Acidic Calcium Sulfate Reduces Thermotolerance of *E. coli* O157:H7 in Ground Beef

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Objective

- Determine rates of thermal inactivation of *E. coli* O157:H7 in frozen and refrigerated ground beef both with and without acidic calcium sulfate with lactic acid

Hypothesis

- The addition of acid to ground beef should increase the sensitivity of pathogens like *E. coli* O157:H7 to thermal inactivation in foods

Properties of Acidic Calcium Sulfate

- pH 1.3
- Has oxidation potential equivalent to concentrated sulfuric acid without corrosiveness associated with strong mineral acids
- High concentrations of H_3O^+ which are biocidal
- When mixed with organic acids, the organic acid is present in its most biologically active form (undisassociated form)

$Safe_2O$™- ACS

Chemical Name:	Acidic calcium sulfate (Mixture of sulfuric acid, calcium hydroxide and calcium sulfate)
Chemical Composition:	2 - 30% H_3O^+
	100 - 800 ppm calcium
	1,100 - 300,000 ppm sulfate

$Safe_2O$™-brand Ground Beef

Chemical Name:	Mixture of acidic calcium sulfate and lactic acid
Chemical Composition:	10% lactic acid
	7 - 22% acidic calcium sulfate [$Safe_2O$™- ACS (5N)]

Experimental Approach

- Spray Acidic Calcium Sulfate with lactic acid (45.4 ml) onto 5 lbs ground beef (24% fat) mixed in Hobart mixer
  - Grind beef three times through 1/8 in plate
- Control ground beef sprayed with sterile deionized water
- Inoculate 100 g ground beef with 1 ml of 10^8 *E. coli* O157:H7 and mix for 2 min with gloved hands
- Freeze 25-g portions at -20^{o}C for up to 41 days and refrigerate 25-g portions at 4^{o}C for up to 10 days

Experimental Approach

- Thaw frozen ground beef at 21^{o}C for 20-30 min
- Lightly pack 1-g portions of refrigerated and thawed treated and control ground beef into Pyrex (10 X 75 mm) test tubes capped with rubber stoppers
- Submerge tubes in circulating water bath preadjusted to appropriate temperature

Experimental Approach

- Heat at 57, 60, 62.8, 64.3 or 68.3^{o}C and withdraw duplicate samples at predetermined times
  - Cool immediately in ice water at 5^{o}C
  - Monitor temperature with thermocouples inserted into ground beef samples
- Enumerate surviving *E. coli* O157 on Rainbow agar and tryptic soy agar incubated at 37^{o}C for 24h
  - Confirm up to 5 isolates from plates of highest dilution as *E. coli* O157 by *E. coli* O157 latex agglutination assay

Thermal inactivation of *E. coli* O157:H7 in ground beef with and without acidic calcium sulfate-lactic acid and stored at 4°C (for up to 10 days)

Temperature (°C)	Control or Treated	*E. coli* O157:H7 (log_{10} CFU/g) at:				
		0	5	10	15	20 min
57	Control	6.2	5.0	4.5	3.9	3.3
	ACS-LA	6.3	4.5	2.8	2.1	2.0
		0	2	5	10	15 min
60	Control	5.6	4.5	2.7	2.3	<1.7
	ACS-LA	6.1	2.2	1.7	1.7	<1.7
		0	1	3	5	7 min
62.8	Control	5.0	3.3	1.7	<1.7	<1.7
	ACS-LA	2.2	1.9	<1.7	<1.7	<1.7

(Continued)

Thermal inactivation of *E. coli* O157:H7 in ground beef with and without acidic calcium sulfate-lactic acid and stored at 4ºC (for up to 10 days) (Cont'd)

Temperature (ºC)	Control or Treated	*E. coli* O157:H7 (log_{10} CFU/g) at:				
		0	0.5	0.75	1	1.25 min
64.3	Control	3.6	3.4	<1.7	<1.7	<1.7
	ACS-LA	<1.7	<1.7	<1.7	<1.7	<1.7
		0	0.17	0.33	0.5	0.67 min
68.3	Control	2.4	2.3	<1.7	<1.7	<1.7
	ACS-LA	<1.7	<1.7	<1.7	<1.7	<1.7

Thermal inactivation of *E. coli* O157:H7 in ground beef with and without acidic calcium sulfate-lactic acid and stored at -20°C (for up to 41 days)

Temperature (°C)	Control or Treated	*E. coli* O157:H7 (log_{10} CFU/g) at:					
		0	1	3	5	10	15 min
57	Control	6.3	6.1	6.0	5.8	5.3	4.3
	ACS-LA	6.2	6.0	5.8	4.7	2.2	1.8
		0	0.5	1	2	5	10 min
60	Control	6.0	5.8	5.6	5.1	3.4	1.7
	ACS-LA	5.6	4.1	3.7	1.7	4.7	<1.7
		0	0.17	0.33	0.5	1	1.5 min
62.8	Control	5.8	5.0	4.5	3.5	1.9	<1.7
	ACS-LA	5.8	4.1	2.6	<1.7	<1.7	<1.7

(Continued)

Thermal inactivation of *E. coli* O157:H7 in ground beef with and without acidic calcium sulfate-lactic acid and stored at -20°C (for up to 41 days) (Cont'd)

Temperature (°C)	Control or Treated	*E. coli* O157:H7 (log_{10} CFU/g) at:					
		0	0.17	0.33	0.5	0.67	1 min
64.3	Control	4.4	2.9	<1.7	<1.7	<1.7	<1.7
	ACS-LA	4.1	<1.7	<1.7	<1.7	<1.7	<1.7
		0	0.17	0.33	0.5	0.67	1 min
68.3	Control	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7
	ACS-LA	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7

D-values of *E. coli* O157:H7 in refrigerated or frozen ground beef with and without acidic calcium sulfate with lactic acid

Refrigerated or frozen	D value (min) at:				
	57°C	60°C	62.8°C	64.3°C	68.3°C
Refrigerated	7.69	3.02	1.11	0.26	ND
Refrigerated With ACS-LA	5.26	0.96	IS[a]	ND[b]	ND
Frozen	5.71	2.07	0.29	0.24	ND
Frozen with ACS-LA	2.7	0.52	0.1	IS	ND

[a] IS, insufficient number of data points to calculate D-value

[b] ND, no detectable *E. coli* O157:H7 at zero time (initial cell counts were ca. 10^7 CFU/g before heating)

D-values of *E. coli* O157:H7 in refrigerated (5°C for up to 10 days) or frozen (-20°C for 3 weeks) ground beef with and without one-half amount of acidic calcium sulfate with lactic acid

Refrigerated	D value (min) at:			
or frozen	57°C	60°C	62.8°C	64.3°C
Refrigerated	10	2.4	1.1	0.26
Refrigerated with 1/2 ACS-LA	5.1	2.1	IS[a]	IS
Frozen	6.3	2.0	0.25	IS
Frozen with 1/2 ACS-LA	3.8	1.6	IS	NS[b]

[a] IS, insufficient number of data points to calculate D-value.

[b] NS, no survivors; no detectable cells at zero time (initial cell counts were ca. 10^7 CFU/g before heating). Ca. 10^5 *E. coli* O157:H7/g were inactivated during the come-up time.

Conclusions

- *E. coli* O157:H7 was consistently more rapidly inactivated at equivalent temperature in ground beef containing Acidic Calcium Sulfate with lactic acid (ACS-LA) than the control
- D-values at equivalent temperature of *E. coli* O157 in ACS-LA-treated ground beef were 1.5 to 4 times less than those in control ground beef

Conclusions

- Initial *E. coli* O157 counts were generally higher in the frozen than in the refrigerated ground beef treated with ACS-LA
- D-values of *E. coli* O157 were higher in refrigerated than in frozen ground beef (ca. 2-fold less) irrespective of the addition of ACS-LA
  - Freezing appears to further sensitize *E. coli* O157 to heat treatment in ACS-LA containing beef