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Growth of *Escherichia coli* and *Salmonella typhimurium* on high-pH beef packed under vacuum or carbon dioxide

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A strain each of *Escherichia coli* and *Salmonella typhimurium*, were inoculated onto samples of high-pH (> 6.0) beef. Samples were packaged under vacuum or CO_2 and stored at 8, 10, 12, 15, 20 or 30 °C. In vacuum packs, *E. coli* and *S. typhimurium* grew at all storage temperatures. At temperatures between 8 and 12 °C inclusive, both organisms grew at rates less or no more than those of the spoilage flora after significant lag periods. At temperatures of 15 °C or above, growth rates were equal to or greater than those of the spoilage flora, and lag periods were insignificant. In CO_2 packs, neither organism grew at 8 °C, and *S. typhimurium* did not grow at 10 °C. Subsequent tests showed that *E. coli* did not grow at 9 °C, nor *S. typhimurium* at 11 °C. At 12 °C, both organisms grew, after prolonged lags, at rates markedly slower than that of the spoilage flora. At 15 °C, their growth rates were similar to that of the spoilage flora. At higher temperatures, both organisms grew without significant lags at rates greater than that of the spoilage flora.

Key words: *Escherichia coli*; *Salmonella typhimurium*; Beef, high-pH; Vacuum pack; CO_2 pack; Temperature abuse

Introduction

As enteropathogens may be transferred to meat surfaces during carcass dressing, their possible presence on all fresh meat must be assumed. The meat-borne pathogens of greatest concern *Salmonella* and enteropathogenic *Escherichia coli*, will usually be initially present on meat only in very small numbers (Grau, 1986). The risks associated with the unavoidable contamination of meat must be considered to be acceptable, provided that potent enteric pathogens have little or no opportunity to proliferate during storage and handling of the product. The proliferation of mesophilic pathogens is prevented by storage of meat at chiller temperatures (< 5 °C). However, during distribution of meat, product temperatures at some times may enter the range permitting growth of mesophiles.

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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 advantaged relative to growth of the spoilage flora by the in-pack condition, the pathogens may grow to very high numbers before spoilage becomes evident (Hintlian and Hotchkiss, 1986). Despite the legitimate concerns regarding relatively advantagous growth of pathogens on meat in preservative packaging (Genigeorgis, 1985), vacuum-packaged meat has been widely traded for many years, and is considered safe for extending the storage life of chilled meats. In recent years, however, the storage life of chilled meat has been greatly increased by packaging product under oxygen-free CO_2 maintained at atmospheric 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 *E. coli* and *Salmonella typhimurium* on beef packaged under vacuum or under CO_2 was therefore compared to better identify any augmented risk to health from mesophilic enteric pathogens that might arise from exposure of meat packaged under CO_2, rather than vacuum, 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 anaerobic conditions (Grau, 1981), and so to discern the greatest potential risks posed by growth of mesophilic enteric pathogens in the preservative packagings.

Materials and Methods

Organisms

The bacteria used were *E. coli*, strain E.10, and *S. typhimurium*, strain S4092. Both strains were originally isolated from offals. Previous studies had shown them to have growth characteristics closely similar to those of other wild-type strains isolated from various meat plant environments (Lowry et al., 1989).

Preparation of inocula

Shaken cultures of each organism were cultivated in Brain Heart Infusion (Difco) at 30 °C. Cultures were grown overnight to the stationary phase. Serial ten-fold dilutions of 1-ml samples from each culture were prepared, and 0.1-ml aliquots of suitable dilutions (10^4, 10^5, 10^6) were spread on duplicate plates of plate count agar (Difco). Plates were incubated for 24 h at 37 °C. During that time, the cultures were stored at 4 °C. The concentration of cells in each culture was ascertained from the plate counts, and cultures were appropriately diluted with sterile 0.1% peptone water to give inocula containing approximately 10^5 cells/ml.

Preparation of meat samples

Beef striploins, from cattle slaughtered 48 h previously, were obtained from a local meat plant. At the plant, the pH values of striploins were determined by direct application of a glass electrode to the muscles, and striploins of high pH (> 6.0) were selected.

The fat was trimmed from the muscle tissue, which was then divided into steaks approximately $100 \times 100 \times 10$ mm, each weighing between 100 and 150 g.

Steaks were either uninoculated or inoculated on one surface with 0.1 ml of one of the diluted cultures, and the inoculum was distributed over the surface using a sterile glass spreader. Each steak was individually packaged in an evacuated pouch measuring 150×150 mm. For samples stored under vacuum, the pouches were composed of polyvinyledene chloride laminate of low gas permeability (Cryovac, W.R. Grace, Porirua, New Zealand). For samples stored under CO_2, the pouches were composed of polyethylene of high gas permeability. Within 30 min of evacuation, these latter packs were further packaged, in groups of eight, in gas-impermeable, aluminum foil laminate pouches (Captech, Printpac-UEB, Auckland, New Zealand), which were first evacuated then filled with CO_2 before sealing.

Storage of samples

Samples were stored in insulated boxes that were held in temperature-controlled rooms operating at 5–10 °C below the required temperature. Each insulated box was fitted with a fan, to circulate air within the box, and a temperature controller connecting to a light fitting that acted as a heating element. The samples were raised above the box floor on racks and did not touch the box walls. Sample temperatures were thus maintained within ± 0.2 °C of the set temperatures. Storage temperatures were 8, 10, 12, 15, 20 or 30 °C. When growth of either test organism did not occur at the lower temperatures, a further set of samples were prepared and incubated at a temperature 1 °C higher than the highest non-permissive temperature that had been observed.

Sampling procedures and microbiological analyses

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 likely differences in growth rates at the different storage temperatures. When packagings for individual samples were opened, the odour of the meat was assessed by a five member panel. Unanimous apprehension of strong, persistent putrid odours was presumed to indicate gross spoilage.

After pack opening, each meat sample was placed in a plastic pouch containing 50 ml of sterile 0.1% peptone water. The pouch and sample were then repeatedly squeezed by hand for 2 min, to vigorously force the rince fluid over the surfaces of the samples. 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; violet red bile agar, VRB-agar (Difco), when the meat had been inoculated with *E. coli*, or not inoculated; or xylose lysine desoxycholate agar, XLD-agar (Difco) when the meat had been inoculated with *S. typhimurium*, 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 inadvertent 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. Entire, smooth colonies on crimson VRB-agar that were surrounded by a zone of precipitated bile were presumed to be *E. coli*. Confirmative reactions were indole +ve, methyl red +ve, Voges-Proskauer −ve. and citrate −ve (ICMSF, 1988a). Black, entire colonies on pink XLD-agar were presumed to be *S. typhimurium*. Confirmative reactions were alkaline slants, acid butts, and blackening of triple sugar iron agar slants, and an alkaline reaction and blackening of lysine iron agar slants (ICMSF, 1988b).

Results

The results presented are the average count for each duplicated determination. The natural flora contaminating samples initially numbered approximately 10^5 bacteria/sample, range 2×10^4 to 3×10^5 bacteria/sample. The approximate composition of the initial floras was micrococci, 50%; acinetobacteria/moraxellae, 40%, pseudomonads, 5% and enterobacteria, 5%.

Spoilage flora numbers were generally similar for inoculated and uninoculated samples that had been packaged and stored identically.

The spoilage floras of vacuum-packaged samples stored at 12 °C and below were composed of lactobacilli and enterobacteria in roughly equal proportion. At higher storage temperatures, the lactobacilli were largely displaced by enterobacteria; lactobacilli forming less than 10% of the spoilage floras of samples stored at 15 and 20 °C, and being undetected in the floras that developed on samples stored at 30 °C (data not included).

The spoilage floras of CO_2-packaged samples were also composed of lactobacilli and enterobacteria, but with lactobacilli predominating at storage temperatures of 12 °C and below. However, the lactobacilli formed less than 20% of the spoilage flora on samples stored at 15 and 20 °C, and were again undetected in the floras that developed on samples stored at 30 °C (data not included).

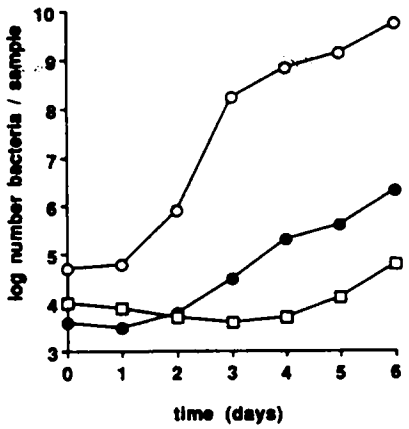

Fig. 1. Growth of bacteria in vacuum packs at 8°C. Counts on PCA, 25°C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

Vacuum packaged samples were invariably spoiled when maximum numbers were attained, the counts per sample then exceeding 1×10^9. Similarly, CO_2-packaged samples stored at 15°C and higher temperatures spoiled as maximum numbers were attained or approached. However, CO_2 packaged samples stored at 12°C and lower temperatures did not spoil until maximum bacterial numbers had persisted for significant periods.

Vacuum packaged samples stored at 8°C were spoiled after 6 days. At that temperature, *E. coli* and *S. typhimurium* grew after lag periods of about 2 and 4 days, respectively, at rates less than that of the spoilage flora, with increases of about 3 and 1 log cycles, respectively, before the samples were spoiled (Fig. 1).

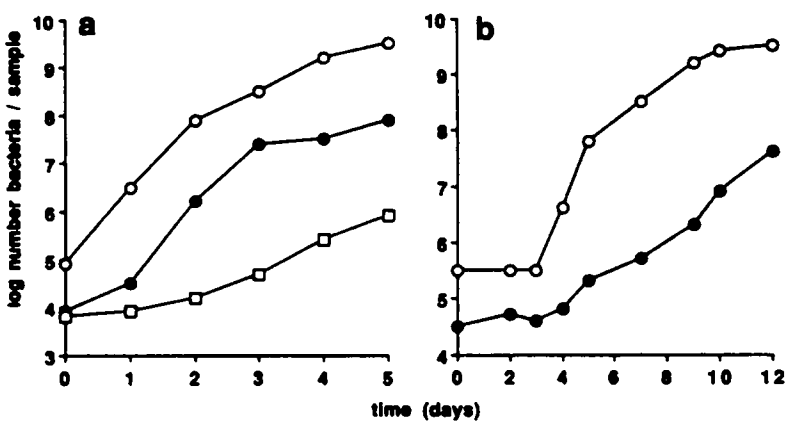

Fig. 2. Growth of bacteria in (a) vacuum and (b) CO_2 packs at 10°C. Counts on PCA, 25°C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

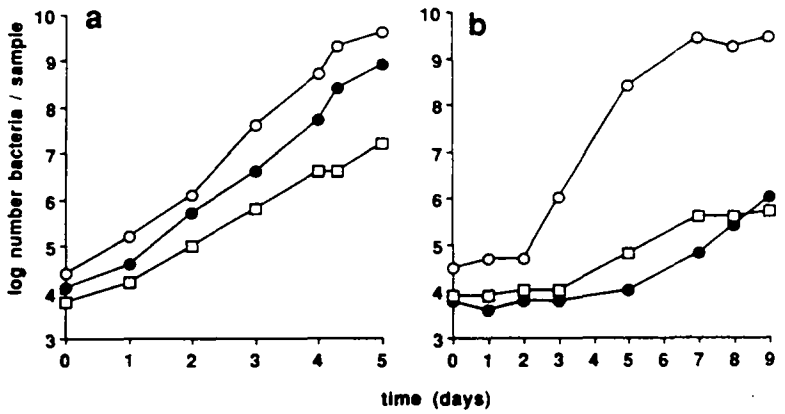

Fig. 3. Growth of bacteria in (a) vacuum and (b) CO_2 packs at 12° C. Counts on PCA, 25° C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

Neither of the test organisms grew in CO_2 packs stored at 8° C before spoilage become evident at 14 days.

In vacuum-packaged samples stored at 10° C spoilage was apparent after 5 days. *E. coli* grew after a lag period of less than 1 day at a rate similar to that of the spoilage flora, the rate of growth declining as the spoilage flora approached maximum numbers. *S. typhimurium* grew after a lag period of about 2 days at a rate less than that of the spoilage flora. Increase in numbers of the test organisms before spoilage became evient were approximately 4 log cycles for *E. coli* and 2 log cycles for *S. typhimurium* (Fig. 2).

In CO_2-packaged samples stored at 10° C, spoilage was evident after 12 days. *E. coli* grew after a lag period of about 4 days at a rate less than that of the spoilage flora, to increase in numbers by about 3 log cycles (Fig. 2). *S. typhimurium* did not grow on CO_2-packaged samples stored at 10° C.

In a subsequent experiment, *E. coli* did not grow on CO_2-packaged samples stored at 9° C (results not included).

In vacuum-packaged samples stored at 12° C, spoilage was apparent after 4 days. Both *E. coli* and *S. typhimurium* grew without significant lag periods, *E. coli* at a rate similar to, and *S. typhimurium* at a rate less than that of the spoilage flora. Numbers of *E. coli* increased by over 4 log cycles, and those of *S. typhimurium* by about 3 log cycles (Fig. 3).

In CO_2-packaged samples stored at 12° C, spoilage was apparent after 9 days. Both *E. coli* and *S. typhimurium* grew after a lag period of at least 3 days at rates less than that of the spoilage flora. However, *S. typhimurium* ceased growth when the spoilage flora attained maximum numbers, whereas growth of *E. coli* was not so inhibited. Consequently, increases in numbers of *E. coli* and *S. typhimurium* were, respectively, about 1.5 and about 1 log cycles (Fig. 3).

In a subsequent experiment, *S. typhimurium* did not grow on CO_2-packaged samples stored at 11° C (data not shown).

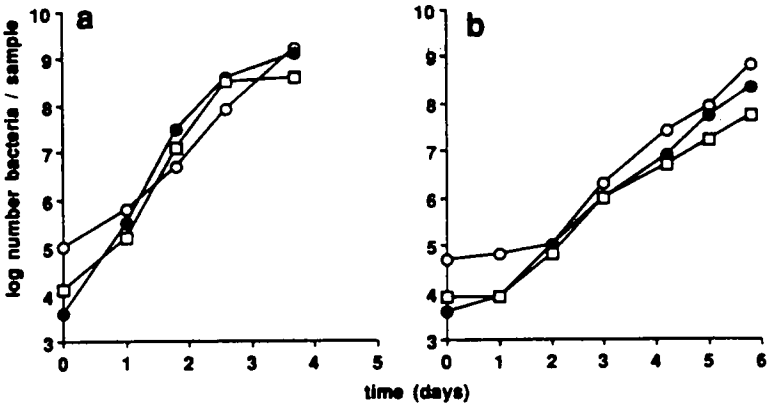

Fig. 4. Growth of bacteria in (a) vacuum and (b) CO_2 packs at 15 °C. Counts on PCA, 25 °C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

Vacuum-packaged samples stored at 15 °C were spoiled at about 4 days. Both *E. coli* and *S. typhimurium* grew without substantial lag periods at rates greater than that of the spoilage flora. Again, the growth of *S. typhimurium* appeared to be affected by the spoilage flora approaching maximum numbers, whereas growth of *E. coli* continued at that time. Consequently, *E. coli* numbers increased by about 5.5 log cycles, while the increase in numbers of *S. typhimurium* was 1 log cycle less (Fig. 4).

CO_2-packaged samples stored at 15 °C were spoiled at about 6 days. Both *E. coli* and *S. typhimurium* grew after a lag period of about 1 day at rates similar to that of the spoilage flora. The increases in numbers of *E. coli* and *S. typhimurium* amounted to about 4 log cycles (Fig. 4).

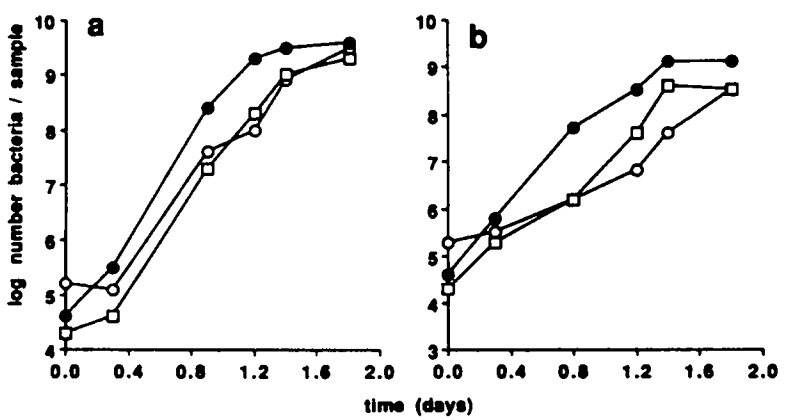

Fig. 5. Growth of bacteria in (a) vacuum and (b) CO_2 packs at 20 °C. Counts on PCA, 25 °C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

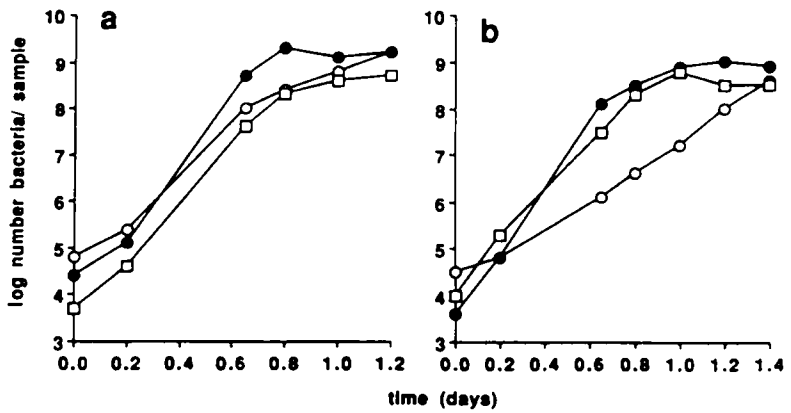

Fig. 6. Growth of bacteria in (a) vacuum and (b) CO_2 packs at 30 °C. Counts on PCA, 25 °C, 48 h (○), *E. coli* (●), *S. typhimurium* (□).

In vacuum-packaged samples stored at 20 °C, spoilage was apparent after about 1.5 days. Both *E. coli* and *S. typhimurium* grew without substantial lag periods at rates greater than that of the spoilage flora. Consequently, numbers of both organisms increased by about 5 log cycles, and were equal to or greater than those of the spoilage flora at the time of spoilage (Fig. 5).

In CO_2-packaged samples stored at 20 °C, spoilage was apparent after about 2 days. However, both *E. coli* and *S. typhimurium* grew in a manner and at rates similar to those observed for vacuum-packaged samples, to reach their maximum numbers before spoilage developed with increases in numbers of about 4.5 log cycles (Fig. 5).

In samples stored at 30 °C in either vacuum or CO_2 packs, the times to spoilage were somewhat less than those observed at 20 °C, but the patterns and rates of growth of both the spoilage flora and the test organisms were similar to those observed, respectively, for samples in the two types of packaging stored at 20 °C (Fig. 6).

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 is 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 when the spoilage flora begins to approach maximum numbers (Gill and Reichel, 1989).

Inhibition of growth of *E. coli* by the spoilage flora was not apparent before spoilage was evident in either vacuum- or CO_2-packs. Inhibition of growth of *S. typhimurium* by the spoilage flora seemingly occurred only in CO_2 packs at lower storage temperatures. 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 period before growth commences at abusive temperatures is the major restriction on proliferation of *E. coli* and *S. typhimurium*. 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, periods of about one day at 15 °C may be tolerable for CO_2-packaged meat with respect to both *E. coli* and *S. typhimurium* proliferation. That tolerable period may extend to about 3 days for CO_2 packs at 12 °C. Obviously, for the strains investigated, a temperature of 9 °C must be regarded as safe for CO_2-packaged meat with respect to *E. coli* proliferation, as the organism did not grow at that temperature. Similarly, 11 °C must be regarded as a safe temperature with regard to proliferation of *S. typhimurium*.

The present study indicates that growth of *E. coli* and *S. typhimurium* will be better controlled in CO_2-packaged meat than in similar vacuum-packaged products at abusive temperatures up to 15 °C. However, the safety advantage for CO_2 packaged products is largely lost at higher temperatures, 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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