Anti-Microbial Effect of $Safe_2O^{®}_{Brand}$RTE 01 Treatment of Smoked Salmon Inoculated with *Listeria monocytogenes*: Dip vs. Spray Application

Objective:

Determine the most effective method of applying $Safe_2O^{®}_{Brand}$RTE 01 for the purpose of killing *Listeria monocytogenes* associated with the surface of smoked salmon.

Material:

- Smoked salmon.
- Five strains of Listeria monocytogenes (ATCC#: 13932, 43256, 7674, 19111 and 19115).
- $Safe_2O^{®}_{Brand}$RTE 01 diluted one part solution with two parts water.
- Sterile dionized H_2O.
- Sterile saline.
- Sterile peptone water.
- Sterile phosphate buffer, pH 7.38.
- Culture Media: BHI (Brain Heart Infusion) broth and Modified Oxford Listeria Selective Agar.

Method:

- *Listeria monocytogenes*, five strains were cultured separately overnight in BHI broth, mixed in equal proportion. The mixture was further diluted 1:1000 with sterile saline before inoculation.
- One piece of smoked salmon was carefully unpacked and removed from the original packages onto a sterile surface inside a bio-safety hood. Fish was then cut into 2.0" X 2.0" X 0.5" size pieces. One face of each piece was covered scales. A total of 15 pieces were prepared for study purposes.
- All cut pieces were laid on a sterile surface with the 2" X 2" non-scale side facing up.
- Previously diluted strain suspension, 20 µl (5.61×10^4/salmon piece), was inoculated onto each piece. All inoculated pieces were kept at room temperature for 1 hour to allow attachment of the *Listeria monocytogenes*.
- Inoculated pieces were divided into 5 groups, DT, DC, SD, SC and C, respectively, having three pieces per group. Group DT pieces were dipped in 1L of $Safe_2O^{®}_{Brand}$RTE 01 (1:2 dilution as described above) for 30 seconds. Group DC pieces were dipped in 1L of deionized H_2O for 30 seconds. Group ST pieces were sprayed with $Safe_2O^{®}_{Brand}$RTE 01 (1:2 dilution as described above) for 10 seconds and group SC pieces were sprayed with deionized H_2O for 10 seconds.

- After treatment, pieces from each group were allowed to drain for 10 seconds then transferred individually into a vacuum bag.
- Group C salmon pieces were directly transferred into vacuum bags without any treatment. All bags were kept in 4^{o}C refrigerator for 24 hours after being vacuum sealed.
- Post-treatment Listeria survival assays were performed after incubation at 4^{o}C for 24 hour. Assays were performed as follows:
    - Ten ml of peptone water was added to each bag.
    - Listeria monocytogenes was physically removed from each salmon piece by two minute hand massage of the bag.
    - Colony forming units (CFU) per piece was determined by plating an aliquot of the rinsate and a portion of the serial dilution thereof on Modified Oxford Listeria Selective Agar plates. After plating, all plates were incubated at 37^{o}C for about ~48 hours before CFU determination.

Result:

Table 1: Anti-Microbial Effect of $Safe_2O^{®}_{Brand}$RTE 01 Treatment of Smoked Salmon Inoculated with *Listeria monocytogenes*

Treatment[§]	CFU/piece	Ā of CFU/piece	Log Value	Log Reduction
"C" Control	6.73E+04	6.80E+04	4.83	
	6.87E+04			
	6.80E+04			
"DC" 30" dip in deionized water	2.60E+04	3.84E+04	4.58	
	4.00E+04			
	4.93E+04			
"DT" 30" dip in $Safe_2O^{®}_{Brand}$RTE 01	1.00E+01	5.77E+02	2.76	1.82*
	5.00E+01			
	1.67E+03			
"SC" 10" spray with deionized water	7.13E+03	9.04E+03	3.96	
	1.06E+04			
	9.40E+03			
"SC" 10" spray with $Safe_2O^{®}_{Brand}$RTE 01	4.80E+02	1.18E+03	3.07	0.89**
	2.84E+03			
	2.30E+02			

[§]Each salmon piece was inoculated with 5.61 X 10^{4} *Listeria monocytogenes* colony forming units.

*Relative to 30 sec dip in deionized water.

**Relative to 10 sec spray with deionized water.

Conclusions:

As can be seen from the results, $Safe_2O^{®}_{Brand}$RTE 01 treatment using a dip process or spray application effectively reduced the number of *Listeria monocytogenes* organisms associated with the smoked salmon pieces. The spray treatment was significantly less effective than the dip process but spray was only applied for 10' as compared to a 30" dip.

With respect to organoleptic effects, no noticeable color differences were noted between treated and untreated samples. It should be noted that in past studies a 30" dip process has been effective in extending the shelf-life of many meat products and likewise for fish.