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Initial Chilling Rate Effects on Bacterial Growth on Hot-Boned Beef[1]

DANIEL Y. C. FUNG*, CURTIS L. KASTNER, CHIA-YEN LEE, MELVIN C. HUNT, MICHAEL E. DIKEMAN and DONALD H. KROPF

Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66506

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ABSTRACT

We studied the chilling rate of hot-boned beef required to control bacterial growth during storage and display. Hot-boned cuts were chilled to 21 C by 3, 5, 9, and 12 h after their removal from the carcass. Cuts were vacuum-stored at 2.2 C for 14 or 21 d, then displayed at 2.2 C for 3 days under natural fluorescent lighting. Initial bacterial loads of hot-boned cuts were low (Log 0-3 CFU/cm²). Conventionally chilled beef (48 h at 2.2 C) and hot-boned cuts chilled to 21 C by 3, 5, and 9 h had lower bacterial counts and more desirable color and odor than hot-boned cuts chilled slower (12 h to 21 C). In general, indicator organisms and potential pathogens (coliforms, fecal coliforms, coagulase-positive *Staphylococcus aureus*, *Clostridium perfringens*, and fecal streptococci) were more numerous for cuts with slower chilling rates (9 and 12 h to 21 C) than for cuts chilled faster (3 and 5 h to 21 C and conventionally chilled beef). No *Salmonella* were detected. Hot-boned beef cuts are in good bacteriological condition (no potential health hazards) for storage if chilled to 21 C in 3 to 9 h.

Centralized beef processing involves animal slaughter, carcass chilling, carcass fabrication and packaging and shipment of cuts (Fig. 1). An alternate process, hot-boning, is of interest because it requires less cooler space and energy (refrigeration), increases cut yield, and facilitates centralized processing (*6*). With hot-boning, the carcass is warm when cut, and the resultant cuts are chilled either before or after packaging (Fig. 1).

In introducing an alternative food processing technique, many factors must be examined to insure that the resultant product is acceptable when compared with conventional processing. To insure that hot-boned meat has an acceptable shelf life and is safe, it is essential to know the numbers and kinds of microorganisms growing on it during processing and storage. This paper deals with microbiological evaluations of hot-boned beef.

MEAT PROCESSING SYSTEMS

Conventional Process	Hot-boning
Slaughter	Slaughter
↓	↓
Refrigerate	Fabricate
↓	↓
Fabricate	Package
↓	↓
Package	Refrigerate
↓	↓
Ship	Ship

Figure 1. *Meat processing systems.*

Hot-boned beef and lamb (*1,7,10,11,12*) had bacterial counts of Log 3-6 CFU (colony forming units) /cm², g, or ml after 1 to 14 days of storage at 1 to 15 C. These hot-boned cuts were considered bacteriologically acceptable. The generally accepted bacterial spoilage index on meats is Log 7 CFU/cm² or g.

We reported (*3*) that hot-boned beef had higher mesophilic and psychrotrophic populations than conventionally chilled beef after 14 days of storage at 2.2 C. Hot-boned cuts had a slower initial temperature decline than conventionally chilled beef. At the center of the box, hot-boned cuts had a slower chilling rate than those near the periphery.

The purpose of this study was to ascertain the effect of initial chilling rate of hot-boned beef on growth of mesophiles, psychrotrophs, indicator organisms and potential pathogens in vacuum-packaged cuts, using conventionally chilled cuts as a comparison. Initial chilling rate of hot-boned beef was related to bacterial

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CFU/cm², data not shown). The levels of bacteria in "External" and "Internal" hot-boned samples were in the intermediate range (Log 4-5 CFU/cm2). After meat samples were repackaged in PVC film and displayed at 2.2 C for 3 days, the mesophilic and psychrotrophic populations were still low (Log 2-3 CFU/cm²) for conventionally chilled meat samples. Bacterial counts of both the "External" and "Internal" hot-boned meat were at the intermediate range (Log 4-5 CFU/cm²). All stored and displayed meat samples in Experiment 2 had acceptable levels of bacteria and color and odor evaluations.

Data from these two experiments indicate that when hot-boned beef was chilled at 21 C within 9 h and continued to chill to below 10 C, the hot-boned meat had acceptable 14-day storage and 3-day display shelf-life. Faster chilling rates will provide meat with better microbiological quality since none of the meat had more than Log 6 CFU/cm² even after 3 days of display in Experiment 2.

Indicator organisms and potential pathogens

At the onset of Experiment 1 meat samples had no detectable coliforms, fecal coliforms, *C. perfringens, S. aureus* and fecal streptococci. In Experiment 2, there were detectable coliforms, fecal coliforms and fecal streptococci at the onset of the experiment, indicating that the meat used in Experiment 2 had lower bacterial quality than that of Experiment 1. Origin of the difference was not investigated, but probably was due to differences in slaughter crew, cattle and climatic conditions. No *Salmonella* were detected at this stage as well as throughout the experiments for all meat samples (Table 1).

When conventionally chilled meat reached 21 C in Experiment 1, coliforms and fecal coliforms were detected and fecal streptococci were found in low numbers (Log 1 CFU/cm²). In Experiment 2, only fecal streptococci were found (Log 2 CFU/cm²) at this stage. Throughout both experiments no indicator organisms and potential pathogens were detected from conventionally chilled meat, except for low numbers of fecal streptococci (Log 0-3 CFU/cm²) and *S. aureus* (in Experiment 2 after 14 days of storage).

When "External" and "Internal" hot-boned meat samples from both experiments reached 21 C, coliforms, fecal coliforms, *S. aureus* and fecal streptococci were detected. Although no *C. perfringens* was detected (except "Internal" hot-boned beef in Experiment 2), we found sulfite-reducing anaerobes in these meat samples at this stage. The identity of those anaerobes was not studied, but they were presumed to be *Clostridium* spp. There were higher numbers of fecal streptococci (Log 3 CFU/cm²) in "Internal" hot-boned samples compared with "External" hot-boned samples (Log 2-3 CFU/cm²) when those reached 21 C in both experiments. After 14 days of cold storage of vacuum-packaged hot-boned samples, coliforms, fecal coliforms, *C. perfringens* (except 14-day "External" hot-boned sample of Experiment 2). *S. aureus* and fecal streptococci were found in both "External" and "Internal" hot-boned meat samples, with the "Internal" hot-boned samples harboring higher numbers of each detected organism. Samples in Experiment 1 had higher counts than comparable samples in Experiment 2. The levels of *C. perfringens* and *S. aureus* were low when found (Log 0-1 MPN/cm²). The levels of fecal streptococci were intermediately high (Log 4 CFU/cm²) for Experiment 2 and high (Log 5-6 CFU/cm²) for Experiment 1. This was not surprising since vacuum-packaged meat favors the growth of facultative anaerobes. The levels of coliforms were presumed to be relatively high since all tubes used in the MPN test were positive for Experiment 1 samples. Coliform counts for Experiment 2 were lower than those of Experiment 1 and the "Internal" samples had higher counts than "External" samples. After these hot-boned samples had been displayed at 2.2 C for 3 days, coliform counts were one Log unit higher than before display. Fecal coliform counts were low for both experiments after 14 days of storage (Log 0-1 MPN/cm²). After 3 days of display there was a 2-Log increase of fecal coliforms in samples from Experiment 1 and no increase for Experiment 2. *C. perfringens* was not detected in either experiment after display, probably because of the poor competitive nature of those organisms with other organisms in the aerobic environment (PVC film is permeable to oxygen). *S. aureus* numbers increased half a Log unit in the displayed samples compared with the 14-day non-displayed samples for Experiment 1 although the numbers were low (Log 1 MPN/cm²). In Experiment 2, *S. aureus* was not detected in the samples after 3 days of display. The levels of fecal streptococci were high (about Log 6 CFU/cm²) for samples in Experiment 1 and intermediate-to-high (Log 4-5 CFU/cm²) for samples in Experiment 2. Again, the numbers of the organisms when found were higher on "Internal" hot-boned samples compared with "External" hot-boned samples in both experiments. Potential pathogens and indicator organisms after 21 days of storage and 3 days of display were not studied.

Summary

These data indicate that conventionally chilled meat had few potential pathogens and indicator organisms before and after storage and display. Apparently the more rapid chilling of these samples prevented growth of these organisms. Fecal streptococci were found in conventionally chilled meats, although in low numbers (Log 0-3 CUF/cm²). *C. perfringens* and *S. aureus*, when found in hot-boned meat, were in low numbers (Log 0-1 MPN/cm²). No *Salmonella* was found on any meat samples in this study. The fecal indicators (fecal streptococci), however, were found in large numbers (Log 5-6 CFU/cm²). Fecal coliforms were in low numbers after 14 days of storage (Log 0 MPN/cm²), but increased to more than Log 2 MPN/cm² after 3 days of display in Experiment 1.

In general, the number of indicator and potential

36.8 C-h) compared with the "External" hot-boned samples. This resulted in bacterial growth of about 1.5 Log units higher for mesophiles and a 0.5 Log unit higher for psychrotrophs for "Internal" compared with "external" hot-boned samples.

Initial bacterial populations of the meat chilled at different rates influenced the shelf life of the meat samples (Fig. 3). Mesophile and psychrotroph counts of conventionally chilled samples were low (Log 3 CFU/cm²) after 14 days of storage. Bacterial levels on "External" hot-boned meat were about Log 6 CFU/cm² and the "Internal" hot-boned meat had Log 7 CFU/cm² after 14 days of storage. At this stage of storage (14 days), both conventionally chilled meat and "External" hot-boned meat were acceptable in color and odor while the "Internal" hot-boned meat was barely acceptable. After meat samples had been repackaged in PVC film and displayed at 2.2 C for 3 days, the mesophilic and psychrotrophic populations were still low (Log 3-4 CFU/cm²) on conventionally chilled meat samples. Bacterial counts of both the "External" and "Internal" hot-boned meats reached Log 7 CFU/cm².

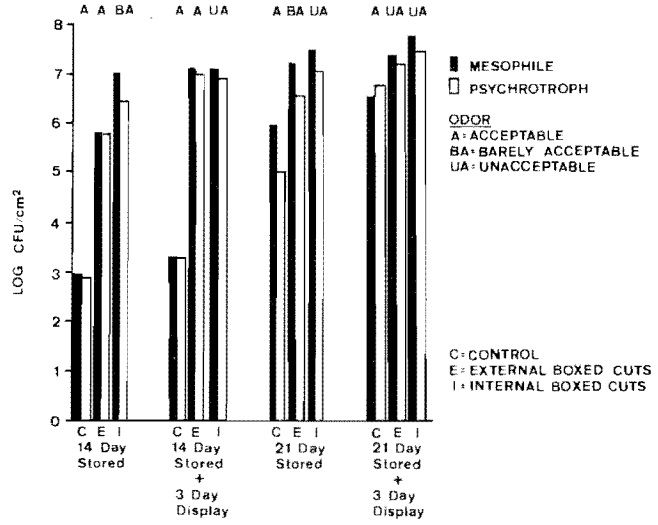

Figure 3. *Mesophile and psychrotroph populations of hot-boned and conventionally chilled beef after 14 and 21 days of storage and 3 days of display (Experiment 1).*

At this stage, both the conventionally chilled meat and "External" hot-boned samples were still acceptable for color and odor, but the "Internal" hot-boned samples were not acceptable. The large number of bacteria (Log 7 CFU/cm²) at the time of repackaging in PVC film created sufficient biochemical changes during the 3 days of display to make the "Internal" hot-boned meat unacceptable. The lower numbers of bacteria (Log 6 CFU/cm²) at the time of repackaging of the "External" hot-boned samples did not initiate enough changes in 3 days to be detected even though the level of bacteria after 3 days of display reached Log 7 CFU/cm².

After 21 days of cold storage, the conventionally chilled samples were acceptable, and the bacterial counts were in the high range (Log 5-6 CFU/cm²). The "External" hot-boned samples were barely acceptable for color and odor, and the "Internal" hot-boned samples were not acceptable. Mesophiles of both "External" and "Internal" hot-boned samples were near Log 7 CFU/cm². The psychrotrophs of "External" hot-boned samples were below Log 7 CFU/cm² while the "Internal" hot-boned samples were above Log 7 CFU/cm². After display for 3 days, only the conventionally chilled samples were acceptable although the bacterial counts were high and approaching Log 7 CFU/cm². The bacterial counts of "External" and "Internal" hot-boned samples after 3 days of display were over Log 7 CFU/cm². These data clearly indicated the important relationship between initial chilling rate and shelf-life of hot-boned meat.

Experiment 2.

The second experiment was made to determine the effect of faster chilling rates (3 and 5 h to 21 C) on microbial growth compared with slower rates (9 and 12 h to 21 C) of Experiment 1. Temperature of plate surfaces of conventionally chilled carcasses declined very rapidly (Fig. 4). When the meat reached 21 C (0.48 C-h units), the mesophilic and psychrotrophic counts were low (Log 2 CFU/cm²). It took the "External" hot-boned samples 3 h to reach 21 C (12.8 C-h units) and the "Internal" hot-boned samples 5 h to reach 21 C (15.9 C-h units). The "External" hot-boned samples had about Log 3 CFU/cm² mesophiles and Log 2 CFU/cm² of psychrotrophs while the "Internal" hot-boned samples had Log 4 CFU/cm² mesophiles and Log 3 CFU/cm² psychrotrophs when the temperature reached 21 C (Fig. 4). After 14 days of cold storage, mesophiles and psychrotrophs of conventionally chilled samples were low (Log 2

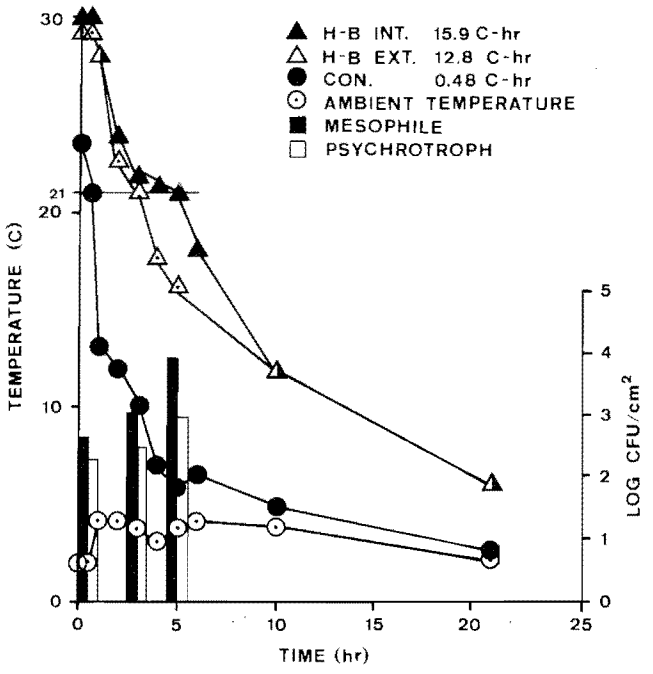

Figure 4. *Effect of initial chilling rate on microbial growth on vacuum-packaged beef from hot-boned and conventionally chilled carcasses (Experiment 2). See Fig. 1 Legend.*

development during the first 12 h postmortem and during storage and display.

MATERIALS AND METHODS

Meat Processing

Three steers were slaughtered within 1 h in each of two experiments. One side of each carcass was hot-boned at 2 h postmortem for hot-boned beef and the other half was chilled at 2.2 C for 48 h and then boned for conventionally processed beef. Meat samples (32.26 cm^2) for initial bacterial examinations were first removed aseptically from the plate region 2 h postmortem and after chilling 48 h at 2.2 C for "0" time evaluations, representing hot-boned and conventionally processed beef, respectively. Thin muscle samples (ca. 2.5 × 15 × 22 cm) from the plate region (cut from the carcass exterior to the first underlying connective tissue septa) were excised aseptically, vacuum-packaged (Cryovac bags SR823) and stored in a box (24 × 56 × 43 cm) at 2.2 C for 14 and 21 days.

Experiment 1

Hot-boned samples were separated into two groups. One group of vacuum-packaged samples from each carcass, referred to as "Internal" chilled hot-boned samples, was placed in the center of the box and surrounded by hot-boned meat. The other group of three vacuum-packaged samples (taken adjacent to "Internal" samples) was placed below the lid of the box on top of the hot-boned meat and was called "External" chilled samples. This arrangement simulated the differential chilling rate of internal and external commercially hot-boned, vacuum-packaged meat placed in a box for chilling.

Samples of meat for microbiological analyses were removed at the following times:

(*a*) Initial bacterial loads were measured on samples (32.26 cm^2) aseptically removed from carcasses at 2 h postmortem (hot-boned) and after 48 h of chilling (conventional). These samples were adjacent to the vacuum-packaged plate samples.

(*b*) A sample (32.26 cm^2) was removed when the internal and external hot-boned samples were chilled to 21 C. The plate samples were subsequently again vacuum-packaged for storage. In a previous publication (*3*), we introduced use of 21 C as a temperature decline marker for chilling rate of hot-boned beef based on the assumption that most bacteria on meat surfaces have fast growth rates above 21 C.

(*c*) Vacuum-stored samples were removed after 14 and 21 days at 2.2 C. Samples were repackaged in polyvinyl chloride (PVC) film and placed in a commercial-type display case, illuminated with continuous natural fluorescent lighting (1,076 lumen/m^2).

(*d*) Post-display samples were removed after 3 days of display at 2.2 C.

All three animals were slaughtered and sampled at practically the same time to avoid differences in subsampling times.

Experiment 2

Protocol for Experiment 2 was identical to that for Experiment 1, except that the meat sandwich arrangement was placed on a metal tray instead of a box to attain faster chilling rates. Only 14 days of storage and display studies were made in Experiment 2.

Temperature measurements and chilling rates

Thermistors were taped to the surface of the plate region of the carcasses chilled for 48 h and to the surface of the bag of the vacuum-packaged hot-boned beef samples, which were placed either "Internally" or "Externally" in the box or on the metal tray. Temperatures of the meat samples were taken at suitable time intervals ranging from 0 to 48 h.

Microbiological procedures

Meat samples (32.26 cm^2) were aseptically excised, put into mason jars containing 100 ml of sterile rinse solution (*6*) and transferred to the microbiology laboratory for analysis. The meat was in the solution for 20 min, blended for 5 sec, and shaken 50 times before liquid samples were drawn from the jar for viable cell counts by *Standard Methods* (*6*). One set of plates was incubated for 48 h at 32 C for the mesophilic count and another set for 10 days at 7 C for the psychrotrophic count. Counts were reported as Log_{10} CFU/cm^2. All data presented were averages of 3 samples. Arithmetic means were calculated before converting them to Log_{10} numbers.

Indicator and potential pathogens

We also monitored coliforms, fecal coliforms, *Clostridium perfringens*, *Salmonella*, coagulase-positive *Staphylococcus aureus*, and fecal streptococci to assess the safety of hot-boned beef. All organisms were identified and enumerated using bacteriological analyses previously reported (*3*).

Evaluation of stored meat

The color and odor of stored samples were examined by 3 laboratory personnel. The meat was ranked as acceptable (normal color and no objectionable odor), barely acceptable (normal color and slightly objectionable odor) and unacceptable (some off-color and objectionable odor).

RESULTS AND DISCUSSION

The importance of initial chilling rate of hot-boned beef on microbial growth during processing, storage, and display is clearly shown in this study.

Experiment 1

Surfaces of plates from conventionally chilled carcasses chilled the fastest (Fig. 2) and the mesophilic and psychrotrophic counts were low (Log 1-2 CFU/cm^2) when the meat reached 21 C [0.48 Centigrade degree-hour (C-h) units]. The "External" hot-boned samples reached 21 C in 9 h (36.8 C-h units) and the "Internal" hot-boned samples reached 21 C in 12 h (63.0 C-h units). The "External" hot-boned samples had about Log 3 CFU/cm^2 while the "Internal" hot-boned samples had more than Log 4 CFU/cm^2 when the meat temperature reached 21 C (Fig. 2). Although there was only a 3-h difference in reaching 21 C, the "Internal" hot-boned samples had about twice the C-h units (63.0 C-h versus

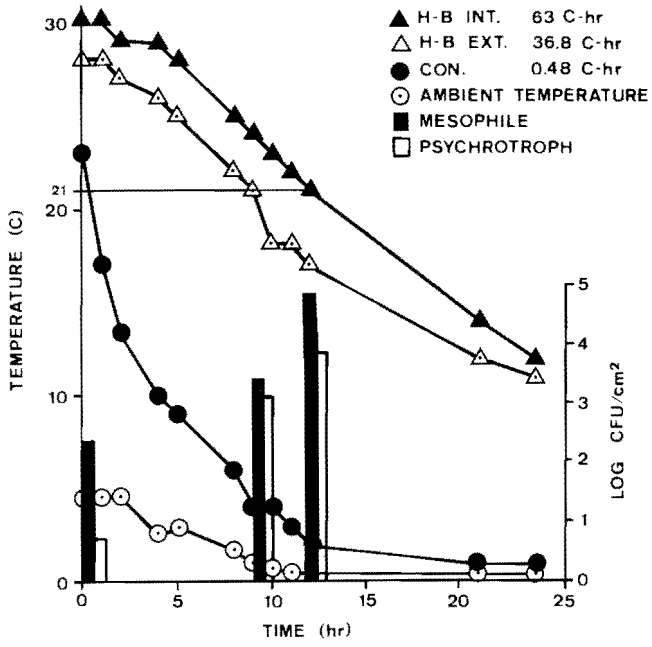

Figure 2. *Effect of initial chilling rate on microbial growth on vacuum-packaged beef from hot-boned and conventionally chilled carcasses (Experiment 1). H-B = hot-boned. Int. = Samples placed in the center of a box. Ext. = Samples placed in the periphery of a box. Con. = Conventionally chilled beef on carcass. C-hr = Centigrade-hour unit.*

TABLE 1. *Effect of chilling rate on occurrence of indicator and potential pathogens on hot-boned and conventionally chilled beef carcasses.*[a]

Meat samples	Coliforms		Fecal Coliforms		*Clostridium perfringens*		*Salmonella*		*Staphylococcus aureus*		Fecal streptococci	
	Exp. 1[b]	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Zero time												
Control (48 h at 2.2 C)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.61	1.72
Hot-boned (2 h post mortem)	ND	D	ND	D	ND	ND	ND	ND	ND	ND	ND	2.87
Sampled at 21 C												
Control	D	ND	D	ND	ND	ND	ND	ND	ND	ND	1.00	2.49
External hot-boned	D	D	D	D	ND	ND	ND	ND	D	D	2.20	2.85
Internal hot-boned	D	D	D	D	ND	D	ND	ND	D	D	3.49	3.62
After 14 days of storage at 2.2 C												
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	-0.40	2.04	1.01
External hot-boned	>1.89	1.72	0.64	1.58	0.28	ND	ND	ND	0.66	-0.03	5.08	4.94
Internal hot-boned	>1.89	>1.89	0.71	1.80	1.53	-0.03	ND	ND	0.67	0.46	6.15	4.65
After 3 days of display at 2.2 C												
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.34	1.30
External hot-boned	>2.89	0.75	>2.89	0.75	ND	ND	ND	ND	1.04	ND	5.94	4.70
Internal hot-boned	>2.89	>1.89	2.60	1.39	ND	ND	ND	ND	1.23	ND	6.15	5.61

[a]All numbers are average of 3 samples, expressed as Log_{10} CFU/cm² (for fecal streptococci) and Log_{10} MPN/cm² for others.

[b]Exp. 1 = hot-boned meat chilled in a box to 21 C at 9 h (External sample) and 12 h (Internal sample) after fabrication.
Exp. 2 = hot-boned meat chilled on a tray to 21 C at 3 h (External sample) and 5 h (Internal sample) after fabrication.
D = target organism detected. ND = target organism not detected.

pathogens, when found, was lower for Experiment 2 than Experiment 1, probably due to the faster chilling rates used in Experiment 2.

We observed that when hot-boned meat is chilled adequately (from carcass temperature to 21 C with 9 h) during the first 24 h, the hot-boned meat is acceptable in color and odor and bacterial quality after 14 days of storage and 3 additional days of display. When meat is not chilled adequately (from carcass temperature to 21 C at 12 h), the shelf-life and storage life will not be acceptable.

It is evident that industry and meat researchers believe that hot-boning of beef has considerable commercial potential. However, our data indicate that it is imperative to chill hot-boned beef at a rate sufficient to produce a bacteriologically acceptable product. Hot-boned beef that is vacuum-packaged and boxed before chilling must be closely monitored from a microbial standpoint. Boxing of hot-boned cuts before chilling may be a more convenient handling system for some processors, but the need for precautions is evident from our data.

Adhering to chilling rates presented in this paper, before or after boxing, will certainly help produce a quality hot-boned product. However, indiscriminate acceleration of the chilling rate of hot-boned meat can lead to other problems, such as cold-induced muscle shortening and resultant toughening.

Muscle toughening should not be a problem, considering chilling rates used in our study. Cold-induced toughening does not occur in beef muscle unless 10 C is reached within 10 h postmortem in the deep tissue of beef carcasses (*8*). We fabricated hot-boned carcasses at 2 h (0 time on Fig. 2 and 4) postmortem and our samples did not reach 10 C in 10 h postmortem. Therefore, even faster chilling rates than the 3-9 h after fabrication of this study may be used to further insure microbial quality, yet avoiding cold-induced toughening. If electrical stimulation is used in conjunction with hot-boning, additional insurance against muscle toughening may be obtained (*4,5*).

Based on our data, we recommend chilling hot-boned meat to 21 C within 3-9 h after fabrication, with continuous chilling to below 10 C within 24 h.

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