GROWTH OF LISTERIA MONOCYTOGENES ON VACUUM PACKAGED BEEF

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SUMMARY

Pieces of beef striploin (ca 400 g) were inoculated with *Listeria monocytogenes*, vacuum packaged and stored at 0°C or 5.3°C. Increases in numbers of listeria occurred on fat and lean, and in the exuded weep during storage. Growth was more rapid at 5.3°C than at 0°C, and faster on striploins of high pH (6.0) than on striploins of low pH (5.6). Higher populations of *L. monocytogenes* were achieved on fatty than on lean tissue. On the fatty tissue of low pH striploins, listeria numbers increased by about 6000-fold in 16 days at 5.3°C but by only about 300-fold in 11 weeks at 0°C. On the fat of high pH striploins stored at 0°C, the count of listeria increased about 6000-fold in 10 weeks.

INTRODUCTION

Following several recent food-borne outbreaks of human listeriosis in North America, *Listeria monocytogenes* has become an old pathogen of new concern. While *L. monocytogenes* is known to occur on meats (Brackett 1988), there is little published information on the growth of this organism on chilled meats. This published data also appears to be conflicting. Knowledge of the ability of *L. monocytogenes* to grow on meats under a variety of conditions is important in any assessment of the possible role of meats in listeriosis.

Although Khan et al. (1973) obtained growth of *L. monocytogenes* in sterile lamb tissue held at 8°C, Gouet et al. (1978) found that the organism could not grow in sterile beef mince during 17 days storage at this temperature. Variable results were obtained when the ability of *L. monocytogenes* to grow at 4°C in sterile muscle "drip" was examined (Khan et al. 1973; 1975). Numbers of inoculated cells could remain relatively static, decrease, or decrease and then increase during incubation at 4°C for up to 40 days. Johnson et al. (1988) observed that listeria survived without detectable growth in vacuum-packed beef mince held at 4°C for 14 days. At 0°C, no significant growth occurred in sterile lamb meat held for 24 days (Khan et al. 1973).

The aim of the experiments reported here was to see if inoculated cells of *L. monocytogenes* strain Murray B could grow on vacuum-packaged beef striploins stored at either 0° or 5.3°C.

EXPERIMENTAL METHODS

Inoculum.

A 0.1 ml aliquot of a stock culture of *L. monocytogenes* strain Murray B, previously grown at 37°C, was added to 10 ml tryptose soya broth (Oxoid; CM 129) supplemented with 0.3% yeast extract (Oxoid; L21). This culture was incubated at 10°C for 3 days, and then 0.06 to 0.25 ml diluted in 1 litre of distilled water to provide the inoculum for the meat.

Meat packaging.

Beef striploins (*M. longissimus dorsi*) with the overlying fat intact were obtained from a commercial boning room and each striploin was cut into 7-8 portions, each about 400 g. The pieces were dipped into 1 litre of distilled water containing the inoculum. After being blotted dry, each portion was placed in a plastic bag (Barrier bag, W gauge; W.R. Grace, Australia) and vacuum-packed (Supervac GK 170; Kieteubl and Assler OHG, Austria). The oxygen transmission rate of the polyvinylidene ethylene-vinylacetate film was 25-30 ml/m^2/24 h/101 kPa measured at 25°C and 75% relative humidity. The packaged meat was stored for up to 11 weeks in rooms maintained at 0°C (range –0.6 to +0.60) or 5.3°C (range 5.0 to 5.50).

Viable count of L. monocytogenes.

Using a cork borer, 5 samples (each 5 cm^2 x ca 0.4 cm deep) were taken from the fatty tissue and 5 from the lean surface. The separate lean and fatty tissue samples were blended with 90 ml 0.1% peptone water in a Colworth Stomacher, Model 400. Aliquots (0.1 ml) of appropriate dilutions in 0.1% peptone were surface plated on tryptone yeast-extract soya glucose agar (TYSG; Grau et al. 1985) and on a modification of the selective agar of Lee and McClain (1986). The addition of 0.1% mannitol, 0.05% aesculin and 0.05% ferric citrate to this selective agar resulted in listeria colonies being black. Plates were incubated at 37°C for 24 h, and suspected listeria colonies counted. Representative colonies were purified and isolates confirmed as being *L. monocytogenes* by the following tests: gram reaction, tumbling and umbrella motility, oxidase, catalase, methyl red, Voges-Proskauer, nitrate reduction, and fermentation of glucose, rhamnose, aesculin, mannitol, and xylose.

pH measurements.

At each sampling time, 5-6 g of lean tissue was blended with 9-times the mass of distilled water, and the pH measured (Radiometer TTT2).

RESULTS

Growth at 5.3°C - lean pH 5.6.

There appeared to be little if any lag period before *L. monocytogenes* began growing on the fatty tissue of vacuum-packed beef stored at 5.3°C. The inoculated organism grew from 5x10^3 CFU/cm^2 to 3x10^7 CFU/cm^2 of fat surface during the first 16 days. Growth in the exuded weep was also marked, with the count increasing from 3x10^4 CFU/ml at 5 days to near 5x10^7/ml after 21 days storage. On the lean surface there was a lag period of 5-6 days before listeria began to grow. From an initial population of about 2x10^3 CFU/cm^2, listeria reached a population of 10^6 CFU/cm^2 after 21 days.

Growth at 0°C - lean pH 5.6.

There was no increase in the count of listeria on the lean tissue of vacuum-packed beef of pH 5.6 until after 9 weeks of storage at 0°C. After 11 weeks, the viable count of listeria on the lean was only 10-fold greater than the initial count. In the weep no growth occurred until after 3 weeks. Subsequent growth was slow. After 11 weeks storage the count had increased by about 100-fold. Growth on fatty tissue began after 1-2 weeks storage and,

after 11 weeks, the population had risen from about $3x10^3$ to 10^6 CFU/cm^2.

Growth at 0°C - lean pH 6.0.

From an initial inoculum of 200-300 CFU of *L. monocytogenes*/cm^2, the viable count of listeria increased to $2x10^5$ and just over 10^6 CFU/cm^2 on the lean and fat surfaces respectively after 10 weeks storage of high pH (6.0) striploins. There appeared to be little lag before the organism began growing on the fat. On the lean the lag period was 2-3 weeks. In the weep the population of listeria increased by about 10^3 between 1 and 10 weeks. Again there was a 2-3 week lag period.

DISCUSSION

The growth of *L. monocytogenes* on vacuum-packaged beef appears to be affected by the temperature of storage, the pH of the lean, and the type of tissue.

When striploins whose lean pH was 5.6 were stored at 5.3°C, the growth of listeria was faster on lean and fat and in the weep than when striploins of this pH were stored at 0°C. The populations of listeria achieved in 2 weeks storage at 5.3°C were greater than achieved in 11 weeks storage at 0°C.

Similarly, growth of listeria was considerably more extensive on lean and fat and in weep from striploins of pH 6.0 than in these tissues from striploins of pH 5.6.

In all storage experiments, whether the temperature was 0° or 5.3°C or whether the pH of the lean was 6.0 or 5.6, listeria maintained higher populations on fat than on lean. Mostly this seemed to be the consequence of a shorter lag phase on fat than on lean. The numbers of listeria/ml of weep were usually at least 30-times the count/cm^2 of lean. While it was difficult to estimate the lag period for growth in weep since the first sample was not obtained until after 5-7 days storage, it appeared from the experiments with striploins of pH 5.6 stored at 0°C that growth in weep could precede that on lean.

This appears to be the first report of *L. monocytogenes* growing on fresh meat at temperatures of 0° to 5.3°C. Khan et al. (1973) observed growth on lamb tissue at 80 but not at 0°C. Gouet et al. (1978) obtained growth of listeria on beef mince at 8°C only after the growth and metabolism of pseudomonads had raised the pH above 6.2. Johnson et al. (1988) found no significant growth in vacuum-packed beef mince (pH 5.6-5.9) held at 4°C. The explanation for the lack of observed growth may be that inocula appear to have been grown at 30° to 37°C. Such inocula may have very long lag periods on lean tissue of pH about 6 at storage temperatures of 0° to 8°C.

CONCLUSIONS

L. monocytogenes strain Murray B can grow on vacuum-packaged beef stored between 0° and 5°C. Growth is influenced by the temperature, pH of the lean, and the type of tissue.

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