final report

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Challenge trials - Listeria

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Contents

1 Introduction

Experiments were undertaken to investigate the reformulation of three cooked processed meat products for greater safety using the model of Mejlholm and Daglaard (2007; 2009; 2010) for prediction of growth rates and growth limits of *Listeria monocytogenes*. The effect of the formulations on the development of populations of *L. monocytogenes* at 4.7-5°C was investigated in vacuum packaged:

- Product 1: Boneless sliced ham,
- Product 2: Sliced giroler (corned beef/silverside)
- Product 3: Boneless bulk ham nuggets (sliced for this study)

The influence of total viable bacteria on the potential for growth of *L. monocytogenes* was also considered.

2 Overview of Trial

All smallgoods were prepared under commercial conditions by a smallgoods manufacturer in Melbourne and sliced and vacuum packed. The samples were appropriately labelled and forwarded to the University of Tasmania, Hobart.

Upon receipt, all samples were labelled and stored at 2°C until ready for inoculation with a cocktail of five strains of *L. monocytogenes* and incubation at 4.7-5°C in a walk-in refrigerator. At appropriate intervals ten replicate samples of each product type were removed from refrigerated storage and *L. monocytogenes* and total viable organisms enumerated.

3 Materials

3.1 Microorganisms

Listeria monocytogenes

Five strains of *L. monocytogenes* were used in combination for all *L. monocytogenes* inoculated samples:

- Scott A (type strain)
- L5/22 (isolated in Tasmania from cold smoked salmon)
- Strains 20425, 20432 and 20423, all isolated from a smallgoods factory and supplied by Silliker Microtech Pty., Ltd., Melbourne, Victoria.

3.2 Culture Media

Listeria monocytogenes were enumerated by spread plating of suspensions of appropriately diluted samples on PALCAM agar (Oxoid CM 877, and SR150 antibiotic supplement).
Total viable aerobic counts were determined on APHA Standard Plate Count Agar (PCA; Oxoid CM463).

Dilutions of homogenised food samples were prepared in 0.1% Bacteriological Peptone (Oxoid L37), hereafter simply referred to as 'diluent' unless otherwise noted.

3.3 Equipment

- Spiral plater: AutoPlate 4000 (Spiral Biotech, Bethesda, USA)
- Vacuum Packer: Tecnovac T60 Gas model (Tecnovac, Azzano S Paolo, Italy)
- Stomacher: Colworth Stomacher 400 (A. J. Seward, London)

4 Methods

4.1 Product preparation, Transport and Receipt

Anti-listerial additives (namely salts of lactic and acetic acids) were applied to the smallgoods during normal commercial processing. Finished smallgoods were sliced by the manufacturer and ~70g lots were dispensed into vacuum barrier bags with a laminate structure typical of those used for consumer retail packs. The bags were then sealed under vacuum.
The treated products were shipped from the manufacturer's premises to Hobart in a large gel-pack freezer bag. Immediately upon receipt all samples were placed at 2°C until the inoculum was ready to be added (~1h).

4.2 Inoculum preparation

The *L. monocytogenes* inoculum was prepared by first inoculating 5 colonies of each strain into individual Tryptone Soya broths (Oxoid CM 129) containing 0.6% Yeast Extract (Oxoid L21), TSB-Ye, and incubating at 37°C for 24 hours. Each culture was serially diluted and added to TSB-YE broth to achieve a concentration of ~ 10^3 CFU.ml^{-1}. The cultures were then incubated in fresh TSB-YE at 8°C until the optical density of the culture had increased to ~30 transmittance, i.e., a cell density of ~10^8 cfu.mL^{-1}, corresponding to late exponential growth.

Two millilitres of each of the five strains were added to a sterile McCartney bottle and vortexed for one minute. This "cocktail" suspension was then diluted in pre-chilled (8°C) diluent to a level of ~10^5 cells per mL (i.e. a 10^{-3} dilution).

The concentration of *L. monocytogenes* in the inoculum "cocktail" was determined by spread-plating 100µl aliquots of appropriate dilutions onto PCA and incubating at 37°C for 24 hours, and colonies counted manually.

4.3 Inoculation of Samples with L. monocytogenes and Incubation

As described previously, upon receipt at the University of Tasmania laboratories samples were stored at 2°C for approximately 1 hour prior to inoculation with the *L. monocytogenes* cocktail and commencement of the challenge trial.

The inoculum, comprising the *L. monocytogenes* "cocktail" prepared as described above, was aseptically added to the sliced products as follows. Sample packages were placed in a laminar flow cabinet and the top surface cut aseptically with sterile scissors and folded back with sterile forceps. The uppermost slice of product was lifted back using sterile forceps and the inoculum pipetted onto the surface of the underlying slice as a series of droplets. The uppermost slice of product in the sample was then gently lowered back into position to facilitate the spread of the

inoculum evenly between the slices. Each inoculated sample was aseptically placed into a new vacuum bag (supplied by the smallgoods manufacturer and of the same type as the original package) and sealed under 95% vacuum. Inoculated and sealed samples were then returned to the large gel-pack freezer bag in the walk-in cold room set to 4.7-5°C.

For each sample type four packages were weighed to allow calculation of the required volume of inoculum. This resulted in an inoculum volume of 0.15ml being added to Product 1: sliced ham. An inoculum volume of 0.16ml was added to both Products 2 and 3 (sliced giroler and ham nuggets).

4.4 Sampling

At appropriate intervals ten samples from each sample type were assessed for levels of *L. monocytogenes* and total viable count. Samples were diluted 1:1 in diluent and further 10-fold dilutions prepared as required. Spread plates were prepared using the 50µL exponential deposition mode on a spiral plater. PALCAM plates were incubated at 37°C for 48 h. PCA plates were incubated at 20°C for 96 h. Colonies were counted manually and $\log_{10}$ (viable cell count) plotted against time. Colony counts falling below the acceptable limit for counting (<30 colonies per plate) are identified as estimates in the accompanying spreadsheet.

4.5 Results

Due to the time taken package and transport the samples from Melbourne to Hobart, the time between preparation of the hams and commencement of the challenge trial was 4 days.

4.6 Inoculum densities

The *L. monocytogenes* "cocktail" was found to contain 5.9×10^5 CFU.mL^{-1}.
Throughout the study the weight of each sample was recorded to determine the amount (g) of diluent to be added when preparing the 1:1 dilution for enumerations. These data are presented in Appendix 1. The average weight of each sample type (based on all samples used for enumerations) is:

- 67.90 (±4.29)gProduct 1: Sliced ham,
- 60.77 (±3.51)gProduct 2: Sliced giroler
- 67.39 (±4.20)gProduct 3: Ham nugget

Using the average weight of *all* samples enumerated then the expected inoculum levels of *L*. *monocytogenes* are:

- 3.11 log cfu/g Product 1: Sliced ham,
- 3.13 log cfu/g Product 2: Sliced giroler
- 3.15 log cfu/g Product 3: Ham nugget

4.7 Microbial Counts

All data for log *L*. *monocytogenes* and log total viable count per gram for each sample, and each sample time, are presented in Appendix 1 and 2. All time measurements reported below are related to the time of *inoculation* of the samples with the *L*. *monocytogenes* 'cocktail'. In accordance with Codex Alimentratius Commission guidelines for management of *L. monocytogenes* (CAC, 2007; 2009), growth was defined as an increase of ≥0.5 log cfu/g compared to the *estimated* inoculum level, determined as detailed above.

When *L. monocytogenes* were inoculated into Product 1: sliced ham and stored at 4.7-5°C, no growth was observed in any of the replicate samples for the duration of incubation (59 days; Figure 1). The total viable count, however, increased with storage time.

Similarly, for *L. monocytogenes* inoculated into Product 2: sliced giroler and stored at 4.7-5°C, no growth was observed in any of the replicate samples for the duration of incubation (59 days; Figure 2). The total viable count increased with storage time.

In contrast to Products 1 and 2, growth was observed in some replicates of Product 3: ham nugget at 49 and 57 days post inoculation. Three of ten samples showed an increase in *L. monocytogenes* numbers on day 49 with counts of 3.84, 3.89 and 4.68 log cfu/g. This equates to estimated growth of 0.69, 0.74 and 1.53 log cfu/g respectively. On day 57 of incubation, two of ten samples showed an increase in *L. monocytogenes* numbers with counts of 3.66 and 4.89 log cfu/g. This equates to estimated growth of 0.51 and 1.74 log cfu/g respectively.

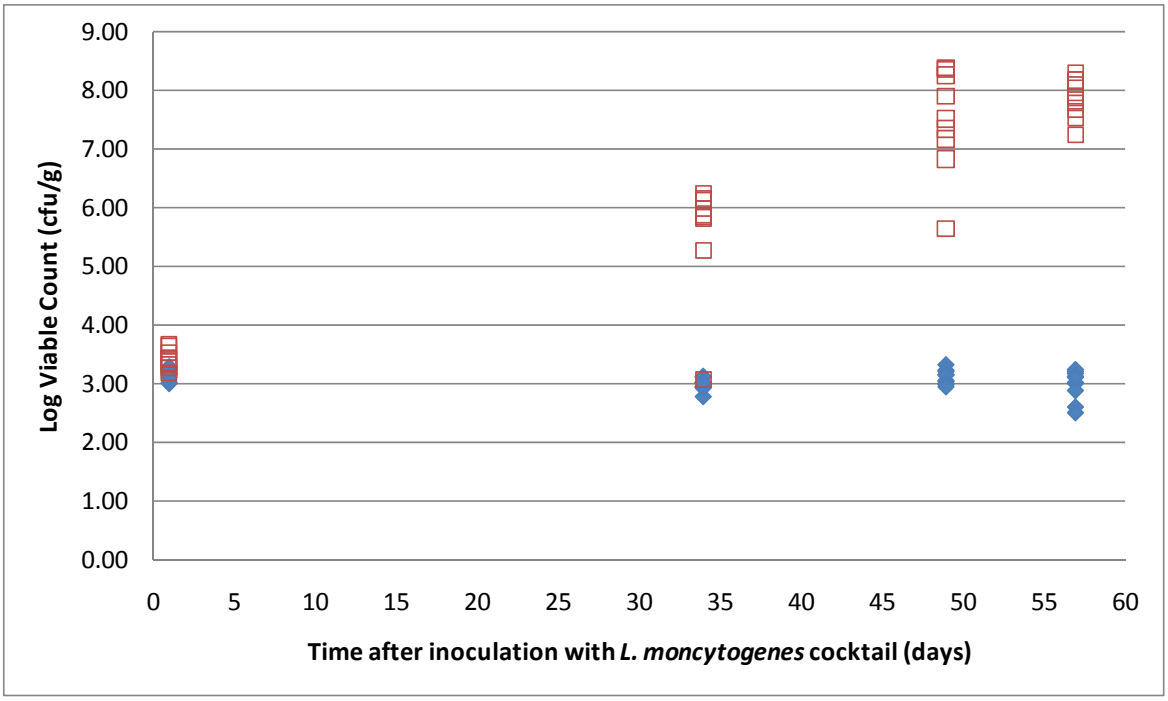

Figure 1: Temporal changes in microbial populations of Product 1: vacuum packaged sliced ham during storage at 4.7-5°C. *L. monocytogenes* (blue symbols) counts from ten replicate samples are shown. Total viable aerobic count (red symbols) are shown and number of replicates is detailed in Appendix 3.

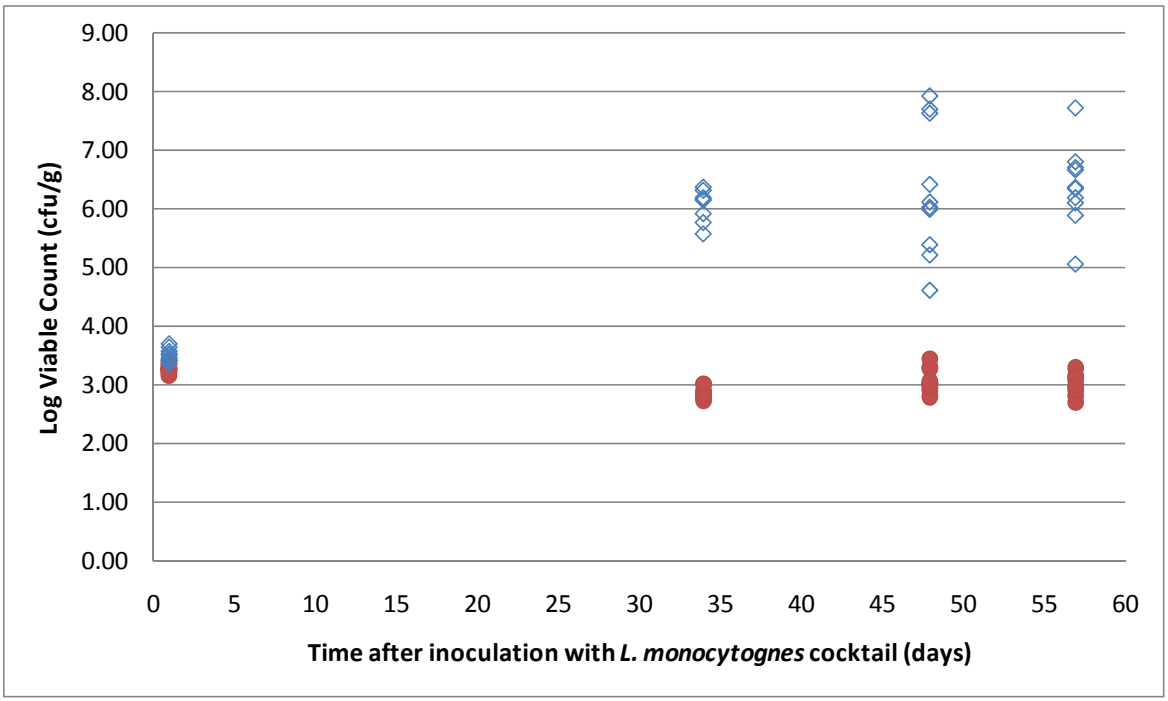

Figure 2: Temporal changes in microbial populations of Product 2: vacuum packaged sliced giroler during storage at 4.7-5°C. *L. monocytogenes* (red symbols) counts from ten replicate samples are shown. Total viable aerobic count (blue symbols) are shown and number of replicates is detailed in Appendix 3.

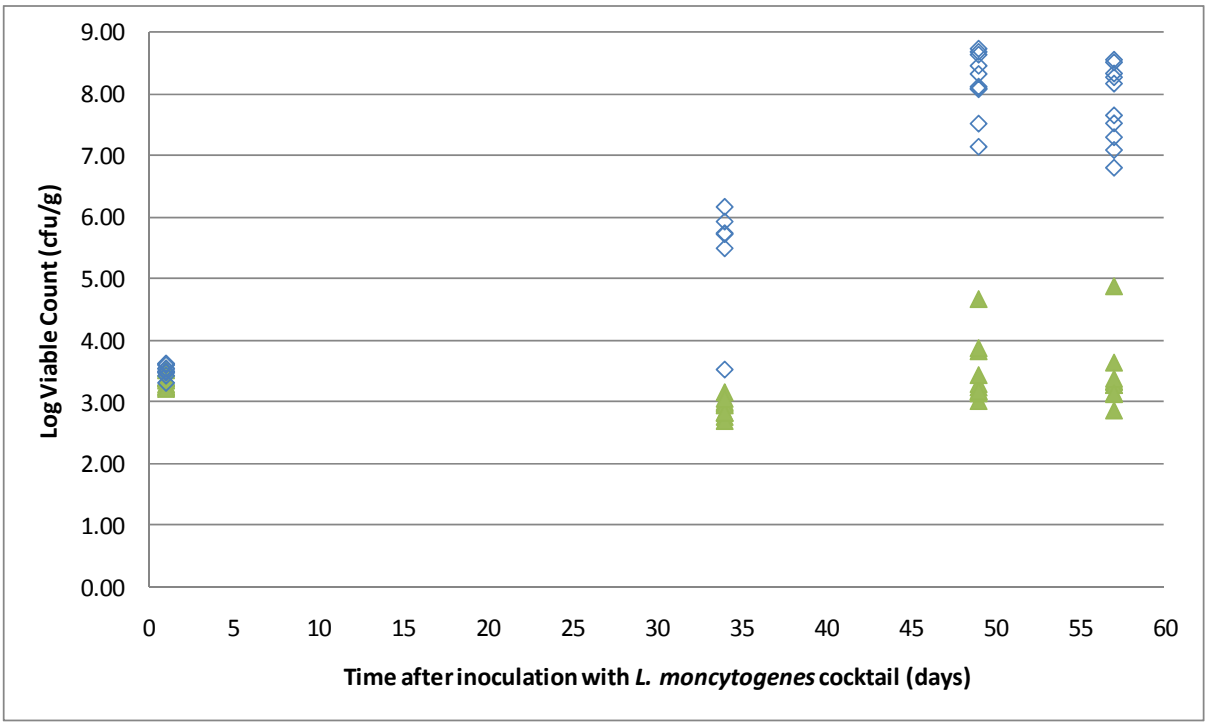

Figure 3: Temporal changes in microbial populations of Product 3: vacuum packaged ham nugget during storage at 4.7-5°C. *L. monocytogenes* (red symbols) counts from nine replicate samples are shown. Total viable aerobic count (blue symbols) are shown and number of replicates is detailed in Appendix 3.

The data for *L. monocytogenes* counts in all sample types are shown in Figure 4 and the average and standard deviation of *L. monocytogenes* counts in all sample types is presented in Table 1.

Table 1: Average log viable counts of *L. monocytogenes* in Product 1: sliced ham, Product 2: sliced giroler and Product 3: ham nugget during vacuum packed storage at 4.7-5°C.

Time (days)	Product 1 LOG VC	Product 1 STDEV	Product 2 LOG VC	Product 2 STDEV	Product 3 LOG VC	Product 3 STDEV
1	3.16	0.10	3.26	0.06	3.37	0.11
34	2.98	0.10	2.87	0.09	2.93*	0.15
48	not sampled		3.09	0.20	not sampled	
49	3.10	0.12	not sampled		3.50	0.50
57	2.98	0.25	3.00	0.18	3.44	0.55

* *n* =9 samples

LOG VC= log viable count/g

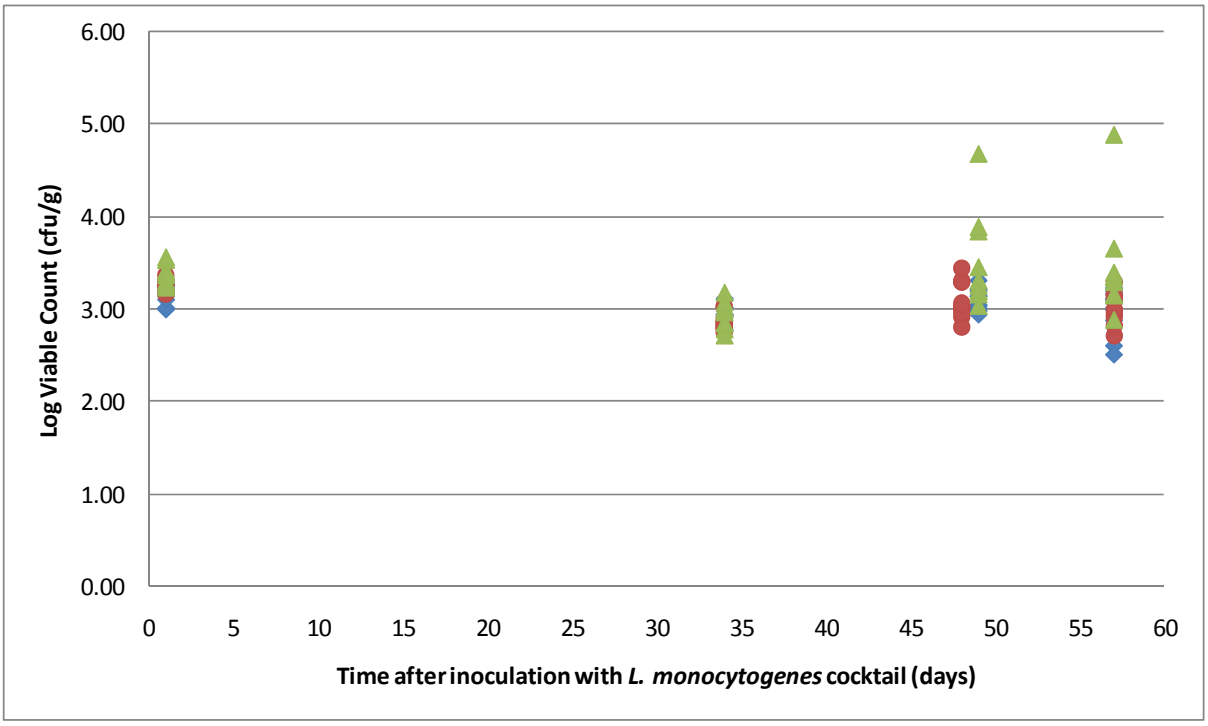

Figure 4: Temporal changes in *L. monocytogenes* counts in all sample types) during vacuum packaged storage at 4.7-5°C: Product 1: sliced ham (blue symbols), Product 2: sliced giroler (red symbols) and Product 3: ham nugget (green symbols). Raw data for each replicate is detailed in Appendix 2.

5 References

CAC (Codex Alimentarius Commission). (2007). Guidelines on the Application of General Principles of Food Hygiene to the Control of *Listeria monocytogenes* in Ready-to-Eat Foods. *CAC/GL 61-2007. (downloaded on 30 July 2010 at:*
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6 Appendix 1

Weight (grams) of samples used for enumeration of L. monocytogenes and total viable count.

	PRODUCT 1		PRODUCT 2		PRODUCT 3
ALL average	**67.90**		**69.77**		**67.39**
ALL stdev	**4.29**		**3.51**		**4.20**
min weight (g)	59.87		60.95		60.80
max weight	76.84		75.77		77.19
Sample Time #0-1	73.90		71.90		70.54
2	75.38		65.78		67.85
3	67.47		74.42		66.33
4	60.52		75.77		72.02
5	65.05		65.25		70.52
6	66.14		65.49		64.09
7	64.13		60.95		66.89
8	65.21		65.07		62.76
9	67.60		64.57		66.40
10	65.58		75.05		64.67
Sample Time #1-1	72.26		72.40		69.22
2	65.56		70.65		72.24
3	63.56		70.55		68.98
4	64.77		68.79		66.00
5	67.69		65.27		67.03
6	74.59		74.67		62.13
7	73.63		68.16		72.90
8	64.38		74.51		60.80
9	66.14		67.10		68.27
10	67.37		67.60		68.36
Sample Time #2-1	68.09		68.19		66.74
2	64.73		71.02		77.19
3	67.16		69.62		71.16
4	72.46		63.72		64.28
5	63.93		75.63		63.74
6	71.43		67.00		61.59
7	60.00		71.93		63.78
8	72.40		71.74		61.74
9	67.20		71.78		65.53
10	75.84		70.33		62.91
Sample Time #3-1	59.87		70.02		65.39
2	71.58		69.79		64.39
3	69.61		68.55		62.80
4	70.80		71.60		71.67
5	76.84		71.30		71.21
6	66.06		71.33		76.23
7	65.76		71.52		62.51
8	66.71		68.32		74.25
9	67.68		72.46		71.25
10	66.73		70.96		69.12

7 Appendix 2

Log viable count (cfu/g) of L. monocytogenes in each sample type. Increases of ≥0.5 log cfu/g compared to the expected inoculum level are highlighted as red cells with red text.

GROWTH= +0.5 log cfu/g		≥3.61		≥3.63		≥3.65
Expected Inoculum level /g		3.11		3.13		3.15
	LOG LISTERIA COUNTS ON PALCAM					
	PRODUCT 1		PRODUCT 2		PRODUCT 3	
	Time (days)	Log VC	Time (days)	Log VC	Time (days)	Log VC
	1	3.18	1	3.26	1	3.41
	1	3.00	1	3.32	1	3.41
	1	3.24	1	3.27	1	3.30
	1	3.02	1	3.38	1	3.26
	1	3.17	1	3.28	1	3.27
	1	3.15	1	3.17	1	3.39
	1	3.26	1	3.17	1	3.53
	1	3.21	1	3.27	1	3.23
	1	3.28	1	3.26	1	3.37
	1	3.11	1	3.21	1	3.56
	34	2.94	34	2.90	34	2.78
	34	3.01	34	2.81	34	2.84
	34	3.11	34	2.85	34	3.02
	34	2.94	34	3.03	34	
	34	2.92	34	2.75	34	2.71
	34	3.12	34	2.82	34	2.99
	34	3.02	34	2.79	34	2.96
	34	2.78	34	2.86	34	2.85
	34	2.94	34	2.87	34	3.18
	34	3.02	34	3.03	34	3.07
	49	3.03	48	2.92	49	3.26
	49	3.20	48	3.02	49	3.03
	49	3.32	48	3.29	49	3.19
	49	3.23	48	3.02	49	3.31
	49	3.05	48	3.31	49	3.45
	49	3.15	48	3.45	49	3.84
	49	3.15	48	3.03	49	3.16
	49	2.98	48	2.81	49	3.89
	49	3.00	48	2.96	49	3.19
	49	2.94	48	3.06	49	4.68
	57	3.20	57	3.29	57	3.29
	57	3.00	57	2.72	57	3.66
	57	3.02	57	2.96	57	3.35
	57	3.17	57	2.83	57	3.34
	57	3.12	57	2.83	57	3.15
	57	3.24	57	2.92	57	4.89
	57	2.88	57	3.09	57	3.29
	57	3.11	57	3.15	57	3.39
	57	2.60	57	3.16	57	3.16

8 Appendix 3:

Log viable count (cfu/g) of total viable count in each sample type. Data highlighted in yellow represents estimates from samples which yielded unexpectedly higher or lower counts not captured by the dilutions plated.

LOG TOTAL AEROBIC PLATE COUNTS ON PCA

Time (days)	PRODUCT 1 Log VC	Time (days)	PRODUCT 2 Log VC	Time (days)	PRODUCT 3 Log VC
1	3.64	1	3.63	1	3.49
1	3.37	1	3.45	1	3.61
1	3.19	1	3.56	1	3.48
1	3.27	1	3.50	1	3.55
1	3.66	1	3.69	1	3.55
1	3.41	1	3.52	1	3.62
1	3.33	1	3.39	1	3.63
1	3.43	1	3.42	1	3.32
1	3.43	1	3.35	1	3.43
1	3.53	1	3.41	1	3.51
34	5.84	34	6.18	34	6.17
34	5.98	34		34	5.93
34	3.08	34	6.15	34	
34	5.82	34	5.57	34	5.50
34	6.12	34	6.31	34	
34	6.23	34	5.76	34	5.73
34	6.14	34		34	
34		34	6.36	34	
34	5.87	34	6.14	34	5.74
34	5.27	34	5.91	34	3.53
49	5.64	48	6.11	49	8.12
49	8.34	48	5.20	49	7.52
49	8.37	48	7.69	49	7.15
49	8.25	48	7.92	49	8.08
49	7.88	48	7.62	49	8.33
49	7.33	48	6.02	49	8.46
49	7.50	48	5.98	49	8.09
49	6.82	48	5.38	49	8.68
49	6.82	48	4.60	49	8.74
49	7.16	48	6.41	49	8.64
57	8.16	57	7.71	57	8.52
57	8.09	57	6.09	57	8.33
57	7.24	57	6.33	57	7.53
57	8.16	57	5.88	57	7.30
57	7.87	57	6.66	57	6.81
57	7.53	57	6.18	57	8.56
57	7.68	57	6.36	57	8.27
57	8.28	57	6.70	57	8.17
57	7.88	57	6.80	57	7.09
57	7.78	57	5.05	57	7.66
	Result reported is an estimate only				