A COMPARATIVE STUDY OF THE MICROBIOLOGY OF COMMERCIAL VACUUM PACKAGED AND HANGING BEEF

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INTRODUCTION

During 1977, the commonplace practice of vacuum packaging of fresh beef was subjected to questions concerning its microbiological safety. These questions were posed in the United States from both consumer groups and from scientists. Research scientists expressed concern about the ability of Yersinia enterocolitica and its growth at refrigeration temperatures (13) while consumer groups were of the opinion that the practice of vacuum packaging encourages high bacterial levels and that the cause of outbreaks of human salmonellosis from cooked beef (1) was due to vacuum packaging of the fresh meat.

Vacuum packaging of raw or cooked meat, poultry and fish has been practiced commercially in the United States since post World War II. During the past 10 years, its application to raw beef has been greatly expanded and represents a modernization of beef distribution having economic advantages (6).

Vacuum packaged raw beef is commonly referred to as boxed beef because it is shipped in boxes as compared to whole or halves of hanging carcasses. It has gained in popularity because it protects against environmental contamination, prevents weight loss and extends shelf life.

The bacterial flora on meat and poultry derived from recently slaughtered domesticated animals is always predominantly mesophilic. Low temperature storage of beef exposed to oxygen, favors the pseudomonads. Evacuation of the atmosphere from the package wrapped in impermeable film enhances shelf life. This effect has been attributed to retention of CO_2 formed by respiration of either the food or the micro-organisms or both or to the exclusion of oxygen (7). Sutherland et al (23) observed that for pseudomonads, the rate of multiplication decreased and the lag phase increased with increasing levels of CO_2. The lactobacilli were similarly affected but to a lesser extent. Empey and Scott (8) demonstrated that the micro-organisms primarily responsible for the spoilage of refrigerated raw beef belonged principally to the genera Pseudomonas and Achromobacter. Sutherland et al (24) observed that during extended storage of vacuum packaged beef, lactic acid bacteria increased profusely while aerobic spoilage organisms increased slowly. Similarly, Pierson et al (22) found that 90-95 percent of the total bacterial count in vacuum packaged beef stored for 15 days at 3.3°C were lactobacilli. These authors also noted that although extremely high levels of lactobacilli developed during storage at 3.3°C for 15 days, the sensory scores remained almost as high as fresh controls.

Most of the work associated with vacuum packaging of beef has pertained to extending shelf life by controlling pseudomonads. During the course of such work, the selective growth of lactic acid types of bacteria has been observed. Most of the scientific work on vacuum packaging did not address the growth of salmonellae since experimental designs controlled storage temperatures below those which permit the growth of salmonellae. In commerce, beef may be abused at various temperatures for various times. Hoke et al (16) discusses the many steps encountered during refrigerated distribution of fresh beef - some of which could lead to temperatures which might permit some growth of salmonellae and other mesophiles. Extensive or even moderate temperature abuse for prolonged periods leads to rapid spoilage and economic losses. For this reason, refrigeration during beef distribution is generally well controlled. The role of vacuum packaging versus aerobic packaging during temperature abuses necessary for the growth of salmonellae has been studied by only a few workers. Goepfert (11) found that aerobic or anaerobic packaging had little or no influence on the growth of salmonellae inoculated into ground beef and held at 1, 4.5, 7 and 12.5°C. In ground, salmonellae inoculated, raw beef held at 12.7°C for 7 days, growth of about two logs was observed in both the aerobically and anaerobically packaged beef. The authors concluded that these results are not surprising and are theoretically predictable. Brown (5) inoculated ground beef with Salmonella newport, packaged it in containers which excluded or exposed the product to oxygen and held these at temperatures of 34, 42 and 58°F. S. newport did not grow in either packaging treatment at 34 and 42°F. At 58°F, growth was evident in both packaging variables after 2 days. The product at this time was organoleptically unacceptable.

The Microbiology Laboratory, FSQS, Science, USDA, was one of the laboratories which in 1977 investigated outbreaks of salmonellosis caused by roast beef and observed that the raw beef used in the incriminated outbreaks was not vacuum packaged but that the cooked product was. We determined the cause of the outbreaks was due to heating the raw beef to temperatures insufficient to kill salmonellae. Based upon our investigation, an emergency rule was passed which required roast beef processors to cook the product to an internal temperature of 145°F (2). Subsequently, Goodfellow et al (12) and Blankenship (4) researched parameters for the thermal destruction of salmonellae in beef. Their work resulted in another change in the requirements for roast beef which recognizes the time and temperature relationship of thermal death of salmonellae in beef (3).

In regard to concerns for vacuum packaged meat and Y. enterocolitica, Hanna et al (13) found Y. enterocolitica like micro-organisms in vacuum packaged beef and lamb and questioned whether their isolates are pathogenic to humans and whether or not vacuum packaging encourages the development of this organism in meat. Y. enterocolitica is psychrotropic thus may proliferate in properly refrigerated meat. In a later report, Hanna et al (14) inoculated beef steaks with Y. enterocolitica and stored these in vacuum packages and in polyvinylchloride (oxygen permeable) containers for 21-35 days. Y. enterocolitica and total aerobic counts were consistently higher in the more oxygen permeable film than in vacuum packages.

Foster et al (10) reviewed the safety of vacuum packaged fresh beef and concluded that on the basis of existing information, vacuum packaging of fresh meat does not result in a decrease in safety.

Although inoculation studies cited here have addressed the relationship of safety and vacuum packaging based upon laboratory trials, no work appears to have been done to relate these factors to product in commerce. The purpose of this study was to survey and compare vacuum packaged and hanging beef in commerce to determine whether a public health and or wholesomeness problem exists.

METHODS AND MATERIALS

Source of beef: Non-frozen, vacuum packaged beef knuckles or knuckles from hanging beef were selected and purchased from central commissaries of three large chain stores. One of these was located in the eastern, one in the southeastern and the other in the western part of the United States.

Samples: Samples were obtained only when both vacuum packaged and hanging beef knuckles were available. When sampled, 10 intact knuckles in vacuum packages and 10 whole knuckles from hanging beef were obtained from each sampling location. Samples were randomly selected by statistical design as follows: Each FSQS inspector involved in sample selection determined an inventory estimate of hind quarters and vacuum packaged knuckles to be processed on the day of collection. Computer generated random numbers corresponding to the estimated inventories were given to the inspector for each week of the study. Ten hanging hind quarters were selected from the on hand inventory using the random numbers provided. These were sampled by plant personnel during the routine cutting and trimming operation. Each sample consisted of a 2 - 2 1/2 pound of tissue portion excised from the knuckle of the hind quarter. After cutting, the sample was placed in a plastic bag and labeled. Ten boxes of vacuum packaged knuckles were selected by using a consistent counting procedure for locating the palletized cartons and the provided random numbers. The cartons were opened and one intact vacuum packaged knuckle from each box was selected and identified.

The unfrozen samples were promptly delivered to the laboratories and analyzed on that day or refrigerated and analyzed the following morning. The samples were analyzed at FSQS laboratories near the point of collection. Initial sampling plans called for equal numbers of samples to be taken at each sampling point, however, limited usage of vacuum packaged beef at the Western sampling point during this study resulted in only one set of 10 paired samples. The other two laboratories extended the weeks of their involvement to bring the total number of samples to the targeted 300 (150 pairs).

Laboratory methods: Sample preparation for all determinations and methods for APC, coliforms, Escherichia coli and salmonellae have been described (25). In all determinations made during this study, plates for aerobic plate counts were incubated at 20°C for 4 days (APC 20), three tube MPNs were conducted for both the coliform group and E. coli, and a 45 gram sample portion (remaining contents in blender) was used for salmonellae rather than the described 25g.

Lactic acid bacteria (lactics), were enumerated by pour plates as described. However, each plate was capped with approximately 4 cc of APT agar to promote growth. After incubation, all colonies were counted. A plate showing well isolated colonies was selected from each sample and 10-12 colonies were picked with a pasteur pipette or toothpick which was then dropped into a small tube of hydrogen peroxide. The percentage factor for catalase negative colonies/colonies tested determined by this procedure was then multiplied by the total counts obtained to arrive at the lactic acid bacteria count/g.

Enterobacteriaceae and gram negative non-fermenter counts, were determined by the method of Mossel (20) with minor modifications. Aliquots from each sample dilution were spread onto duplicate agar plates containing MacConkey agar with 1 percent glucose and 0.5 percent agar added. One set of plates was incubated at 35°C for 48 hours for enterobacteriaceae counts and the duplicate set at 20°C for gram negative non-fermenter counts. Following incubation, the 35°C plates were examined. Colonies that were brick red by reflected light, with or without a surrounding zone of precipitated bile were counted. A plate showing well isolated colonies was selected from each sample and 10-12 colonies of those counted were selected and transferred to Triple Sugar Iron Agar Slants (TSI). Isolates showing fermentation were then tested for their oxidase reaction using the API system. Colonies selected because of their appearance on MacConkey agar and which fermented glucose in TSI and which were oxidase negative were considered to be enterics. The percentage factor for colonies confirmed/colonies picked was multiplied by the counts determined by visual observation. The result was reported as the enterobacteriaceae per gram.

Following incubation, the 20°C plates were examined. Transluscent-to-pink colonies were counted and the number of each colonial type recorded. Representative colonies of each colonial type (usually 10-12 per plate counted) were tested for glucose fermentation and oxidase reactions. Colonial types which did not ferment glucose and were oxidase positive were considered to be members of the Pseudomonas/Acinetobacter/Moraxella (PAM) group of bacteria. Previous workers have referred to this group of bacteria as the Pseudomonas/Achromobacter group. The reported counts were adjusted using the same procedure as for lactics and enterics.

Yersinia enterocolitica strains were isolated by adding 4 ml (0.4g) of the homogenate which had been frozen for 7 days at -20°C to 100 ml of a modified selenite broth made up by combining filter sterilized solutions as follows: 2.5g/l sodium selenite, 20 mg/l malachite green and 10 mg/l carbicillin into pH 7.5, 1% phosphate buffer and also to an alkaline, sodium tripolyphosphate cold enrichment broth (17). The selenite enrichment broth was streaked after 48 and 72 hours incubation at 25°C. The cold enrichment broth was streaked after 7 and 14 days incubation at 4°C. All enrichments were streaked onto plates of bismuth sulfite agar (which were prepared and refrigerated for 3 days prior to use) (15) and DNAse agar with sorbitol and tween 80 added (18). Characteristic colonies were selected and transferred to TSI agar slants and motility agar (26) then identified by the API-20 system (17). Additional biochemical reactions of Y. enterocolitica were tested according to the methods of Nilehn (21) and Wauters (26).

At least one Y. enterocolitica isolate from each positive sample was tested for HeLa cell invasiveness (19).

RESULTS

There were great variations in the levels of bacteria found between vacuum packaged and hanging beef, in results

obtained from week to week and between the three widely separated U.S. sampling points. Fields (9) reported on similar variations. Generally, of all of the tests conducted in this study, the APC 20/g appears to reflect the length of time that beef had been under refrigeration since slaughter and or temperature abuses to which it may have been exposed or both. Unfortunately, the APC 20/g will not differentiate between these. Table 1 shows the variations encountered in this study. Vacuum packaged beef had higher APC 20/g counts than did hanging beef. This difference ranged from 1-3 logs in magnitude. The highest levels appeared in vacuum packaged beef sampled in the East. It is unfortunate that samples from the West were only available for one of the sampling weeks since there appears to be a consistent microbiological difference in vacuum packaged beef between the other two sampling points. This difference is not evident when the results from hanging beef from the same two points are compared. All 300 samples collected were examined organoleptically prior to analysis and all had a normal odor and appearance. This observation appears to be consistent with the purported advantage of vacuum packaging in extending shelf life since high aerobic plate counts were frequently observed.

Another significant set of results from this study concerns the levels of lactics and PAM group found in the two sets of samples. These results are shown in Figure 1. From this it is apparent that lactics constitute the majority of the APC 20/g as presented in Table 1. This is true of both vacuum packaged and hanging beef. Since lactics are notoriously psychrotropic, it is possible that the high levels present in the vacuum packaged beef occurred as a result of extended refrigeration. The PAM group appears to have slightly increased levels in the vacuum packaged as compared with hanging beef. When one compares ratios, however, the classical selection of lactics and repression of PAM members appears to have occurred consistently in vacuum packaged beef.

The Enterobacteriaceae group of bacteria includes a large number of gram negative rods some of which are psychrotropic and others which are mesophilic. Salmonellae and E. coli for example are mesophilic and do not grow in properly refrigerated meat. Y. enterocolitica and some members of the coliform group are psychrotropic. Because some of these particularly the salmonellae are important human pathogens, total enterobacteriaceae counts were conducted to determine comparative growth in the two variables. Figure 2 shows that total enterobacteriaceae counts in vacuum packaged meat in commerce were generally between 1-2 logs higher than those obtained from hanging beef in commerce. The results for the coliform MPN/g were similar as shown in Table 2.

Overall, the coliform MPN for vacuum packaged meat was about 2 logs higher than that found in the hanging beef.

Of all of the determinations made, those for salmonellae and E. coli relate most directly to the issue of safety. Both of these are mesophilic and if present in large numbers would indicate growth at temperatures above those used in commercial refrigeration. Table 2 shows that there is no perceptible difference for the E. coli MPNs of the two variables. This appears to be significant since the other five determinations listed, each of which contain psychrotropic members, all show clear evidence of higher levels for vacuum packaged product as opposed to hanging beef. The results for salmonellae are similar to those for E. coli. Although the sample size used in this study was nearly twice that normally used by these laboratories (25g), only one of the 300 samples contained salmonellae. This sample contained Group E Salmonella and was obtained from one of the 150 vacuum packaged samples. All 150 hanging beef samples were negative. Overall, there was no evidence of any difference between the variables and the incidence of salmonellae was lower than anticipated.

Typical Y. enterocolitica and/or enterocolitica like strains were isolated from 70 of the 300 samples tested. Vacuum packaged beef samples showed 60 positive samples out of 150 and the hanging beef showed 4 positive samples out of 150. The majority of the positive samples yielded only atypical Y. enterocolitica (64) while 6 samples were positive for both the atypicals and biotype I.

At the Eastern sampling point, all isolates were atypical, rhamnose positive Y. enterocolitica and all were obtained from the vacuum packaged beef as shown in Table 3. Samples collected at the Southeastern sampling point showed both the atypical and the rhamnose negative biotype I Y. enterocolitica from both vacuum packaged and hanging beef. These data suggest that the types of Y. enterocolitica found in beef may be dependent upon the source or geographical origin of the carcass.

The types of Y. enterocolitica found in this study have not been known to cause food-borne infections or intoxications in the U.S. Little is known about the environmental strains of Y. enterocolitica isolated in this country, therefore, we felt it important to subject this diverse group of isolates to HeLa cell invasiveness. At least one culture of each Y. enterocolitica type isolated from each positive sample was examined for HeLa cell invasiveness. All isolates were negative for HeLa cell invasiveness except two. The results from these two positive cultures were unexpected and surprising. Both isolates belong to the rhamnose positive atypical biotype and this is the first report of HeLa cell invasiveness for this group of Y. enterocolitica. Both isolates were strongly invasive and further invasive studies using other test systems are being planned. One of these invasive isolates was obtained from vacuum packaged beef collected at the Eastern sampling point and the other was obtained from hanging beef collected at the Southeastern sampling point.

DISCUSSION

This study was undertaken to determine whether there were differences in the safety of raw commercial vacuum packaged beef as compared with unpackaged hanging beef particularly in regard to their salmonellae content. Only one of the total of 300 samples was positive for salmonellae indicating not only that there is no evidence of any difference between the salmonellae content of vacuum packaged and hanging beef but that the salmonellae content for both variables was lower than expected. In addition, no evidence of any difference was observed when the Y. enterocolitica strains invasive to HeLa cells were compared for the two variables. The odor and appearance for all 150 pairs of samples tested was normal thus no evidence of a difference was observed for this determination. For microbiological determinations related to microbial quality but not safety, vacuum packaged beef contained on the average much higher levels of APC 20/g and lactic acid bacteria. Total enterobacteriaceae counts, coliform MPNs and the PAM group were about one log higher in the vacuum packaged beef variable. E. coli levels were similar for both variables. Y. enterocolitica isolates which were not invasive to HeLa cells showed a higher percentile incidence in the vacuum packaged variable.

There was no indication that either variable had been subjected to abuse temperatures since E. coli and salmonellae which are mesophilic remained at low levels in both variables. Overall, these results show no difference as regards food safety between the two variables tested. At the same time, these results have shown the presence of low levels of pathogens in both variables indicating again that proper handling and cooking procedures in the food service industry and in the home are essential steps in the prevention of food-borne illnesses and intoxications from meat including beef.

The commercial vacuum packaged beef tested in this study generally had higher levels for bacterial groups with known psychrotrophic members than did the hanging beef. These findings appear to indicate that the vacuum packaged beef was older than the hanging beef and that the microbial elevations observed were caused by the growth of psychrotropic bacteria. There was no relationship observed between elevated levels of bacteria and the presence of pathogens.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Ronald E. Engel, Science; Merlin Nelson, Technical Services; and Bert Levy, Statistical Services Staff, FSQS for their advice, counsel and assistance in planning this study. We also wish to acknowledge the efforts of George Krumm, Athens Interdisciplinary Laboratory; Richard Jacobs, San Francisco Interdisciplinary Laboratory and Stanley Green, Science, Washington, DC, for their coordination and supervision of laboratory activities. Additionally, we acknowledge the competent, professional analytical services rendered by Joseph Macaluso, Martha Workman, Joseph Chiu, Carl Custer, Carol Vincent, Dennis McClain and James Eye, Beltsville, FSQS, Paul Uhler, Richard Cook and Ruth Simmons, San Francisco, FSQS and Larry Dillard and Jane Evans, Athens, FSQS.

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TABLE 1. Variations in APC 20/g in commercial vacuum-packaged and hanging beef as influenced by sampling location and week sampled - APC 20/g x 10^3

Week	Vacuum Packaged Beef			Hanging Beef		
	Sampling Location			Sampling Location		
	Eastern	Southeastern	Western	Eastern	Southeastern	Western
1 Gm	13,700	612	4	406	< 1	14.4
Hi	60,000	18,000	29	250,000	44	110
Lo	4,500	3.4	1	12	< 1	5.3
2 Gm	32,000	1,240		66	9.2	
Hi	130,000	22,000	NT	42,000	69	NT
Lo	3,500	< 1		< 1	< 1	
3 Gm	13,000	18.6		255	11	
Hi	80,000	600	NT	2,700	1,800	NT
Lo	770	< 1		30	< 1	
4 Gm	4,190	3,770		1.7	4.7	
Hi	38,000	14,000	NT	290	120	NT
Lo	220	500		< 1	< 1	
5 Gm	240	< 1		22.8	< 1	
Hi	27,000	27	NT	54	2.8	NT
Lo	15	< 1		13	< 1	
6 GM	1,390	3.7		1.1	< 1	
Hi	61,000	77	NT	12	3.6	NT
Lo	190	< 1		< 1	< 1	
7 Gm	60.2			< 1		
Hi	1,700	NT	NT	1	NT	NT
Lo	7.4			< 1		
8 Gm	4,800			21.7		
Hi	18,000	NT	NT	110	NT	NT
Lo	480			2.1		

NT = None Tested

Gm = Geometric mean

Hi = Highest level found

Lo = Lowest level found

TABLE 2. Geometric means of counts per g or MPN's/g of all samples of vacuum packaged and hanging beef

Determination	Vacuum packaged beef	Hanging beef
APC 20/g	5,000,000	54,000
lactics/g	3,400,000	8,200
enterobacteriaceae	62,000	6,200
PAM group/g	12,000	1,000
coliform MPN/g	6,700	48
E. coli MPN/g	34	30

TABLE 3. Isolation of Yersinia enterocolitica from 150 samples each of vacuum packaged and hanging beef

Sampling point	Samples	+ samples	% +	Samples + for atypical rhamnose positive strains only	Samples + for Y. enterocolitica biotype I only	Samples + for both atypical and biotype I strains
WESTERN						
Vacuum packaged	10	0	0	0	0	0
Hanging	10	0	0	0	0	0
EASTERN						
Vacuum packaged	80	48	60	48	0	0
Hanging	80	0	0	0	0	0
SOUTHEASTERN						
Vacuum packaged	60	18	30	13	0	5
Hanging	60	4	6.6	3	0	1
TOTALS	300	70	23	64	0	6

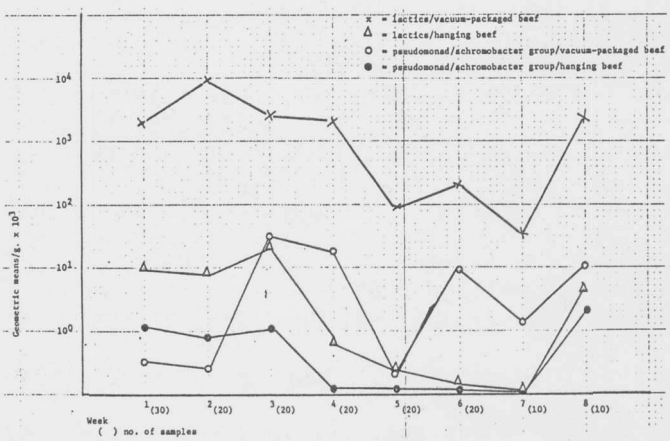

FIG. 1. Geometric means/g. of lactic acid bacteria and PAM group in vacuum packaged and hanging beef by week

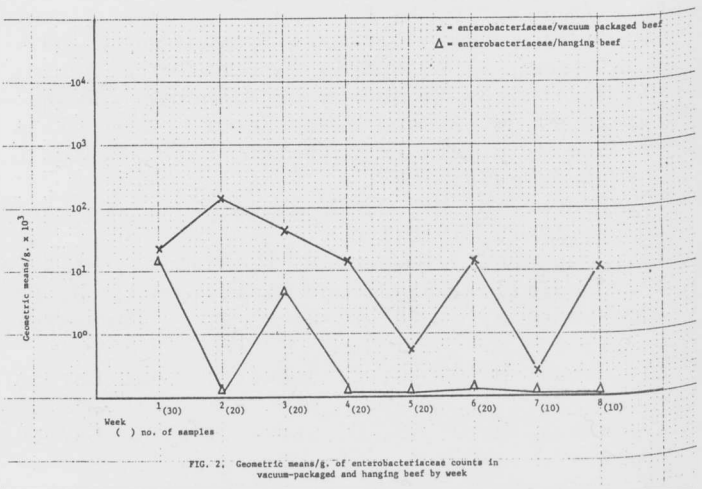

FIG. 2. Geometric means/g. of enterobacteriaceae counts in vacuum-packaged and hanging beef by week