



VALIDATION OF DRY-AGING AS AN EFFECTIVE INTERVENTION STEP AGAINST *ESCHERICHIA COLI* O157:H7 ON BEEF CARCASSES

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Study 1. Use of NY strip steaks as models for beef carcass

Organism Treatments Involved:

1. Normal meat microflora (no inoculation)
2. Generic *E. coli* cocktail
3. *E. coli* O157:H7 cocktail

Meat

- New York strip steaks with twine in one end for hanging. The twine was labeled with the treatment no.

Target environmental conditions

- Trial 1: 41°F (target; 35°F was achieved) and 80% RH (target; 50-60% was achieved)
- Trial 2: 41°F (target; 38°F was achieved) and 80% RH (target; 84 – 89% was achieved)

Inoculation Procedure

- Cells grown as per standard procedure, centrifuged, and re-suspended in Butterfield's phosphate diluent
- Each culture for organism treatment 2 combined to make a cocktail e.g. all generic *E. coli* cultures combined to make a cocktail. This process was also done for organism treatment 3.
- Each cocktail was diluted so that there were 6 logs (one million) cells per ml
- Each steak was turned on edge and 0.1 ml was pipetted onto the fat surface and spread with a hockey stick. The steak was held on edge until we were sure that inoculum wouldn't drip off.
- The steak was then laid flat and 0.1 ml of cocktail was pipetted onto the lean (horizontal surface) and spread over a 2 inch x 2 inch area in the center. We made sure the inoculum soaked in. The steak was then flipped over and the other side was inoculated.

- Each steak was carefully put in a bag for transport to Biotron environmental chamber. Three bags for each treatment were put into the refrigerator.
- After 1 hour three steaks for each treatment in the refig were sponge-sampled. The lean (both sides, one sponge) and fat (one sponge) were separately sampled. There were 9 samples total – these were the ones in refig. Analytical methods used were: treatment 1 (APC) = Petrifilm Aerobic Count, treatment 2 (generic *E. coli*) = Petrifilm *E. coli*, treatment 3 (*E. coli* O157:H7) = Sorbitol MacConkey agar.

Storage Procedure

- The steaks were hung in Biotron with plenty of room for air movement.
- After 2 days (Trial 1 and 2) and 6 days (Trial 2) of incubation, the twine was carefully cut on 3 steaks per treatment (9 total) and those steaks were returned to the lab for analysis.

Results

- After incubation of Petrifilms and plates at 35°C, typical colonies were counted. Pinpoint colonies on Petrifilm Aerobic Count are not considered typical (based on our experience).
- The CFU/ml was calculated and multiplied by 25 to determine total number of CFU on surface.

Table 1. Log CFU per surface on lean and fat tissue of New York strip steaks during dry refrigerated storage TRIAL 1.

Time/Surface	Aerobic Plate Count	Generic <i>E. coli</i>	<i>E. coli</i> O157:H7	
0 lean	4.5	5.7	6.0	
	4.4	5.7	5.8	
	4.6	5.7	5.6	
	Mean (SD)	4.5 (0.1)	5.7 (0)	5.8 (0.2)
0 fat	4.7	5.3	5.6	
	4.6	5.5	5.6	
	4.8	5.4	5.5	
	Mean (SD)	4.7 (0.1)	5.4 (0.1)	5.6 (0.1)
2 lean	4.0	3.8	5.1	
	3.6	4.0	5.5	
	2.7	4.0	5.5	
	Mean (SD)	3.4 (0.7)	3.9 (0.1)	5.4 (0.2)
2 fat	4.0	3.9	4.1	
	3.2	3.6	4.3	
	3.2	4.1	4.4	
	Mean (SD)	3.5 (0.5)	3.9 (0.3)	4.3 (0.1)
Net change	lean	-1.1	-1.8	-0.4
	fat	-1.2	-1.5	-1.3

Table 2. Log CFU per surface on lean and fat tissue of New York strip steaks during dry refrigerated storage TRIAL 2.

Time/Surface		Aerobic Plate Count	Generic <i>E. coli</i>	<i>E. coli</i> O157:H7
0	lean	5.7	5.0	5.5
		5.7	5.1	5.4
		5.7	5.0	5.8
	Mean (SD)	5.7 (0)	5.0 (0)	5.6 (0.2)
0	fat	5.5	4.3	5.0
		5.5	4.3	5.2
		5.7	4.5	5.1
	Mean (SD)	5.6 (0.1)	4.4 (0.1)	5.1 (0.1)
2	lean	5.7	4.6	5.0
		5.6	4.1	4.9
		5.9	4.4	5.3
	Mean (SD)	5.7 (0.2)	4.4 (0.3)	5.1 (0.2)
2	fat	5.2	3.2	4.0
		5.5	3.5	4.5
		6.0	4.1	4.2
	Mean (SD)	5.6 (0.4)	3.6 (0.4)	4.2 (0.2)
6	lean	5.7	4.2	4.5
		5.4	3.6	4.1
		5.5	3.3	4.1
	Mean (SD)	5.5 (0.2)	3.7 (0.5)	4.2 (0.2)
6	fat	5.1	3.3	3.9
		5.0	3.3	3.6
		5.4	2.8	3.4
	Mean (SD)	5.2 (0.2)	3.1 (0.3)	3.6 (0.3)
Net change	lean	-0.2	-1.3	-1.4
	fat	-0.4	-1.3	-1.5

Study 2. Use of flank, brisket, and plate pieces as models for beef carcass

Organism Treatments Involved

1. Normal meat microflora (no inoculation)
2. Generic *E. coli* cocktail combined with *E. coli* O157:H7 cocktail

Meat

- flank, brisket, plate from each half of a freshly slaughtered Jersey cow

Target environmental conditions

- 41°F (target; 35 - 38°F was achieved) and 80% RH (target; 86-92% was achieved)

Inoculation Procedure

- Same as for Study 1, except generic and pathogenic *E. coli* cocktails were combined.
- Flank, brisket and plate cuts were placed on a metal mesh cart. One cart held pieces that were not to be inoculated; a second cart held pieces to be inoculated.
- 1.5 ml of inoculum was distributed over a 12 x 5 inch area on each cut placed on the second cart. Inoculum was allowed to dry 15 min.
- Sponge-samples were taken from two or three 1.5 x 5 inch sections from cut on days 0, 2, and 6. After taking 2 or 3 samples, a knife cut was made to indicate where sampling had been done (to avoid repeatedly sampling the same area)
- Microbiological analyses were the same as in Study 1.

Results

- After incubation of Petrifilms and plates at 35°C, typical colonies were counted. Pinpoint colonies on Petrifilm Aerobic Count are not considered typical (based on our experience).
- The CFU/ml was calculated and multiplied by 25 to determine total number of CFU on the sampled surface.

Table 3. Log CFU per surface on flank, brisket, and plate cuts of freshly slaughtered beef during dry refrigerated storage

Time/Cut	Aerobic Plate Count	Generic <i>E. coli</i>	<i>E. coli</i> O157:H7	
0	flank	4.2	4.8	5.0
		4.9	4.9	5.3
		4.9	4.6	5.0
	Mean (SD)	4.7 (0.4)	4.8 (0.2)	5.1 (0.2)
0	brisket	3.9	5.0	4.3
		4.4	5.0	4.8
		4.4	5.1	4.5
	Mean (SD)	4.2 (0.3)	5.1 (0.1)	4.5 (0.2)
0	plate	3.0	4.3	5.2
		3.2	4.9	4.5
		4.0	4.7	4.1
	Mean (SD)	3.4 (0.5)	4.6 (0.3)	4.6 (0.6)
2	flank	4.2	3.5	4.3
		4.3	3.8	3.7
		Mean (SD)	4.3 (0.1)	3.7 (0.2)
2	brisket	4.2	3.7	3.4
		4.1	3.8	4.0
		Mean (SD)	4.2 (0.1)	3.7 (0.1)
2	plate	4.0	2.7	3.2
		4.3	2.7	2.7
		Mean (SD)	4.1 (0.2)	2.7 (0)
6	flank	5.2	3.1	2.4
		4.2	3.5	3.2
		4.7	3.8	3.0
	Mean (SD)	4.7 (0.5)	3.5 (0.4)	2.9 (0.4)
6	brisket	3.3	2.9	2.4
		4.7	1.7	1.1*
		3.2	1.4	1.1*
	Mean (SD)	3.7 (0.8)	2.0 (0.8)	1.5 (0.8)
6	plate	3.9	1.1*	1.4
		4.3	1.1*	1.1*
		3.3	1.7	1.1*
	Mean (SD)	3.9 (0.5)	1.3 (0.3)	1.2 (0.2)

*= None detected. Arbitrary value assigned.

Mean change

Flank	0	-1.3	-2.2
Brisket	-0.5	-3.1	-2.6
Plate	+0.5	-3.3	-3.4

Conclusions

- Dry-aging (temperature below 41°F, % RH below 90%, at least 6 days duration) will effectively reduce populations of generic *E. coli* and *E. coli* O157:H7.
- Processors using this intervention strategy are advised to monitor temperature and %RH (or wet bulb temperature) during the 6+ day process.
- Aerobic Plate Count does not correlate well with counts of generic *E. coli* and *E. coli* O157:H7. Microbiological verification must be done by testing for generic *E. coli* and/or *E. coli* O157:H7.