NB) at 37^{o}C overnight (up to 24h), the three cultures for each organism were combined and centrifuged to concentrate the levels. A stock solution of the MS2 phage was produced and kept frozen.

2.3 Sample Inoculation

The viral and bacterial trials were carried out separately. A repeat vial trial using chilled and frozen raspberries was carried out on a separate occasion.

Samples were inoculated in accordance with TES-MB-196.

For the bacterial trial, triplicate 500g samples of fresh raspberries were inoculated with 1.5ml of each of the concentrated cultures, (*Salmonella, Listeria and E. coli* O157) to give a target level of 10^5 - 10^6 cfu per gram.

For the viral trial, triplicate 500g samples of fresh raspberries were inoculated with 5 ml of diluted MS2 bacteriophage stock solution to give a target level of 10^5 - 10^6 pfu (plaque forming units) per gram.

For the repeat viral trial, six 200g samples of fresh raspberries were inoculated with 2 ml of diluted MS2 bacteriophage stock solution to give a target level of 10^5 - 10^6 pfu (plaque forming units) per gram.

Note: the final level in the samples could be up to 1 log unit above or below the target level although usually it will be within 0.5log units.

2.4 Storage conditions

For the bacterial trial, after inoculation the samples were stored at 5°C ± 1°C overnight and then each 500g batch was dispensed into 5 x 100g amount and stored at -18°C ± 1 °C for 4 to 6 hours.

For the viral trial, after inoculation the samples were stored at 5°C ± 1°C for 2 hours and then each 500g batch was dispensed into 5 x 100g amount and stored at -18°C ± 1 °C for 6 days.

For the repeat viral trial, post inoculation the samples were stored at 5°C ± 1°C for 2 hours and then 3 of the 6 x 200g samples were transferred and stored at -18°C ± 1 °C overnight, the remaining 3 x 200g samples remained at 5°C ± 1°C for the overnight. Each of the 200g samples was then dispensed into 2 x 100g amounts, 1 x 100g for the non-treated controls and 1 x 100g to undergo the decontamination procedure.

Following storage the decontamination procedure was carried out in the same way for the bacterial and first viral trial and then adapted for the repeat viral trial. For the first trials this was carried out in the Campden BRI aerobiology laboratory. For each treatment a model treatment tunnel was prepared by cutting vents into a large stomacher bag, the raspberries to be treated were placed into the bag and the opening to the bag was held over the vent to the dry fogging system. The solution of 140ppm hypochlorus acid was applied to the produce via the fogging system which applied 8 microns of the solution, for the duration of the treatment time. Triplicate batches of raspberries were treated for each time.

The decontamination procedure was also carried out on frozen non inoculated raspberries for 0, 1, 3 and 5 minutes, the samples were kept and stored at 5°C ± 1°C for 7 days and checked for visual and odour signs of deterioration.

For both trials, and for each batch of product, triplicate samples were tested after 0, 1, 3 and 5 minutes of treatment. For batch 3 of the viral trial triplicate samples were also tested after 10 minutes of treatment.

Total number of samples tested was 36 for the bacterial trial, and 39 for the viral trial.

For the repeat viral trial the decontamination procedure was carried out using a pilot-scale 250l produce washer, which has a class II safety cabinet attached. For each batch of the frozen and chilled raspberries 1 x 100g was placed into a clean basket which was transferred into the produce washer. The vent of the dry fogging system was replaced with a flexible hose, with the opening placed into the produce washer this was then sealed using polyethene autoclave bags, tape, and elastic bands. The solution of 140ppm hypochlorus acid was applied to the produce via the fogging system as before for 5 minutes. Triplicate batches of chilled and frozen raspberries were treated. The batches of raspberries were weighed pre and post treatment and the weights recorded.

The repeat decontamination procedure was also carried out on chilled non inoculated raspberries for 5 minutes. The samples were stored 5°C ± 1°C for 7 days and checked for visual and odour signs of deterioration.

The total number of samples tested for the repeat trial was 36.

2.5.1 Microbiological/chemical analysis

Samples of product for the bacterial trial were evaluated for levels of *Salmonella, Listeria* and *E. coli* O157 and samples of product for the viral trials were evaluated for levels of MS2 using the following methods:

Table 2: Microbiological tests

Organism	Test method	Method Summary*
Salmonella enumeration	TES-MB-201	Spread plate with XLD. Incubation at 37°C for 24h
Listeria enumeration	TES-MB-186	Spread plate with LCA. Incubation at 37°C for 48h
E. coli 0157 enumeration	TES-MB-224	Spread plate with CT-SMAC 37°C for 18-24h
MS2 enumeration	Plaque assay (Dawson *et al.* 2005) amended**	Plaque assay with NZCYM 37°C for 24h

* full details of tolerances on method time and temperatures are given in the full methods.
** the plaque assay followed the method quoted, with the following amendment, a 0.2% Maltose supplement was added to the NZCYM top agar.

3. RESULTS

3.1 Study Data

The log reductions for each batch are calculated by working out the mean of the triplicate 0 minute results and transforming this value to log cfu/g. Each of the individual after treatment results is then subtracted from this mean to give the log reduction. The mean of each of these reductions is then calculated. A summary of the mean log reductions and the mean 0 minute levels is given in Table 3.

The same information is given for the repeat virus trial in Table 4. Graphs showing the mean levels of organisms on the products before and after washing are shown in Figures: 1 to 6. The full result and log reductions are shown in Tables 6 to 19 in the Appendices. The sensory results for the first and second trials are given in Tables 20 and 21 in the Appendices respectively. The data showing the product weight pre and post treatment in the repeat trial can be found in the Appendices (Table 22).

As can be seen in Table 3, the mean log reductions achieved for *Salmonella* ranged from 0.3 to 2.8 log cfu, for *Listeria* the reductions ranged from 0.1 to 0.9 and for *E. coli* O157 (non toxigenic) the mean log reductions ranged from 0.2 to 2.3 log cfu. In the first viral trial no significant log reductions were observed with a range between of an increase of 1.5 logs cfu to a reduction of 1.3 logs. In the repeat viral trial (Table 4) the Mean log reductions observed for the frozen product were about 1.0 log cfu and 1.4 – 2.8 for the chilled raspberries.

Table 3: Log reductions achieved

Mean levels of bacteria and MS2 bacteriophage before treatment (log cfu/g) and mean log reduction (log cfu) after treatment for each batch of Raspberries

Batch	Treatment	*Salmonella*	*E. coli* O157	*Listeria*	MS2
A	Before	5.3	4.9	4.5	6.8
	1 min	1.3	0.5	0.1	1.0
	3 min	0.3	0.8	0.9	1.3
	5 min	1.3	0.9	0.7	1.3
B	Before	5.6	5.1	4.6	5.9
	1 min	0.4	0.2	0.5	0.0
	3 min	1.8	1.1	0.1	-1.5
	5 min	2.8	1.1	0.8	-0.8
C	Before	5.2	5.4	4.4	6.6
	1 min	1.6	1.7	0.3	0.3
	3 min	2.0	2.1	0.3	-0.6
	5 min	2.5	2.3	0.8	0.1

As can be seen in Figure 1 and Tables 6 – 8 the reduction of *Salmonella* after 1 minute of treatment was between 0.2 and 1.8 log with a standard deviation of 0.2 for each batch, 2 out of the 3 batches produced a mean log reduction of >1log (the target log reduction). For the 3 minute treatment the reduction of *Salmonella* was between an increase of 1.7 and a decrease of 2.2 logs, the increase of 1.7 is an anomalous result in comparison to the other results received and was from 1 sample in batch A, if this result is omitted then the 3 minute treatment would have shown a reduction of 1.3 to 2.2 logs and a standard deviation of 0.2 for each batch, 2 out of the 3 batches produced a mean log reduction of >1 log. After 5 minutes of treatment the reduction of *Salmonella* was between 0.8 and 3.1 (with standard deviations of between 0.3 and 0.5). All 3 of the 3 batches produced a mean log reduction of > 1log.

Figure 1: *Salmonella*

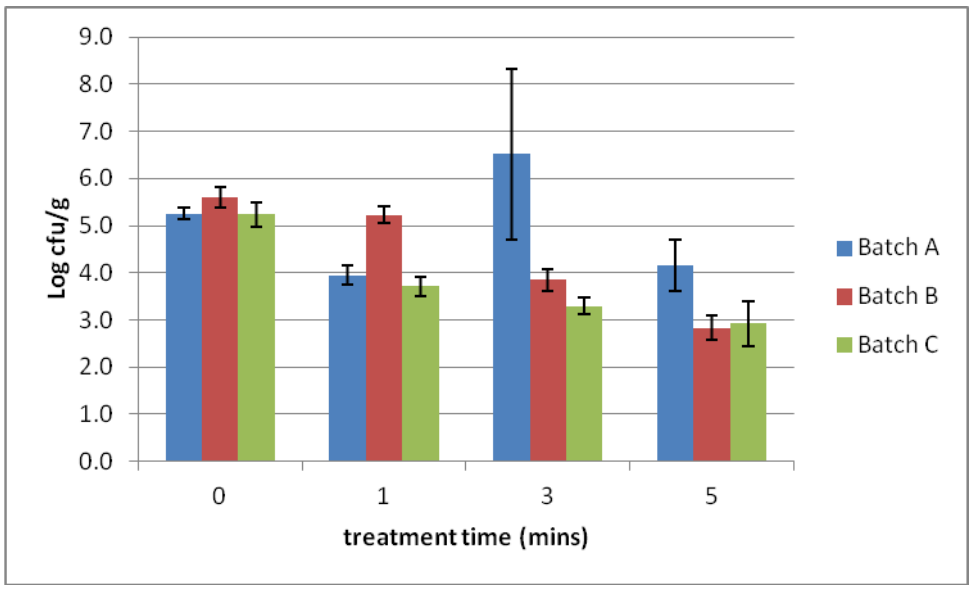
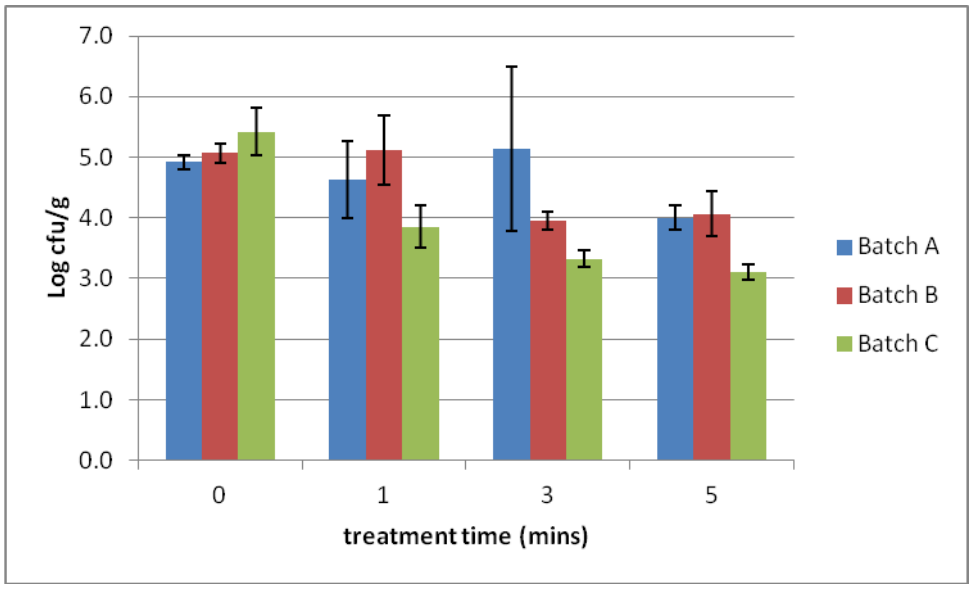


Figure 2 and Tables 9 to 11 show the *E. coli* O157 results, after 1 minute a reduction of between -0.4 and 2 logs was observed, with a standard deviation of 0.4 – 0.6, 1 out of the 3 batches obtained a greater than 1 mean log reduction. For the 3 minute treatment the reduction of *E. coli* was between an increase of 0.7 logs to a reduction of 2.2, again this was due to an anomalous result in batch A and was observed in the same sample, if this result was omitted then the 3 minute treatment would have shown a reduction of 1.0 to 2.2 logs and standard deviations of 0.1 – 0.5. 2 out of the 3 batches of produced a mean log reduction of >1 log. The 5 minute treatment provided a log reduction of between 0.7 and 2.5 with standard deviations between 0.1 and 0.4. 2 out of the 3 batches produced a mean log reduction of >1 log after 5 minutes.

Figure 2: *E. coli*



As can be seen in Figure 3 and Tables 12 to 14 the reduction of *Listeria* across all the batches and all the treatment times were very low, for 1 minutes an increase of 0.6 to a decrease of 0.7 was observed, an increase of 0.1 to a decrease of 1.1 log for 3 minutes treatment and an increase of 0.2 to a decrease of 1.5 logs was observed for the 5 minute treatment. None of batches for any of the treatment reached a mean of 1 log reduction.

Figure 3: *Listeria*

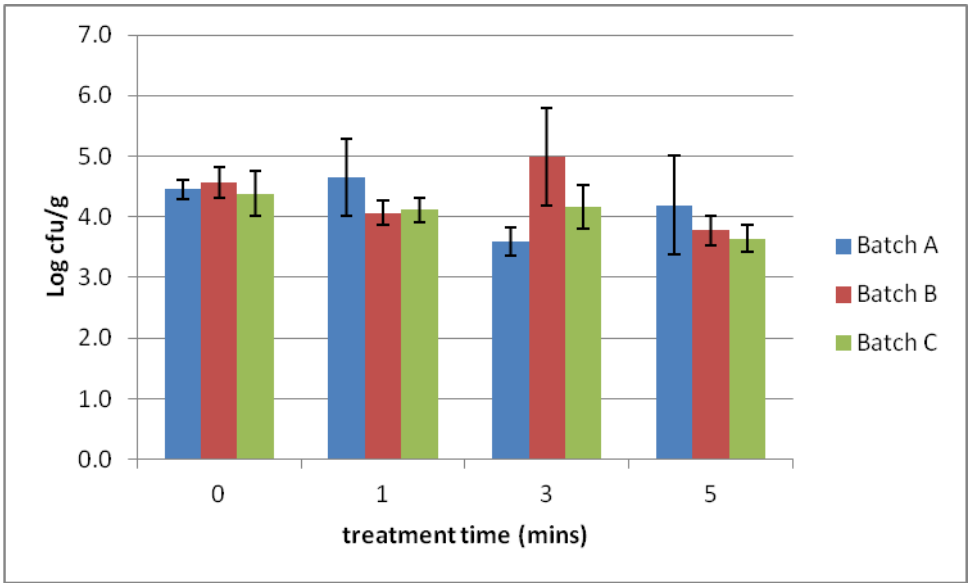


Figure 4 and Tables 15 to 17 provide the data for the MS2 bacteriophage, the full results show that there was a lot of variation in levels on all of the samples and the standard deviations observed are high it is probable that no true log reduction was observed. It was decided that it would better to repeat the experiment using frozen and chilled raspberries and to change the inoculation method so that a smaller amount of raspberries (200g rather than 500g) was to be inoculated and a higher proportion of the inoculated sample was to be sampled to generate the untreated results (0 minute), the treatment was done for 5 minutes. This was carried out as a repeat trial and the results are shown in Tables 4, 18 and 19 and also in Figures 5 and 6. The log reductions observed for the frozen product were 0.7 to 1.4 logs and -0.2 – 3.2 for the chilled raspberries. There is a high variation in results for batch B of the chilled product although all batches of both the chilled and frozen product gave a mean log reduction of ≥ 1 log.

Figure 4: MS2

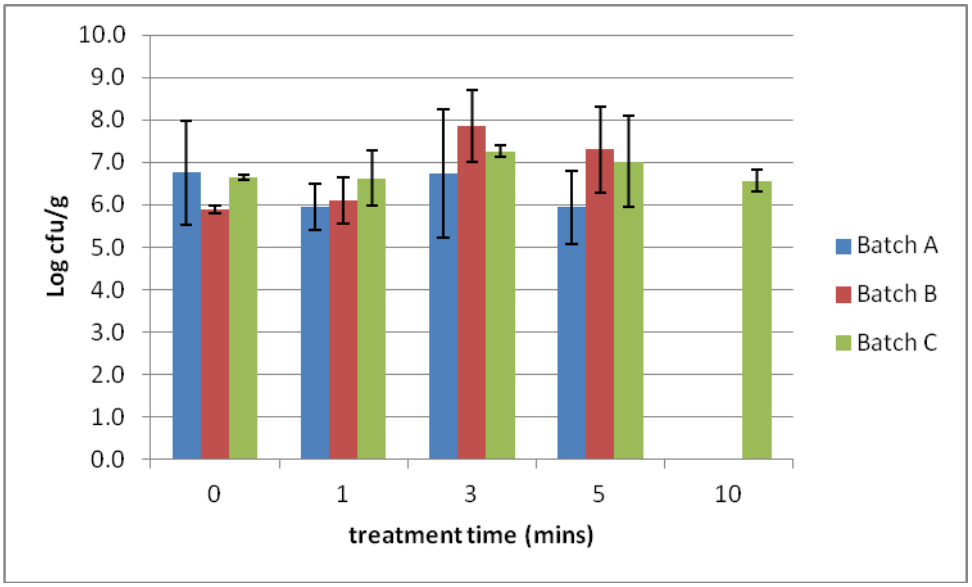


Table 4: Log reductions achieved – repeat trial

Summary table showing Mean levels MS2 bacteriophage before treatment and mean log reduction after treatment for each batch of Raspberries – repeat trial.

Product	Batch	Treatment	MS2
Chill	A	Before	6.5
		5 min	2.6
	B	Before	6.4
		5 min	1.4
	C	Before	6.2
		5 min	2.8
Frozen	A	Before	6.7
		5 min	1.1
	B	Before	6.7
		5 min	1.0
	C	Before	6.2
		5 min	1.1

Figure 5: MS2 repeat trial – chilled

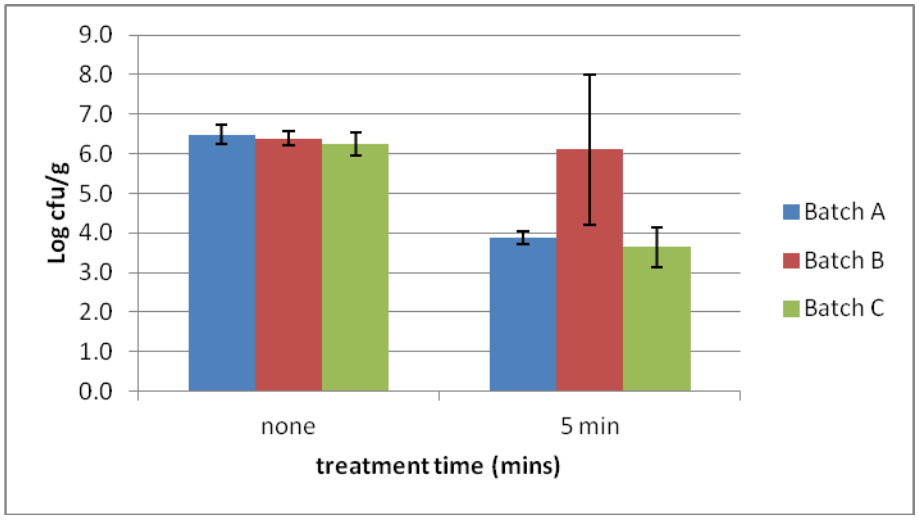
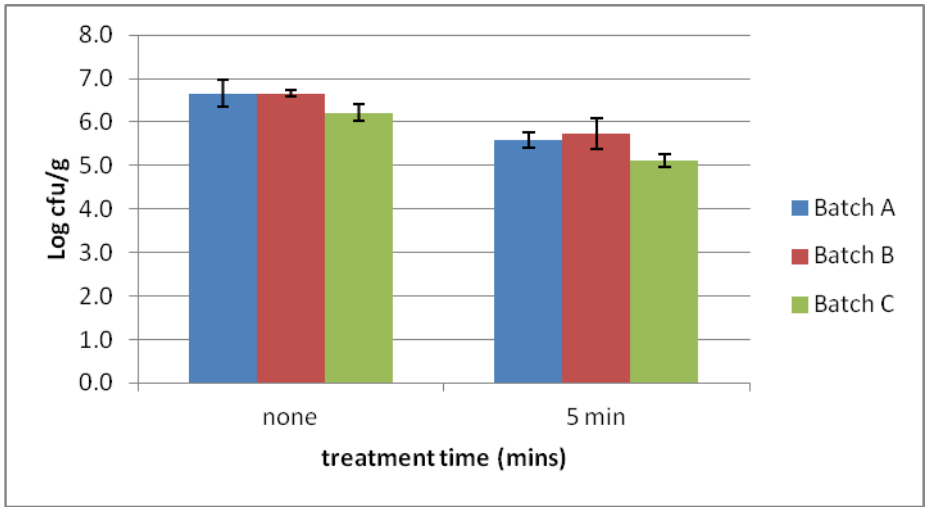


Figure 6: MS2 repeat trial – frozen



Tables 20 and 21 give the sensory data and this showed that the frozen product was slightly affected by all treatments with the product showing deterioration 1 day earlier than the untreated product. With the chilled product no significant difference was observed between the treated and untreated products.

4. CONCLUSION

The pre-determined criteria for this trial is a target log reduction of 1- 2 log of each of the test organisms. Table 5 given below shows the number of batches for each treatment reaching or exceeding the target reduction.

Table 5: Number of treatments obtaining target mean log reduction of a minimum of 1 log

Treatment time (mins)	Number of treatments obtaining target mean log reduction				
	Salmonella	*E. coli* O157	*Listeria*	MS2 batcteriophage, frozen product Trial 2	MS2 batcteriophage, frozen product Trial 2
1	2/3	1/3	0/3		
3	2/2*	2/2*	0/3		
5	3/3	2/3	0/3	3/3	3/3

Key = * Batch A omitted due to anomalous result with 1 sample.

Note: These results are valid for batches of product produced and stored under identical conditions. Any changes in product formulation or storage conditions may change the results expected to be obtained.

5. REFERENCE

Dawson, D.J. *et al.* (2004) Survival of Viruses on Fresh Produce using MS2 as a Surrogate for Norovirus. Journal of Applied Microbiology, 98, 203-209.

Appendix:

Glossary of Microbiological Media

Agar/broth	Full name	Media Manufacturers and Codes
BGAm	Brilliant Green Agar-modified	Oxoid CM0329 LabM LAB 034-A
CTSMAC	Cefixime Tellurite Sorbitol MacConkey Agar	Oxoid CM 813, SR 172
LCA	Listeria Chromogenic Agar (called ALOA in BS EN ISO 11290-2)	Oxoid CM1080B plus supplements SR0227E and SR0228E
MRD	Maximum Recovery Diluent	LabM LAB103 Oxoid CM0733
NA	Nutrient Agar	Oxoid CM0003
NB	Nutrient Broth	Oxoid CM0001
NZCYM broth	Recipe	
NZCYM B	Recipe	
NZCYM T	Recipe	
PCA	Plate Count Agar	LabM LAB149 Oxoid CM0325
SM Buffer	Recipe	
TSB	Tryptone Soy Broth	CM0129
XLD	Xylose Lysine Desoxycholate Agar	Oxoid CM 469 LabM LAB 032

Microbiological Media - Recipes

Agar/Broth	Ingredients	Method
NZCYM Broth	950 ml deionised H_2O 10g NZ amine (sigma C-0626) 5g NaCl (VWR 27810.295) 5g Bacto-yeast extract (Lab M mc1) 1g Casamino acids (Difco – 0230-01-1) 2g $MgS0_4$ 7 H_2O (BDH-1015144)	Shake until dissolved Adjust pH to 7.0 with 5M NaOH Adjust volume to 1 litre with deioised H_2O Autoclave 20mins @ 15 psi
NZCYM B	Recipe as for NZCYM broth But add 15g/litre bacto-agar (Lab M)	Shake until dissolved Adjust pH to 7.0 with 5M NaOH Adust volume to 1 litre with deioised H_2O Autoclave 20mins @ 15 psi
NZCYM T	Recipe as for NZCYM broth But add 7g/litre bacto-agar (Lab M)	Shake until dissolved Adjust pH to 7.0 with 5M NaOH Adust volume to 1 litre with deioised H_2O Autoclave 20mins @ 15 psi
SM buffer	5.8g NaCl (VWR 27810.295) 2g $MgSO_4$ $7H_20$ (BDH-101514y) 5 mls 2% Gelatin solution 50mls 1M Tris Cl (pH 7.5) (Sigma T1503)	Dissolve the NaCl and $MgSO_47H_2O$ in 800 ml of H_2O; add the Tris-Cl and gelatin; and adjust the volume to 1 litre with H_2O. Sterilize the buffer by autoclaving for 20 minutes at 15 psi.

Table 6: Batch A *Salmonella* results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	A	a	1.3E+05	5.1	
		b	2.1E+05	5.3	
		c	2.0E+05	5.3	
		Mean	**1.8E+05**	**5.3**	
		Stdev		0.1	
1 min	A1	a	5.9E+03	3.8	1.5
		b	6.8E+03	3.8	1.4
		c	1.4E+04	4.1	1.1
		Mean	**8.9E+03**	**3.9**	**1.3**
		Stdev		0.2	0.2
3 min	A2	a	1.0E+07	7.0	-1.7
		b	1.0E+04	4.0	1.3
		c	5.5E+03	3.7	1.5
		Mean	**3.3E+06**	**6.5**	**0.3**
		Stdev		1.8	1.8
5 min	A3	a	2.6E+03	3.4	1.8
		b	3.2E+04	4.5	0.8
		c	7.7E+03	3.9	1.4
		Mean	**1.4E+04**	**4.1**	**1.3**
		Stdev		0.5	0.5

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 7: Batch B *Salmonella* results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	B	a	2.7E+05	5.4	
		b	2.6E+05	5.4	
		c	6.4E+05	5.8	
		Mean	**3.9E+05**	**5.6**	
		Stdev		0.2	
1 min	B1	a	1.2E+05	5.1	0.5
		b	2.6E+05	5.4	0.2
		c	1.3E+05	5.1	0.5
		Mean	**1.7E+05**	**5.2**	**0.4**
		Stdev		0.2	0.2
3 min	B2	a	9.5E+03	4.0	1.6
		b	3.5E+03	3.5	2.0
		c	8.1E+03	3.9	1.7
		Mean	**7.0E+03**	**3.8**	**1.8**
		Stdev		0.2	0.2
5 min	B3	a	3.2E+02	2.5	3.1
		b	7.5E+02	2.9	2.7
		c	9.8E+02	3.0	2.6
		Mean	**6.8E+02**	**2.8**	**2.8**
		Stdev		0.3	0.3

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 8: Batch C *Salmonella* results

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	C	a	2.7E+05	5.4	
		b	8.2E+04	4.9	
		c	1.6E+05	5.2	
		Mean	**1.7E+05**	**5.2**	
		Stdev		0.3	
1 min	C1	a	6.0E+03	3.8	1.5
		b	6.6E+03	3.8	1.4
		c	2.7E+03	3.4	1.8
		Mean	**5.1E+03**	**3.7**	**1.6**
		Stdev		0.2	0.2
3 min	C2	a	1.2E+03	3.1	2.2
		b	1.9E+03	3.3	2.0
		c	2.8E+03	3.4	1.8
		Mean	**2.0E+03**	**3.3**	**2.0**
		Stdev		0.2	0.2
5 min	C3	a	1.8E+03	3.3	2.0
		b	4.9E+02	2.7	2.5
		c	2.0E+02	2.3	2.9
		Mean	**8.3E+02**	**2.9**	**2.5**
		Stdev		0.5	0.5

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = plaque forming units per gram

Table 9: Batch A *E.* coli O157 (non toxigenic) results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	A	a	1.1E+05	5.0	
		b	7.4E+04	4.9	
		c	6.4E+04	4.8	
		Mean	**8.3E+04**	**4.9**	
		Stdev		0.1	
1 min	A1	a	5.3E+03	3.7	1.2
		b	2.5E+04	4.4	0.5
		c	1.0E+05	5.0	-0.1
		Mean	**4.3E+04**	**4.6**	**0.5**
		Stdev		0.6	0.6
3 min	A2	a	4.0E+05	5.6	-0.7
		b	4.5E+03	3.7	1.3
		c	1.0E+03	3.0	1.9
		Mean	**1.4E+05**	**5.1**	**0.8**
		Stdev		1.4	1.4
5 min	A3	a	7.9E+03	3.9	1.0
		b	1.6E+04	4.2	0.7
		c	6.4E+03	3.8	1.1
		Mean	**1.0E+04**	**4.0**	**0.9**
		Stdev		0.2	0.2

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 10: Batch B *E.* coli O157 (non toxigenic) results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	B	a	8.3E+04	4.9	
		b	1.0E+05	5.0	
		c	1.7E+05	5.2	
		Mean	**1.2E+05**	**5.1**	
		Stdev		0.2	
1 min	B1	a	2.1E+04	4.3	0.7
		b	2.9E+05	5.5	-0.4
		c	8.2E+04	4.9	0.2
		Mean	**1.3E+05**	**5.1**	**0.2**
		Stdev		0.6	0.6
3 min	B2	a	1.3E+04	4.1	1.0
		b	7.0E+03	3.8	1.2
		c	7.5E+03	3.9	1.2
		Mean	**9.2E+03**	**4.0**	**1.1**
		Stdev		0.1	0.1
5 min	B3	a	6.1E+03	3.8	1.3
		b	5.0E+03	3.7	1.4
		c	2.4E+04	4.4	0.7
		Mean	**1.2E+04**	**4.1**	**1.1**
		Stdev		0.4	0.4

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 11: Batch C *E.* coli O157 (non toxigenic) results

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	C	a	7.7E+04	4.9	
		b	3.8E+05	5.6	
		c	3.4E+05	5.5	
		Mean	**2.7E+05**	**5.4**	
		Stdev		0.4	
1 min	C1	a	4.8E+03	3.7	1.7
		b	1.4E+04	4.1	1.3
		c	2.8E+03	3.4	2.0
		Mean	**7.2E+03**	**3.9**	**1.7**
		Stdev		0.4	0.4
3 min	C2	a	2.8E+03	3.4	2.0
		b	2.0E+03	3.3	2.1
		c	1.5E+03	3.2	2.2
		Mean	**2.1E+03**	**3.3**	**2.1**
		Stdev		0.1	0.1
5 min	C3	a	9.0E+02	3.0	2.5
		b	1.3E+03	3.1	2.3
		c	1.6E+03	3.2	2.2
		Mean	**1.3E+03**	**3.1**	**2.3**
		Stdev		0.1	0.1

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = plaque forming units per gram

Table 12: Batch A *Listeria* results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	A	a	2.2E+04	4.3	
		b	4.1E+04	4.6	
		c	2.2E+04	4.3	
		Mean	**2.8E+04**	**4.5**	
		Stdev		0.2	
1 min	A1	a	6.0E+03	3.8	0.7
		b	1.8E+04	4.3	0.2
		c	1.1E+05	5.0	-0.6
		Mean	**4.5E+04**	**4.6**	**0.1**
		Stdev		0.6	0.6
3 min	A2	a	2.3E+03	3.4	1.1
		b	6.4E+03	3.8	0.6
		c	2.9E+03	3.5	1.0
		Mean	**3.9E+03**	**3.6**	**0.9**
		Stdev		0.2	0.2
5 min	A3	a	1.0E+03	3.0	1.5
		b	4.1E+04	4.6	-0.2
		c	5.0E+03	3.7	0.8
		Mean	**1.6E+04**	**4.2**	**0.7**
		Stdev		0.8	0.8

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 13: Batch B *Listeria* results

Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
none	B	a	2.6E+04	4.4	
		b	6.3E+04	4.8	
		c	2.2E+04	4.3	
		Mean	**3.7E+04**	**4.6**	
		Stdev		0.2	
1 min	B1	a	1.0E+04	4.0	0.6
		b	7.0E+03	3.8	0.7
		c	1.8E+04	4.3	0.3
		Mean	**1.2E+04**	**4.1**	**0.5**
		Stdev		0.2	0.2
3 min	B2	a	1.0E+04	4.0	0.6
		b	1.2E+04	4.1	0.5
		c	2.7E+05	5.4	-0.9
		Mean	**9.7E+04**	**5.0**	**0.1**
		Stdev		0.8	0.8
5 min	B3	a	3.0E+03	3.5	1.1
		b	6.0E+03	3.8	0.8
		c	8.8E+03	3.9	0.6
		Mean	**5.9E+03**	**3.8**	**0.8**
		Stdev		0.2	0.2

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = colony forming units per gram

Table 14: Batch C *Listeria* results

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	C	a	4.4E+04	4.6	
		b	8.0E+03	3.9	
		c	2.1E+04	4.3	
		Mean	**2.4E+04**	**4.4**	
		Stdev		0.4	
1 min	C1	a	7.1E+03	3.9	0.5
		b	1.6E+04	4.2	0.2
		c	1.6E+04	4.2	0.2
		Mean	**1.3E+04**	**4.1**	**0.3**
		Stdev		0.2	0.2
3 min	C2	a	5.5E+03	3.7	0.6
		b	9.7E+03	4.0	0.4
		c	2.8E+04	4.4	-0.1
		Mean	**1.4E+04**	**4.2**	**0.3**
		Stdev		0.4	0.4
5 min	C3	a	5.7E+03	3.8	0.6
		b	2.3E+03	3.4	1.0
		c	5.2E+03	3.7	0.7
		Mean	**4.4E+03**	**3.6**	**0.8**
		Stdev		0.2	0.2

KEY: 4.8E+03 = 4.8×10^3 = 4800 colony forming units. cfu/g = plaque forming units per gram

Table 15: Batch A MS2 bacteriophage results – trial 1

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	A	a	1.0E+05	5.0	
		b	1.8E+05	5.3	
		c	1.7E+07	7.2	
		Mean	**5.8E+06**	**6.8**	
		Stdev		1.2	
1 min	A1	a	1.5E+05	5.2	1.6
		b	1.9E+06	6.3	0.5
		c	5.9E+05	5.8	1.0
		Mean	**8.8E+05**	**5.9**	**1.0**
		Stdev		0.6	0.6
3 min	A2	a	1.0E+05	5.0	1.8
		b	1.6E+07	7.2	-0.4
		c	2.0E+04	4.3	2.5
		Mean	**5.4E+06**	**6.7**	**1.3**
		Stdev		1.5	1.5
5 min	A3	a	1.8E+05	5.3	1.5
		b	5.0E+04	4.7	2.1
		c	2.4E+06	6.4	0.4
		Mean	**8.8E+05**	**5.9**	**1.3**
		Stdev		0.9	0.9

KEY: 4.8E+03 = 4.8×10^3 = 4800 plaque forming units. pfu/g = plaque forming units per gram

Table 16: Batch B MS2 bacteriophage results – trial 1

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	B	a	9.8E+05	6.0	
		b	6.9E+05	5.8	
		c	6.3E+05	5.8	
		Mean	**7.7E+05**	**5.9**	
		Stdev		0.1	
1 min	B1	a	2.6E+05	5.4	0.5
		b	6.1E+05	5.8	0.1
		c	2.9E+06	6.5	-0.6
		Mean	**1.3E+06**	**6.1**	**0.0**
		Stdev		0.5	0.5
3 min	B2	a	3.8E+06	6.6	-0.7
		b	1.9E+08	8.3	-2.4
		c	1.8E+07	7.3	-1.4
		Mean	**7.1E+07**	**7.8**	**-1.5**
		Stdev		0.9	0.9
5 min	B3	a	5.7E+07	7.8	-1.9
		b	5.5E+05	5.7	0.1
		c	3.1E+06	6.5	-0.6
		Mean	**2.0E+07**	**7.3**	**-0.8**
		Stdev		1.0	1.0

KEY: 4.8E+03 = 4.8×10^3 = 4800 plaque forming units. pfu/g = plaque forming units per gram

Table 17: Batch A MS2 bacteriophage results – trial 1

Treatment	Campden Code	Replicate	pfu/g	log pfu/g	Log reduction pfu
none	C	a	5.3E+06	6.7	
		b	4.2E+06	6.6	
		c	3.9E+06	6.6	
		Mean	**4.5E+06**	**6.6**	
		Stdev		0.1	
1 min	C1	a	4.9E+05	5.7	1.0
		b	3.5E+06	6.5	0.1
		c	8.8E+06	6.9	-0.3
		Mean	**4.3E+06**	**6.6**	**0.3**
		Stdev		0.6	0.6
3 min	C2	a	1.4E+07	7.1	-0.5
		b	2.6E+07	7.4	-0.8
		c	1.5E+07	7.2	-0.5
		Mean	**1.8E+07**	**7.3**	**-0.6**
		Stdev		0.1	0.1
5 min	C3	a	2.1E+05	5.3	1.3
		b	6.3E+06	6.8	-0.1
		c	2.5E+07	7.4	-0.7
		Mean	**1.1E+07**	**7.0**	**0.1**
		Stdev		1.1	1.1
10 min	C4	a	4.2E+06	6.6	0.0
		b	5.1E+06	6.7	-0.1
		c	1.7E+06	6.2	0.4
		Mean	**3.7E+06**	**6.6**	**0.1**
		Stdev		0.3	0.3

KEY: 4.8E+03 = 4.8×10^3 = 4800 plaque forming units. pfu/g = plaque forming units per gram

Table 18: MS2 bacteriophage results - chilled – trial 2

Batch	Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
A	none	A1	a	1.5E+06	6.2	
			b	4.7E+06	6.7	
			c	3.0E+06	6.5	
			Mean	**3.1E+06**	**6.5**	
			Stdev		0.2	
	5 min	A1	a	8.0E+03	3.9	2.6
			b	5.0E+03	3.7	2.8
			c	1.0E+04	4.0	2.5
			Mean	**7.7E+03**	**3.9**	**2.6**
			Stdev		0.2	0.2
B	none	B1	a	3.2E+06	6.5	
			b	2.6E+06	6.4	
			c	1.5E+06	6.2	
			Mean	**2.4E+06**	**6.4**	
			Stdev		0.2	
	5 min	B1	a	7.1E+02	2.8	3.5
			b	3.5E+06	6.5	-0.2
			c	3.1E+05	5.5	0.9
			Mean	**1.3E+06**	**6.1**	**1.4**
			Stdev		1.9	1.9
C	none	C1	a	2.7E+06	6.4	
			b	1.8E+06	6.3	
			c	7.0E+05	5.8	
			Mean	**1.7E+06**	**6.2**	
			Stdev		0.3	
	5 min	C1	a	2.0E+03	3.3	2.9
			b	1.0E+04	4.0	2.2
			c	1.0E+03	3.0	3.2
			Mean	**4.3E+03**	**3.6**	**2.8**
			Stdev		0.5	0.5

KEY: 4.8E+03 = 4.8×10^3 = 4800 plaque forming units. pfu/g = plaque forming units per gram

Table 19: MS2 bacteriophage results - frozen – trial 2

Batch	Treatment	Campden Code	Replicate	cfu/g	log cfu/g	Log reduction cfu
A	none	A2	a	8.7E+06	6.9	
			b	2.5E+06	6.4	
			c	2.4E+06	6.4	
			Mean	**4.5E+06**	**6.7**	
			Stdev		0.3	
	5 min	A2	a	2.5E+05	5.4	1.3
			b	5.4E+05	5.7	0.9
			c	3.5E+05	5.5	1.1
			Mean	**3.8E+05**	**5.6**	**1.1**
			Stdev		0.2	0.2
B	none	B2	a	4.5E+06	6.7	
			b	3.8E+06	6.6	
			c	5.4E+06	6.7	
			Mean	**4.6E+06**	**6.7**	
			Stdev		0.1	
	5 min	B2	a	2.0E+05	5.3	1.4
			b	1.0E+06	6.0	0.7
			c	3.9E+05	5.6	1.1
			Mean	**5.3E+05**	**5.7**	**1.0**
			Stdev		0.4	0.4
C	none	C2	a	1.9E+06	6.3	
			b	9.5E+05	6.0	
			c	2.1E+06	6.3	
			Mean	**1.7E+06**	**6.2**	
			Stdev		0.2	
	5 min	C2	a	1.5E+05	5.2	1.0
			b	1.6E+05	5.2	1.0
			c	8.7E+04	4.9	1.3
			Mean	**1.3E+05**	**5.1**	**1.1**
			Stdev		0.1	0.1

KEY: 4.8E+03 = 4.8×10^3 = 4800 plaque forming units. pfu/g = plaque forming units per gram

Table 20: Sensory evaluation – appearance and odour, Trial 1, frozen product

Treatment time (mins)	Days							
	0	1	2	3	4	5	6	7
0	fine	fine	a little liquid, no visible spoilage, odour fine	half of raspberries in liquid, no visible spoilage, odour fine	half of raspberries in liquid, no visible spoilage, odour fine	half of raspberries in liquid, visibly, wet squashy, odour fine	Same as day 5	Same as day 5
1	fine	lots of liquid, no visible spoilage	half of raspberries in liquid, no visible spoilage, odour fine	3/4 of raspberries in liquid, no visible spoilage, odour fine	3/4 of raspberries in liquid, visibly lighter, wet squashy, odour fine	Same as day 4	Same as day 4	3 Same as day 4
3	slightly thawed but fine	lots of liquid, no visible spoilage	all raspberries in liquid , no visible spoilage, odour fine	all raspberries in liquid , no visible spoilage, odour fine	all raspberries in liquid, visibly lighter, wet squashy, odour fine	Same as day 4	Same as day 4	Same as day 4
5	thawed but fine	lots of liquid, no visible spoilage	lots of liquid, no visible spoilage, odour fine	all raspberries in liquid , no visible spoilage, odour fine	all raspberries in liquid, visibly lighter, wet squashy, odour fine	Same as day 4	Same as day 4	Same as day 4

Table 21: Sensory evaluation – appearance and odour, Trial 2, chilled product

Treatment	Day					
	0	1	4	5	6	7
treated	fine	fine	fine	1 discoloured raspberry, no liquid, odour fine	as day 5	as day 5
untreated	fine	fine	fine	fine with some liquid (< 1ml), odour fine	as day 5	More liquid (4.8 ml), wet appearance, odour fine

Table 22: Weights of Samples before and after treatment – trial 2

Product	Campden Code	Weight (grams)		
		before	after	Variation
Chill	A1	100.74	102.9	2.16
	B1	100.54	102.88	2.34
	C1	101.6	103.35	1.75
Frozen	A2	98.99	100.56*	1.57*
	B2	98.32	101.96	3.64
	C2	101.56	107.92	6.36

*Sample loss occurred post treatment

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