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ORIGINAL ARTICLE

Growth of *Yersinia enterocolitica* O : 3 on modified atmosphere packaged lamb

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The growth of pathogenic Yersinia enterocolitica *serotype 0:3 was investigated on lamb pieces and lamb mince, packaged in air and a range of modified atmospheres, 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.* Y. enterocolitica *0:3 counts were examined at 28 days in each of the atmosphere/temperature combinations.*

Growth of Y. enterocolitica *0:3 occurred on lamb pieces stored at 5°C in air and all the modified atmospheres tested. On lamb pieces stored at 0°C, pathogen growth was noted in air, vacuum packs and 50% CO_2/50% N_2. In all atmospheres except air, growth of* Y. enterocolitica *0:3 on minced lamb at 5 and 0°C was reduced compared to growth on lamb pieces. At 5°C on minced lamb, growth of the organism occurred in air, vacuum packs and 50% CO_2/50% N_2. However, at 0°C, growth was observed in air only. An atmosphere containing a high O_2 concentration was inhibitory to the growth of* Y. enterocolitica *0:3. In addition, the combination of a high CO_2 (100%) atmosphere and low temperature (0°C) was inhibitory to growth.*

Introduction

Concerns have been expressed regarding the safety of modified atmosphere packaged foods (Farber 1991). Such products are frequently stored at refrigeration temperatures as an additional hurdle to microbial growth. Under these conditions, psychrotrophic bacterial pathogens may outgrow the spoilage bacteria before any signs of overt spoilage are evident. Thus consumers could judge these products wholesome even though they contain clinically significant levels of pathogens.

Yersinia enterocolitica serotype 0 : 3 has been frequently isolated from pigs (Nesbakken 1988, de Boer and Nouws 1991) and pork products (Wauters et al. 1988, Toora et al. 1994). It is a psychrotrophic, Gram-negative, facultative anaerobic bacterium belonging to the family *Enterobacteriaceae* (Palumbo 1986). The main clinical symptoms of *Y. enterocolitica* infection include fever, diarrhoea and abdominal pain (Carniel and Mollaret 1990).

To date, the growth of *Y. enterocolitica* 0 : 3 has been examined on pork (Fukushima and Gomyoda 1986, van Laack et al. 1993) and beef (Kleinlein and Untermann 1990, Gibbs et al. 1993). There is very little information available on the growth of this organism on lamb, or indeed the potential dangers posed by the survival and growth of this organism under modified atmosphere packaging. Thus 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.

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Materials and Methods

Animals

Lambs purchased in local markets were rested overnight on straw, given water *ad libitum* and slaughtered the next day in the pilot scale abattoir at The National Food Centre. After dressing, carcasses were not washed and were chilled for 16 h at 4°C at an air speed of $<0{\cdot}2$ m s^{-1} and at a relative humidity (RH) of 85–90% until the temperature of the deep round reached 4°C. The pH of the striploin was measured after chilling. Muscles with a pH in the range 5·4–5·8 were selected from carcasses, dissected into small pieces and all visible fat was removed. The meat was stored in sterile plastic bags at 0°C before use. A sample was removed from this bulk stored material and tested for the presence of *Yersinia* spp. as described below.

Organism

A culture of *Y. enterocolitica* GER, serotype 0:3 strain was obtained from Dr S. Bhaduri, USDA, ERRC, Philadelphia, PA, USA. It was maintained on tryptone soya agar (TSA; Oxoid, Unipath Ltd, Basingstoke, UK) at 0°C.

Inoculation

Before inoculation, the lamb pieces were examined for the presence of *Yersinia* spp. A random 10 g sample of lamb pieces was homogenized in a Colworth Stomacher (Model No. BA6021, A. J. Seward & Company Ltd, London, UK) for 1 min with 90 ml of maximal recovery diluent (MRD, BBL Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). A 1 ml aliquot of the homogenate was inoculated in duplicate onto *Yersinia* selective agar plates (CIN; Oxoid, Unipath Ltd, Basingstoke, UK) containing the following supplements in 500 ml of agar: cefsulodin (7·5 mg), irgasan (2 mg) and novobiocin (1·25 mg). The inoculum was spread on the surface using a sterile glass rod. Before incubation, the CIN plates were placed in a laminar air flow cabinet (Nuaire, Class II, type A, Plymouth, MN, USA) for approximately 30 min to dry excess liquid from the surface of the agar plate and were then incubated at 37°C for 24 h.

Approximately 14 kg of lamb pieces per replicate were inoculated by immersion for 5 s in MRD containing 1000–1500 *Y. enterocolitica* 0:3 organisms ml^{-1}. The inoculum was prepared from an overnight culture of *Y. enterocolitica* 0:3 grown in brain heart infusion broth (Oxoid) at 37°C. Excess liquid was allowed to drain off the meat. Minced lamb was prepared by double mincing approximately 7 kg of inoculated lamb pieces per replicate through a 10 mm plate and then through a 5 mm plate using a Crypto-Peerless mincing machine (Model EB12F, Crypto-Peerless Ltd, London, UK), sterilized by autoclaving at 121°C for 15 min.

Packaging

The inoculated lamb pieces or mince were weighed out in 100 g lots into Dynopack (Dynopack AS, Kristiansand, Norway) high density polyethylene trays with an oxygen transmission rate (OTR) of 3·5 $cm^3 m^{-2}$ 24 h^{-1} atm at 25°C and a RH of 50%. The meat was modified atmosphere packaged with a Transoplan (A&R, Flexible, Lund, Sweden) top web film with an OTR of 8·0 cm^3 m^{-2} 24 h^{-1} atm at 25°C and a RH of 50%. The packs were filled with a modified gas atmosphere or air and sealed using a Mecapac 500 semi-automatic packaging machine (rue Diderot, Bagnolet, France). A KM 100-3M (Witt Gasetechnik, Postfach, Germany) gas mixer was used to mix food grade CO_2, O_2 and N_2 (Air Products PLC, Walton-on-Thames, UK) to obtain pack atmospheres of: 80% O_2/20% CO_2; 50% CO_2/50% N_2 and 100% CO_2. The final pack volume was 475 ml giving a gas : meat ratio in excess of 3 : 1.

Vacuum packaging bags with a capacity of approximately 200 g were made from Cryovac BB6 bags (W.R. Grace Ltd, Dublin, Ireland) with an OTR of 48 cm^3 m^{-2} 24 h^{-1} atm at 25°C and a RH of 50%. The bags were filled with 100 g of inoculated lamb pieces or mince and heat sealed using a Swissvac vacuum packaging machine [Model 380, Swissvac (GB) Ltd, Langley, UK]. After packing, the vacuum packs were dipped for 5 s in water held at 90°C to shrink the bags.

Storage

All packs were stored at 5 or 0°C for up to 4 weeks. At 7 day intervals, modified atmosphere (n = 16) and air (n = 4) packs were removed and examined. The inoculated meat pieces or mince were randomly distributed over each atmosphere/temperature/time combination. The air experiments were set up separately using different animals. Three replicates were set up for air and each modified atmosphere/temperature/time combination.

Gas analysis

A Gow-Mac gas chromatograph (Spectra 250, Gow-Mac Instrument Co. Ltd, Co. Clare, Ireland) fitted with an Alltech Speciality CTRI column, was used to confirm the individual gas ratios in the gas mixtures before packaging and to analyse head space contents when packs were opened for microbiological examination. A CI-4100 Integrator (Milton Roy, Co. Clare, Ireland) was used to plot the chromatographs and calculate the gas percentages from the areas under the curves using the normalization method (Anon 1987).

Microbiology

A 10 g sample of pieces or mince was removed from the pack and homogenized in a Colworth Stomacher for 1 min with 90 ml of MRD. Counts for *Y. enterocolitica* 0:3 were obtained by surface inoculating duplicate CIN plates with 1 or 0·1 ml of the homogenate or successive 10-fold dilutions in MRD. The inoculum was spread on the surface using a sterile glass rod. Before incubation, the 1 ml plates were placed in a laminar air flow cabinet for approximately 30 min to dry excess liquid from the surface of the agar plate. All plates were incubated at 37°C for 24 h.

A presumptive *Y. enterocolitica* 0:3 colony from each CIN plate was confirmed if it was a Gram-negative rod, produced an acid slope and butt with no gas in triple sugar iron agar (Oxoid), displayed positive reactions for catalase, urease (Oxoid) and ornithine decarboxylase (Oxoid), a negative reaction for lysine decarboxylase (Oxoid) and was motile at 25°C in SIM medium (Oxoid) but not at 37°C.

Total counts were obtained on all purpose tween (APT; BBL) agar. Duplicate plates were surface inoculated with the homogenate or successive 10-fold dilutions in MRD and incubated at 25°C for 3 days.

Statistical analysis

The data obtained from lamb pieces and mince stored under modified atmospheres were analysed as a split-plot design in which meat type was the main plot effect and pack type and storage temperature were the sub-plot effects. The air data were analysed separately, also as a split-plot design. Differences between means were determined using one or two tailed *t*-tests as appropriate.

Results

The level of *Yersinia* spp. on the meat before inoculation was found to be < 5 cfu g^{-1}. After inoculation, the lamb pieces and mince packaged under each of the four modified atmospheres had an initial *Y. enterocolitica* 0:3 count of $\log_{10}$ 1·6 cfu g^{-1}. Lamb pieces packaged in air had an initial *Y. enterocolitica* 0:3 count of $\log_{10}$ 1·7 cfu g^{-1}, while minced lamb packaged in air had an initial *Y. enterocolitica* 0:3 count of $\log_{10}$ 1·2 cfu g^{-1}. Since the modified atmosphere and air experiments were set up using different animals, the bacterial counts from both experiments could not be compared directly. Counts from each of the modified atmosphere/temperature combinations are compared at 28 days. Likewise, the counts from lamb pieces and mince packaged in air at 5 and 0°C are compared at 28 days.

The mean *Y. enterocolitica* 0:3 counts on lamb pieces and mince are shown in Table 1. *Y. enterocolitica* 0:3 grew extensively at 5°C on lamb pieces in all the packaging atmospheres examined, including air. Of particular interest was the high count obtained in 100% CO_2 ($\log_{10}$ 5·56 cfu g^{-1}). Differences between atmospheres were small, occurring only between vacuum packs and 100% CO_2 and 50% CO_2/50% N_2 and 100% CO_2 ($P < 0·05$).

Table 1. Mean *Yersinia enterocolitica* 0:3 counts ($\log_{10}$ cfu g^{-1}) at 28 days on lamb pieces and mince packaged in air and different gas atmospheres at 5 or 0°C

Meat type	Pieces		Mince	
Storage temperature (°C)	5	0	5	0
Gas atmosphere				
Air	9·54	5·82	9·40	4·75
Vacuum pack	8·11	5·88	6·50	2·68
80% O_2/20% CO_2	6·84	1·16	2·40	0·78
50% CO_2/50% N_2	8·52	3·86	5·25	1·29
100% CO_2	5·56	1·56	1·05	0·00

Standard error of difference between means = 1·47 (air), 1·17 (gas atmospheres), except when comparing pieces vs mince at the same temperature (air) = 1·15, in the same gas atmosphere = 1·34. Degrees of freedom = 4 (air), 27 (gas atmospheres).

At 0°C, growth of *Y. enterocolitica* 0:3 on lamb pieces was observed in air, vacuum packs and 50% CO_2/50% N_2. In general, counts in vacuum packs and 50% CO_2/50% N_2 were higher than in 80% O_2/20% CO_2 and 100% CO_2 ($P < 0.05$).

A comparison of *Y. enterocolitica* 0:3 counts on lamb pieces at 5 and 0°C showed that the 5°C counts were higher than at 0°C in all packaging atmospheres including air ($P < 0.05$).

Growth of *Y. enterocolitica* 0:3 on minced lamb at 5°C occurred in air, vacuum packs and 50% CO_2/50% N_2. Examination of the data showed that the counts in 80% O_2/20% CO_2 and 100% CO_2 were significantly lower than in vacuum packs and 50% CO_2/50% N_2 ($P < 0.05$).

At 0°C, *Y. enterocolitica* 0:3 grew on minced lamb packaged in air but did not grow in any of the modified atmospheres tested.

A reduction in *Y. enterocolitica* 0:3 counts at 0°C, compared to 5°C, was observed for minced lamb packaged in air, vacuum packs and 50% CO_2/50% N_2, ($P < 0.05$).

The *Y. enterocolitica* 0:3 counts on lamb pieces stored at 5°C were higher than the corresponding counts on minced lamb in all the packaging treatments examined except air and vacuum packs ($P < 0.05$). At 0°C, a difference in *Y. enterocolitica* 0:3 counts between pieces and mince was shown for vacuum packs only ($P < 0.05$).

Table 2. Mean *Yersinia enterocolitica* 0:3 counts ($\log_{10}$ cfu g^{-1}) at 28 days on lamb pieces packaged in different gas atmospheres at 0°C

Gas atmosphere	*Y. enterocolitica*
Vacuum pack	5·14
80% O_2/20% Co_2	1·54
50% CO_2/50% N_2	2·37
100% CO_2	0·25

Standard error of difference between means = 0·43. Degrees of freedom = 6.

The very low *Y. enterocolitica* 0:3 counts on lamb pieces stored in 80% O_2/20% CO_2 at 0°C (Table 1) were considered unusual in that this atmosphere/temperature combination appeared to be inhibitory to pathogen growth. In contrast significant growth was observed on lamb pieces stored under this atmosphere at 5°C. Because of this apparent anomaly, a repeat experiment was carried out in which lamb pieces were inoculated with *Y. enterocolitica* 0:3, packaged in the four atmospheres and stored at 0°C. The results obtained confirmed the inhibitory nature of the 80% O_2/20% CO_2 atmosphere initially observed (Table 2).

A typical profile showing the relationship between bacterial counts and time is shown for lamb pieces and mince packaged under 80% O_2/20% CO_2 at 5°C (Fig. 1). Similar profiles were observed in the other atmospheres.

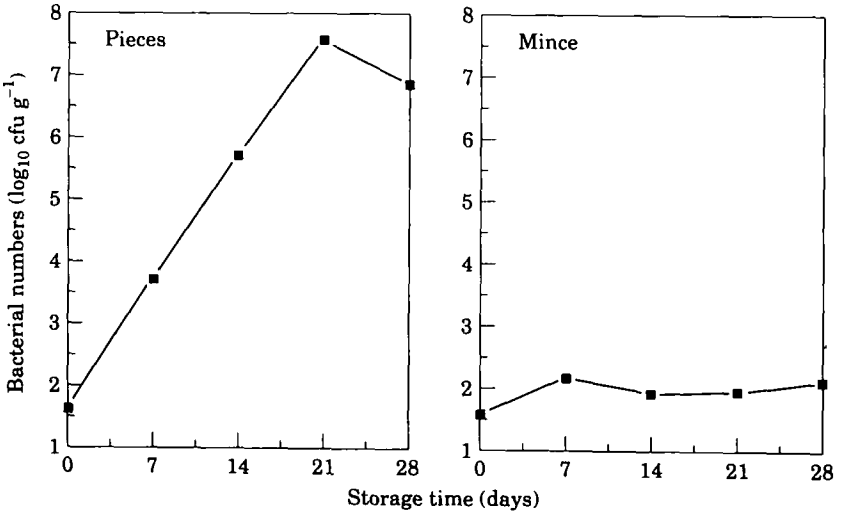

Figure 1. Growth of *Yersinia enterocolitica* on lamb pieces and mince packaged in an atmosphere containing 80% O_2/20% CO_2 at 5°C.

Table 3. Mean total aerobic counts (log_{10} cfu g^{-1}) at 28 days on lamb pieces and mince packaged in air and different gas atmospheres at 5 or 0°C

Meat type	Pieces		Mince	
Storage temperature (°C)	5	0	5	0
Gas atmosphere				
Air	12·27	9·41	11·98	8·20
Vacuum pack	8·55	6·14	9·07	5·25
80% O_2/20% CO_2	9·37	5·40	8·95	3·87
50% CO_2/50% N_2	8·93	5·11	7·75	4·59
100% CO_2	7·70	4·03	6·44	2·68

Standard error of difference between means = 1·58 (air), 0·78 (gas atmospheres), except when comparing pieces vs mince at the same temperature (air) = 1·45, in the same gas atmosphere = 0·82. Degrees of freedom = 4 (air), 28 (gas atmospheres).

The total counts obtained on lamb pieces and mince at 28 days are shown in Table 3. As for the *Y. enterocolitica* 0:3 counts, the total counts in air appeared to be higher than in the modified atmospheres but this could not be confirmed statistically.

Small differences between atmospheres at 5 and 0°C were noted for the total counts obtained on lamb pieces. These occurred between 80% O_2/20% CO_2 and 100% CO_2 at 5°C and between vacuum packs and 100% CO_2 at 0°C ($P < 0·05$). Total counts at 0°C were lower than at 5°C in all the packaging treatments tested except air ($P < 0·01$).

On minced lamb stored at 5°C, the total counts in 100% CO_2 were lower than in vacuum packs and 80% O_2/20% CO_2 ($P < 0·01$). At 0°C, small differences in total counts were recorded between vacuum packs and 100% CO_2 and between 50% CO_2/50% N_2 and 100% CO_2 ($P < 0·05$). The total counts on minced lamb stored at 5°C were higher than at 0°C in air and all the modified atmospheres ($P < 0·05$).

There were no significant differences between the total counts from pieces and mince at 5 and 0°C in any of the packaging atmospheres including air.

Discussion

With the exception of minced lamb at 0°C, *Y. enterocolitica* grew in all vacuum pack storage regimes. The ability of this pathogen to grow in vacuum packs has been reported by a number of other workers (Hanna et al. 1977b, Eklund and Jarmund 1983, Manu-Tawiah et al. 1993). However, van Laack et al. (1993) reported 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 in this case may have been related to the lower storage temperature and shorter storage time. Alternatively, components of the pig faeces, incorporated in the inoculum, may have been responsible for inhibiting growth. Using 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 or 0°C for 36 or 91 days. Indeed these authors also showed complete inhibition of pathogen growth in 80% O_2/20% CO_2, 50% CO_2/50% N_2 and 100% CO_2 at both temperatures.

High levels of O_2 inhibited growth of *Y. enterocolitica* on pieces and mince at 5 and 0°C, particularly on mince at 5°C. Other workers have noted similar effects on beef steaks inoculated with *Y. enterocolitica*, stored at 0°C (Gibbs et al. 1993). Hudson et al. (1994) indicated that O_2 appears to be inhibitory to the growth of *Y. enterocolitica*. Clark and Burki (1972) and Clark and Lentz (1973) showed that high O_2 concentrations were inhibitory to a number of other organisms including *Pseudomonas* and members of the *Moraxella-Acinetobacter* group.

In the present study *Y. enterocolitica* was capable of growth in an atmosphere containing 50% CO_2/50% N_2, at 5°C. This is broadly in agreement with the report of Manu-Tawiah et al. (1993) who observed growth of this organism on pork in an atmosphere containing 40% CO_2/60% N_2, at 4°C. In 100% CO_2 at 5°C, growth was significantly less than in 50% CO_2/50% N_2, suggesting the presence of a CO_2 concentration effect. In both these high CO_2 atmospheres, lower holding temperatures enhanced the inhibitory effects of CO_2. The influence of increased CO_2 concentration in growth inhibition has been previously reported (Gill and Penney 1988), as has the effect of low temperature storage (Eklund and Jarmund 1983, Gill and Harrison 1989).

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. Similar inhibition was noted by Fukushima and Gomyoda (1986) on minced pork at 6°C. Hudson and Mott (1993) working on smoked salmon also reported inhibition of *Y. enterocolitica* under aerobic storage at 5°C. These results are at variance with the present data and that of other workers (Hanna et al. 1977a,b, Eklund and Jarmund 1983, Molin 1983).

Mincing lamb prior to inoculation had the effect of reducing final counts obtained under all storage atmospheres. Mincing may have an effect on the microflora or its distribution, such that the pathogen is at a competitive disadvantage. Kleinlein and Untermann (1990) examining minced beef, noted a marked inhibition of *Y. enterocolitica* growth by the background flora. On minced pork at 6°C, Fukushima and Gomyoda (1986) demonstrated that growth of *Y. enterocolitica* 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 inhibitory effect of the lactic acid bacteria and the normal spoilage flora of vacuum packaged cooked sausage on *Y. enterocolitica* was noted by Nielsen and Zeuthen (1985). Schiemann and Olson (1984) also reported an antagonistic effect of the microflora to *Y. enterocolitica*.

The inhibition observed with minced meat may be due to components of the meat itself, which are released during mincing. Minced beef exudate has been reported to display antibacterial activity (Mattila-Sandholm and Skyttä 1991). These authors showed that *Y. enterocolitica* growth was completely inhibited in a medium prepared from minced meat. Further work will be necessary to confirm the presence of such meat components and to identify their nature and mechanism of action.

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