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Effect of the Addition of $Safe_2O^{®}$ Brand -GB on the Thermal Inactivation of E. *coli.* O157:H7 at 57.5°C: D-value Determination

Despite the best efforts of the meat industry, microbial organisms including pathogens such as *E. coli* O157:H7 and/or *Salmonella spp.* persist in infiltrating the food supply. Since total elimination of these organisms is unlikely, especially with minimally processed foods, such as fresh ground beef, the meat industry has turned to a hurdle approach to limit the dangers. This technique sets up as many roadblocks as possible to microbial survival and growth. This not only includes processing, HACCP programs, SSOPs, and cGMPs, but also involves designing formulas that suppress and/or prevent replication of microbial organisms. The studies presented herein demonstrate that $Safe_2O^{®}$ Brand -GB when added to ground beef significantly alters the D-value of the ground beef, thereby increasing the thermal effectiveness in eliminating microbial pathogens from the meat. Hence, addition of $Safe_2O^{®}$ Brand -GB represents an additional hurdle and decreases the risk of transmission of pathogens to the consumer.

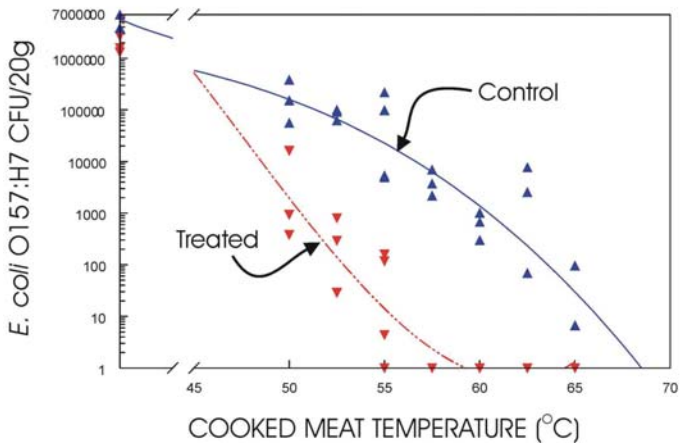

Fig. 1: $Safe_2O^{®}$ Brand -GB effects on E. *coli* O157:H7 survival in ground beef during cooking

The flesh of meat animals prior to slaughter has a pH value of ~7.1. After slaughtering, some of the glycogen in the meat turns into lactic acid. As a result, the pH value is lowered. The increasing acidity of the maturing carcass varies in its speed, depending on a number of factors such as type of animal, breed, rearing characteristics and treatment of the animal prior to slaughter.

Nevertheless, beef normally reaches its lowest pH value of 5.4 to 5.7 at 18-24 hours after slaughter. After the lowest pH level is reached, the pH starts to rise again slowly but steadily until it reaches a pH of ~6.5.

Many factors affect microbial growth in foods such as meat - temperature, oxygen, presence of salts, microbial load and competition, antimicrobial agents, water activity and pH are among the most important. All factors aside other than pH, meat having a pH of 6.5 is subject to microbial decomposition.

In seeking to prevent microbial replication in ground meats Mionix demonstrated that $Safe_2O^{®}$ Brand –GB added to ground beef reduced the temperature to which ground beef had to be cooked in order to completely eliminate viable *E. coli* organisms (see Fig. 1).

Based on these studies Dr. Michael Doyle, University of Georgia, carried out a study entitled "Effect of $Safe_2O^{®}$ Brand -Ground Beef Additive and heating on the inactivation of *E. coli* O157:H7 in ground beef: Determination of D-values of *E. coli* O157:H7 in ground beef". This study showed that the addition of $Safe_2O^{®}$ Brand -GB to ground beef substantially increased the rate of thermal inactivation of *E. coli* O157:H7 in ground beef, with D-values reduced by approximately 1.5- to 4-fold. In addition, D-values of *E. coli* O157:H7 were approximately 2-fold less in frozen than in refrigerated. $Safe_2O^{®}$ Brand -GB -treated ground beef, indicating that freezing further sensitized the pathogen to heat and $Safe_2O^{®}$ Brand -GB treatments.

Mionix subsequently carried out additional studies to determine the effect of the addition of $Safe_2O^{®}$ Brand -GB on bind and meat color as well as additional D-value studies. The studies presented in Fig. 1 and those of Dr. Doyle, utilized a $Safe_2O^{®}$ Brand –GB formula comprised of 20% ACS50 and 10% lactic acid. It was shown that the addition of the additive caused discoloration of the meat and prevented binding negating the beneficial effects.

A second study was performed by Dr. Doyle using the $Safe_2O^{®}$ Brand -GB formula described herein entitled “Effect of KLS-1/2 and heating on the inactivation of *E. coli* O157:H7 in ground beef: Determination of D-values of *E. coli* O157:H7 in ground beef.” The study showed that *E. coli* O157:H7 (OH1395) in ground beef containing KLS-1/2 was more rapidly inactivated at an equivalent temperature than E. coli O157:H7 in the control ground beef (without KLS-1/2). Furthermore, holding *E. coli* O157:H7-contaminated beef frozen (at $-20^{O}C$) for 3 weeks prior to heat treatment increased the pathogen’s sensitivity to thermal inactivation resulting in lower D-values compared with studies using ground beef held refrigerated prior to heat treatment.

Ground beef treated as described in the second study carried out by the University of Georgia had a pH of 5.2 and from an organoleptic viewpoint it was acceptable from a consumer viewpoint. Moreover, in the making of ground beef patties bind was not an issue.

Presented herein is an objective method whereby it can be scientifically demonstrated that the addition of Mionix $Safe_2O^{®}$ Brand -GB effectively suppresses in part the viability of contaminating pathogens, including *E. coli* O157:H7, and lowers the D-Value, i.e.,

changes the time-temperature combination that ground beef has to be cooked to in order to kill all associated *E. coli* O157:H7.

Materials and Methods:

Equipment

- Water bath: LAB-LINE SHAK-R-BATH
- Glass culture tubes with cap (13X100 mm)
- 2 Omega Digital Thermometers HH82 with 4 type K thermocouples

Bacteria and Culture Media

- 5 strains of *E. coli* O157:H7 (ATCC#: 43895, 43895, 83888, 43889 plus a strain obtained from the Pennsylvania State University *E.coli* reference center)
- *E. coli* Growth Media and Fluorocult *E. coli* O157:H7 Agar (EM Science)

Treatment solution

$Safe_2O^{®}$ Brand -GB - 10% Lactic Acid solution prepared from a lactic acid concentrate having an 85% concentration and 10% ACS50 (V/V)

Meat treatment

Ground beef having a fat content of 15% and 20% fat was purchased from a local supermarket. An equal amount of the 15 and 20% ground beef was blended to achieve ground beef having a fat content of ~17.5%.

E. coli O157:H7 strains were cultured overnight at $37^{O}C$ in shaking water bath. Equal amount from the cultures were combined in a 1:1 ratio then the mixture was serially diluted. An aliquot from each dilution was plated on Fluorocult *E. coli* O157:H7 agar plates to determine the number of *E. coli* O157:H7 CFU present in the culture.

For study purposes the ground beef prepared as described above was divided into two 100 g portions. Two ml of $Safe_2O^{®}$ Brand -GB was blended into one of the ground beef portions, whereas nothing was added to the other portion. Thus the samples were labeled $Safe_2O^{®}$ Brand -GB**-**treated and Control.

$Safe_2O^{®}$ Brand -GB**-**treated and Control meat was inoculated with 2 ml of an *E. coli* O157:H7 mixture and in each case the combined materials were then thoroughly mixed by hand. After treatment and inoculation samples were incubated at $4^{O}C$ for 1 hour.

Before analysis one gram aliquots from the *E. coli* O157:H7 inoculated $Safe_2O^{®}$ Brand -GB-treated and Control meat samples were placed in glass culture tubes. In each case the meat sample was packed in the bottom of each tube using a sterile stainless steel spatula.

It is essential that the meat be firmly attached to the glass wall. Air bubbles prevent heat conduction. After packing all tubes were tightly capped and stored at -20^{o}C for at least three days.

Thermo Inactivation

Addition of heat to the samples was achieved by binding three tubes (GB_A, GB_B, GB_C) from the $Safe_2O^{®}_{Brand}$ -GB group and three tubes (C_A, C_B and C_C) from the control group to a plastic or steel rod (3/8" X 13") using rubber bands to produce one bundle. All tubes were bound to a rod such that they could not touch the bottom of the water bath, nor slip during thermo inactivation.

A total of 15 bundles were produced for three repeats. Each test was comprised of five bundles. The tubes for each test were labeled: 0'GB_A, O'GB_B, O'GB_C, O'C_A, O'C_B and O'C_C …….7'GB_A, 7'GB_B, 7'GB_C, 7'C_A, 7'C_B and 7'C_C , respectively. Three additional sets consisting of a GB and C tube were prepared to be used to monitor the internal temperature of the meat during heating (thermal inactivation). After assembling all bundles and sets were stored at -20^{O}C until used.

Care was taken to make sure that the water bath was filled such that the capped test bundles would be completely immersed in the water. In addition, a test tube rack was placed in the water bath to make sure the water level was such that it would come to a level well below the neck of the open test tubes containing the meat samples. In addition, it was assured that the test tubes do not float when placed into the rack.

Water was pre-heated to 57.5^{o}C (care was taken to not exceed 58.5^{o}C). One thermal couple probe was placed into the water bath as a temperature indicator.

The caps were removed from a set of tubes consisting of a GB and C tube prepared for monitoring the internal temperature of the meat during heating. The rack containing these tubes was placed into the water bath. A thermocouple was inserted into the middle of the meat sample at the bottom of each tube when the meat had slightly melted. Five bundles of tubes were labeled 0'GB_A, O'GB_B, O'GB_C, O'C_A, O'C_B and O'C_C …….7'GB_A, 7'GB_B, 7'GB_C, 7'C_A, 7'C_B and 7'C_C, respectively for the time periods of zero, 1, 3, 5, and 7 minutes. These were totally immersed into the water bath at the same time. The time required for the GB and C samples with the thermocouple inserts to reach the desired temperature, i.e., 57.5^{O}C was recorded. This time will be used to facilitate subsequent repeats of the study.

As soon as the temperature reached 57.5^{o}C, the tubes labeled 0'GB_A, O'GB_B, O'GB_C, O'C_A, O'C_B and O'C_C were removed and immediately transferred to an ice water bath to stop heat transfer. The 1', 3', 5' and 7' bundles were removed at the appropriate times and processed accordingly.

Enumeration of *E. coli* O157:H7

Phosphate buffer, 4.5 ml, was added to each test sample tube. The meat pellet was then disrupted and the contents decanted into a sterile 50 ml tube. An additional 4.5 ml of phosphate buffer was added to the sample tube. The tube was vortexed and the contents transferred to a 50 ml tube.

An aliquot for analyis was removed and assayed as foolows: The tube was vortexed briefly to suspend, disrupt and evenly distribute the particulates. An aliquot of the suspensuion was removed and serially diluted. Fifty microliters from each dilution tube was plated in triplicate onto Fluorocult *E. coli* O157:H7 agar plates. To increase detection limits, 1 ml from the higher dilutions was evenly divided and plated on four plates.

Inoculated plates were incubated at 37^{O}C for 48 hours. After incubation the colonies were counted and the titer for each test sample was calculated.

Results:

As shown in Table 1, *E. coli* O157:H7 was thermally inactivated at 57.5^{O}C more rapidly when ground beef was treated with Safe$_2$O$^{®}$ $_{Brand}$ -GB as compared to untreated ground beef (Table 2).

Table 1: Thermal inactivation of *E. coli* O157:H7 inoculated into ground beef treated with Safe$_2$O$^{®}$$_{Brand}$ -GB

	E. coli O1567:H7 titer (cfu log10/g)				
Test Set	0 min*	1 min*	3 min*	5 min*	7 min*
Test 1	6.28	5.67	0.00	0.00	0.00
	6.42	5.21	0.00	0.00	0.00
	6.12	5.71	0.00	0.00	0.00
Test 2	6.23	5.37	3.42	0.00	0.00
	5.98	5.88	3.65	0.00	0.00
	6.12	5.56	2.32	0.00	0.00
Test 3	5.76	4.36	2.38	0.00	0.00
	5.70	5.00	2.46	0.00	0.00
	5.74	5.08	3.25	0.00	0.00
Statistics					
Mean	6.039	5.316	1.942	0.000	0.000
Std Deviation	0.259	0.467	1.530	0.000	0.000
Variance	0.067	0.218	2.341	0.000	0.000
Std Error	0.086	0.156	0.510	0.000	0.000

*Time of thermal treatment

The mean number of surviving organisms was reduced from log 6.039 to 1.942/g following thermal treatment for ground beef containing the Safe$_2$O$^{®}$$_{Brand}$ –GB. additive as compared to a reduction of log 6.021 to 3.968/g for meat containing no additive, i.e., a 4.097 vs. 2.053 log reduction. Further analysis showed Safe$_2$O$^{®}$ $_{Brand}$ –GB decreased the D-value as compared to untreated ground beef (Figure 2).

The D-value for ground beef mixed with Safe$_2$O$^{®}$$_{Brand}$ -GB was determined to be $D_{57.5}$=1.044 whereas the D-value for untreated ground beef was shown to be $D_{57.5}$=1.755 (see Figure 2). The Safe$_2$O$^{®}$$_{Brand}$ -GB additive effectively reduced the D-value by a factor of 1.68. It is expected that the shift in D-value would even be more had the inoculated meat samples been frozen for a longer period of time per the observations presented in

"Influence of Freezing and Freezing plus Acidic Calcium Sulfate Addition on Thermal Inactivation of *Escherichia coli*."

Table 2: Thermal inactivation of *E. coli* O157:H7 inoculated into ground beef

	E. coli O1567:H7 titer (cfu log10/g)				
Test Set	0 min*	1 min*	3 min*	5 min*	7 min*
Test 1	6.11	5.65	3.48	1.48	0.00
	5.98	5.57	3.65	1.30	1.30
	6.19	5.81	3.14	1.48	1.30
Test 2	6.00	5.69	4.23	3.94	3.00
	6.39	5.80	4.58	3.67	3.03
	6.13	5.65	4.10	3.32	2.70
Test 3	5.82	5.12	4.10	1.78	2.57
	5.77	5.05	4.34	2.40	2.49
	5.80	4.80	4.09	2.40	2.48
Statistics					
Mean	6.021	5.460	3.968	2.419	2.097
Std Deviation	0.206	0.370	0.455	1.007	1.015
Variance	0.042	0.137	0.207	1.014	1.030
Std Error	0.069	0.123	0.152	0.336	0.338

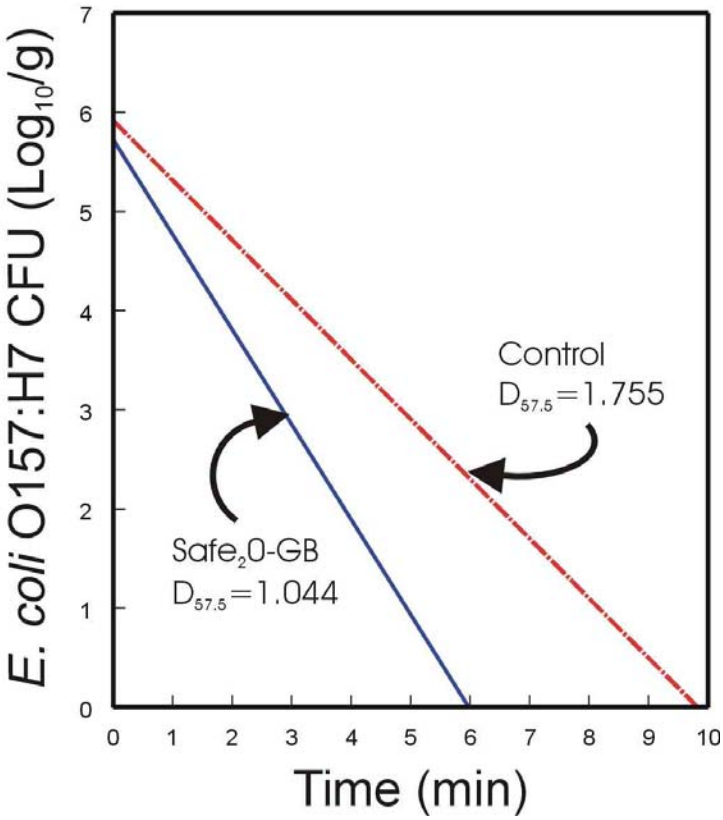

Figure 2: Effect of the addition of $Safe_2O^{®}_{Brand}$-GB on the thermal inactivation of *E. coli.* O157:H7 at 57.5^{O}C: D-value determination