# Changes in Bacterial Counts on Hot Boned Boxed Beef Trimmings

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# Story in Brief

Total aerobic, psychrotrophic, and anaerobic bacterial counts were determined on meat samples placed at three positions (top, middle, and bottom) within boxes of different depth (4, 8, and 12 inches) filled with hot boned beef trimmings, after cooler storage of the boxed meat for each of the following periods: 0, 24, 48, 72, and 96 hours. The counts were lower in the 4-inch than in the 8 or 12-inch depth boxes, and increased as the depth of the box increased. These results were attributed to the faster decline in meat temperature with decreased box depth.

After the 0-hour storage period, total aerobic and psychrotrophic counts were higher on the meat at the top position within the boxes than at the middle position, where the temperature decline was slower. In contrast, anaerobic counts were higher at the middle position in the 8 and 12-inch depth boxes. These results were likely produced by a higher oxygen availability at the top position than at the middle position within the boxes. Rapid chilling of the meat seemed to have a more significant effect than aerobic condition in determining growth of anaerobic bacteria after 24 hours of storage within the 4-inch depth boxes.

### Introduction

Raw beef is increasingly being distributed in a box. Currently, about 50 percent of all choice beef is marketed in the form of boxed vaccum packaged primal cuts (Ayres et al., 1980). Additionally, beef trimmings used for preparation of ground beef or hamburger are also being distributed boxed.

On the other hand, hot boning of the beef carcass (boning of the carcass while the meat is still warm) and then boxing of the meat has several advantages. Removal of excess fat and bone results in considerable savings in refrigeration energy, cooler space, and transportation costs (Henrickson, 1975). When hot boning is combined with electrical stimulation of the carcass there is also a saving in time because the meat can be chilled faster with reduced risk of cold shortening. However, hot boning has some disadvantages, one of which is the possibility for increased microbial growth due to the high temperature and high surface moisture of the warm tissues.

The objective of this study was to ascertain changes in total aerobic, psychrotrophic, and anaerobic bacterial counts at three different positions inside boxes of electrically stimulated hot boned beef trimmings, chilled aerobically for different periods of time.

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# **Materials and Methods**

Fifteen commercial Hereford steers of approximately 900 lb live weight were slaughtered at weekly intervals. Each animal was electrically stimulated immediately after exsanguination by using an "Electro-Stem" electrical stimulator (Doube J Products, Wichita, KS) which supplied an electrical alternating current of 48 volts and less than 0.5 amperes, with a pulsation time of one second, during a 90-second period, through a spring loaded clamp attached to the nostril and positive ground probes inserted into the tissues near the hocks of the animal.

After dressing, splitting, and washing of each carcass, one of the sides was randomly designated to be hot boned within three hours after slaughtering. Using a sterile knife, the semitendinosus muscle was excised and sectioned transversally so that twelve slices approximately two cm in thickness were obtained (Figure 1). Three of the meat slices were randomly designated for analysis to determine the initial bacterial load on the muscle surface. The remaining nine meat slices were randomly assigned to three different positions (top, middle, and bottom) within each of three single-wall cardboard boxes differing only in depth (12x12x4, 12x12x8, and 12x12x12 inches, respectively). The boxes were filled respectively with 20, 40, and 60 pounds of meat trimmings obtained from the side forequarter. Before filling, the interior of each box was lined with Cryovac film in order to avoid adherence of the meat to the walls of the box.

After filling, the boxes were sealed and stored in a cooler  $(36^{\circ}F)$  at the O.S.U. Meat Laboratory for one of the following randomly assigned storage periods: 0, 24, 48, 72 or 96 hours. Thus, meat boxes from three beef sides were randomly assigned to each storage period. At each storage period, the temperature at each position within each box was determined at different time intervals (0, 6, 12, 24, 48, 72 or 96 hours, depending upon the storage period) by inserting a thermocouple close to each meat slice. A free thermocouple was used to record the air temperature.

After the respective storage period, the boxes were removed from the cooler, and the meat slices were aseptically removed from the boxes. For the zero-hour storage period the meat slices in each box were left in contact with the rest of the meat trimmings for five minutes before being removed from the boxes for analysis. The slices were individually placed in sterile pre-weighed Mason jars (1-qt capacity) and their weight was determined. The jars were then placed in ice to prevent or delay additional bacterial growth on the meat slices, and transported to the laboratory, where microbiological analysis was immediately initiated.

A pre-measured volume of sterile 0.1 percent peptone water equal to twice the weight of the meat slice was delivered into each jar. The jar was then shaken, making 25 back-and-forth movements of about one foot in seven seconds to permit removal of bacteria from the meat surface. Each ml of "rinse" thus prepared represented 0.5g of sample. Serial dilutions, as needed, were prepared from each jar using sterile 99 ml dilution bottles containing 0.1 percent peptone water as diluent. Preparation of the dilutions and further platting and bacterial enumeration were made according to the *Compendium of Methods for the Microbiological Examination of Foods* (Speck, 1976).

The total aerobic, psychrotrophic, and anaerobic bacterial counts were determined for each of the meat slices in duplicate Petri dishes by the pour plate technique using plate count agar. Total aerobic and psychrotrophic counts





Figure 1. Schematic drawing of meat slices utilized for microbiological analysis and their position within the boxes during storage.

were obtained after incubation of the plates at 32°C for 48 hours, and at 5°C for seven days, respectively. The anaerobic count was determined by incubating the plates in anaerobic Gas-Pak jars at 32°C for 48 hours. After the respective incubation period, the colonies were counted using a Quebec colony counter. The average number of colonies on the selected plates was multiplied by the appropriate dilution factor, multiplied by two, and referred to as count per gram (count/g). The bacterial counts thus obtained were converted to common logarithms (Log<sub>10</sub>) for statistical analysis. Bacterial counts on the meat slices utilized for determination of the initial level of contamination were not considered further in this study because they were similar to those obtained at the zero-hour storage period.

### **Results and Discussion**

Mean  $\log_{10}$  bacterial counts determined on the meat within the boxes at different storage periods are presented in Table 1. Except for the counts at 72 hours of storage, statistically significant differences (P<0.05) in mean  $\log_{10}$  total aerobic and anaerobic counts were found among box sizes after the 0-hour storage period. Mean  $\log_{10}$  psychrotrophic counts were significantly different (P<0.05) among box sizes only at the 24- and 48-hour storage periods. However, after 0 hours, all bacterial counts were lower on meat in the 4-inch deep boxes than on meat in the 8-inch deep boxes and these in turn had lower bacterial counts than meat in the 12-inch deep boxes. These results are associated with a faster temperature decline observed in the meat as the depth of the box decreased (Figures 2, 3, and 4 respectively), which allows for a more effective control of bacterial growth.

Tables 2 and 3 contain the mean  $\log_{10}$  total aerobic and psychrotrophic bacterial counts, respectively, determined at each position within the boxes at all storage periods. Total aerobic and psychrotrophic bacteria were more abundant (but not necessarily statistically different) on meat at the top than at the middle or bottom positions within the boxes at all storage periods beyond 0 hours. These results were more noticeable as the depth of the boxes increas-

Bacterial Count	Box Depth (inches)	Storage (hours)					
		0	24	48	72	96	
the decline	4	2.94a <sup>2</sup>	3.44a	4.64a	4.80a	5.35a	
Total aerobic	8	3.04a	4.31b	5.11a	5.70a	5.99ab	
	12	3.14a	5.01c	6.25b	6.43a	6.57b	
	4	1.70a	3.29a	4.39a	4.80a	5.46a	
Psychrotrophic	8	2.02a	3.93b	5.10ab	5.69a	6.10a	
	12	2.19a	4.72c	6.02b	6.30a	6.25a	
	4	2.35a	2.82a	3.98a	4.00a	4.15a	
Anaerobic	8	2.47a	3.91b	4.98ab	4.79a	5.38b	
	12	2.47a	5.07c	5.84b	5.68a	5.79b	

#### Table 1. Bacterial counts<sup>1</sup> in boxes of hot boned beef trimmings.

<sup>1</sup>Each value is the average log<sub>10</sub> count/g of 3 determinations.

<sup>2</sup>Means within each group followed by the same letter are not significantly different (P > 0.05) according to Duncan's Multiple Range test.

Box Depth (inches)	Position <sup>2</sup>	Storage (hours)					
		0	24	48	72	96	
	Т	2.74a <sup>3</sup>	3.66a	4.73a	5.23a	5.95a	
4	M	3.08a	3.74a	4.67a	4.64a	5.28a	
	В	3.00a	3.59a	4.51a	4.51a	4.81a	
	Т	3.19a	4.70a	6.21a	6.81a	6.26a	
8	M	2.99a	4.47a	5.34a	5.72b	6.23a	
	В	2.94a	3.77b	4.45a	4.58c	5.48a	
	Т	2.94a	5.71a	7.09a	7.26a	7.29a	
12	M	3.13a	5.22a	6.47b	6.68a	6.53a	
	В	3.37a	4.10b	5.19c	5.34a	5.88a	

### Table 2. Total aerobic bacteria<sup>1</sup> on meat at three positions within boxes of hot boned beef trimmings.

<sup>1</sup>Each value is the average log<sub>10</sub> count/g of 3 determinations.

 ${}^{2}T$  = top position, M = middle position, B = bottom position.

<sup>3</sup>Means within each group followed by the same letter are not significantly different (P>0.05) according to Duncan's Multiple Range test.

#### Table 3. Psychrotrophic bacteria<sup>1</sup> on meat at three positions within boxes of hot boned beef trimmings.

Box Depth (inches)	Position <sup>2</sup>	Storage (hours)					
		0	24	48	72	96	
t as the dej	A DO TO DO	1.65a3	3.51a	4.61a	5.26a	6.09a	
4	М	1.77a	3.41a	4.29a	4.53a	5.47a	
	В	1.69a	2.94a	4.28a	4.61a	4.81a	
8	T	2.35a	4.62a	6.27a	6.90a	6.81a	
	М	1.77a	3.94b	4.89b	5.61b	6.11a	
	В	1.93a	3.25c	4.14¢	4.56c	5.39a	
	T	1.95a	5.63a	7.16a	7.29a	7.23a	
12	М	2.23a	4.78b	6.08b	6.29b	6.06b	
	В	2.38a	3.76c	4.83c	5.32c	5.45c	

<sup>1</sup>Each value is the average  $\log_{10}$  count/g of 3 determinations. <sup>2</sup>T = top position, M = middle position, B = bottom position.

<sup>3</sup>Means within each group followed by the same letter are not significantly different (P>0.05) according to Duncan's Multiple Range test.

ed. Thus, the meat at the top position had higher total aerobic and psychrotrophic counts than at the middle position, where the decline in temperature was slower than at the other positions (Figures 2, 3, and 4). Although the most important single factor governing microbial growth is temperature, bacterial population density may be determined by the rate at which oxygen becomes available to the cells (Gill and Newton, 1977). Therefore, it is likely that the aerobic environment favored a higher oxygen tension in meat at the top position within the boxes, producing a more significant effect upon bacterial growth than that expected to be produced by the higher temperature at the middle position. The results obtained at 0 hours of storage may be explained by the fact that bacteria had not yet grown and increased in numbers.

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Figure 2. Average temperature change during storage of hot boned beef trimmings in 12-inch depth boxes.







Figure 4. Average temperature change during storage of hot boned beef trimmings in 4-inch depth boxes.

Box Depth (inches)	Position <sup>2</sup>	Storage (hours)					
		0	24	48	72	96	
	Т	2.27a3	3.02a	3.94a	3.83a	4.05a	
4	M	2.28a	3.26a	4.00a	3.85a	3.99a	
	В	2.50a	2.86a	4.01a	4.31a	4.41a	
8	Т	2.69a	4.07a	5.34a	4.82a	5.14a	
	М	2.45a	4.36a	5.41a	5.11a	5.80a	
	В	2.28a	3.31a	4.18b	4.44a	5.20a	
12	Т	2.44a	5.14a	5.91a	5.38a	5.27a	
	М	2.40a	5.83b	6.53a	6.56a	6.35a	
	В	2.57a	4.22c	5.09a	5.09a	5.75a	

## Table 4. Anaerobic bacteria<sup>1</sup> on meat at three positions within boxes of hot boned beef trimmings.

<sup>1</sup>Each value is the average  $\log_{10}$  count/g of 3 determinations. <sup>2</sup>T = top position, M = middle position, B = bottom position.

<sup>3</sup>Means within each group followed by the same letter are not significantly different (P > 0.05) according to Duncan's Multiple Range Test.

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Unlike total aerobic and psychrotrophic bacteria, anaerobic bacterial numbers were higher (but not necessarily statistically different) on the meat at the middle position than at the top or bottom positions within the 8 and 12-inch deep boxes at all storage periods except at 0 hours (Table 4). Again, oxygen availability would explain these results. At the middle position, the amount of oxygen may be reduced in such a way that it may provide partial anaerobic conditions when combined with the higher temperature at this position. This condition would favor the growth of facultative anaerobes and/or microaerophilic bacteria. In contrast, the faster meat temperature decline seemed to be responsible for the different growth pattern within the 4-inch deep boxes, where after 24 hours of storage, the anaerobic count on the meat was higher at the bottom than at the middle or top positions.

# Literature Cited

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