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CHALLENGE STUDY REPORT

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TITLE

Survival of *Salmonella* spp., *Listeria monocytogenes*, and
Escherichia coli O157:H7 on Prunes

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1.0 OBJECTIVE

The overall objective of this study is to evaluate the survival of pathogens on dried plums under varying conditions (including high and low moisture, presence of potassium sorbate, dehydration, and steam hydration).

2.0 PROTOCOL OVERVIEW

Whole and pitted prunes were received by Food Safety Net Services and surface inoculated with an acid-adapted cocktail of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 (depending on the section of the study one or more of the pathogens were used). Prunes were sampled until pathogen levels were reduced below the limit of detection. Samples were stored at 20°C and bacterial recoveries were performed at set time points. At each test point, the amount of pathogen observed was compared to the initial inoculation amount to determine the relative effect of the prunes on survival of each applicable pathogen.

3.0 MATERIALS AND METHODS

3.1 Strains

Whole and pitted prunes were independently inoculated with the designated cocktails of the foodborne pathogens as shown in Table 1.

Table 1. Experimental Strains

Organism	Strain	Source
<i>Salmonella</i>	Balidon	University of Georgia; Tomato isolate associated with human illness
<i>Salmonella</i>	Meunchen	University of Georgia; Orange juice isolate associated with human illness
<i>Salmonella</i>	Phage Type 30	University of California; Almond isolate associated with human illness
<i>Salmonella</i>	Senftenberg	ATCC 8400
<i>Salmonella</i>	Tennessee	Isolated from peanut butter sample
<i>E. coli</i> O157:H7	C7927	University of Georgia; Apple cider isolate associated with human illness
<i>E. coli</i> O157:H7	SEA 13B88	University of Georgia; Apple cider isolate associated with human illness
<i>E. coli</i> O157:H7	Outbreak Strain	FDA; Spinach isolate associated with human illness

**Table 1. continued**

Organism	Strain	Source
<i>Listeria monocytogenes</i>	F8255 (serotype 1/2b)	University of Georgia; Peach/plum isolate
<i>Listeria monocytogenes</i>	G1091 (serotype 4b)	University of Georgia; Coleslaw isolate associated with human illness
<i>Listeria monocytogenes</i>	Scott A	University of Wisconsin – Madison Food Research Institute; Human isolate

3.2 Preparation of strains for product inoculation

All strains were prepared according to acid adaptation procedures in conjunction with procedures previously described by Danyluk *et al.* (2005).

Fresh cultures of test organisms were revived from -80°C freezer stocks and prepared as follows: a fresh subculture of each organism was streaked onto Tryptic Soy Agar (TSA, BD Diagnostic Systems, Sparks, MD) and incubated for 24 hours at 35°C. A single isolated colony of each culture was transferred into Tryptic Soy Broth (TSB, BD) containing 1% glucose and incubated for 24 hours at 35°C, followed by two consecutive transfers under the same conditions. According to the procedures of Danyluk *et al.* (2005), this culture was used to inoculate 150 mm x 15 mm TSA plates to produce a bacterial lawn after incubation for 24 h at 35°C. Three plates per 400-g of product sample were prepared. Following incubation, approximately 10 ml of Butterfield's Phosphate Buffer (BPB, BD) was added to each TSA plate. The bacterial lawn was loosened with a sterile spreader and a sterile pipette was used to collect the cells (approximately 25 ml). Prior to inoculating the product, the appropriate number of 25-ml preparations (depending upon the total amount of prunes inoculated) was pooled, thoroughly mixed, and inoculated onto the product in 30-45 minutes. Inoculum levels were assayed by spectrophotometry and determined by plating serial dilutions of the inoculum onto TSA and selective medias: XLT4 Agar (XLT4, BD) for *Salmonella*, Modified Oxford Agar (MOX, BD) for *Listeria*, and Sorbitol MacConkey Agar with Cefixime and Tellurite (CT-SMAC, BD) for *E. coli* O157:H7.

3.3 Product inoculation

Prunes were inoculated according to procedures described by Danyluk *et al.* (2005). Briefly, prune samples (400 ± 1 g) were aseptically weighed into sterile, plastic bags (approximately 30.5 cm x 30.5 cm) and 25 ml of the pooled inoculum was added within 30-45 minutes of removal from the Petri dish. The bag was closed and inverted by hand for 60 seconds. Prunes were poured out of the bag and spread onto two sheets of 46 x 57-cm filter paper (Fisherbrand Qualitative P8; Fisher Scientific, Pittsburgh, PA) which were



folded in half. The filter paper was placed on a metal drying rack inside a large, sanitized plastic tub. Prunes were stored for 24 ± 2 h at $23 \pm 2^{\circ}\text{C}$ with the lid ajar in a biological safety cabinet. Inoculated prunes were pooled into one plastic, sterile polyethylene bag (approximately 16" x 16"). The pooled prunes were then thoroughly mixed by manual inversion for 1 minute. The target inoculum was approximately 10^6 - 10^7 CFU/g per pathogen strain in each cocktail.

3.4 High moisture testing

Pitted prunes were inoculated with cocktails of all three pathogens. Bacterial recoveries were performed after the inoculum dries (T_0) while the remainder of was stored at 23°C and 7°C at held at approximately 30% relative humidity. Prunes were sampled after 6, 12, 24, 30, 36, 42, and 48 days. The primary approach for pathogen enumeration was direct plate analysis. In the event that a pathogen was not detected by direct plate enumeration, a qualitative, genomic assay (using enrichment) was performed to verify the pathogen's presence. Should the qualitative analysis also show that the organism is not present in the samples over two consecutive test points, testing for that organism was stopped.

For enumeration of pathogen at each test point, a 100 gram, representative sample was collected and mixed with an equal amount of BPB. Samples were blended in a stomacher (Stomacher[®] 400, Seward, Thetford, Norfolk, UK) for 2 minutes prior to direct plating on selective agars as shown above. Six total counts, plated in duplicate, were enumerated using an automated counting system (Flash and Go, IUL Instruments, Barcelona, Spain). Average total counts per sample and the corresponding \log_{10} values were determined. Representative, typical growth was culturally and biochemically confirmed after enumeration to ensure the colonies enumerated represent the inoculum originally applied rather than any possible confounding organisms naturally present on the prunes.

All uninoculated control samples and inoculated samples with no observable count were evaluated for the presence of the relevant pathogen using a qualitative assessment performed the Qualicon[®] PCR-BAX system following the manufacturer's procedure as described below (negative control).

Listeria monocytogenes: Samples were enriched for the presence of *Listeria monocytogenes* by mixing a 25 gram portion of the inoculated product with 225 ml of buffered *Listeria* enrichment broth (BLEB; BD) without antibiotics. Samples were incubated for 22 – 24 hours at 30°C . After incubation, a 100 μl volume of the enrichment was transferred to 9.9 ml of MOPS-BLEB (BD) and incubated at 35°C for 18 – 24 hours.



Subsequently a 5 µl aliquot of this enrichment was analyzed for the presence of *Listeria monocytogenes* using the PCR-BAX assay.

E. coli O157:H7: Samples were enriched for the presence of *E. coli* O157:H7 by mixing a 25 gram portion of the inoculated product with 225 ml of enterohemorrhagic *E. coli* (EHEC) broth. Samples were incubated at 37°C for 22 – 26 hours after which a 5 µl aliquot was analyzed for the presence of *E. coli* O157:H7 using the PCR-BAX assay.

Salmonella spp.: Samples were enriched for the presence of *Salmonella* spp. by mixing a 25 gram portion of the inoculated product with 225 ml of Buffered Peptone Water (BPW, BD). Samples were incubated at 35°C for 22 – 26 hours after which a 5 µl aliquot was analyzed for the presence of *Salmonella* spp. using the PCR-BAX assay.

3.5 Potassium sorbate testing

Pitted prunes prepared with and without potassium sorbate were steamed, pitted, and inoculated with the *Salmonella* cocktail and held at 23°C and 30% relative humidity. Samples were enumerated for *Salmonella* at the testing intervals shown above (0, 6, 12, 24, 30, 36, 42, and 48 days). As with the samples above, testing was suspended when pathogen is no longer detectable. Relative counts for each sample group was compared to the *Salmonella* samples above to determine the effect of potassium sorbate on the inoculum. Samples were that fell below the limit of detection were tested by BAX PCR.

3.6 Steam hydration

Whole prunes were inoculated with the *Salmonella* cocktail only and were exposed to steam for 22 minutes (approximately 180°F). After exposure, pits were removed from the exposed prunes. Whole (the positive control) and steam-treated pitted prunes were sampled for *Salmonella* after they return to room temperature and every 6 days after until there is no recovery of *Salmonella*. Prunes were stored at 23°C and 30% relative humidity.

3.7 Impact of dehydration

Whole prunes were inoculated with the *Salmonella* cocktail only and dried in a fashion similar to the process used in industry. Bacterial recovery was performed before the dehydration process (positive control) and after the prunes had returned to room temperature. Relative counts for each sample group were compared to the *Salmonella* samples above to determine the effect of dehydration on the inoculum.



3.8 Limit of detection testing

Whole prunes were inoculated with the *Salmonella* cocktail only and were held at the conditions shown above (23°C, 30% relative humidity). Samples were enumerated for *Salmonella* after inoculation, and after 4, 8, and 12 days. Samples were enumerated for *Salmonella* each day thereafter, to determine the precise day when *Salmonella* was no longer detectable in the room temperature samples.

4.0 RESULTS

4.1 High moisture results

Results from the high moisture testing are shown in Tables 2-4, below including time of storage, storage condition (room temperature or refrigerated), the raw enumeration (in CFU/g) for each replicate, the log₁₀ result for each replicate, the average result for all replicates, and the reduction (in log₁₀ CFU/g) from the amount of pathogen initially observed at Day 0.

Table 2. High moisture prunes inoculated with *Salmonella*

Day 0	XLT4 (CFU/g)	Log ₁₀ (CFU/g)		
1	499,000,000	8.70		
2	524,000,000	8.72		
3	531,000,000	8.73		
4	521,000,000	8.72		
5	531,000,000	8.73		
6	561,000,000	8.75		
Average	527,833,333	8.72		
Day 6	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	7,150,000	6.85	5,810,000	6.76
2	8,180,000	6.91	35,400,000	7.55
3	17,100,000	7.23	3,020,000	6.48
4	10,200,000	7.01	7,110,000	6.85
5	3,110,000	6.49	36,500,000	7.56
6	2,110,000	6.32	31,600,000	7.50
Average	7,975,000	6.90	19,906,667	7.30
Reduction		1.82		1.42

**Table 2. continued**

Day 12	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	1,950,000	6.29	1,030	3.01
2	2,580,000	6.41	1,120	3.05
3	1,700,000	6.23	3,200	3.51
4	2,550,000	6.41	3,130	3.50
5	1,100,000	6.04	1,100	3.04
6	1,100,000	6.04	1,010	3.00
Average	1,830,000	6.26	1,765	3.25
Reduction		2.46		5.48
Day 24	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	36,600	4.56	126	2.10
2	181,000	5.26	<1	0.00
3	5,170	3.71	<1	0.00
4	133,000	5.12	<1	0.00
5	25,600	4.41	<1	0.00
6	16,900	4.23	<1	0.00
Average	66,378	4.82	22	1.34
Reduction		3.90		7.38
Day 30	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	9,150	3.96	<1	0.00
2	3,110	3.49	<1	0.00
3	2,890	3.46	<1	0.00
4	1,010	3.00	<1	0.00
5	8,180	3.91	<1	0.00
6	2,100	3.32	<1	0.00
Average	4,407	3.64	<1	0.00
Reduction		5.08		8.72
Day 36	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	1,570	3.20	<1	0.00
2	2,990	3.48	<1	0.00
3	1,370	3.14	<1	0.00
4	1,140	3.06	<1	0.00
5	1,280	3.11	<1	0.00
6	990	3.00	<1	0.00
Average	1,557	3.19	<1	0.00
Reduction		5.53		8.72

Table 2. continued

Day 42	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	216	2.33	<1	0.00
2	250	2.40	<1	0.00
3	219	2.34	<1	0.00
4	854	2.93	<1	0.00
5	385	2.59	<1	0.00
6	125	2.10	<1	0.00
Average	342	2.53	<1	0.00
Reduction		6.19		8.72
Day 48	Refrigerated		Room Temperature	
	XLT4 (CFU/g)	Log ₁₀ (CFU/g)	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	947	2.98	<1	0.00
2	85	1.93	<1	0.00
3	138	2.14	<1	0.00
4	32	1.51	<1	0.00
5	11	1.04	<1	0.00
6	11	1.04	<1	0.00
Average	204	2.31	<1	0.00
Reduction		6.41		8.72

Table 3. High moisture prunes inoculated with *E. coli* O157:H7

Day 0	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)		
1	251,000,000	8.40		
2	371,000,000	8.57		
3	40,000,000	7.60		
4	97,000,000	7.99		
5	86,100,000	7.94		
6	40,800,000	7.61		
Average	147,650,000	8.17		
Day 6	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	24,500,000	7.39	31,100,000	7.49
2	31,400,000	7.50	24,500,000	7.39
3	3,030,000	6.48	3,110,000	6.49
4	40,100,000	7.60	41,500,000	7.62
5	10,200,000	7.01	3,120,000	6.49
6	16,100,000	7.21	24,900,000	7.40
Average	20,888,333	7.32	21,371,667	7.33
Reduction		0.85		0.84

Table 3. continued

Day 12	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	358,000	5.55	5,610	3.75
2	824,000	5.92	623	2.79
3	480,000	5.68	538	2.73
4	376,000	5.58	342	2.53
5	345,000	5.54	2,950	3.47
6	431,000	5.63	3,280	3.52
Average Reduction	469,000	5.67	2,224	3.35
		2.50		4.82
Day 24	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	310,000	5.49	317	2.50
2	3,120	3.49	1,300	3.11
3	2,080	3.32	5,670	3.75
4	323,000	5.51	351	2.55
5	171,000	5.23	362	2.56
6	2,580	3.41	532	2.73
Average Reduction	135,297	5.13	1,422	3.15
		3.04		5.02
Day 30	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	41,900	4.62	105	2.02
2	81,800	4.91	<1	0.00
3	70,000	4.85	300	2.48
4	29,000	4.46	<1	0.00
5	130,000	5.11	<1	0.00
6	79,900	4.90	<1	0.00
Average Reduction	72,100	4.86	68	1.83
		3.31		6.34
Day 36	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	226,000	5.35	<1	0.00
2	173,000	5.24	<1	0.00
3	67,000	4.83	<1	0.00
4	10,000	4.00	<1	0.00
5	3,280	3.52	<1	0.00
6	851	2.93	<1	0.00
Average Reduction	80,022	4.90	<1	0.00
		3.27		8.17

**Table 3. continued**

Day 42	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	20,700	4.32	<1	0.00
2	17,400	4.24	<1	0.00
3	13,800	4.14	<1	0.00
4	18,400	4.26	<1	0.00
5	18,500	4.27	<1	0.00
6	11,400	4.06	<1	0.00
<i>Average Reduction</i>	<i>16,700</i>	<i>4.22</i> <i>3.95</i>	<i><1</i>	<i>0.00</i> <i>8.17</i>
Day 48	Refrigerated		Room Temperature	
	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)	CT-SMAC (CFU/g)	Log ₁₀ (CFU/g)
1	1,390	3.14	<1	0.00
2	2,530	3.40	<1	0.00
3	4,740	3.68	<1	0.00
4	3,240	3.51	<1	0.00
5	3,130	3.50	<1	0.00
6	1,390	3.14	<1	0.00
<i>Average Reduction</i>	<i>2,737</i>	<i>3.44</i> <i>4.73</i>	<i><1</i>	<i>0.00</i> <i>8.17</i>

Table 4. High moisture prunes inoculated with *Listeria*

Day 0	MOX (CFU/g)	Log ₁₀ (CFU/g)		
1	95,800,000	7.98		
2	72,600,000	7.86		
3	76,900,000	7.89		
4	84,300,000	7.93		
5	77,900,000	7.89		
6	109,000,000	8.04		
<i>Average</i>	<i>86,083,333</i>	<i>7.93</i>		
Day 6	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	2,300,000	6.36	31,000,000	7.49
2	1,650,000	6.22	2,110,000	6.32
3	3,120,000	6.49	1,710,000	6.23
4	26,700,000	7.43	1,850,000	6.27
5	1,710,000	6.23	26,700,000	7.43
6	18,500,000	7.27	1,210,000	6.08
<i>Average Reduction</i>	<i>8,996,667</i>	<i>6.95</i> <i>0.98</i>	<i>10,763,333</i>	<i>7.03</i> <i>0.90</i>

Table 4. continued

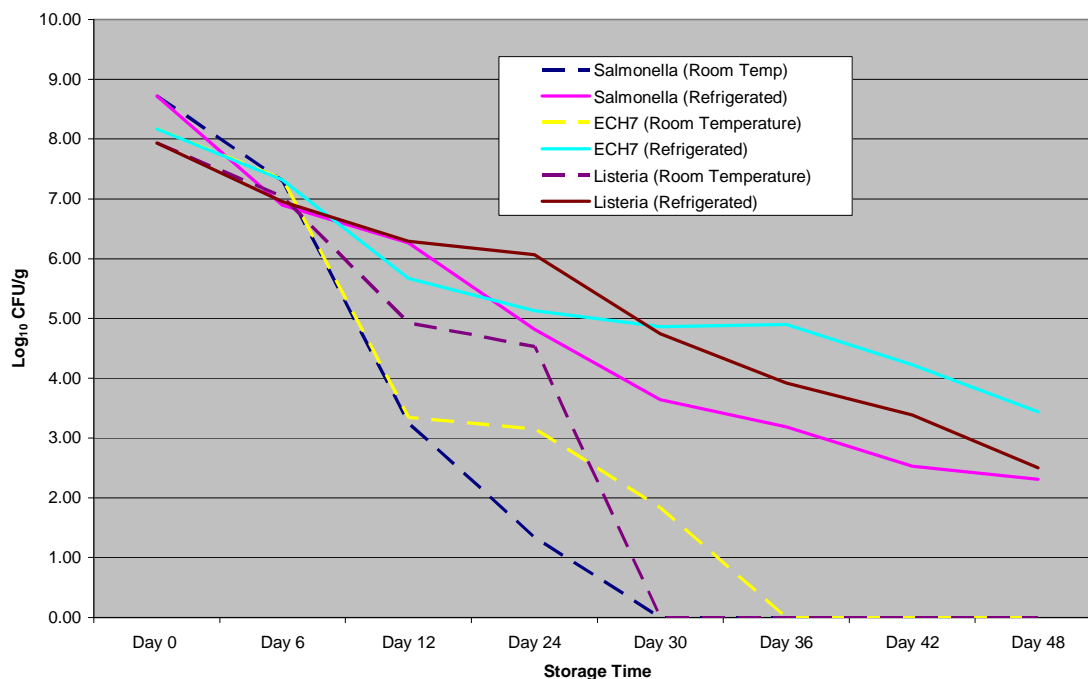
Day 12	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	2,530,000	6.40	129,000	5.11
2	1,100,000	6.04	113,000	5.05
3	1,600,000	6.20	25,500	4.41
4	2,800,000	6.45	31,900	4.50
5	2,620,000	6.42	87,200	4.94
6	1,030,000	6.01	123,000	5.09
Average Reduction	1,946,667	6.29	84,933	4.93
		1.65		3.01
Day 24	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	877,000	5.94	49,400	4.69
2	1,160,000	6.06	18,400	4.26
3	1,380,000	6.14	13,100	4.12
4	1,292,000	6.11	49,400	4.69
5	884,000	5.95	61,800	4.79
6	1,390,000	6.14	12,300	4.09
Average Reduction	1,163,833	6.07	34,067	4.53
		1.87		3.40
Day 30	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	97,200	4.99	<1	0.00
2	110,000	5.04	<1	0.00
3	25,600	4.41	<1	0.00
4	46,000	4.66	<1	0.00
5	43,900	4.64	<1	0.00
6	13,200	4.12	<1	0.00
Average Reduction	55,983	4.75	<1	0.00
		3.19		7.93
Day 36	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	11,000	4.04	<1	0.00
2	2,940	3.47	<1	0.00
3	6,600	3.82	<1	0.00
4	7,020	3.85	<1	0.00
5	17,000	4.23	<1	0.00
6	5,250	3.72	<1	0.00
Average Reduction	8,302	3.92	<1	0.00
		4.02		7.93

Table 4. continued

Day 42	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	5,770	3.76	<1	0.00
2	1,870	3.27	<1	0.00
3	512	2.71	<1	0.00
4	904	2.96	<1	0.00
5	301	2.48	<1	0.00
6	5,160	3.71	<1	0.00
<i>Average</i>	<i>2,420</i>	<i>3.38</i>	<i><1</i>	<i>0.00</i>
<i>Reduction</i>		<i>4.55</i>		<i>7.93</i>
Day 48	Refrigerated		Room Temperature	
	MOX (CFU/g)	Log ₁₀ (CFU/g)	MOX (CFU/g)	Log ₁₀ (CFU/g)
1	1,780	3.25	<1	0.00
2	11	1.04	<1	0.00
3	<1	0.00	<1	0.00
4	<1	0.00	<1	0.00
5	53	1.72	<1	0.00
6	85	1.93	<1	0.00
<i>Average</i>	<i>322</i>	<i>2.51</i>	<i><1</i>	<i>0.00</i>
<i>Reduction</i>		<i>5.43</i>		<i>7.93</i>

In samples stored at refrigerated temperatures, pathogen was reduced from 4.73 to 6.41 logs over the course of 48 days of storage. Samples stored at room temperature were cleared of observable pathogen after 30-36 days of storage. Pathogen reduction is shown graphically in Figure 1, below.

Figure 1. Reduction of pathogens in high moisture prunes



4.2 Potassium sorbate results

Results from the potassium sorbate testing are shown in Table 5 including time of storage, the raw enumeration (in CFU/g) for each replicate, the \log_{10} result for each replicate, the average result for all replicates, and the reduction (in \log_{10} CFU/g) from the amount of pathogen initially observed at Day 0.

Samples treated with potassium sorbate showed a greater reduction than control samples, with an additional 1 log reduction at Day 6, and an additional 1.5 log reduction on Day 12. *Salmonella* was not detected by plating after Day 12 in treated or control samples; however qualitative analysis by BAX showed that the prunes were positive through to Day 42.

Table 5. Prunes inoculated with *Salmonella* and treated with potassium sorbate

Day 0	Without Sorbate	With Sorbate	Log₁₀ + Sorbate	Log₁₀ - Sorbate
1	1602000	6181000	6.20	6.79
2	8356000	5069000	6.92	6.70
3	7940000	8102000	6.90	6.91
4	2113000	5101000	6.32	6.71
5	3121000	3821000	6.49	6.58
6	4115000	2113000	6.61	6.32
<i>Average</i>	<i>4541167</i>	<i>5064500</i>	<i>6.58</i>	<i>6.67</i>
Day 6	Without Sorbate	With Sorbate	Log₁₀ + Sorbate	Log₁₀ - Sorbate
1	2110	110	3.32	2.04
2	1010	230	3.00	2.36
3	31220	110	4.49	2.04
4	1000	210	3.00	2.32
5	2110	300	3.32	2.48
6	6130	410	3.79	2.61
<i>Average</i>	<i>7263</i>	<i>228</i>	<i>3.49</i>	<i>2.31</i>
Log ₁₀ Reduction			3.09	4.36
Day 12	Without Sorbate	With Sorbate	Log₁₀ + Sorbate	Log₁₀ - Sorbate
1	510	10	2.71	1.00
2	630	20	2.80	1.30
3	210	10	2.32	1.00
4	300	20	2.48	1.30
5	800	10	2.90	1.00
6	610	20	2.79	1.30
<i>Average</i>	<i>510</i>	<i>15</i>	<i>2.67</i>	<i>1.15</i>
Log ₁₀ Reduction			3.91	5.52

Table 5. continued

Day 24	Without Sorbate	With Sorbate	Log ₁₀ + Sorbate	Log ₁₀ - Sorbate	Day 24 Bax	Without Sorbate	With Sorbate
1	<2	<2	0	0	1	positive	positive
2	<2	<2	0	0	2	positive	positive
3	<2	<2	0	0	3	negative	positive
4	<2	<2	0	0	4	positive	positive
5	<2	<2	0	0	5	negative	positive
6	<2	<2	0	0	6	negative	negative
Average	<2	<2	0	0			
Log ₁₀ Reduction			6.58	6.67			
Day 30	Without Sorbate	With Sorbate	Log ₁₀ + Sorbate	Log ₁₀ - Sorbate	Day 30 Bax	Without Sorbate	With Sorbate
1	<2	<2	0	0	1	negative	positive
2	<2	<2	0	0	2	negative	negative
3	<2	<2	0	0	3	negative	negative
4	<2	<2	0	0	4	negative	negative
5	<2	<2	0	0	5	negative	positive
6	<2	<2	0	0	6	negative	positive
Average	<2	<2	0	0			
Log ₁₀ Reduction			6.58	6.67			
Day 36	Without Sorbate	With Sorbate	Log ₁₀ + Sorbate	Log ₁₀ - Sorbate	Day 36 Bax	Without Sorbate	With Sorbate
1	<2	<2	0	0	1	negative	positive
2	<2	<2	0	0	2	negative	negative
3	<2	<2	0	0	3	negative	negative
4	<2	<2	0	0	4	negative	negative
5	<2	<2	0	0	5	negative	positive
6	<2	<2	0	0	6	negative	negative
Average	<2	<2	0	0			
Log ₁₀ Reduction			6.58	6.67			
Day 42	Without Sorbate	With Sorbate	Log ₁₀ + Sorbate	Log ₁₀ - Sorbate	Day 42 Bax	Without Sorbate	With Sorbate
1	NA	<2		0	1	NA	negative
2	NA	<2		0	2	NA	negative
3	NA	<2		0	3	NA	positive
4	NA	<2		0	4	NA	positive
5	NA	<2		0	5	NA	negative
6	NA	<2		0	6	NA	positive
Average		<2		0			
Log ₁₀ Reduction				6.67			



4.3 Steam hydration results

Results from the steam hydrated pitted prunes are shown in Table 6, below including time of storage, the raw enumeration (in CFU/g) for each replicate, the log₁₀ result for each replicate, the average result for all replicates, and the reduction (in log₁₀ CFU/g) from the amount of pathogen initially observed at Day 0.

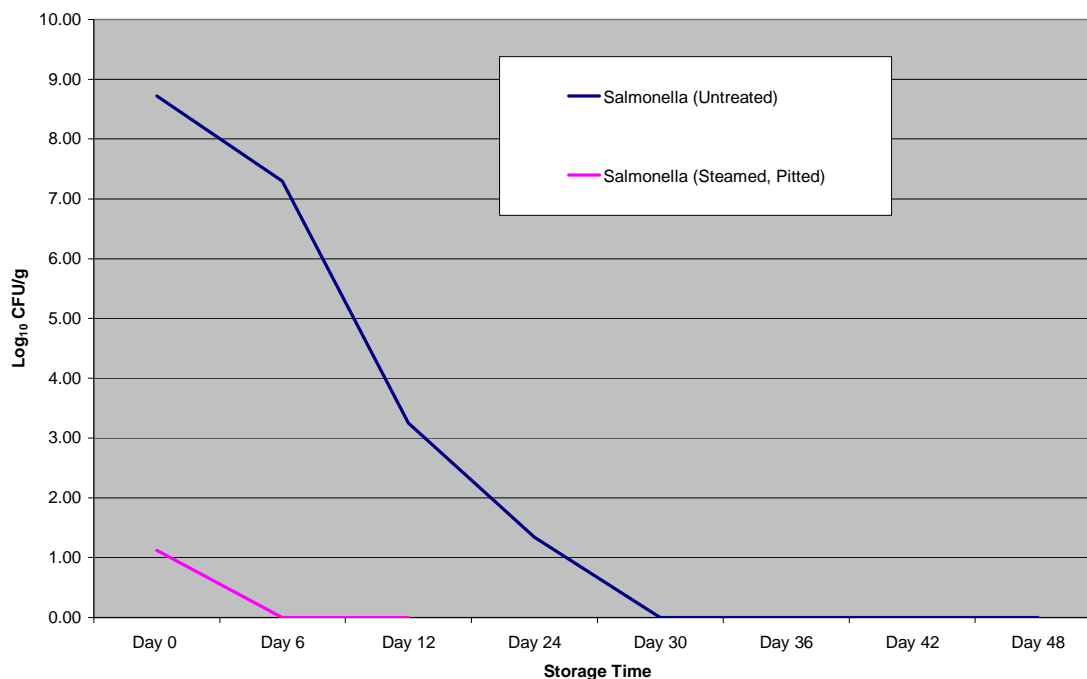
Table 6. Prunes inoculated with Salmonella and steam hydrated

Day 0	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	18	1.26
2	10	1.00
3	6	0.78
4	20	1.30
5	18	1.26
6	8	0.90
<i>Average</i>	<i>13</i>	<i>1.12</i>

**Table 6. continued**

Day 6	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	<1	0.00
2	<1	0.00
3	<1	0.00
4	<1	0.00
5	<1	0.00
6	<1	0.00
<i>Average</i>	<1	0.00
Day 12	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	<1	0.00
2	<1	0.00
3	<1	0.00
4	<1	0.00
5	<1	0.00
6	<1	0.00
<i>Average</i>	<1	0.00

When compared to prunes inoculated with *Salmonella* and held without steam treatment (See Table 2, Room Temperature column), steam hydrated prunes had a greater initial reduction of pathogen, and had no recoverable *Salmonella* after 6 days of storage. Results of the steam hydration testing are shown graphically in Figure 3, below.

Figure 3. Reduction of *Salmonella* on prunes using steam hydration

4.4 Dehydration results

Results from the dehydration testing are shown in Table 7, below including the raw enumeration (in CFU/g) for each replicate, the log₁₀ result for each replicate, and the average result for all replicates.

Table 7. Prunes inoculated with *Salmonella* and dehydrated

Replicate	Before Dehydration (CFU/g)	Log ₁₀ (CFU/g)	After Dehydration (CFU/g)	Log ₁₀ (CFU/g)
1	54,000,000	7.73	78	1.89
2	32,400,000	7.51	90	1.95
3	345,000,000	8.54	74	1.87
4	54,000,000	7.73	60	1.78
5	10,800,000	7.03	77	1.89
6	32,400,000	7.51	51	1.71
Average	88,100,000	7.94	72	1.86

Prunes inoculated with *Salmonella* and dehydrated showed a reduction of 6.09 log₁₀ CFU/g of pathogen.



4.5 Limit of detection results

Results from the limit of detection testing are shown in Table 8, below including the raw enumeration (in CFU/g) for each replicate, the log₁₀ result for each replicate, and the average result for all replicates.

Table 8. High moisture prunes (limit of detection)

Day 0	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	812,000,000	8.91
2	601,000,000	8.78
3	365,000,000	8.56
4	471,000,000	8.67
5	654,000,000	8.82
6	301,000,000	8.48
Average	534,000,000	8.73
Day 4	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	2,750,000	6.44
2	1,160,000	6.06
3	1,610,000	6.21
4	1,100,000	6.04
5	1,630,000	6.21
6	1,820,000	6.26
Average	1,678,333	6.22
Day 8	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	262,000	5.42
2	111,000	5.05
3	159,000	5.20
4	369,000	5.57
5	434,000	5.64
6	553,000	5.74
Average	314,667	5.50
Day 12	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	4,020	3.60
2	1,260	3.10
3	7,260	3.86
4	2,190	3.34
5	3,620	3.56
6	7,020	3.85
Average	4,228	3.63
Day 13	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	3,490	3.54
2	746	2.87
3	285	2.45
4	954	2.98
5	4,180	3.62
6	965	2.98
Average	1,770	3.25

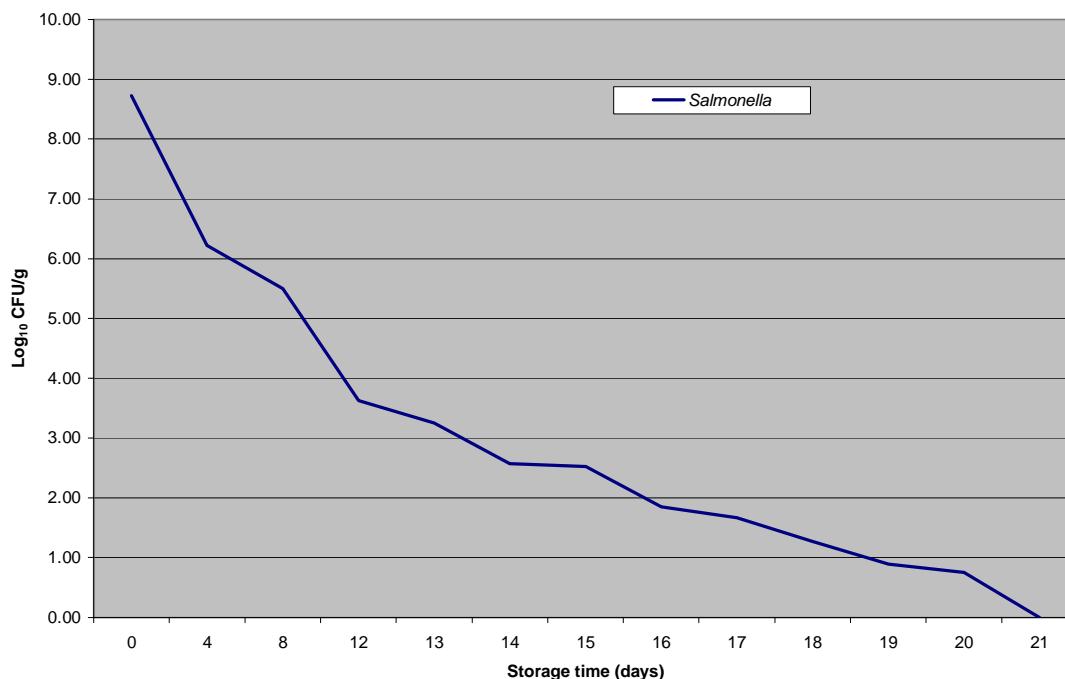
**Table 8. continued**

Day 14	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	212	2.33
2	725	2.86
3	215	2.33
4	301	2.48
5	394	2.60
6	392	2.59
<i>Average</i>	373	2.57
Day 15	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	238	2.38
2	207	2.32
3	104	2.02
4	301	2.48
5	892	2.95
6	277	2.44
<i>Average</i>	337	2.53
Day 16	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	120	2.08
2	80	1.90
3	60	1.78
4	50	1.70
5	30	1.48
6	80	1.90
<i>Average</i>	70	1.85
Day 17	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	105	2.02
2	21	1.32
3	11	1.04
4	11	1.04
5	117	2.07
6	11	1.04
<i>Average</i>	46	1.66
Day 18	XLT4 (CFU/g)	Log₁₀ (CFU/g)
1	10	1.00
2	10	1.00
3	10	1.00
4	41	1.61
5	10	1.00
6	31	1.49
<i>Average</i>	19	1.27

**Table 8. continued**

Day 19	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	<1	0.00
2	11	1.04
3	<1	0.00
4	<1	0.00
5	32	1.51
6	<1	0.00
Average	8	0.89
Day 20	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	<1	0.00
2	<1	0.00
3	<1	0.00
4	<1	0.00
5	10	1.00
6	20	1.30
Average	6	0.75
Day 21	XLT4 (CFU/g)	Log ₁₀ (CFU/g)
1	<1	0.00
2	<1	0.00
3	<1	0.00
4	<1	0.00
5	<1	0.00
6	<1	0.00
Average	<1	0.00

Salmonella was not detectable in the prunes after 21 days of storage. Reduction of *Salmonella* is shown graphically in Figure 4, below.

Figure 4. Limit of Detection testing for *Salmonella* in whole prunes

5.0 CONCLUSIONS

The data in this study shows that *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* are reduced more quickly in prunes held at room temperature than in prunes held refrigerated. *Salmonella* was reduced to undetectable levels in high moisture prunes in as little as 21 days when stored at room temperature, while *Listeria* was undetectable after 30 days and *E. coli* O157:H7 was undetectable after 36 days. When stored refrigerated, high moisture prunes still had detectable levels of all three pathogens after 48 days of storage, although the overall amounts of each pathogen had been reduced considerably (6.41 logs for *Salmonella*, 4.73 logs for *E. coli* O157:H7, and 5.43 logs for *Listeria*).

Several methods of reducing *Salmonella* on prunes were also investigated. The addition of potassium sorbate, steaming for 22 minutes and dehydration were effective. Steaming resulted in an initial 7.60 log reduction as compared to the standard high moisture prunes and no detectable pathogen in the prunes after 6 days of storage. Dehydration also produced a significant reduction of 6.09 logs of *Salmonella* after treatment.



6.0 REFERENCES

Danyluk, M. D., Uesugi, A. R. and L. J. Harris. 2005. Survival of *Salmonella* Enteritidis PT30 on inoculated almonds after commercial fumigation with propylene oxide. *J Food Prot* 68:1613 – 1622.



7.0 FINAL REPORT APPROVAL

Prepared By:

A handwritten signature in black ink, appearing to read "William C. Centrella II".

6/24/09

Bill Centrella
Study Director

Date

Approved By:

Food Safety Net Services Management:

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6/24/09

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Date

Study Sponsor:

Gary Obenauf

Date

NOTE: All results contained in the above report relate only to the items tested.