Effect of Treatment Of Roast Beef With $Safe_2O^{®}_{brand}$RTE 01 on Replication of *Listeria monocytogenes:* Post-lethality and Growth Inhibitor

Objective:

Determine whether a 30 sec. treatment with $Safe_2O^{®}_{brand}$RTE 01 can effectively extend the shelf-life and inhibit *Listeria monocytogenes* growth when incubated for seven weeks at 4^oC.

Materials and Methods

1. Five strains of *Listeria monocytogenes* were cultured separately in BHI broth overnight at 37^oC 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 serial dilutions onto TSA plates.
2. Roast beef obtained from manufacturer was carefully unpacked and removed from the original package to a sterile surface in a laminar flow bio-safety hood. Eighty one pieces, approximately 1.2" X 1.2" X 0.3" were excised from the surface of the same piece of roast beef.
3. All pieces were irradiated with UV light for 30 minutes before inoculation.
4. After irradiation, 20 microliters of the *Listeria monocytogenes* suspension described above, was inoculated onto the exterior side of each one of the roast beef meat pieces. All inoculated pieces were kept in hood for an additional 30 minutes to allow bacteria to attach.
5. Inoculated pieces were divided into 3 groups (T, C_1 & C_2), having twenty seven pieces each. Group T pieces were treated by dipping them into 1000 ml of $Safe_2O^{®}_{brand}$RTE 01 (1:2 dilution) for 30 seconds. Group C_1 pieces were treated by submersion into 1000 ml of sterile dH_2O for 30 seconds. After the treatment, excess solution was allowed to drip off for 10 sec and all pieces were then individually sealed in a vacuum pouch.
6. Group C_2 was directly transferred and sealed in vacuum pouches without treatment.
7. All roast beef pieces were incubated at 4^oC refrigerator. Listeria determinations were carried out at one hour post-treatment and at weekly intervals thereafter.
8. For each set time period, three pouches from each group were unpacked, and 5 ml of peptone water was added to each pouch. *Listeria monocytogenes* organisms were washed off the surface of each roast beef piece by two minutes of hand massage in the pouch. The number of *Listeria monocytogenes* colony forming units (CFU) per piece of roast beef 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^oC for 40-48 hours before CFU determination.

Results:

The objective of the study was to determine whether a 30 sec. treatment with $Safe_2O^{®}_{brand}$RTE 01 can effectively kill more than two logs of Listeria monocytogenes on contact and prevent *Listeria monocytogenes* outgrowth for more than seven weeks when roast beef is incubated at 4^oC. The effect of treatment on initial kill and outgrowth of *Listeria monocytogenes* was assessed by CFU determination on selective agar (see **Table 1**).

Table 1: Effect of $Safe_2O^{®}_{brand}$RTE 01 treatment of roast beef on replication of *Listeria monocytogenes*

Time in 4^oC (weeks)	Treatment	CFU/piece	Average CFU/piece	Log Value	Log Reduction*
0	Without Any Treatment	2.20E+02	3.28E+02	2.52	
		3.75E+02			
		3.90E+02			
	Sterile H_2O X 30" Dip	2.95E+02	2.82E+02	2.45	
		2.40E+02			
		3.10E+02			
	RTE 01 X 30" Dip	0.00E+00	1.67E+00	0.22	2.23
		5.00E+00			
		0.00E+00			
1	Without Any Treatment	5.00E+03	1.32E+04	4.12	
		8.97E+03			
		2.55E+04			
	Sterile H_2O X 30" Dip	1.41E+04	1.58E+04	4.20	
		2.51E+04			
		8.07E+03			
	RTE 01 X 30" Dip	1.00E+01	6.67E+00	0.82	3.38
		1.00E+01			
		0.00E+00			
2	Without Any Treatment	1.49E+05	2.61E+05	5.42	
		2.04E+05			
		4.30E+05			
	Sterile H_2O X 30" Dip	1.53E+06	3.28E+06	6.52	
		3.31E+06			
		5.00E+06			
	RTE 01 X 30" Dip	0.00E+00	<1.67E+00	<0.22	>6.3
		0.00E+00			
		0.00E+00			

3	Without Any Treatment	2.33E+07	3.62E+07	7.56	
		5.07E+07			
		3.47E+07			
	Sterile H_2O X 30" Dip	2.55E+09	1.66E+09	9.22	
		1.30E+08			
		2.31E+09			
	RTE 01 X 30" Dip	0.00E+00	<1.67E+00	<0.22	>9.00
		0.00E+00			
		0.00E+00			

4	Without Any Treatment	2.63E+08	3.52E+08	8.55	
		5.60E+07			
		7.37E+08			
	Sterile H_2O X 30" Dip	3.07E+08	2.35E+09	9.37	
		2.45E+09			
		4.30E+09			
	RTE 01 X 30" Dip	0.00E+00	1.67E+00	0.22	9.15
		5.00E+00			
		0.00E+00			

6	Without Any Treatment	6.40E+09	7.15E+09	9.85	
		5.43E+09			
		9.63E+09			
	Sterile H_2O X 30" Dip	6.93E+09	1.27E+10	10.10	
		1.25E+10			
		1.87E+10			
	RTE 01 X 30" Dip	0.00E+00	<1.67E+00	<0.22	>9.88
		0.00E+00			
		0.00E+00			

7	Without Any Treatment	5.87E+09	9.36E+09	9.97	
		1.09E+10			
		1.13E+10			
	Sterile H_2O X 30" Dip	9.43E+09	8.93E+09	9.95	
		8.63E+09			
		8.73E+09			
	RTE 01 X 30" Dip	0.00E+00	1.67E+00	0.22	9.73
		0.00E+00			
		5.00E+00			

8	Without Any Treatment	8.43E+09	9.11E+09	9.96	
		9.43E+09			
		9.47E+09			
	Sterile H_2O X 30" Dip	6.33E+09	8.81E+09	9.94	
		6.30E+09			
		1.38E+10			
	RTE 01 X	0.00E+00	<1.67E+00	<0.22	>9.72

	30" Dip	0.00E+00			
		0.00E+00			

* Log reduction relative to sterile deionized H_2O Treated Group.

For study purposes 81 roast beef pieces were excised from the surface of a piece of roast beef and inoculated with *Listeria monocytogenes* at a level of 6.46 X 10^2 CFU/piece. The 81 pieces were divided into three groups, T, C_1 and C_2, comprised of 27 pieces each. Group T pieces were treated by dipping them into 1000 ml of $Safe_2O^{®}_{brand}$RTE 01 (1:2 dilution) for 30 seconds. Group C_1 pieces were treated by submersion into 1000 ml of sterile deionized H_2O for 30 seconds. After the treatment, excess solution was allowed to drip off for 10 sec and all pieces were then individually sealed in a vacuum pouch. Group C_2 was directly transferred and sealed in vacuum pouches without treatment.

As can be seen from Table 1, detectable levels on untreated and water treated roast beef pieces were in excess of 2 logs, whereas the number of CFU associated with the treated pieces was on average reduced by >2 logs. Over the next eight weeks the CFU associated with the control and water treated pieces increased from 2.52 and 2.45 logs to 9.96 and 9.94 logs respectively. Treatment with $Safe_2O^{®}_{brand}$RTE 01 reduced the number of Listeria monocytogenes CFU from inoculation log levels of 2.81 to 0.22, near detectable limits. Moreover, treatment maintained and/or prevented outgrowth throughout the study cycle of eight weeks.

Conclusions:

The studies presented above show treatment with $Safe_2O^{®}_{brand}$RTE 01 kills more than two logs of *Listeria monocytogenes* on contact and totally prevents outgrowth for at least eight weeks. From the regulatory perspective these results allow a manufacturer to go from an “Alternative 3” status to an “Alternative 1” status, i.e., the treatment functions as a post-lethality step and acts as a growth inhibitor, i.e., it prevents Listeria outgrowth. An additional benefit is the extension shelf-life relative to decay organisms, i.e., aerobic organisms (see Effect of $Safe_2O^{®}_{brand}$RTE 01 Treatment on Shelf-Life of Roast Beef).