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## **Effect of Safe<sub>2</sub>O<sup>®</sup> RTE-01 Intervention Treatment on *Listeria monocytogenes* on RTE Roasted Chicken**

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### **Objective:**

Determine the effectiveness of a Mionix intervention in reduction of *L. monocytogenes* on roasted chicken.

### **Materials and Methods:**

1. The experiment was done by Hillary lead team in Food Safety and Research Laboratory, 3609 Johnson Rd., Springdale, AR 72762-6999. Dr. Maurice Kemp, Chief Technical Officer, and Yao Yu, Research Scientist, of Mionix Corporation, 4031 Alvis Court, Rocklin, CA 95677, prepared the Safe2O-RTE-01 solution for treatment.
2. Bacteria Used:  
A five strain cocktail of *Listeria monocytogenes* provided by Tyson.
3. Treatment Solutions Used:
  - 1) RTE 01 (1:1 dilution with Springdale's city water), pH 1.44, Lactate 176721.77ppm, Sulfate 8656.13ppm, Phosphate 7034.89ppm (see HPLC analysis report).
  - 2) City water (pH 8.0)
  - 3) Without treatment
4. Procedure:
  - 1) The skin side of roasted chicken breast was cut off by 10g each piece. Total 120 pieces. 40 pieces for untreated group; 40 pieces for control group treated with city water; 40 pieces for the group treated with Mionix RTE 01.

- 2) About 4 log of *Listeria* cocktail (100ul) was inoculated onto each piece of chicken breast at 20 minutes before the treatment.
- 3) At 20 minute inoculation, 40 pieces were transferred into stomach bags (one piece chicken breast per bag). Sealed the bags and kept in 4°C. 40 pieces were sprayed with city water for 20 seconds and transferred into stomach bags (one piece chicken breast per bag), and sealed and kept in 4°C. 40 pieces were sprayed with Mionix RTE 01 for 20 seconds and transferred into stomach bags (one piece chicken breast per bag), and sealed and kept in 4°C.
- 4) After 1 hour in 4°C, 20 bags from each treatment group were taken out of the 4°C refrigerator. 90 ml of buffer were added into each bag. All the chicken breasts were stomached and then plated onto *Listeria* Selective Agar plates for CFU count and data analysis.
- 5) After 24 hours in 4°C, rest of the bags from each treatment group were taken out of the refrigerator and done the same as that in step 4).

Note:

- 1) Because the city water in Springdale is alkaline, more RTE 01 was added in the treatment solution to target final pH 1.5. (10L city water + 10L RTE 01).
- 2) Chicken breasts were transferred into stomacher bags after treatment without dripping off the extra solution.
- 3) The *Listeria* attachment time actually was about 1 hour for Mionix treatment because of the delay of solution preparation.

## Results:

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A study was conducted to determine the effectiveness of a Mionix intervention in reduction of *L. monocytogenes* in roasted chicken. The roasted chicken breast meat was inoculated with a *L. monocytogenes* five strain cocktail to an initial level of approximately  $1.2 \times 10^4$  cfu/ml per piece of breast meat. The inoculated chicken was then subjected to a Mionix treatment, a water treatment, or no treatment. Samples were collected at both one and twenty-four hours after treatment. This resulted in the following six treatment groups: A. no treatment (control) sampled one hour after treatment, B. water treatment sampled one hour after treatment, C. Mionix treatment sampled one hour after treatment, D. no treatment (control) sampled twenty-four hours after treatment, E. water treatment sampled twenty-four hours after treatment, and F. Mionix treatment sampled twenty four hours after treatment.

Count data were analyzed using the GLM procedure of SAS, (Barr, et.al., 1982). Data were log<sub>10</sub> transformed prior to analysis in order for the data to meet the underlying assumption of the Analysis of Variance (ANOVA). Counts denoted <10 were given a value of 1 before transformation. Data were analyzed using a one-way analysis of variance. The statistical model was:

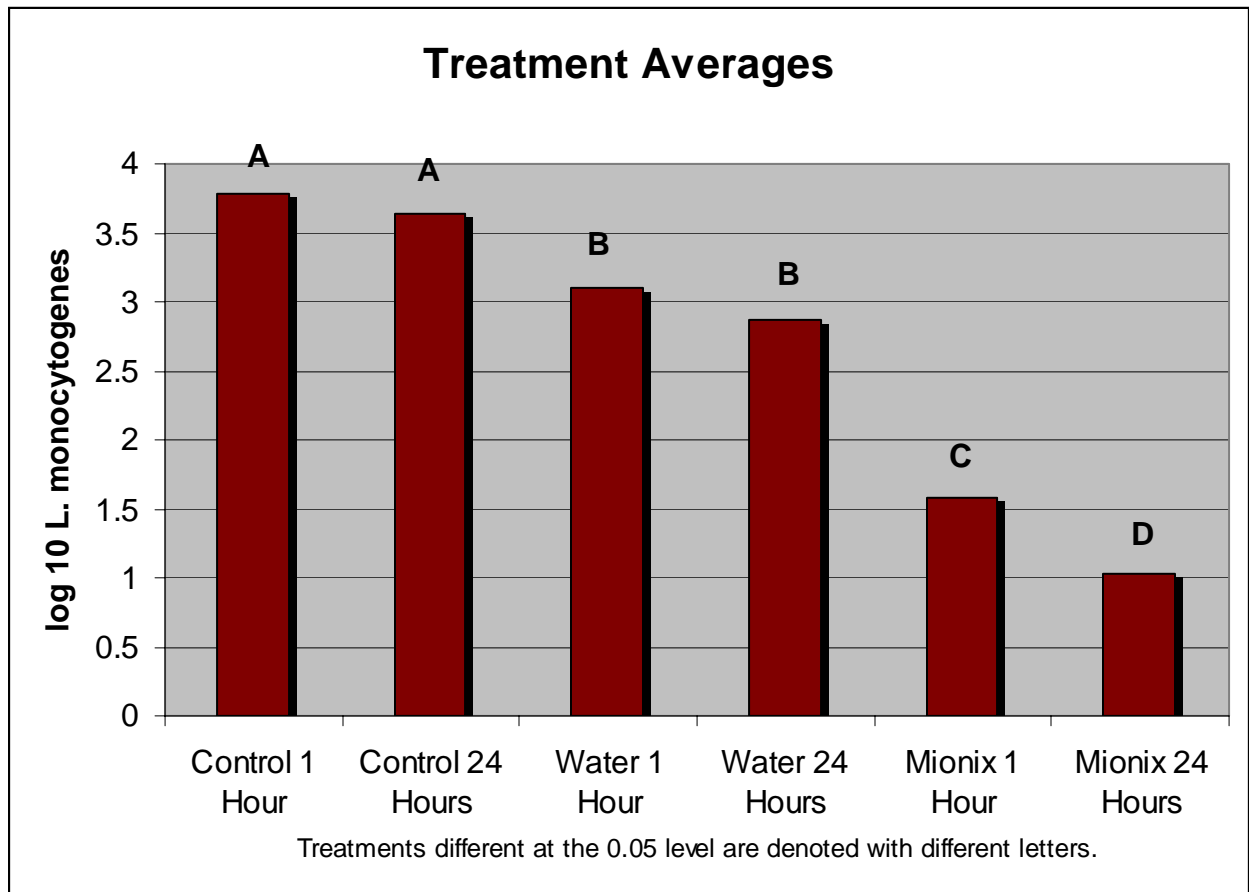
$$y_{ij} = \mu + \alpha_i + e_{ij}$$

Where  $y_{ij}$  is equal to the overall mean, ( $\mu$ ), plus the effect due to the treatment, ( $\alpha_i$ ), plus random error, ( $e_{ij}$ ).

Table 1. Mean *L. monocytogenes* Counts by Treatment.

<u>Treatment</u>	<u>Log10</u>	<u>Natural Number</u>
Control 1 Hour After Treatment	3.79 <sup>a</sup>	6107
Control 24 Hours After Treatment	3.63 <sup>a</sup>	4293
Water 1 Hour After Treatment	3.10 <sup>b</sup>	1257
Water 24 Hours After Treatment	2.88 <sup>b</sup>	753
Mionix 1 Hour After Treatment	1.58 <sup>c</sup>	38
Mionix 24 Hours After Treatment	1.03 <sup>d</sup>	11

<sup>abcd</sup> Means having different superscripts were significantly ( $p \leq .05$ ) different.



## Conclusions:

Treatment effects were significant, ( $p \leq .05$ ). The chicken that received no treatment had the highest counts. The chicken treated with water had significantly lower levels of *L. monocytogenes* on average than the group receiving no treatment. The chicken treated with Mionix had the lowest levels of *L. monocytogenes*. The groups receiving no treatment (the controls) and the groups receiving the water treatment were not significantly different from 1 hour to 24 hours after treatment. However, of the two groups receiving the Mionix treatment, were significantly different from the Control and water treatments at 1 hour and 24 hour. In addition, the Mionix treated group sampled 24 hours after treatment had significantly lower counts than the group sampled just one hour after treatment, (Table 1).

In summary, Safe<sub>2</sub>O-RTE-01 treatment has a significant biocidal effect causing >2 than a two log reduction in the number of *Listeria monocytogenes* organisms associated with RTE roasted chicken breasts at 1 hour post-treatment.

## SAS OUTPUT:

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The MEANS Procedure

Analysis Variable : I result

trt	N Obs	N	Mean	Std Dev	Minimum
Control 1	20	20	3.7858117	0.0829553	3.6127839
Control 24	20	20	3.6328452	0.0997012	3.4913617
Mionix 1	20	20	1.5821407	0.8801272	0
Mionix 24	20	20	1.0276213	0.9535547	0
Water 1	20	20	3.0991770	0.2939232	2.6532125
Water 24	20	20	2.8766670	0.2823678	2.3979400

Analysis Variable : I result

trt	N Obs	Maximum
Control 1	20	3.8976271
Control 24	20	3.9084850
Mionix 1	20	2.9138139
Mionix 24	20	2.3617278
Water 1	20	3.5682017
Water 24	20	3.2648178

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The GLM Procedure  
Class Level Information

Class	Levels	Values
trt	6	Control 1 Control 24 Mionix 1 Mionix 24 Water 1 Water 24

Number of observations 120

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The GLM Procedure

Dependent Variable: I result

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	125.5962978	25.1192596	80.73	<.0001
Error	114	35.4698607	0.3111391		
Corrected Total	119	161.0661584			

R-Square 0.779781  
Coeff Var 20.91187  
Root MSE 0.557798  
I result Mean 2.667377

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	5	125.5962978	25.1192596	80.73	<.0001

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The GLM Procedure

Duncan's Multiple Range Test for I result

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05  
Error Degrees of Freedom 114  
Error Mean Square 0.311139

Number of Means	2	3	4	5	6
Critical Range	.3494	.3678	.3799	.3889	.3958

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	3.7858	20	Control 1
A	3.6328	20	Control 24
B	3.0992	20	Water 1
B	2.8767	20	Water 24
C	1.5821	20	Mionix 1
D	1.0276	20	Mionix 24