GROWTH OF *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* ON HIGH PH BEEF PACKAGED UNDER VACUUM OR CARBON DIOXIDE

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SUMMARY: Both *Salmonella typhimurium* and *Escherichia coli* grew on vacuum packaged high-pH beef at all temperatures between 8 and 30°C. Increases in numbers before spoilage developed ranged, for *S. typhimurium*, from 1.1 log cycles at 8°C to about 5 log cycles at temperatures above 15°C, and for *E. coli*, from 2.8 log cycles at 8°C to 5 log cycles at 15°C and higher temperatures. When high pH beef was packaged under CO_2, the minimum temperatures for growth of *S. typhimurium* and *E. coli* were respectively 12 and 10°C. Increases in numbers before spoilage developed ranged, for *S. typhimurium*, from 1.4 log cycles at 12°C to about 4 log cycles at 20 and 30°C, and for *E. coli*, from 2.1 log cycles at 10°C to about 5 log cycles at 30°C.

INTRODUCTION: As enteropathogens may be transferred to meat surfaces during carcass dressing, their possible presence on all fresh meat must be assumed for public health purposes. The meat borne pathogens of greatest concern, *Salmonella* and enteropathogenic *Escherchia coli*, will usually be initially present on meat only in very small numbers. The risks associated with such unvoidable contamination must be considered to be acceptable, provided that the pathogens have little or no opportunity to proliferate during storage and handling of product. Proliferation of mesophilic pathogens is generally prevented by storage of meat at temperatures below 7°C. However, during distribution of meat, product temperatures at some times may enter the range permitting growth of mesophiles.

The risks arising from such temperature abuse are contained, to some extent, by rapid growth of the spoilage flora at abusive temperatures, which will result in relatively early spoilage and rejection of the product. However, the growth of a spoilage flora can be retarded by preservative packaging. If the growth of pathogens is relatively advantaged by the in-pack condition, the pathogens may grow to very high numbers before spoilage becomes evident. No incident of food poisoning clearly arising from risk of that type has been identified for raw meat in the most common form of preservative packaging, vacuum packaging. However, in recent years the storage life of chilled meat has been greatly increased by packaging product under oxygen-free CO_2 maintained at atmosphere pressure after the meat has been saturated with the

gas (Gill and Penney, 1988). This system of CO_2 packaging is coming into increasing commercial use (Gill, 1989). The lengthened storage life attainable with that type of packaging might allow significant hazards from mesophilic enteric pathogens to develop during storage at abusive temperatures.

The growth of Salmonella typhimurium and E. coli on meat packaged under vacuum or under CO_2 was therefore compared, to better identify any augmented risk to health from enteric pathogens that might arise from exposure of CO_2 packaged meat to abusive temperatures. Studies were confined to high-pH beef, to ensure that the tissue pH was sufficiently high to allow uninhibited growth of the test organisms under anoxic conditions (Grau, 1981).

MATERIALS AND METHODS: High-pH (>6.0) beef striploins were obtained from a local meat plant. Fat tissue was trimmed from the meat, then it was divided into steaks 100 x 100 x 50 mm weighing between 100 and 150 g each.

Each steak was either uninoculated or inoculated on one surface with 0.1 ml of a stationary phase culture of one of the test organisms, both of which had originally been isolated from meat plant environments, diluted to a cell concentration of approximately 10^5 cells/ml. Each steak was packaged in an evacuated pouch, composed of polyvinylidine chloride laminate of low gas permeability (Cryovac, W.R. Grace, Porirua, New Zealand) for samples held under vacuum, or of polyethylene of high gas permeability (4000 cc $O_2/m^2/24h/atm$) for samples held under CO_2. Within 30 min of evacuation, those latter packs were further packaged, in groups of six, in gas impermeable aluminium foil laminate pouches (Captech, Printpac-UEB, Auckland, New Zealand) filled, after evacuation, with 2 l of CO_2.

Samples were stored at 8, 10, 12, 15, 20 and 30°C, with temperatures maintained within $\pm$ 0.2°C of the set temperature. When growth of either test organism did not occur at a particular temperature, a further set of samples were prepared and incubated at a temperature 1°C higher than the non-permissive temperature.

Duplicate samples from each sample series (inoculum/no inoculum-storage temperature-packaging type) were examined at zero time and at subsequent times that were chosen to take account of differences in growth rates at the different storage temperatures. When packages were opened, the odour of the meat was assessed. Strong, persistant putrid odours were presumed to indicate gross spoilage.

After pack opening, each meat sample was vigorously massaged with 50 ml of 0.1 % peptone water. The rinse fluid was serially diluted, and 0.1 ml portions of suitable dilutions were spread on duplicate plates of Plate Count Agar, PCA (Difco) for all meat samples; Xylose Lysine Desoxycholate Agar, XLD-Agar (Difco) when the meat had been inoculated with S. typhimurium, or not inoculated; or violet Red Bile Agar, VRB-Agar (Difco), when the meat had bee inoculated with E. coli, or not inoculated. PCA plates were incubated at 25°C for 48 h; XLD and VRB plates were incubated at 37°C for 24 h.
The compositions of the natural floras were assessed from PCA plates bearing at least 100 colonies that had been derived from uninoculated meat samples. Numbers of each distinctive colony type were estimated. At each estimation, three representative colonies of each type were picked from each plate and identified to the generic level by the criteria of Cowan (1974). Numbers of

the spoilage flora were determined from similar PCA plates bearing 20 to 100 colonies.

Pathogen numbers were determined from counts on selective agar plates. At each enumeration, the appearances of colonies of the natural flora on the same selective medium were compared with the appearance of the test species colonies on the enumeration plates. This was done to avoid inadvertant counting of elements of the natural flora that give colonies somewhat similar to the test species on the selective agar.

At each count, two representative, presumptive colonies were picked from each plate and the identities of the isolates confirmed by further tests. Black, entire colonies on pink XLD-Agar were presumed to by S. typhimurium. Colonies on VRB-Agar that were entire and smooth on crimson agar surrounded by a zone of precipitated bile were presumed to be E. coli.

RESULTS: Initial natural floras, at numbers about 10^5 bacteria/sample, were dominated by entrococci, with substantial fractions of acinetobacteria and moraxellae. The spoilage floras that developed in all vacuum packaged samples were composed of lactobacilli and enterobacteria in roughly equal proportions at lower temperatures, but with enterobacteria greatly predominating at 15°C and higher temperatures. Vacuum packaged samples spoiled as maximum numbers in excess of 1×10^9 bacteria/sample were attained.

The spoilage floras of CO_2 packaged samples were greatly dominated by lactobacilli at lower temperatures, but at 15°C and above enterobacteria formed the major fraction of the floras. Spoilage of CO_2 packaged samples at lower temperatures did not occur until maximum flora numbers had presisted for significant periods, but at higher temperatures spoilage became apparent as maximum numbers were attained.

The storage life of CO_2 packaged samples was about twice that of vacuum packaged samples at the lower temperatures, but little more than that of the vacuum packaged samples at 20 and 30°C.

Table 1. The effect of storage temperature on the storage life of vacuum and CO_2 packaged samples of high-pH beef stored at abusive temperatures.

Temperature (°C)	Storage life (days)	
	Vacuum pack	CO_2 pack
8	6	14
10	5	12
12	4.3	9
15	3.7	5.8
20	1.4	1.8
30	1.2	1.4

In vacuum packs, S. typhimurium grew at 8° only after a lag of about 4 days, at a rate substantially less than that of the spoilage flora. With increasing temperature the lag decreased, to about 2 days at 10 and 12°C and a few hours at 15 and 20°C, and the growth rate increased relative to that of the spoilage flora. At 15°C and higher temperatures, the growth rate of S. typhimurium was comparable with that of the spoilage flora. The reducing lag time and increasing relative rate of growth with increasing temperature resulted in greater increases of S. typhimurium numbers as the temperature increased. At 15°C and higher temperatures, S. typhimurium remained a major fraction of the total flora at all times, with maximum increases in numbers of about 5 log cycles (Table 2).

In vacuum packs, E. coli behaved similarly to S. typhimurium, but with substantially shorter lag periods, 2 days at 8°C and < 1 day at 10°C, and somewhat faster growth at lower temperatures. Consequently, increases in E. coli numbers were substantially greater than those of S. typhimurium at lower temperatures, and somewhat greater at higher temperatures (Table 2).

Table 2. The effect of storage temperature on the maximum increases in numbers of S. typhimurium and E. coli on vacuum-packaged, high-pH beef.

Temperature (°C)	Log increase	
	S. typhimurium	E. coli
8	1.1	2.8
10	2.1	4.0
12	2.8	4.3
15	4.6	5.6
20	4.7	5.0
30	5.1	4.9

In CO_2 packs, S. typhimurium did not grow at 11°C or lower temperatures. Growth at 12°C was characterized by a long lag, about 5 days, and relatively slow growth. A significant lag, of about 1 day, persisted at 15°C, but at that temperature the growth rate was comparable with that of the spoilage flora. At higher temperatures, S. typhimurium outgrew the spoilage flora to become the predominant organism in inoculated samples. In such samples, spoilage developed as S. typhimurium approached numbers of 10^9 bacteria/sample, substantially before spoilage developed in uninoculated samples. Consequently, the maximum increases in S. typhimurium numbers at higher temperatures were somewhat less than the increases in similar vacuum-packaged samples (Table 3).

In CO_2 packs, E. coli did not grow at 9°C. Growth at 10 and 12°C was characterized by a substantial lag, about 3 days, and relatively slow growth. The behaviour of E. coli at higher temperatures was very similar to that of S. typhimurium. Consequently, at 12°C the maximum increase in E. coli numbers was substantially greater than the increase in S. typhimurium numbers, but increases in numbers at higher temperatures were similar for the two species (Table 3).

Table 3. The effect of storage temperature on the maximum increases in numbers of S. typhimurium and E. coli on CO_2 packaged, high-pH beef.

Temperature (°C)	Log increase	
	S. typhimurium	E. coli
8	-	-
9	ND	-
10	-	2.1
11	-	ND
12	1.4	2.3
15	3.8	4.4
20	4.3	4.5
30	4.4	4.9

- No growth ND No data

DISCUSSION: It is obviously desirable that preservative packagings for raw meat should restrict the possibilities for pathogens to grow to high numbers before the product in spoiled, not only at optimal chiller storage temperatures but also during limited periods of temperature abuse likely to be encountered during retail distribution. Restriction of pathogen proliferation at abusive temperatures would be obtained when, irrespective of variation in product composition, the in-pack conditions either prevent pathogen growth, or impose on pathogens a lag phase of substantial duration relative to the storage life, a growth rate slower than that of the spoilage flora, and inhibition of pathogen growth at an early stage of the spoilage flora's approach to maximum numbers (Gill and Reichel, 1989).

Inhibition of growth of S. typhimurium and E. coli by the spoilage flora was not significant before spoilage was apparent, with either vacuum- or CO_2-packs and, in vacuum packs, the growth rates of the pathogens were similar to those of the spoilage flora at 10°C and higher temperatures. Therefore, with vacuum packages, the lag before growth commences at abusive temperatures is the major restriction on proliferation of S. typhimurium and E. coli. From the maximum increases in numbers it is apparent that temperatures up to 12°C may be tolerable for perhaps two days with respect to growth of salmonellae in vacuum packaged meat, but that temperatures much in excess of 8°C would not be tolerable for any significant time with respect to proliferation of E. coli.

In contrast, with CO_2 packaged meat, the growth rates of the pathogens were similar to those of the spoilage flora only at 15°C and higher temperatures, with significant lags still apparent for both species at 15°C. Therefore, relatively long periods at temperatures as high as 15°C may be tolerable, and temperatures up to 11°C would seem to be completely safe, with respect to Salmonella proliferation. Similarly, CO_2 packaged product could well tolerate some two days at 12°C, and would seem safe at 9°C, with respect to E. coli proliferation.

Overall, it appears that growth of Salmonella and E. coli will be better controlled in CO_2-packaged meat than in similar vacuum packaged product at abusive temperatures up to 15°C. However, the safety advantage for CO_2 packaged product is largely lost at higher temperature, and with the rapid, advantaged proliferation of enterobacteria at those temperatures (Gill and Newton, 1980) meat in either type of packaging should not experience temperatures above 15°C for even brief periods.

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