GROWTH OF YERSINIA ENTEROCOLITICA IN MODIFIED ATMOSPHERE PACKAGED LAMB

SHERIDAN J.J.*, ALICE DOHERTY A.*, McDOWELL D.A.** and BLAIR I.S.**

* The National Food Centre, Dublin, Ireland. ** University of Ulster, Northern Ireland.

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SUMMARY

The safety of modified atmosphere packaged (MAP) lamb was examined by following the growth of pathogenic Yersinia enterocolitica on lamb, as pieces and mince, packaged in different modified atmospheres and in air, and stored at 5 or 0°C. The modified atmospheres investigated were (i) vacuum pack; (ii) 80% O_2/20% CO_2; (iii) 50% CO_2/50% N_2 and (iv) 100% CO_2.

It was noted that carbon dioxide concentration and storage temperature had an effect on pathogen growth in atmospheres containing high levels of this gas. In an atmosphere containing 80% O_2/20% CO_2, the high oxygen concentration was found to be inhibitory. Growth of Y.enterocolitica generally occurred in vacuum packs and air. One of the major effects on pathogen growth in all atmospheres was the inhibition observed with mincing the meat.

Introduction

Concerns have been expressed regarding the safety of modified atmosphere packaged foods (Farber 1991). In such foods the normal spoilage organisms may be inhibited, thus consumers could judge these products wholesome even though pathogens may be present in very high numbers (Marshall et al. 1992). One such pathogen which has raised concern is Yersinia enterocolitica, a gram-negative, facultative anaerobic bacillus belonging to the family Enterobacteriaceae. Y.enterocolitica has been isolated from a variety of foods including milk, pork, chicken, vegetables and vacuum-packaged refrigerated beef and lamb (Hanna et al. 1976; Tsay & Chou 1989). This organism is capable of growth at refrigeration temperatures (Palumbo 1985). The aim of this study was to obtain information on the growth of Y.enterocolitica on lamb packaged under a range of modified atmospheres and storage temperatures.

Materials and Methods

Lambs (mean live weight 34kg) were slaughtered in the pilot scale abattoir at The National Food Centre (NFC). Carcasses with a pH below 5.8 were dissected into small pieces. All visible fat was removed. The resulting lean meat was cut into approximately 100g lots. All meat was stored in sterile plastic bags at 0°C before use.

A Y.enterocolitica GER, serotype 0:3 strain, containing a stable virulence plasmid, was obtained from Dr S. Bhaduri, USDA, ERRC, Philadelphia, Pennsylvania, USA.

Meat pieces were inoculated by immersion for 5 s in maximal recovery diluent (MRD; BBL) containing 1000-1500 Y.enterocolitica organisms/ml. Excess liquid was allowed to drain off the meat. Minced meat was prepared by mincing inoculated meat pieces using a Crypto-Peerless mincing machine. It was minced through a 10mm plate, followed by a second mincing through a 5mm plate.

Meat pieces or mince were weighed out in 100g lots into Dynopack high density polyethylene trays and modified atmosphere packed with a Transoplan top web film. The modified gas atmospheres and air were filled and sealed using a Mecapac 500 semi-automatic packaging machine. A KM 100-3M gas mixer was used to mix food grade CO_2, O_2 and N_2 to obtain pack atmospheres of: 80% O_2/20% CO_2; 50% CO_2/50% N_2 and 100% CO_2. A Gow-Mac gas chromatograph fitted with an Alltech Speciality CTRI column was used to check gas mixtures. A CI-4100 Integrator was used to plot the chromatographs and calculate the gas percentages from the areas under the curves using the normalisation method (Anon 1987).

Small vacuum packaging bags with a capacity of approximately 200g were made from Cryovac BB6 bags. Packs were heat sealed using a Swissvac vacuum packaging machine. After packing, the vacuum packs were dipped in water at 90°C for 5sec to shrink the bags.

Packs were stored at 0 or 5°C and examined at 7 day intervals over a four week period. For the modified amospheres, the meat from several animals was bulked together and sub-samples were randomly assigned to the different modified atmosphere/temperature treatments. The air experiments were set up separately using different animals. Three replicates were set up for air and each modified atmosphere/temperature combination.

For microbiological examination, a 10g sample was removed from a pack and homogenised in a Colworth Stomacher for 1min with 90ml of MRD. Bacterial numbers were estimated from plates surface inoculated with 0.1ml of the neat solution or successive ten-fold dilutions of this in MRD. All plates were inoculated in duplicate and the inoculum spread on the surface using a sterile glass rod. Counts for Y.enterocolitica were obtained on Yersinia selective agar containing the following supplements in 500ml of agar: cefsulodin (7.5mg), irgasan (2mg) and novobiocin (1.25mg). The plates were incubated at 37°C for 24h.

At the outset of the experiment, the meat was checked to ensure it was Yersinia free. A 10g sample was homogenised in 90ml of MRD and plated out as above on Yersinia selective agar. The absence of Yersinia on these plates after incubation confirmed that the meat was pathogen free.

Presumptive Y.enterocolitica colonies were confirmed if they were gram negative rods, produced an acid slope and butt with no gas in triple sugar iron agar, displayed positive reactions for catalase, urease and ornithine decarboxylase, a negative reaction for lysine decarboxylase and were motile at 25°C in SIM medium but not at 37°C.

Results

When lamb pieces were inoculated with Y.enterocolitica, extensive growth occurred in all atmospheres at 5°C (Table 1). Even in an atmosphere containing 100% CO_2 growth was quite substantial. When comparing atmospheres at this temperature, significant differences ($P<0.05$) in growth were noted only between 100% CO_2 and 50% CO_2/50% N_2 and between 100% CO_2 and vacuum packs.

At 0°C, growth occurred in vacuum packs and in an atmosphere containing 50% CO_2/50% N_2. There was no growth in 80% O_2/20% CO_2 and 100% CO_2. Significant differences in growth were observed between the different atmospheres at this temperature.

In all atmospheres, counts at 5°C were higher than at 0°C. These differences were significant ($P<0.05$) in all atmospheres, except in vacuum packs.

The mean counts for Y.enterocolitica on minced lamb are shown in Table 2. At 5°C, there was no significant difference between counts in vacuum packs and 50% CO_2/50% N_2. Counts in these two atmospheres were significantly different from 80% O_2/20% CO_2 and 100% CO_2. Suppression of growth occurred in all atmospheres at 0°C.

A comparison of pathogen growth on pieces and mince at 5°C is shown in Table 3. Differences between counts for pieces and mince, held under identical conditions was significant in all atmospheres, except in vacuum packs.

When the samples were stored at 0°C the inhibitory effect of minced samples was again evident but a significant difference in counts was shown only for vacuum packaged samples ($P<0.05$) (Table 4).

The counts of Y.enterocolitica on lamb pieces and mince in air were much higher after storage at 5 than after storage at 0°C (Fig.1). At 5°C, growth in air at 28 days was higher than in any other atmosphere tested (e.g. $\log_{10}$ 9.54 cfu/g versus $\log_{10}$ 8.52 cfu/g in 50% CO_2/50% N_2).

Counts in air at 0°C were similar to those in vacuum packs on lamb pieces which was the highest growth at this temperature ($\log_{10}$ 5.82 cfu/g versus $\log_{10}$ 5.88 cfu/g).

The inhibition observed with mincing in the other atmospheres was not evident in air at either temperature. There was no significant difference between counts for pieces and mince at 5 and 0°C.

Discussion

In the present study Y. enterocolitica was capable of growth in an atmosphere containing 50% CO_2 / 50% N_2, at 5°C. In 100% CO_2 at this temperature, growth was significantly less, suggesting the presence of a CO_2 concentration effect. In both these high CO_2 atmospheres the effect of temperature on growth inhibition was also evident. The influence of increased CO_2 concentration in growth inhibition has been observed in the past

(Gill & Penney 1988), as has the effect of lowering the temperature (Eklund and Jarmund 1983; Gill & Harrison 1989).

The inhibitory effect of high O_2 concentrations was evident for pieces and mince at 5 and 0°C, particularly in mince at 5°C. This effect has been noted by other workers on beef steaks inoculated with Y.enterocolitica and stored at 0°C (Gibbs et al. 1993). Clark & Burki (1972) and Clark & Lentz (1973) also showed that high O_2 concentrations were inhibitory to a number of organisms including Pseudomonas and the Moraxella-Acinetobacter group.

With the exception of minced lamb at 0°C, growth of Y.enterocolitica occurred in all vacuum pack storage regimes. The ability of this pathogen to grow in vacuum packs has been observed by others (Hanna et al. 1976; Seelye & Yearbury 1979; Eklund & Jarmund 1983; Manu-Tawiah et al. 1993). However, Van Laack et al. (1993) found that the pathogen failed to grow over a 9-day period on vacuum packaged inoculated pork loins stored at 1°C. The lack of growth may have been related to the low storage temperature and time or to the fact that the pathogen was mixed with pig faeces as an inoculum, which may have been inhibitory. Working on the same organism as in the present study, Gibbs et al. (1993) showed that growth on beef steaks was inhibited on vacuum packs stored at 5 and 0°C. Indeed these authors also showed complete inhibition of pathogen growth in 50% CO_2/50% N_2, 100% CO_2 and 80% O_2/20% CO_2, at both temperatures.

Manu-Tawiah et al. (1993) investigated the survival of Y.enterocolitica on pork chops packaged in air. They showed that growth was inhibited at 4°C. This result would be at variance with the present data and that of other workers (Hanna et al. 1977; Eklund & Jarmund 1983; Molin 1983).

One of the major effects observed with mincing the meat was that counts were lowered in all atmospheres. Mincing may have an effect on the microflora or its distribution, such that the pathogen is at a competitive disadvantage. In the present work there is no evidence to suggest that the microflora was involved in pathogen inhibition, but other workers have observed this effect (Kleinlein & Untermann 1990). These authors noted a marked inhibition of Y.enterocolitica growth by the background flora, using minced beef. On minced pork at 6°C, Fukushima & Goymoda (1986) demonstrated that serotype 0:3, similar to that used in the present work, was completely inhibited. These authors suggested that the inhibition was due to members of the Enterobacteriaceae, particularly Hafnia alvei. The antagonistic effect of the microflora to Y.enterocolitica has been observed by Schiemann & Olson (1984). They suggest that a certain critical space is required for cell multiplication as demonstrated by the inhibition of multiplication of Y.enterocolitica by Klebsiella pneumoniae during growth in fresh media. The antibacterial activity of minced beef has also been reported by Mattila-Sandholm and Skyttä (1991). These authors showed that Y.enterocolitica growth was completely inhibited in a medium prepared from minced meat.

The present work has shown that high levels of CO_2 combined with low temperature can prevent the growth of pathogenic Y.enterocolitica in lamb. An additional inhibitory effect was observed by mincing the meat. This effect may have potential in future efforts to control the growth of this and other pathogens.

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