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Effect of Safe$_2$O$^{®}_{\text{Brand}}$-GB Additive on the Replication of Gram Negative Microbial Pathogens in Ground Beef: Temperature Abuse

Notwithstanding 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$_2$O$^{®}_{\text{Brand}}$-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. Addition of Safe$_2$O$^{®}_{\text{Brand}}$-GB represents an additional hurdle and decreases the risk of transmission of pathogens to the consumer as demonstrated in the report entitled "Effect of the Addition of Safe$_2$O$^{®}_{\text{Brand}}$-GB on the Thermal Inactivation of *E. coli.* O157:H7 at 57.5^{O}C: D-value Determination" (see attachment).

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.

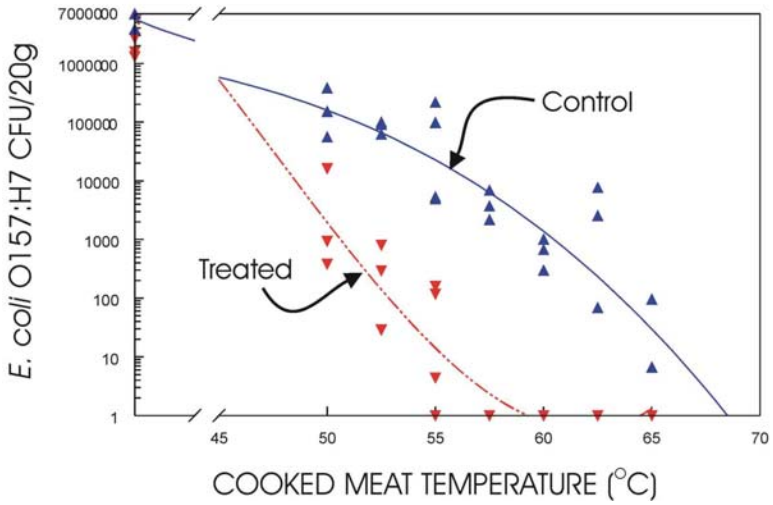

Fig. 1: $Safe_2O^{®}_{Brand}$-GB effects on E. *coli* O157:H7 survival in ground beef during cooking

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 (Fig. 1: $Safe_2O^{®}_{Brand}$-GB additive effects on E. coli O157:H7 survival in ground beef during cooking)and attachments entitled: Effect of $Safe_2O^{®}_{Brand}$-Groubd 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; 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 and Effect of the Addition of $Safe_2O^{®}_{Brand}$-GB on the Thermal Inactivation of *E. coli.* 0157:H7 at 57.5°C: D-value Determination.

The increased emphasis on production of case ready meats and need for longer distribution times dramatically increases the chances for product temperature abuse. For example, it is not well understood that the refrigeration unit (or reefer unit) on a truck or trailer does not have the refrigeration capacity to cool product, i.e., the reefer is simply designed to remove the heat coming into the truck or trailer via the ceiling, the walls and the floor. Thus, when product is placed against the walls or ceiling or placed directly on the floor, heat is conducted into the refrigerated product and the resultant effect is a condition that can not be overcome by the reefer. Thus, the product may be subjected to temperature abuse and as distribution times increase, the problem becomes ever more severe. Likewise, upon arrival at the supermarket product is transferred to display cabinets. Display cabinets are the link in the cold chain where ground meat is displayed to the consumer. They are intended to be used for displaying meat and

not for lowering product temperature, thus like the reefer they are to hold the product temperature, not lower it. If meat is contaminated with pathogens such as *E. coli* O157:H7 and/or *Salmonella spp*., the display of meat represents another step in the process whereby pathogens can multiply. Thus, the ground beef the consumer purchases represents a potentially hazardous food due in part to lack of proper temperature control.

Whether it be at the reefer or display cabinet level there needs to be a hurdle that can prevent and or suppress replication of pathogens. Mionix $Safe_2O^{®}_{Brand}$-GB when ground into beef changes the D-value. In addition as demonstrated herein it also prevents replication of microbial organisms under temperature abuse that are of significant concern, i.e., *E. coli* O157:H7 and/or *Salmonella spp*.

I. Materials and Methods

A. Bacteria and Culture Media

1. Bacterial strains used for this study included *E. coli* O157:H7 (ATCC#: 43894) and Salmonella choleraesuis (ATCC# 10708)

2. *E. coli O1*57:H7 was cultured using *E. coli* Growth Media and Fluorocult *E. coli* O157:H7 Agar (EM Science) for plate titration assays.

3. *Salmonella choleraesuis* (ATCC# 10708) was cultured using BHI media and Hektoen agar for plate titration assays.

B. 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)

C. Meat treatment and inoculation

1. 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%.

2. The meat was divided into two lots. Half of the meat was treated with $Safe_2O^{®}_{Brand}$-GB at 2ml per 100g of meat. After the addition of $Safe_2O^{®}_{Brand}$-GB the meat was run through a grinder to evenly distribute the additive.

3. For inoculation purposes *E. coli* O157:H7 and *Salmonella choleraesuis* were cultured overnight at 37^{O}C in shaking water bath. In each case, the respective cultures were diluted 1:1000 and separate 100 g treated and untreated meat samples were inoculated with 1 ml of either the E. *coli* O157:H7 or *Salmonella choleraesuis* culture per 100 g of meat. After inoculation the combined materials were then thoroughly mixed by hand to evenly distribute the bacteria.

4. Twenty gram aliquots of the inoculated treated and control meat were placed in sterile containers. Sets of inoculated treated and control meat samples were incubated at three different temperatures: 4^{O}C, 11^{O}C and 24^{O}C. Colony forming units/g inoculated meat

(cfu/g) of *E. coli* O157:H7 and *Salmonella choleraesuis* were determined at 0, 24, 48, 72 and 96 hr of incubation, respectively, for both treated and control meats.

II. Results

As shown in Tables 1 and 2, the addition of Mionix $Safe_2O^{®}_{Brand}$-GB prevents and/or suppresses replication of *E. coli* O157:H7 and *Salmonella choleraesuis*, respectively, even under temperature abuse conditions. The addition of

Table 1: Effect of $Safe_2O^{®}_{Brand}$-GB additive on the replication of *E. coli* O157:H7 in ground beef incubated at 4, 11 and $24^{O}C$

Treatment	Incubation Time (hr)	Incubation Temperature $24^{O}C$	$11^{O}C$	$4^{O}C$
Control	0	7.2×10^3	7.2×10^3	7.2×10^3
$Safe_2O^{®}_{Brand}$-GB	0	6.5×10^3	6.5×10^3	6.5×10^3
Control	24	8.2×10^8	3.0×10^4	4.8×10^3
$Safe_2O^{®}_{Brand}$-GB	24	1.4×10^4	4.6×10^3	4.0×10^3
Control	48	7.6×10^9	7.2×10^3	5.5×10^3
$Safe_2O^{®}_{Brand}$-GB	48	5.0×10^3	6.5×10^3	4.6×10^3
Control	72	ND*	2.5×10^7	5.3×10^3
$Safe_2O^{®}_{Brand}$-GB	72	ND*	5.0×10^3	8.4×10^2
Control	96	ND*	2.9×10^7	5.5×10^3
$Safe_2O^{®}_{Brand}$-GB	96	ND*	6.0×10^3	6.9×10^2

*Besides *E. coli* O157:H7, numerous decay bacteria were present in sample making it impossible to differentiate colony types.

$Safe_2O^{®}_{Brand}$-GB lowered the pH of the meat from approximately 6.5 to 5.2. At this pH the observed effect is bacteriastatic not bacteriacidal. The bacteriastatic effect on replication of *E. coli* O157:H7 is much more significant at all temperatures tested as compared to that observed for *Salmonella choleraesuis* (see

Fig. 2).

As can be seen from Table 1 and Fig. 2 even at an incubation temperature of 24^{O}C, replication of *E. coli* O157:H7 was completely blocked for 48 hr. This temperature represents extreme temperature abuse. However, an abuse temperature of 11^{O}C frequently occurs. Under these conditions it can be seen

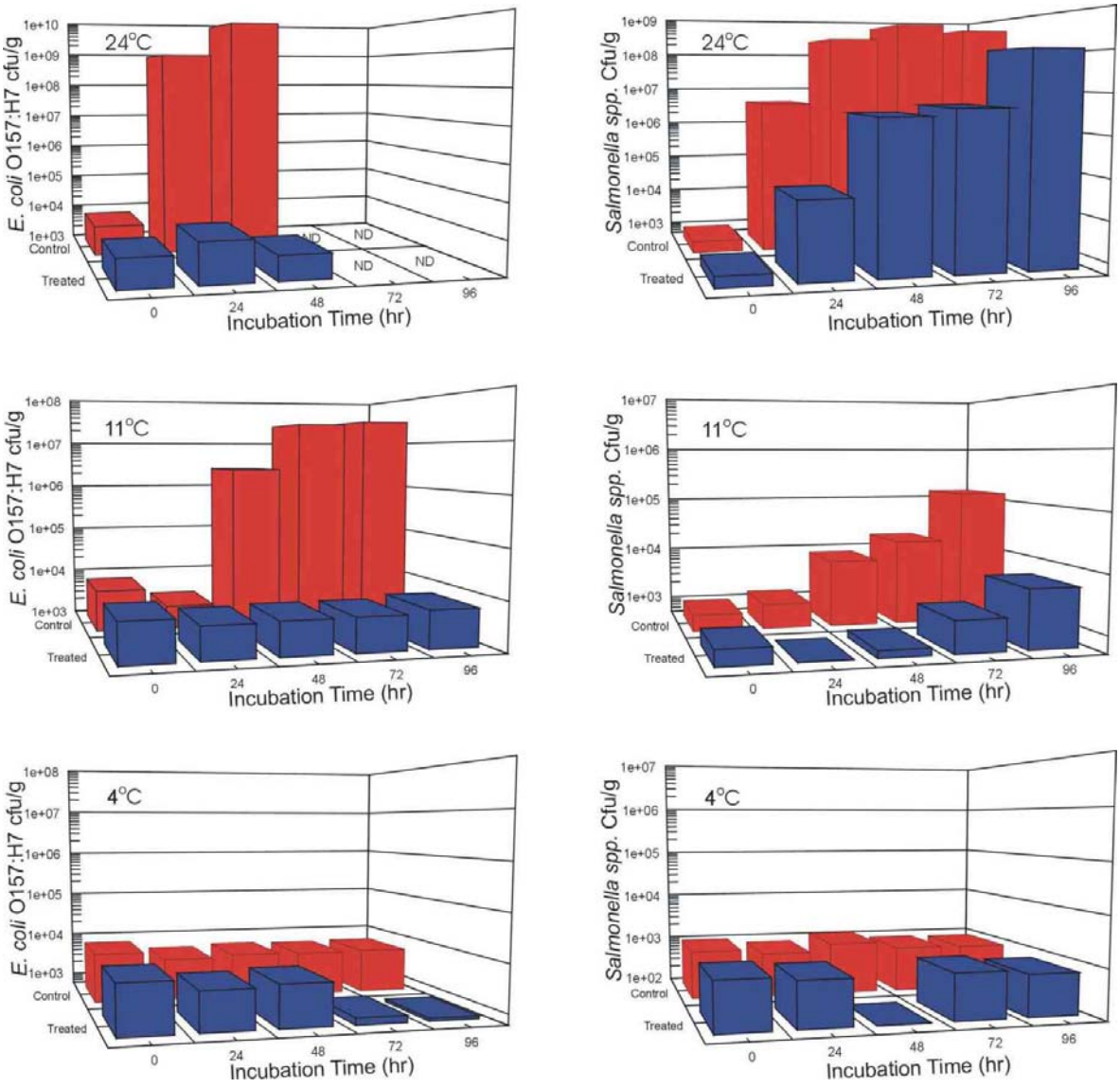

Figure 2: Comparison of the effect of Safe$_2$O$^{®}_{Brand}$-GB additive on the replication of *E. coli* O157:H7 and *Salmonella choleraesuis* in ground beef

from Table 1 and Fig 2, replication *E. coli* O157:H7 was likewise prevented all the way out to 96 hr.

As would be expected replication of *E. coli* O157:H7 did not occur in untreated or beef treated with $Safe_2O^{®}_{Brand}$-GB at $4^{O}C$. But, by 72 hr as can be seen from Table 1, the addition of $Safe_2O^{®}_{Brand}$-GB was somewhat biocidal as compared to the control even at $4^{O}C$.

Table 1: Effect of $Safe_2O^{®}_{Brand}$-GB additive on the replication of *Salmonella choleraesuis* in ground beef incubated at 4, 11 and $24^{O}C$

Treatment	Incubation Time (hr)	Incubation Temperature $24^{O}C$	$11^{O}C$	$4^{O}C$
Control	0	1.3×10^3	1.0×10^3	1.0×10^3
$Safe_2O^{®}_{Brand}$-GB	0	9.4×10^2	9.4×10^2	9.4×10^2
Control	24	4.3×10^6	1.4×10^3	8.1×10^2
$Safe_2O^{®}_{Brand}$-GB	24	4.2×10^5	5.1×10^2	4.1×10^2
Control	48	2.3×10^8	8.2×10^3	1.2×10^3
$Safe_2O^{®}_{Brand}$-GB	48	2.9×10^6	6.7×10^2	10.0×10^2
Control	72	5.8×10^8*	1.8×10^4	8.7×10^2
$Safe_2O^{®}_{Brand}$-GB	72	4.4×10^6*	1.8×10^3	8.4×10^2
Control	96	4.2×10^8*	1.4×10^5	8.5×10^2
$Safe_2O^{®}_{Brand}$-GB	96	1.1×10^8*	5.4×10^3	6.9×10^2

*Besides *Salmonella choleraesuis*, numerous decay bacteria were present in sample making it difficult to differentiate colony types.

Salmonella is often first detected during grinding of beef trimmings, in fact the USDA in the past has pulled its inspectors from a grinding operation that could not meet Salmonella standards. With this observation in mind it is an important observation that $Safe_2O^{®}_{Brand}$-GB when added to ground beef prevents the replication of *Salmonella choleraesuis* out to 96 hr when the meat is incubated at $11^{O}C$ (Table 2 and Fig. 2). Overall the bacteriastatic effect on the replication of *Salmonella choleraesui* is less dramatic than it is on *E. coli* O157:H7.

Nevertheless, as can be seen from Table 2 and Fig. 2, replication of *Salmonella choleraesuis* is prevented at a commonly observed temperature of abuse, $11^{O}C$.