GROWTH OF COLD-TOLERANT PATHOGENS ON HIGH-pH BEEF PACKAGED UNDER CO_2

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INTRODUCTION

The cold-tolerant pathogens that commonly contaminate meat, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes*, may grow on product during chiller storage (Palumbo, 1986). The degree of risk to consumers from the presence of these organisms on meat is uncertain, but any risks must be increased by an increase in pathogen numbers before the meat is spoiled. Packaging conditions that enhance the growth of a cold-tolerant pathogen relative to the rate of spoilage development are therefore likely to result in an augmented health hazard.

The storage life of chilled meat is substantially extended by packaging the product under vacuum in a pouch composed of film of low gas permeability (Newton & Rigg, 1979). An even longer storage life can be achieved by packaging meat under oxygen-free CO_2 maintained at atmospheric pressure after the meat is saturated with the gas (Gill & Penney, 1988).

Because vacuum packaged meat has been traded for many years without the emergence of a clear health risk from cold-tolerant pathogens, the increase of these organisms during storage of vacuum packaged meat may indicate their practically tolerable proliferation. Some increases are to be expected, despite uncertainty about the behaviour of *L. monocytogenes*, as studies on the microflora of vacuum packaged meat indicate that growth of *Y. enterocolitica* and *A. hydrophila* may be relatively advantaged in that packaging (Myers *et al*; 1982). In contrast, the behaviour of all three cold-tolerant pathogens on CO_2-packaged meat is unknown. As CO_2-packaging is coming into increasing commercial use (Gill, 1988), and the very long storage life attainable with that packaging might allow a significant health hazard from cold-tolerant pathogens to develop, the growth of cold-tolerant pathogens on meat packaged under CO_2 was examined.

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, *Y. enterocolitica* (isolated from vacuum packaged lamb), *A. hydrophila* (ATCC 7966) or *L. monocytogenes* (ATCC 19111), diluted to a cell concentration of approximately 10^5 cells/ml. Each steak was packaged in an evacuated pouch, composed of polyvinylidene 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 $O_2/m_2/24$ h/atm) for samples held under CO_2. Within 30 min of evacuation, these latter packs were further packaged, in groups of eight, 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 -2, 0, 2, 5, or 10°C, with temperatures maintained within ±0.2°C of the set temperature. Air temperatures were recorded throughout the experiment.

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 packagings were opened, the odour of the meat was assessed. Strong, persistent 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; Cefsulodin-Irgasan-Novobiocin Agar, CIN-Agar (Difco, *Yersinia* media) when the meat had been inoculated with *Y. enterocolitica*; Starch-Ampicillin Agar, SA-Agar (Palumbo *et al.*, 1985) when the meat had been inoculated with *A. hydrophila*; or LiCl-Phenylethanol-Moxalactam Agar, LPM-Agar (Lee & McClain, 1986) when the meat had been inoculated with *L. monocytogenes*. All plates were incubated at 25°C for 48 h.

The composition of the natural spoilage flora was assessed from PCA plates bearing at least 100 colonies that had been derived from uninoculated meat samples. Pinhead or small, white, glossy colonies were assumed to be lactobacilli, and large opaque or pigmented colonies were assumed to be enterobacteria (Gill and Penney, 1988). Numbers of the spoilage flora were determined from PCA plates derived from the same meat samples but bearing 20 to 100 colonies.

Pathogen numbers were determined from counts on selective agar plates. At each count, two representative, presumptive colonies were picked from each plate and the identities of the isolates confirmed by further tests. Colonies on CIN-Agar that were small to medium and smooth with a dark red centre surrounded by transparent borders were presumed to be *Y. enterocolitica* (Schiemann, 1982). Colonies on SA-Agar that showed as amylase-positive after plates were flooded with Lugol's iodine were presumed to be *A. hydrophilia* (Palumbo *et al.*, 1985). Colonies on LPM-Agar that appeared blue with an irregular, lacy surface pattern when viewed using Henry's oblique light system (transillumination at a 45°C angle) were assumed to be *L. monocytogenes* (Lee and McClain, 1986).

RESULTS

Spoilage development

Spoilage flora numbers were generally similar for inoculated and uninoculated samples that had been packaged and stored identically. The spoilage floras of all vacuum-packaged samples were composed of lactobacilli and enterobacteria in roughly equal proportions. Vacuum-packaged samples were invariably spoiled when maximum numbers were attained, with the total counts increasing by 5 log cycles to exceed 1×10^7 bacteria/sample. The spoilage floras of all CO_2-packaged samples were greatly dominated by lactobacilli, enterobacteria forming less than 1% of the flora when maximum numbers were attained. Spoilage of CO_2-packaged samples did not occur until maximum bacterial numbers had persisted for significant periods. The storage life of CO_2-packaged samples was twice as long as that of vacuum-packaged samples at the higher storage temperatures, and three times as long at the lower temperatures (Table 1).

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

Temperature (°C)	Storage life (days)	
	Vacuum pack	CO_2 pack
-2	63	182
0	49	126
2	35	70
5	17	35
10	5	10

Pathogen growth in vacuum packs

In vacuum packs, *Y. enterocolitica* grew at -2 and 10°C only after significant lags but without significant lags at intermediate temperatures. At all temperatures, growth rates were similar to those of the spoilage flora. Higher maximum number were attained at 10°C than at -2°C because, in contrast to growth at lower temperatures, growth of *Y. enterocolitica* at 10°C was not obviously inhibited as the spoilage flora

approached maximum numbers. However, increases in numbers, of 5 log cycles, were greatest at intermediate temperatures because of the absence of significant lags (Table 2).

At 5°C and lower temperatures, *A. hydrophila* grew in vacuum packs after significant lags, the lag at -2°C being very prolonged, at rates similar to those of the spoilage flora. Growth was inhibited at an early stage of the spoilage flora's approach to maximum numbers. The lag period, and early inhibition of growth, generally limited increases of *A. hydrophila* to about 4 log cycles, while the prolonged lag at -2°C allowed only modest proliferation. However, at 10°C, *A. hydrophila* grew without lag at a rate greater than that of the spoilage flora to increase by 5 log cycles (Table 2).

L. monocytogenes grew in vacuum packs only after significant lags at rates less than that of the spoilage flora. At all temperatures, growth was inhibited as the spoilage flora approached maximum numbers. These factors limited increases of *L. monocytogenes* to less than 3 log cycles at 2°C and higher temperatures, and to less than 2 log cycles at 0°C. *L. monocytogenes* did not grow at -2°C (Table 1).

Table 2. The effect of storage temperature on the maximum increase in numbers of *Y. enterocolitica (Yer)*, *A. hydrophila (Aer)* and *L. monocytogenes (Lis)* on vacuum-packaged, high-pH beef.

Temperature (°C)	Log increase		
	Yer	*Aer*	*Lis*
-2	2.6	1.2	-
0	4.7	3.9	1.6
2	5.2	4.3	2.8
5	5.3	3.5	2.8
10	3.9	5.5	2.9

Pathogen growth under CO_2

In CO_2 packs, none of the test organisms grew at 2°C or at lower temperatures. *Y. enterocolitica* grew at 5 and 10°C after substantial lags at rates similar to those of the spoilage flora. *A. hydrophila* and *L. monocytogenes* grew only at 10°C after an insignificant and a relatively short lag respectively. *A. hydrophila* grew at a rate similar to that of the spoilage flora, but the growth rate of *L. monocytogenes* was somewhat slower than that of the spoilage flora. Growth of all three species was inhibited as the spoilage flora approached maximum numbers, with inhibition of *A. hydrophila* preceeding inhibition of the other species. At 10°C, numbers of *A. hydrophila* increased by 4 log cycles, and numbers of *Y. enterocolitica* and *L. monocytogenes* by 3 log cycles (Table 3).

Table 3. The effect of storage temperature on the maximum increase in numbers of *Y. enterocolitica (Yer)*, *A. hydrophila (Aer)* and *L. monocytogenes (Lis)* on CO_2-packaged, high-pH beef.

Temperature (°C)	Log increase		
	Yer	*Aer*	*Lis*
2	-	-	-
5	2.2	-	-
10	3.1	4.1	3.5

- no growth

CONCLUSIONS

It is obviously desirable that a packaging for extending the storage life of meat should restrict the possibilities for cold-tolerant pathogens to grow to high numbers before the product is spoiled. The restriction of growth should not be dependent on a low muscle tissue pH as, except with some beef products, such a condition cannot be assured for all meat that is packaged.

Without specific data relevant to the infection of humans by each of the cold-tolerant pathogens, it is not possible to define the numbers of these bacteria that would be tolerable on raw meats. However, it can reasonably be suggested that storage conditions may be hazardous if numbers of a potential pathogen can increase by 5 log cycles before spoilage becomes evident. By that crude criterion, storage of vacuum-packaged meat at temperatures much above 0°C would be considered hazardous because of possible growth

of *Y. enterocolitica* or *A. hydrophila* to high numbers. In contrast, storage of CO_2-packaged meat at 5°C or below would be considered safe with respect to growth of the cold-tolerant pathogens, and even storage at 10°C might be considered adequate for control of cold-tolerant pathogen proliferation.

Meat must be held at or below 0°C for prolonged chiller storage (Gill *et al.*, 1988). The storage of CO_2-packaged, chilled meat for very long periods is therefore highly unlikely to lead to the development of any health hazard from the cold-tolerant pathogens *Y. enterocolitica, A. hydrophila* or *L. monocytogenes* because these species apparently cannot grow at low chiller temperatures. Even at higher storage temperatures, growth of the cold-tolerant pathogens will be better controlled in CO_2-packaged meat than in similar vacuum-packaged product, so risks associated with temperature abuse will be smaller for product in the former packaging.

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