THE BACTERIOLOGICAL QUALITY OF MEAT FROM ELECTRICALLY STIMULATED CARCASSES

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INTRODUCTION

It has been established that postmortem electrical stimulation of beef carcasses is important for improving muscle tenderness (Gilbert et al 1976; Raccach and Henrickson, 1979). It accelerates the onset of rigor mortis (Gilbert and Davey, 1976; Will et al, 1979) allowing for early boning of muscles. On-the-rail hot boning complements electrical stimulation. It speeds up the fabrication of carcasses (7-10% less labor requirements) and reduces both refrigeration energy (30-50%) and cooler space (70-80%).

Last but not least on-the-rail early boning may reduce recontamination of meat from surfaces and considerably decrease the time for bacterial proliferation (Kastner et al; 1976).

It is estimated that 3.1 million ton of hamburger and ground beef have been processed in the U.S. during 1978 with a corresponding consumption of 14.5kg/capita (AMI, 1978).

The bacteriological quality of beef and its ground product is of concern from both of the industrial and public health stand points. Reduced shelf life and discoloration of the product due to bacterial growth are often encountered. The source of meat for ground beef and its holding time may affect the bacteriological quality of the product, its safety and storage stability. Proposed bacteriological standards for ground beef in some states set a maximum range of 1.0×10^6 to 5.0×10^6 bacteria/g (Geopfert, 1976). The United Kingdom has proposed a level of 10^6/g (Green, 1976) while in Canada the maximum limit is 10^7/g (Pivnick et al, 1975). It is well established that most psychrotropic meat-spoilage bacteria are of gram-negative type (Davidson et al; 1973; Mossel, 1968). The nonpigmented *Pseudomonas* organisms predominate the flora at spoilage (Ayres, 1960; Herbert, 1975).

The purpose of this work was to study the effect of prefabrication procedures such as electrical stimulation and hot boning on the bacteriological quality and wholesomeness of both beef and ground beef. In addition the stability of refrigerated ground beef and its spoilage flora were studied.

MATERIALS AND METHODS

ELECTRICAL STIMULATION (ES). The electrical stimulation (a square wave pulse of 300 V, 400 cpm with a duration of 0.5 msec and a current of 1.6 to 1.8 A) of beef sides (commercial Angus and Hereford steers) started at 30 min postmortem and continued for 15 min. Both sides, stimulated and control (non-stimulated), were held at 16C during the stimulation period and up to 1.5 h postmortem. Six carcasses (290 kg average weight) were used in this study.

BONING OF MUSCLES/PORTIONS. The Biceps femoris (BF), Semimembranosus (SM), Longissimus dorsi (LD) and portions of the chuck were boned on-the-rail at 1.5 h postmortem from the ES side ("Hot-Boned-Hot"), and after 24 h at 1.1C from the control (non ES) side.

PROCESSING AND STORAGE. About one third of each BF, SM and LD was stored at 1.1C for 15 h ("Hot-Boned-Chilled"). One third of the BF was vacuum packaged (72.5 mm Hg) in a polymylar film (E.I. DuPont, Wilmington, Delaware) and sotred at 16C for 18-20 h. The portions of the chuck were ground and frozen (-25C) until used. Twelve 250g portions of ground chuck (six of each ES and control) were packed using polystyrene foam tray wrapped with polyvinyl chloride and stored at 5 ± 1C.

SAMPLING. The exterior and interior parts of the BF and the exterior parts of the SM and the LD were sampled. One half of a cm of the surface of each muscle and cores of 5 cm diameter were bored only from the interior part of the BF.

Sampling was done manually using sterile scalpels. The bacteriological examinations were done on 50g pooled samples from either the surface meat tissue or the meat cores. A 10g sample from each portion of the ground chuck was removed every 22-24 h for the different examinations.

Aseptic measures (lighted burner, sanitized working surfaces, frequent changes of sterile scalpels etc.) were taken throughout the sampling process to prevent recontamination of the samples.

BACTERIOLOGICAL EXAMINATIONS. The homogenates of the BF, SM, and LD were prepared by blending 50g samples with 450 ml of 0.1% peptone (Difco) water. The 10g samples of the ground chuck were blended each with 90 ml of the same diluent. Further decimal dilutions were prepared (as required) with the same diluent.

The Aerobic Plate Count (APC) was done by spreading 0.1 ml aliquots of the appropriate dilutions of prepoured dried surface plate count agar (PCA) (Difco) plates. Incubation was at 22C for 40-48 h. The same procedure and medium were used for the Psychrotrophic Bacterial Count (PBC). The plates were incubated at 7C for 10 days. The Anaerobic Bacterial Count was determined by the pour plate method using tryptic soy agar (Difco) plates incubated (35C, 48 h) in a GasPak Anaerobic System (BBL). Total coliform and total Enterobacteriaceae were determined using violet red bile agar and MacConkey agar (supplemented with 1% glucose) respectively, incubated at 35C for 24 h. Both media were from BBL.

Clostridium perfringens, *Salmonella* and *Staphylococcus aureus* were determined according to the Bacteriological Analytical Manual for Foods. (FDA, 1976).

NUMBER OF MICROBIAL GENERATIONS AND SHELF LIFE DETERMINATION. The number of generation (G) was calcuated according to the following formula: $G=3.3 \log b/B$ in which b= number of bacteria at the end of a given time period, and B=Intial number of bacteria.

The meat samples were organoleptically evaluated for "off odors" by a three-people panel. A sample envolving "off odors" was considered spoiled.

IDENTIFICATION OF SPOILAGE ORGANISMS. Thirty colonies were randomly selected from PCA plates of each of the ES and control ground chuck samples. Each colony was purified and transferred to a nutrient agar (Difco) slant (incubated at 22C) which served as the stock culture.

The following tests were conducted for the taxonomical classification: gram stain, motility, catalase, benzidine (Deibel and Evans, 1960) cytochrome oxidase (Kovacs, 1957) oxidation-fermentation (glucose) (Hugh and Leifson, 1954) pigment production (King et al, 1954) reducing compounds from gluconate (Haynes, 1951) starch hydrolysis (Cowan and Steel, 1965) and hydrogen sulfide production (Thornley, 1967). The isolates were broadly classified in a scheme based upon the work of Shewan, (1963) and Shewan et al, (1960). All tests were conducted at 22C.

STATISTICAL ANALYSIS. Results were subjected to the analysis of variance and the Least Significance Difference test (Steel, 1960). The correlation and the linear regression between the APC and the PBS were calculated.

RESULTS AND DISCUSSION

The aerobic plate counts (Table 1) of the exterior parts of the different "Hot-Boned-Hot" muscles were in the range from 1.0×10^1 to 3.8×10^3/g. Chilling (15 h, 1.1C) did not significantly ($P<0.05$) change the bacterial counts. The exterior parts of the control muscles had an aerobic plate count that ranged from 1.0×10^1 to 1.0×10^3/g. These bacterial levels are 10^4 to 10^6 fold lower than the documented spoilage level of 10^7 bacteria/g (Kraft and Ayres, 1952).

"Hot-Boning" (boning before chilling the carcass) and on-the-rail-boning of muscles expose a relatively small surface area to bacterial growth and recontamination. The processing time is shortened, becoming a limiting factor, for bacterial growth. In all treatments, the interior part of the BF had an aerobic plate count of 1.0×10^1g (Table 1).

The combination of ES, and on-the-rail "Hot Boning" prevented recontamination of the product especially with pathogens. As a result *C. perfringens* and *Salmonella* were not detected in the exterior and interior parts of the BF (Table 2), while *S. aureus*, total Enterobacteriaceae and the coliform group were each at a low level of $<1.0 \times 10^1$/g. The absence of pathogens (and the low level of *S. aureus*) is in accordance with a proposed standard (Green, 1976).

It should be emphasized that a meat product with a bacterial level as low as 10^3/g may become potentially dangerous because pathogens (if present) may grow with less competition from the "indigenous" flora (Baird-Parker, 1971; Mack and Lippitz, 1962; Troller and Frazier 1963). Therefore, it is extremely important to prevent bacterial recontamination of the product, and this can be achieved useing a sanitary process.

Vacuum packaging of "Hot-Boned" meat (Valin et al, 1976) and storage at elevated temperatures (Fields et al, 1976; Smith et al., 1973) stimulated the postmortem metabolism and conversion of the animal flesh to post-rigor meat. Under the conditions used in this work (72.5 mm of Hg, 16C for 18-20 h) no significant ($P<0.05$) bacterial growth took place either on the exterior or in the interior parts of the BF (Table 3). These results indicate that "Hot-Boning" and vacuum-storage at 16C for as long as 20 h will not increase the aerobic and anaerobic plate counts and will not cause deterioration of the product. One should be very cautious to adequately control the temperature of storage and the storage period.

The linear regression between the APC and the PBC of the ES ground chuck samples (n=6) is shown in Fig. 1. The equation of the regression line was $Y=0.78 + 0.85\ X$ with a correlation coefficient of 0.96, significant at $P<0.01$. Figure 2 shows the linear regression between the APC and the PBC of the control (non ES) samples (n=6). The equation of the regression line was $Y= -0.3 + 1.06\ X$ with a correlation coefficient of 0.99, significant at $P<0.01$. These results show that one can get an accurate estimation of the psychrotrophic bacterial population of ground chuck from ES carcasses by using an incubation temperature of 22C. This elevated temperature (22C) shortens the incubation time from 10 days (7C) to 48 h. Since the psychrotrophic bacterial population of ground chuck is responsible for spoilage (Ayres, 1960), thus it is controlling the storage stability of the refrigerated product.

It is important to the industry, for quality control purposes, to have rapid means to estimate the psychrotrophic bacterial population in order to take adequate measures concerning processing and marketing.

A 22C incubation temperature was used for monitoring the APC of the ES and control samples of ground chuck stored at 5C (Fig. 3). The initial bacterial count of ground chuck from both ES carcasses and control was as low as $8.0-9.0 \times 10^3$/g. This level was lower by 10^2 to 10^3- fold than currently proposed bacteriological standards for ground beef (Geopfert, 1976: Green, 1976; Pivnick, et al., 1975). As can be seen (Fig. 3), the bacterial population of the samples from ES carcasses had a lag phase of 3 days as compared to 1 day with the control bacterial population. It is probable that the electrical stimulation may have impaired the bacterial cell metabolism (Munnert and Gradman, 1976) causing an extended lag phase. A significant difference ($P<0.05$) was found among the APC of the samples from ES carcasses and the control on the third, fifth, sixth, and seventh days of storage. Between the third and fifth day of storage, the bacterial population of ground chuck from ES carcasses formed 12.2 generations while the control population formed only 9.9 generations.

The shelf life (i.e. time to "off odor") of a refrigerated product in general and of a meat product in particular is determined, among other factors, by bacterial growth. Any processing or preservation procedure that will induce a long lag phase and will slow the grow rate of the bacterial population will also extend the shelf life of the product. Electrical stimulation extended the shelf life of ground chuck by 3 days, "off odors" were detected after 4-5 days in the control samples but after 7-8 days in the samples from ES carcasses with a corresponding bacterial count of 8×10^8/g ground chuck. The rapid growth of the bacterial population of the electrically-stimulated samples did not compensate for the 3-day lag phase (Fig. 3) resulting with a lower population level than that of the control samples (up to the eighth day of storage). This low population level did not impair the organoleptic properties of the product from ES carcasses while the control samples spoiled after 4-5 days.

At spoilage, 30 colonies were picked from PCA plates inoculated with either the ES samples or the control. Most colonies (22-23 isolates) from both samples had the properties described in Table 4. These properties are characteristic to the nonpigmented *Pseudomonas* group II (Shewan et al, 1960) or *Pseudomonas fragi* (Davidson et al, 1973). Four isolates from both the ES carcasses and the control were gram negative, nonmotile, and nonpigmented and oxidative in Hugh-Leifson medium. These isolates were designated *Moraxella*-oxidative in accordance with the description of Thornley (1967). A gram positive, catalase-positive, benzidine- and hydrogen sulfide negative

cocobacillus organism was isolated from the control sample; it was identical to a culture of *Microbacterium thermosphactum* (Davidson et al, 1968; McLean and Sulzbacher, 1953). These results show that electrical stimulation did not affect the nature of the spoilage flora of ground chuck. The nonpigmented *Pseudomonas* predominated the flora at spoilage.

C. perfringens, *Salmonella* and coliform organisms were not detected in the samples from either ES carcasses or the control. *S. aureus* was detected at a level as low as 10 cells/g of ground chuck. This level coincides with proposed bacteriological standards (Geopfert, 1976; Green, 1976; Pivnick et al, 1975). These results point out again one of the advantages of electrical stimulation and on-the-rail hot-boning: reducing sanitation problems especially preventing bacterial contamination of the product from surfaces as with on-the-table boning.

This study showed that both the samples from ES carcasses and the control were free of pathogens except *S. aureus*. The ground samples from ES carcasses had a prolonged shelf life as compared with the control with no unique problem concerning the nature of the spoilage flora.

REFERENCES

1. AMI (American Meat Institute) 1978. How much hamburger? Weekly report, Vol. 21, No. 39. September 29, 1978. 2. Ayres, J.C. 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. J. Appl. Bacteriol. 23:471-486. 3. Baird-Parker, A.C. 1971. Factors affecting the production of bacterial food poisoning toxins. J. Appl. Bacteriol. 34:181-197. 4. Cowan, S.T. and K.J. Steel, 1965. Manual for the Identification of medical bacteria. Cambridge University Press, England. 5. Dack, G.M., and G. Lippitz. 1962. Fate of staphylococci and enteric microorganisms introduced into slurry of frozen pot pies. Appl. Microbiol. 10:472-479. 6. Davidson, C.M., M.J. Dowdell, and R.G. Board. 1973. Properties of gram negative aerobes isolated from meats J. Food Sci. 38:303-305. 7. Davidson, C.M., P. Mobbs, and J.M. Stubbs. 1968. Some morphological and physiological properties of *Microbacterium thermosphactum*. J. Appl. Bacteriol. 31:551-559. 8. Deibel, R.H., and J.B. Evans. 1960. Modified benzidine test for the detection of cytochrome containing respiratory systems in microorganisms J. Bacteriol. 79:356-360. 9. Fields, P.A., Z.L. Carpenter, and G.C. Smith. 1976. Effects of elevated temperature conditioning on youthful and mature beef carcasses. J. Anim. Sci. 42:72. 10. Food and Drug Administration. 1976. Bacteriological analytical manual, Washington, D.C. 11. Geopfert, J.M. 1976. The aerobic plate count, coliform and *Escherichia coli* content of raw ground beef at the retail level. J. Milk Food Technol. 39:175-178. 12. Gilbert, K.V., and C.L. Davey. 1976. Carcass electrical stimulation and early boning of beef. N.Z.J. Agric. Res. 19:429-434. 13. Gilbert, K.V., C.L. Davey and K.G. Newton 1976. Electrical stimulation and the hot boning of beef. N.Z.J. Agric. Res. 20:139-143. 14. Green, S.I. 1976. Microbiological aspects of quality control. Food Man. p. 55 & 71, July, 1976. 15. Haynes, W. C. 1951. *Pseudomonas aeruginosa* - its characterization and identification. J. Gen. Microbiol. 5:939-950. 16. Herbert, R.A. 1975. Microbial ecology of fish spoilage. Proc. Biochem. December 1975: 9-11. 17. Hugh, R., and E. Leifson. 1954. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-26. 18. Kastner, C.L., L.O. Luedecke, and T.S. Russel, 1976. A comparison of microbial counts of conventionally and hot-boned bovine carcasses. J. Milk Food Technol. 39:684-685. 19. King, E.O., M.K. Ward, and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 54:301-308. 20. Kovacs, W. 1957. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178-703. 21. Kraft, A.A. and J.C. Ayres. 1952. Bostmortem changes in stored meats. IV. Effect of packaging materials on keeping quality of self service meats. Food Technol. 6:8-12. 22. McLean, R.A., and W.L. Sulzbacher. 1953. *Microbacterium thermosphactum* spec. nov. a nonheat resistant bacterium from fresh pork sausage. J. Bacteriol. 65:428-433. 23. Mossel, D. A.A. 1968. Microbiological qualities. J. Food Technol. 3:401-421. 24. Munnert, H., and Gradman. 1976. Voltage dependent potassium fluxes and the significance of action potentials in Acetabularia. Biochim. Biophys. Acta 443:443-450. 25. Pivnick, H., J.E. Erdman, D. Collins-Thompson, and G. Roberts. 1975. Microbiological standards for meat in Canada J. Milk Food Technol. 38:638. 26. Raccach, M., and R.L. Henrickson. 1979. The combined effect of electrical stimulation and blade tenderization on some bovine muscles. J. Food Proc. Pres. 3:43-58. 27. Shewan, J.M. 1963. The differentiation of certain bacteria frequently encountered in marine environments. pp. 499-521. In: C.H. Oppenheimer (ed.) Marine microbiology. Charles C. Thomas, Springfield, IL. 28. Shewan, J.M., G. Hobbs, and W. Hodgkiss. 1960. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the *Pseudomonadaceae*. J. Appl. Bacteriol. 23:379-390. 29. Smith, G.C., R.C. Hostetler, and Z.L. Carpenter. 1973. Effects of elevated storage temperature and changes in method of carcass suspension of beef tenderness. Proc. European Meeting of Meat Research Workers. 19:139. 30. Steel, R.G.D. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, New York, NY. 31. Thornley, M.J. 1967. A taxonomic study of *Acinetobacter* and related genera. J. Gen. Microbiol. 49:211-257. 32. Troller, J.A., and W.C. Frazier. 1963. Repression of *Staphylococcus aureus* by food bacteria. II. Causes of inhibition. Appl. Microbiol. 11:163-165. 33. Valin, C., J. Fournaud, A. Lacourt, and C. Touraille. 1976. Evolution en cours de conservation das caracteristique de tendreté de muscles de bovins mis sous vide ante rigor mortis. Ann. Technol. Agric. 25:357-372. 34. Will, P.A., R.L. Henrickson, R.D. Morrison and G.V. Odell 1979. Effect of electrical stimulation on ATP depletion and sarcomere length in delay-chilled bovine muscle. J. Food Sci. 44:1646-1648.

Table 1. Aerobic plate count of electrically stimulated beef.

Treatment	BF		SM[a]	LD[a]
	Exterior part	Interior part		
		(count/g)[b]		
"Hot-Boned-Hot"	1.9×10^2	1.0×10^1	3.8×10^3	1.0×10^1
"Hot-Boned-Chilled" (1.1C, 15 h)	7.5×10^3	1.0×10^1	3.9×10^3	6.5×10^2
Control[c]	1.0×10^3	1.0×10^1	6.0×10^2	1.0×10^1

[a]The exterior part only.
[b]Each result represents the arithmetic average six determinations.
[c]Not electrically stimulated.

Table 2. Occurence of pathogenic and indicator microorganisms in electrically stimulated Biceps femoris.

Organism/Group	Count[a]
Clostridium perfringens	Absent/50 g
Salmonella[b]	Absent/50 g
Staphylococcus aureus[b]	$<1.0 \times 10^1$/g
Total Enterobacteriaceae	$<1.0 \times 10^1$/g
Total coliform	$<1.0 \times 10^1$/g

[a]Each result represents the arithmetic average of six determinations.
[b]Examined by the 5 tube MPN method.

Table 3. Effect of vacuum packaging on the bacteriological quality of electrically stimulated Biceps femoris.[a]

Sample	Aerobic plate count		Anaerobic count	
	Before storage	After storage	Before storage	After storage
		(count/g)[b]		
Exterior part	2.2×10^3	3.6×10^3	5.0×10^2	1.6×10^3
Interior part	1.0×10^1	1.0×10^1	1.0×10^1	1.0×10^1

[a]Vacuum packaged and stored for 18-20 h at 16C.
[b]Each result represents the arithmetic average of six determinations.

Table 4. Properties common to most[a] isolates.

Character	Observation
Gram reaction	-
Morphology	Short rods
Motility	+
Oxidase	+
Oxidation/fermentation test (glucose)	Oxidative
Pigmentation	-
Reducing compounds from gluconate	+
Starch hydrolysis	-
Growth at 4C	+
Growth at 41C	-

[a]55 isolates (23 and 22 isolates from ground chuck from ES carcasses and the control respectively).

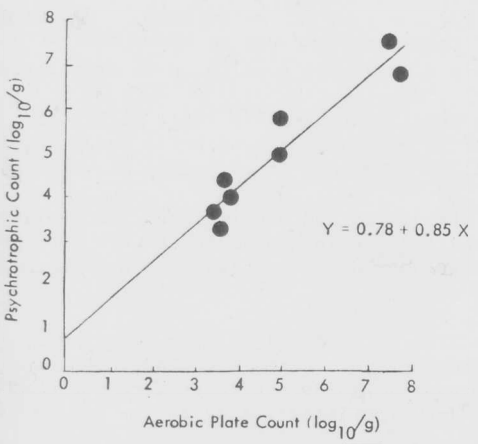

Figure 1. *The linear regression between the Aerobic Plate Count and Psychrotrophic Bacterial Count of ground beef from electrically stimulated carcasses.*

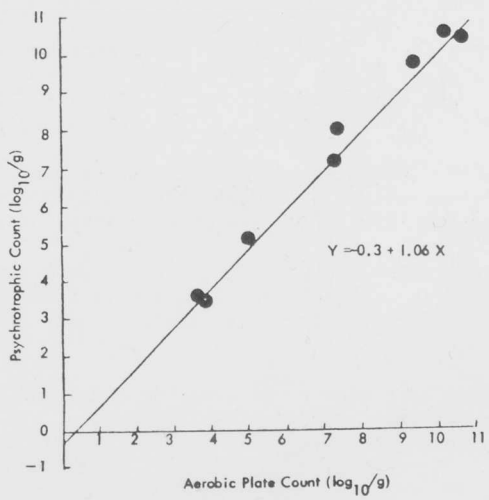

Figure 2. *The linear regression between the Aerobic Plate Count and Psychrotrophic Bacterial Count of ground beef from non-stimulated carcasses.*

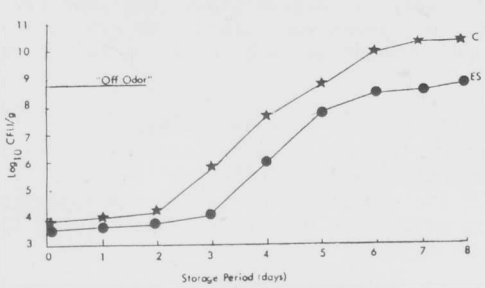

Figure 3. *The growth of the endogenous flora of ground beef from electrically stimulated carcasses stored at 5 C. (C = control; ES = electrically stimulated. CFU = colony forming unit).*