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Study Assessing the Effect of Safe₂O[®] brand RTE 01 Treatment on Comminuted Turkey Ham Products Inoculated With *Listeria monocytogenes* And Incubated at 4°C for Eleven Weeks

Objective:

Determine whether a 30 second treatment with Safe₂O[®] brand RTE 01 effectively prevents *Listeria monocytogenes* outgrowth for eleven weeks at 4°C.

Materials and Methods

1. Five strains of *Listeria monocytogenes* were cultured separately in BHI broth overnight at 37°C in a shaking water bath. Prior to use cultures were mixed in equal proportions. The mixture was further diluted 1:100,000 with sterile saline to produce a strain suspension for use. Meat inoculation level was determined by removing an aliquot of the mixture, making a serial dilution and plating same onto Tryptose Soy Agar (TSA) plates.
2. Turkey hams, prepared and shipped directly to Mionix Corporation, were carefully removed from the original packages to a sterile surface in a laminar flow bio-safety hood. Cured turkey ham pieces (108) approximately 1.2" X 1.2" X 0.3", were excised from the surface of the turkey hams.
3. All pieces were irradiated with UV light for 30 minutes before inoculation.
4. After irradiation, 20 micro liters of the *Listeria monocytogenes* suspension was inoculated onto the exterior side of each one of the pieces. All inoculated pieces were kept in the bio-safety hood for an additional 30 minutes to allow bacteria to attach.
5. Inoculated pieces were evenly divided into 3 groups (T, C₁ & C₂). Group T and group C₁ pieces were transferred into UV light sterilized nylon nets. Group T pieces were treated by dipping them into Safe₂O[®] brand RTE 01 (1:2 dilution) for 30 seconds. Group C₁ pieces were treated by submersion into sterile deionized water

- for 30 seconds. The amount of treatment solution used for each group was recorded in Table 1. After treatment, excess solution was allowed to drip off each piece for 15 sec and all pieces were then individually vacuum packed and sealed.
6. Group C₂ pieces were directly transferred and sealed in vacuum bags without treatment.
 7. All turkey pieces were incubated at 4°C. *Listeria* determinations were carried out at one hour post-treatment and at weekly intervals thereafter (see Table 1).
 8. For each set time period, three bags from each group were unpacked, and 5 ml of peptone water was added to each bag. *Listeria monocytogenes* organisms were washed off the surface of each turkey piece by a two minute hand massage of the bag. The number of *Listeria monocytogenes* colony forming units (CFU) per piece of turkey was determined by serial dilution of an aliquot from each rinsate followed by plating on Modified Oxford Selective Agar plates. All plates were incubated at 37°C for 40-48 hours before CFU determination.

Table 1: Treatment and Testing Schedule for Turkey Pieces

Product	Number of Pieces			Volume of Treatment Solution	Frequency of Micro-determination
	T	C ₁	C ₂		
Cured turkey ham	36	36	36	30 ml/piece	24 hrs and weekly

Results:

All turkey pieces were inoculated with 5.7×10^2 CFU/piece of turkey product the results are presented in Table 2.

Table 2: Effect of Safe₂O[®] brand RTE 01 treatment of Cured Turkey Ham on replication of *Listeria monocytogenes*

Time in 4°C (weeks)	Treatment	CFU/piece	\bar{A} of CFU/piece	Log Value	Log Reduction+
0	Without Any Treatment	2.15E+03	2.08E+03	3.32	
		2.24E+03			
		1.85E+03			
	Sterile H ₂ O X 30" Dip	4.45E+02	3.32E+02	2.52	
		3.10E+02			
		2.40E+02			
	RTE 01 X 30" Dip	0.00E+00	0.00E+00	<0.00	>2.52
		0.00E+00			
		0.00E+00			

1	Without Any Treatment	1.00E+05	8.35E+04	4.92	
		6.56E+04			
		8.50E+04			
	Sterile H ₂ O X 30" Dip	7.83E+03	9.29E+03	3.97	
		8.87E+03			
		1.12E+04			
	RTE 01 X 30" Dip	0.00E+00	0.00E+00	<0.00	>3.97
		0.00E+00			
		0.00E+00			

2	Without Any Treatment	8.70E+05	8.99E+05	5.95	
		9.47E+05			
		8.80E+05			
	Sterile H ₂ O X 30" Dip	3.87E+04	4.98E+04	4.70	
		3.87E+04			
		7.20E+04			
	RTE 01 X 30" Dip	0.00E+00	3.33E+00	0.52	4.18
		0.00E+00			
		1.00E+01			

3	Without Any Treatment	1.39E+07	2.04E+07	7.31	
		2.65E+07			
		2.09E+07			
	Sterile H ₂ O X 30" Dip	1.28E+06	8.44E+05	5.93	
		5.17E+05			
		7.40E+05			
	RTE 01 X 30" Dip	0.00E+00	0.00E+00	<0.22	>5.71
		0.00E+00			
		0.00E+00			

4	Without Any Treatment	2.97E+08	2.08E+08	8.32	
		2.39E+08			
		8.87E+07			
	Sterile H ₂ O X 30" Dip	9.73E+06	1.15E+07	7.06	
		6.90E+06			
		1.79E+07			
	RTE 01 X 30" Dip	5.00E+00	2.90E+02	2.46	4.60
		2.00E+01			
		8.45E+02			

5	Without Any Treatment	8.43E+08	5.69E+08	8.76	
		4.50E+08			
		4.13E+08			
	Sterile H ₂ O X 30" Dip	9.57E+07	1.06E+08	8.03	
		7.40E+07			
		1.47E+08			
	RTE 01 X 30" Dip	5.00E+00	3.33E+00	0.52	7.51
		0.00E+00			
		5.00E+00			

6	Without Any Treatment	4.37E+08	6.10E+08	8.79	
		9.17E+08			
		4.77E+08			
	Sterile H ₂ O X 30" Dip	7.77E+08	5.17E+08	8.71	
		2.97E+08			
		4.77E+08			
	RTE 01 X 30" Dip	0.00E+00	1.17E+01	1.07	7.64
		0.00E+00			
		3.50E+01			

7	Without Any Treatment	1.19E+09	1.11E+09	9.05	
		1.09E+09			
		1.06E+09			
	Sterile H ₂ O X 30" Dip	2.37E+08	3.06E+08	8.49	
		4.53E+08			
		2.27E+08			
	RTE 01 X 30" Dip	0.00E+00	0.00E+00	<0.22	>8.27
		0.00E+00			
		0.00E+00			

8	Without Any Treatment	1.44E+09	1.36E+09	9.13	
		1.41E+09			
		1.22E+09			
	Sterile H ₂ O X 30" Dip	3.80E+08	7.09E+08	8.85	
		8.00E+08			
		9.47E+08			
	RTE 01 X 30" Dip	0.00E+00	0.00E+00	<0.22	>8.63
		0.00E+00			
		0.00E+00			

9	Without Any Treatment	1.47E+09	1.38E+09	9.14	
		1.44E+09			
		1.23E+09			
	Sterile H ₂ O X 30" Dip	4.77E+08	6.57E+08	8.82	
		9.43E+08			
		5.50E+08			
	RTE 01 X 30" Dip	0.00E+00	1.37E+03	3.14	5.68
		0.00E+00			
		4.11E+03			

10	Without Any Treatment	9.97E+08	1.19E+09	9.08	
		9.03E+08			
		1.66E+09			
	Sterile H ₂ O X 30" Dip	8.57E+08	7.53E+08	8.88	
		7.23E+08			
		6.80E+08			
	RTE 01 X 30" Dip	0.00E+00	2.33E+01	1.37	7.51
		6.50E+01			
		5.00E+00			

11	Without Any Treatment	9.87E+08	9.85E+08	8.99	
		9.83E+08			
	Sterile H ₂ O X 30" Dip	6.13E+08	5.63E+08	8.75	
		5.60E+08			
		5.17E+08			
	RTE 01 X 30" Dip	5.50E+01	2.25E+04	4.35	4.40
		6.73E+04			
		1.00E+01			

*Relative to Group C₂ (sterile deionized water).

Conclusions:

As is evidenced by the studies presented herein, a 30 second treatment with Safe₂O[®]_{brand} RTE 01 effectively extends the shelf-life and prevents *Listeria monocytogenes* outgrowth when turkey ham pieces are incubated at 4°C (Table 2).

It is noteworthy that sporadic outgrowth occurred on one Safe₂O[®]_{brand} RTE 01 treated piece, at weeks 9 and 11, respectively. For reasons that are not understood results of this nature occur regularly in studies of this nature. Overall, a post-lethality effect of >2 logs was observed for the Safe₂O[®]_{brand} RTE 01 treated pieces and outgrowth was held to <2 logs. Therefore, according to FSIS DIRECTIVE 10,240.4 issued 10/2/03 a product so treated would be classified as Alternative 1.