

MICROBIAL CONTROL FOR A READY TO EAT (RTE) COMMINUTED HAM PRODUCT: EFFECT OF A POST-LETHALITY TREATMENT WITH Safe₂O[®] Brand RTE 03 ON SHELF-LIFE AND LISTERIA OUTGROWTH

Objective:

Determine if treatment with Safe₂O[®] Brand RTE 03 can extend the shelf-life of RTE ham products and prevent outgrowth of *Listeria monocytogenes*.

Methods and Materials:

Shelf-Life Study

1. Comminuted hams were cut into 111 pieces.
2. Each piece was weighted, placed in a zip-lock bag and labeled.
3. The microbial level associated with three pieces was determined to establish a baseline.
4. The remaining pieces (108) were divided into two groups. Each piece in the control (C) group consisting of 54 pieces of ham was individually placed in bags and vacuum-packed. Likewise, after treatment with Safe₂O[®] Brand RTE 03 by total immersion for 30 sec followed by a drip period of 10 sec the 54 pieces comprising the treatment group (T) group were placed in bags and vacuum-packed.
5. Both C and T group pieces were then incubated at 10°C prior to testing at designated intervals.
6. Microbial levels associated with three pieces from the C and T groups were evaluated at weekly intervals up to 17 weeks.
7. For microbial evaluation each piece was removed from the vacuum packaging material and transferred to a zip-lock bag. One ml/g of ham product of phosphate buffer (pH 7.38) was added to each ham piece and ham pieces were shaken 100 times to wash associated bacteria from the surface of each piece. An aliquot of the rinsate from each piece was serially diluted and an aliquot of each dilution was plated on aerobic plate count (APC) agar (Casein Peptone Dextrose Yeast Agar). Plates were incubated for 72 hours at 37°C before APC determination.

***Listeria monocytogenes* Challenge Study**

1. Seventy-two pieces of comminuted ham (200-300g) were received and kept at 4°C for about two months.
2. Individual ham pieces were unwrapped and allowed to air dry for 15 minutes. An overnight culture of *Listeria monocytogenes* ATCC 19111 was prepared and ham

- pieces were inoculated with 100 microliters ($2.41E+08$ CFU/ham piece). After inoculation pieces were air dried for an additional 3 hrs at room temperature.
- Inoculated pieces were divided into 2 groups; control group (C) and treated group (T). C and T pieces were then processed as follows: C pieces were immersed in 150ml of deionized water for 30 seconds, removed, allowed to drip for 5 seconds and individually packaged in zip lock bags. T pieces were immersed in 150ml Safe₂O[®]_{Brand} RTE 03 for 30 seconds, allowed to drip for 10 seconds and individually packaged in zip-lock bags.
 - Control and treated ham pieces were incubated at 10°C.
 - One set of samples was tested at 24 hr while the remainder was assayed at 2-week intervals.
 - At each test interval, three C and T ham pieces were removed from the incubator and transferred to zip lock bags containing 100ml of phosphate buffer (pH 7.38). Ham pieces were shaken 100 times to wash associated bacteria from the surface of each piece. An aliquot from each rinsate was serially diluted and an aliquot of each dilution was plated on Oxford Listeria Selective Agar plates.
 - Plates were incubated for 72 hours at 37°C prior to determination of the number of *Listeria monocytogenes* colony-forming units (CFU).

Results:

One week after the shelf-life study was initiated the total aerobic plate count (TPC) for control and treated ham pieces was the same (Table 1). However, by the third week there was a significant increase in the TPC level for the control pieces (Table 1 and Figure 1). By the fourth week TPC levels associated with the control pieces were such that they would be considered spoiled.

Table 1: Effect of treatment with Safe₂O[®]_{Brand} RTE 03 on the total number of aerobic bacteria associated with a ready to eat (RTE) comminuted ham product

Incubation Time (Weeks)	Control (CFU/100g)	Treated (CFU/100g)
0	2.89×10^3	2.89×10^3
1	2.20×10^2	2.20×10^2
2	6.60×10^4	2.20×10^2
3	3.72×10^7	$<2.20 \times 10^2$
4	2.07×10^6	5.10×10^3
5	2.06×10^7	4.44×10^2
6	8.34×10^8	1.20×10^4
7	1.69×10^7	2.20×10^2
8	4.10×10^8	3.56×10^3
9	1.12×10^8	2.20×10^2
10	6.76×10^8	2.20×10^2
11	3.04×10^8	2.67×10^3
12	1.59×10^8	2.56×10^4
13	3.53×10^9	4.44×10^2
14	6.05×10^7	2.13×10^4
15	1.93×10^9	$<2.20 \times 10^2$
16	9.60×10^6	3.56×10^3
17	6.11×10^8	1.47×10^4

The TPC levels associated with the ham pieces treated with Safe₂O[®]_{Brand}RTE 03 remained at levels initially detected at the start of the study. In fact, as can be seen from Table 1 and Figure 1, the TPC levels after 17 weeks of incubation were well under the threshold number at which meat products are considered spoiled and not fit for consumption.

The treatment with Safe₂O[®]_{Brand}RTE 03 effectively prevented outgrowth of aerobic bacteria over a 17 week period extending the shelf-life of the comminuted ham product. Beyond shelf-life extension this study was extended to assess the effects of treatment from the perspective of Listeria control, i.e., does treatment with Safe₂O[®]_{Brand}RTE 03 bring about a biocidal effect with respect to *Listeria monocytogenes* and/or prevent outgrowth of this organism?

Ham pieces were inoculated with 2.41×10^8 CFU of *Listeria monocytogenes*/ham piece and treated with water or Safe₂O[®]_{Brand}RTE 03 for 30 sec. As can be seen from Table 2, the water treatment alone removed <1 log of Listeria from the surface of the ham pieces as determined at 24 hr post-treatment. However, treatment with Safe₂O[®]_{Brand}RTE 03 brought about an additional reduction of >2 logs. It is concluded, therefore, that Safe₂O[®]_{Brand}RTE 03 treatment is biocidal and is effective as a post-lethality treatment.

Beyond the instant kill properties of Safe₂O[®]_{Brand}RTE 03 treatment it can be seen from Table 2 and Figure 2, treatment with Safe₂O[®]_{Brand}RTE 03 in addition to suppressing replication aerobic bacteria (Table 1 and Figure 1) prevents the outgrowth of *Listeria monocytogenes* on ham pieces incubated at 10⁰C for at least 12 weeks. Moreover, the number of viable *Listeria monocytogenes* organisms continues to diminish over the time of incubation and it appears that this is not due to competition from aerobic bacteria (see Table 1).

Figure 1: Effect of treatment with SAFE₂O[®] Brand RTE 03 on total number of aerobic bacteria associated with a ready to eat (RTE) comminuted ham product

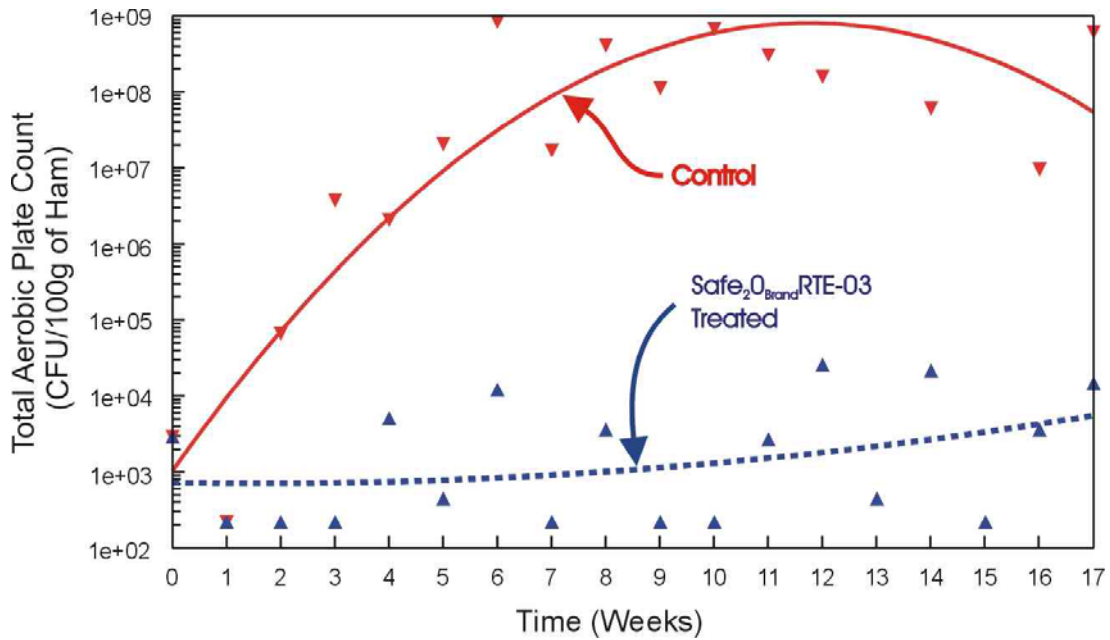


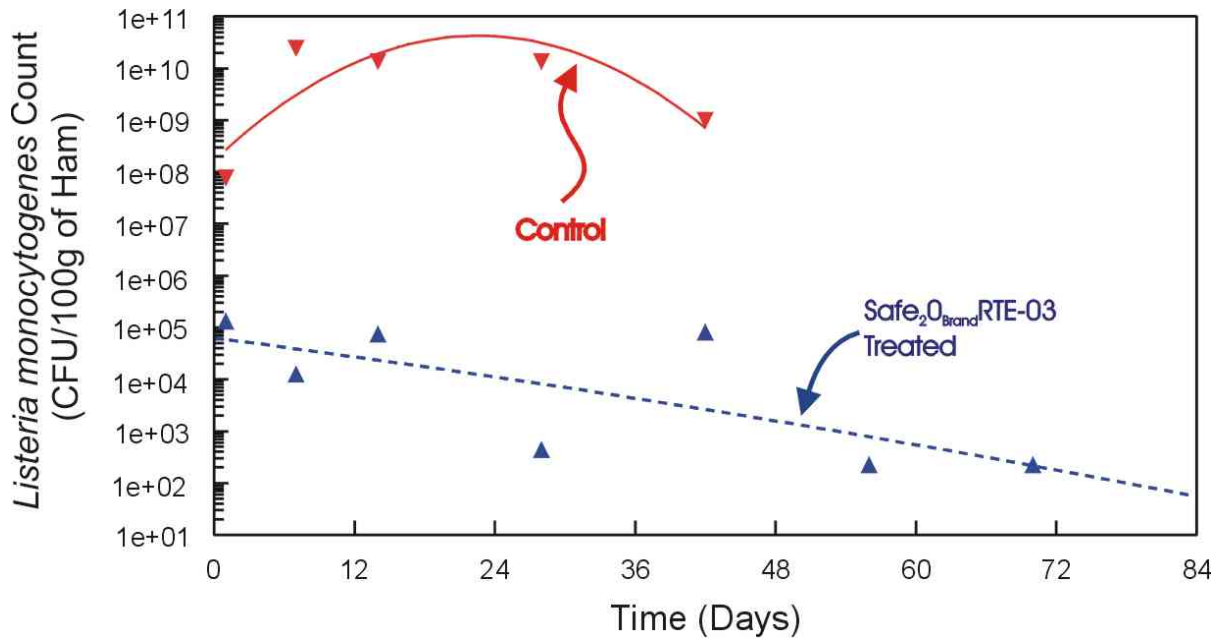
Table 2: Effect of treatment with Safe₂O[®] Brand RTE 03 on ready to eat (RTE) comminuted ham products inoculated with *Listeria monocytogenes**

Time Post-Treatment (Days)	Control (CFU/ham piece)	Treated (CFU/Ham Piece)
24hours	7.77 X 10 ⁷	1.31 X 10 ⁵
Week 2	2.44 X 10 ¹⁰	1.25 X 10 ⁴
Week 4	1.35 X 10 ¹⁰	7.40 X 10 ⁴
Week 6	1.37 X 10 ¹⁰	4.44 X 10 ²
Week 8	1.00 X 10 ⁹	8.07 X 10 ⁴
Week 10	ND**	2.22 X 10 ²
Week 12	ND**	<2.22 X 10 ²

*Inoculation level, 2.41 X 10⁸ CFU/ham piece

**Not determined because meat was spoiled

Figure 2: Effect of treatment with Safe₂O[®] Brand RTE 03 on ready to eat (RTE) comminuted ham products inoculated with *Listeria monocytogenes*



Conclusion

Treatment with Safe₂O[®] Brand RTE 03 not only prevents replication of decay bacteria it also functions as a post lethality treatment relative to *Listeria monocytogenes* and prevents outgrowth of this organism as well. Therefore, Safe₂O[®] Brand RTE 03 treatment would allow the manufacturer to claim Alternative 1 status for products so treated under the new USDA guidelines for the control of Listeria per the final rule effective October 6, 2003 [see Federal Register: June 6, 2003 (Volume 68, Number 109)][Rules and Regulations][Page 34207-34254]