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Survival and growth of *Aeromonas hydrophila* on modified atmosphere packaged normal and high pH lamb

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Abstract

The growth of *A. hydrophila* on normal pH (5.5–5.8) and high pH (> 6.0) lamb stored under modified atmospheres was examined. Lamb pieces and mince, inoculated with *A. hydrophila* were packaged in air, vacuum pack, 80% O_2/20% CO_2, 50% CO_2/50% N_2 or 100% CO_2 and stored at 5 or 0°C for up to 42 days. Samples were examined for the survival and/or growth of *A. hydrophila* by enrichment in alkaline peptone water and/or direct plating on starch ampicillin agar. The pH of each sample was estimated. On lamb pieces and mince of normal pH, *A. hydrophila* numbers decreased during storage at 5 and 0°C under all the packaging conditions. The organism was not detected after 21 days storage. In contrast, *A. hydrophila* numbers were maintained or increased on high pH lamb under most storage regimes. Storage at 5°C allowed significant increases in *A. hydrophila* numbers on high pH lamb under all the atmospheres, except 100% CO_2. In lamb held at 5°C under 100% CO_2 for up to 42 days, *A. hydrophila* was recovered from most samples, although cell numbers decreased during storage. After storage at 0°C, *A. hydrophila* was recovered from high pH packs stored under all atmospheres. Significant increases in cell numbers were only observed in minced lamb of high pH stored under air or vacuum. The pH values of lamb pieces and mince held at 5 or 0°C under any of the packaging atmospheres did not change in a uniform manner during storage.

Keywords: *A. hydrophila*; MAP; Lamb, Normal pH; High pH

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1. Introduction

Aeromonas hydrophila is a Gram-negative, motile, facultative anaerobe belonging to the family Vibrionaceae. It can produce a number of potential virulence factors including cytotoxin, haemolysin, and enterotoxins (Krovacek et al., 1991) and has, in recent years, become recognised as a potential pathogen of commercial and clinical significance. *A. hydrophila* is well known as an important cause of disease in fish, amphibia and reptiles (Austin and Allen-Austin, 1985). More recently, it has received increased attention as an emerging human pathogen (Altwegg and Geiss, 1989).

A. hydrophila occurs widely in the environment and is frequently associated with water supplies (Cunliffe and Adcock, 1989). It has been isolated from a variety of foods including vegetables (Callister and Agger, 1987), meat (Okrend et al., 1987), fish (Gobat and Jemmi, 1993) and chicken (Hänninen, 1993). In a survey by Palumbo et al., (1985), *A. hydrophila* was detected in the range of foods examined including red meat, chicken, raw milk and seafood. Perhaps more significantly, this study noted that *A. hydrophila* numbers increased in these products during storage at 5°C. Thus, like *Listeria monocytogenes* and *Yersinia enterocolitica*, *A. hydrophila* is capable of growth in foods at refrigeration temperatures (Palumbo, 1986). As a facultative psychrotrophic anaerobe, *A. hydrophila* may multiply under storage conditions which suppress the aerobic flora, leading to the development of clinically significant numbers of *A. hydrophila* in organoleptically acceptable food products. Modified atmosphere packaging (MAP), frequently used to extend product shelf life, creates such conditions. There have, however, been few studies of the growth and survival of *A. hydrophila* under MAP.

Palumbo (1988) reported this species as particularly sensitive to pH values less than 6.0 and therefore not likely to be an organism of concern in foods of lower pH. While most meat products would have pH values of less than 6.0, such products as high pH lamb may however support the growth of *A. hydrophila*. The objective of the present study was to examine the survival and growth of *A. hydrophila* on normal pH (5.5–5.8) and high pH (> 6.0) lamb pieces and mince packaged under a range of modified atmospheres, i.e., air, vacuum pack, 80% O_2/20% CO_2, 50% CO_2/50% N_2 or 100% CO_2 and stored at 5 or 0°C.

2. Materials and methods

2.1. Lamb of normal pH

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 chilled for 16 h in air of 4°C, r.h. 85–90% and speed of < 0.2 m/s, until the temperature of the deep round reached 4°C. 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. Muscle pH was

measured using the procedure described below. The muscle pieces were mixed and stored in sterile plastic bags at 0°C before use. Samples were removed from the bulked material and tested for the presence of *Aeromonas* spp. by the enrichment method described below.

2.2. Lamb of high pH

Lambs were purchased and rested overnight as described above. Meat of high pH was produced by injecting the lambs with approximately 0.15 mg/kg of adrenaline (Antigen Pharmaceuticals Ltd., Roscrea, Ireland), 2 h before slaughter. Carcasses were chilled as described above. Muscles with a pH above 6.0 were selected from carcasses, dissected into small pieces and all visible fat was removed. Muscle pH was measured using the procedure described below. The muscle pieces were mixed and stored in sterile plastic bags at 0°C before use. Samples were removed from the bulked material and tested for the presence of *Aeromonas* spp. by the enrichment method described below.

2.3. Organism

The organism used was *A. hydrophila* 8049 NCTC supplied by Mr P. van Netten, Public Health Laboratory, Colindale, London, England. This organism was confirmed as a Gram-negative, catalase positive, oxidase positive rod, displaying a fermentative reaction in Hugh and Leifson medium, producing gas from glucose in glucose broth, and hydrolysing aesculin (Havelaar et al., 1992). Colonies displayed the typical *A. hydrophila* morphology on starch ampicillin agar, i.e., yellow-honey coloured colonies, 3 to 5 mm in diameter, surrounded by a clear zone (Palumbo et al., 1985). The organism was maintained on tryptone soya agar plates (Oxoid, Unipath Ltd., Basingstoke, Hampshire, England) at 0°C.

2.4. Microbiological analysis

Two methods were used in the analysis of meat samples.

2.4.1. Direct plating

Samples (10 g) were homogenised in a Colworth Stomacher (Model BA6021, A.J. Seward and Company Ltd., London, England) for 1 min with 20 ml of maximal recovery diluent (MRD, Becton Dickinson Microbiology Systems (BBL), Cockeysville, MD 21030, USA). Numbers of *A. hydrophila* were estimated by spreading duplicate samples of 1 ml or 0.1 ml of homogenate, or successive 10-fold dilutions in MRD, on the surface of starch ampicillin agar plates (Palumbo et al., 1985) with a sterile glass rod. Before incubation, the 1 ml plates were placed in a laminar air flow cabinet (Nuaire, Class II, type A, 2100 Fernbrook Lane, Plymouth, Minnesota 55447, USA) for approximately 30 min to remove excess liquid from the surface of the agar.

2.4.2. Enrichment method

Samples (25 g) were homogenised with 225 ml of alkaline peptone water (pH 8.6, Oxoid) in a Colworth stomacher for 1 min and incubated at 25°C for 24 h. Following incubation, a loopful of the homogenate was successively streaked on two starch ampicillin agar plates using a sterile inoculating loop.

Starch ampicillin agar plates were placed in a 11 anaerobic jar containing two deoxidisers (Ageless SS-200, Mitsubishi Corporation, 6-3 Marunouchi 2-Chome Chiyoda-Ku, Tokyo, Japan), a moist piece of paper and an anaerobic indicator (BBL 70504). Immediately after sealing, the jar was subjected to a vacuum of −0.8 bar for 1 min.

After incubation at 30°C for 48 h, the plates were removed and examined for colonies exhibiting typical *A. hydrophila* morphology.

2.5. Aerobic plate counts

Aerobic plate counts were obtained on All Purpose Tween (APT; BBL) agar. Duplicate plates were inoculated with the homogenate, or successive 10-fold dilutions in MRD, using a spiral plate maker (Don Whitley Scientific Ltd., 14 Otley Road, Shipley, West Yorkshire, England) and incubated at 25°C for 3 days.

2.6. Inoculation

Approximately 14 kg each of normal or high pH lamb pieces per replicate were inoculated by immersion for 5 s in MRD, containing 1000–1500 *A. hydrophila* organisms/ml. Excess liquid was allowed to drain off the meat. Minced lamb was prepared by double mincing approximately 7 kg of inoculated normal or high pH 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, England), sterilised by autoclaving at 121°C for 15 min. These procedures yielded lamb pieces and mince of normal or high pH with initial *A. hydrophila* counts of approximately $\log_{10}$ 1–2 cfu/g. The inoculated lamb pieces and mince of normal or high pH were randomly distributed over each atmosphere/temperature/time combination. Three replicates were prepared for each atmosphere/temperature/time combination using normal and high pH lamb.

2.7. Packaging

The inoculated lamb pieces and mince of normal or high pH were weighed out in 100 g lots into Dynopack (Dynopack AS, Postboks 1514, Valhalla N-4602, Kristiansand, S. Norway) high density polyethylene trays which had an oxygen transmission rate (OTR) of 3.5 $cm^3/m^2/24$ h/atm at 25°C and 50% r.h.. 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/atm at 25°C and 50%

r.h.. The packs were flushed and filled with a modified gas atmosphere or air and sealed using a Mecapac 500 semi-automatic packaging machine (rue Diderot, 93170 Bagnolet, France). A KM 100-3 M gas mixer (Witt Gasetechnik, D-5810 Witten 1, Postfach 2550, Germany) was used to mix food grade CO_2, O_2, and N_2 (Air Products PLC., Molesey Road, Walton-on-Thames, England) 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 stated by the manufacturer was 475 ml, giving a meat to gas 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., Beech Road, Unit 400, Western Industrial Estate, Naas Road, Dublin, Ireland) with an OTR of 48 $cm^3/m^2/24$ h/atm at 25°C and 50% r.h.. The bags were filled with 100g of inoculated lamb pieces or mince of normal or high pH and heat sealed using a Swissvac vacuum packaging machine (Model 380, Swissvac (GB) Ltd., Marish Wharf, St. Mary's Road, Langley, Berkshire, England). After packing, the vacuum packs were dipped in water at 90°C for 5 s to shrink the bags.

Table 1
Survival of *A. hydrophila* over 42 days on lamb pieces of normal pH packaged in air and different modified atmospheres at 5°C

Air	Time (days)						
Replicate	0	7	14	21	28	35	45
I	1.18 [a]	+ [b]	+	–	–	–	–
II	1.08	0.65	+	–	–	–	–
III	1.94	1.54	1.76	+	+	–	–
Vacuum pack							
I	1.18	+	–	–	–	–	–
II	1.08	0.18	–	0.18	–	–	–
III	1.94	+	–	–	–	–	–
80% O_2/20% CO_2							
I	1.18	+	–	–	–	–	–
II	1.08	0.65	2.38	2.63	–	–	–
III	1.94	+	–	–	–	–	–
50% CO_2/50% N_2							
I	1.18	+	+	–	–	–	–
II	1.08	0.48	0.18	+	–	–	–
III	1.94	0.18	0.18	+	–	+	–
100% CO_2							
I	1.18	+	+	–	–	–	–
II	1.08	–	–	+	–	–	–
III	1.94	0.18	0.49	+	–	–	–

[a] $\log_{10}$ cfu/g.
[b] + = positive for *A. hydrophila* after enrichment; – = negative for *A. hydrophila* after enrichment.

2.8. Storage

All packs were stored at 5 or 0°C for up to 42 days. At 7-day intervals, 20 packs each of normal or high pH lamb pieces or mince were removed and examined.

2.9. Gas analysis

A Gow-Mac gas chromatograph (Spectra 250, Gow-Mac Instrument Co. Ltd., Industrial Estate, Shannon, Co. Clare, Ireland) fitted with an Alltech Speciality CTRI column, was used to confirm the individual gas ratios of the gas mixtures before packaging and to analyse head space contents when packs were opened for microbiological examination. A CI-4100 Integrator (Milton Roy, LDC Division, Building 89, Industrial Estate, Shannon, Co. Clare, Ireland) was used to plot the chromatographs and calculate the gas percentages from the areas under the curves using the normalisation method (Anon, 1987).

Table 2
Growth/survival of *A. hydrophila* over 42 days on lamb pieces of high pH packaged in air and different gas atmospheres at 5°C

Air	Time (days)						
Replicate	0	7	14	21	28	35	42
I	1.29 [a]	3.20	4.21	7.26	6.52	5.86	6.98
II	1.87	4.69	5.57	7.46	6.02	7.06	7.56
III	1.50	2.32	5.34	5.22	7.00	– [b]	5.95
Vacuum pack							
I	1.29	2.46	3.38	3.48	5.83	4.45	5.02
II	1.87	5.12	6.59	5.29	5.06	6.25	6.08
III	1.50	2.74	4.16	2.48	–	1.48	+
80% O_2 /20% CO_2							
I	1.29	2.46	3.43	5.72	6.97	6.71	0.78
II	1.87	1.82	3.41	5.48	5.64	7.59	6.32
III	1.50	3.06	3.95	4.76	4.64	6.70	5.18
50% CO_2 /50% N_2							
I	1.29	1.13	2.11	1.13	2.48	2.44	2.18
II	1.87	2.26	2.88	1.48	3.14	3.23	4.35
III	1.50	1.02	2.39	5.02	–	1.54	+
100% CO_2							
I	1.29	0.78	+	0.78	0.18	0.18	0.65
II	1.87	1.81	1.77	1.08	0.78	1.02	0.48
III	1.50	1.71	1.22	0.88	+	+	0.18

[a] $\log_{10}$ cfu/g.
[b] + = positive for *A. hydrophila* after enrichment; – = negative for *A. hydrophila* after enrichment.

2.10. pH measurement

When packs were opened, a 2 g sample was removed and added to 10 ml of a solution of sodium iodoacetate (5 mM; BDH Chemicals Ltd., Poole, England) and potassium chloride (150 mM; AnalaR*, BDH) (Bendall, 1973). The sample was homogenised (Silverson Machines Ltd., Waterside, Chesham, Bucks, England) for two 15-s intervals with a 5-s interval between treatments. A combination electrode (Model 91-06, ATI Orion Europe, York Street, Cambridge, England) was inserted into the homogenate and the pH recorded using an Orion pH meter (Model 221). Before each determination the meter was calibrated using standard phosphate buffers of pH 4.01 and pH 7 (Radiometer Analytical A/S, Bagsvaerd, Denmark).

2.11. Statistical analysis

The data for the aerobic counts were analysed as a split-plot design in which meat type was the main plot effect and pack type and storage temperature were

Table 3
Growth/survival of *A. hydrophila* over 42 days on minced lamb of high pH packaged in air and different gas atmospheres 0°C

Air	Time (days)						
Replicate	0	7	14	21	28	35	42
I	1.08 [a]	1.13	+ [b]	3.22	4.41	3.68	5.58
II	2.06	1.92	1.86	+	+	−	−
III	1.56	1.41	1.43	1.13	1.43	+	2.31
Vacuum pack							
I	1.08	0.18	0.18	0.18	3.51	4.24	5.12
II	2.06	1.95	1.52	1.18	1.43	1.45	+
III	1.56	1.43	2.54	2.78	5.08	3.18	6.24
80% O_2/20% CO_2							
I	1.08	0.48	0.18	+	−	+	0.48
II	2.06	1.73	1.02	1.73	1.29	1.38	1.13
III	1.56	1.54	1.08	0.18	0.18	2.79	+
50% CO_2/50% N_2							
I	1.08	1.08	0.48	0.48	+	+	+
II	2.06	1.52	3.25	1.82	1.81	1.72	1.26
III	1.56	1.29	0.48	1.18	1.26	1.18	1.02
100% CO_2							
I	1.08	0.65	0.48	+	+	−	+
II	2.06	1.56	1.35	1.29	1.57	1.71	1.77
III	1.56	1.48	0.95	1.02	0.18	−	+

[a] $\log_{10}$ cfu/g.
[b] + = positive for *A. hydrophila* after enrichment; − = negative for *A. hydrophila* after enrichment.

the sub plot effects. Differences between means were determined using one and two tailed *t*-tests as appropriate.

3. Results

The results for headspace gas analysis are not presented. However, several generalisations can be made for lamb of normal and high pH, stored at 5 or 0°C in the different gas atmospheres. The percentage of CO_2 in 80% O_2/20% CO_2 increased to approximately 95% at 5°C and 28% at 0°C over 42 days. N_2 was detected in these packs during storage but could not be measured by the calibration method. In 50% CO_2/50% N_2, CO_2 decreased to about 37% during storage and there was only a small difference between the two temperatures. The level of CO_2 also declined to about 90% in packs initially containing 100% CO_2 with only a small difference being noted between 5 and 0°C. Small concentrations of O_2 were detected in the 50% CO_2/50% N_2 (< 0.1%) and 100% CO_2 (< 0.2%) packs during storage.

Aeromonas hydrophila was not detected in the normal pH meat examined before inoculation by either the direct or the enrichment method. The direct method determined that replicate I of the high pH meat had an initial count of $\log_{10}$ 0.72 cfu/g. Replicate III of the high pH meat was found to contain *A. hydrophila* before inoculation using the enrichment method.

Growth and/or survival of *A. hydrophila* on lamb pieces and mince of normal or high pH was very variable within and between replicates. In consequence, the results were not analysed statistically. They are presented as direct counts from starch ampicillin agar plates ($\log_{10}$ cfu/g) or as positive (+) or negative (−) after enrichment in alkaline peptone water. *A. hydrophila* numbers did not increase on lamb pieces of normal pH, in any of the atmospheresat 5°C (Table 1) . Similar patterns of suvival were observed for lamb pieces of normal pH at 0°C and for minced lamb of normal pH at 5 or 0°C (not shown).

Table 4
Mean aerobic plate counts ($\log_{10}$cfu/g) at 42 days on lamb pieces of normal pH packaged in different gas atmospheres at 5 or 0°C

Gas atmosphere	Storage temperature (°C)	
	5	0
Air	9.06	8.77
Vacuum pack	8.24	7.37
80% O_2 /20% CO_2	9.24	8.40
50% CO_2 /50% N_2	8.14	7.56
100% CO_2	8.12	6.12

Standard error of difference between means = 0.42.
Degrees of freedom = 36.

Table 5
Mean aerobic plate counts (log_{10}cfu/g) at 42 days on minced lamb of normal pH packaged in different gas atmospheres at 5 or 0°C

Gas atmosphere	Storage temperature (°C)	
	5	0
Air	8.86	8.78
Vacuum pack	8.34	7.84
80% O_2 /20% CO_2	10.25	8.89
50% CO_2 /50% N_2	8.72	8.54
100% CO_2	8.29	7.36

Standard error of difference between means = 0.42.
Degrees of freedom = 36.

Increases in *A. hydrophila* counts were observed on high pH lamb pieces stored at 5°C in all atmospheres, except 100% CO_2 (Table 2). Under this atmosphere, although *A. hydrophila* survived, numbers remained low. A similar pattern of survival and/or growth was observed for high pH minced lamb stored at 5°C (not shown). In general, *A. hydrophila* counts on high pH lamb were lower in samples stored under higher CO_2 concentrations.

At 0°C, *A. hydrophila* counts increased on minced lamb of high pH in some cases in some atmospheres, i.e., air –replicate I only; vacuum pack –replicates I and III only (Table 3). On lamb pieces of high pH at 0°C, there was no crease in *A. hydrophila* counts in any of the atmospheres (not shown). However, the organism generally survived on normal pH lamb pieces and mince in all the atmospheres at 0°C.

In general, on lamb pieces of normal pH stored at 5°C, small but significant differences in aerobic plate counts were observed between atmospheres after 42 days ($p < 0.05$) (Table 4). Differences between atmospheres were also noted at 0°C, with the largest difference occurring between air and 100% CO_2 ($p < 0.001$). Although counts at 0°C appeared to be lower than at 5°C, the differences were not significant in all the atmospheres ($p < 0.05$).

Table 6
Mean aerobic plate counts (log_{10}cfu/g) at 42 days on lamb pieces of high pH packaged in different gas atmospheres at 5 or 0°C

Gas atmosphere	Storage temperature (°C)	
	5	0
Air	9.09	8.71
Vacuum pack	8.28	7.90
80% O_2 /20% CO_2	9.22	8.18
50% CO_2 /50% N_2	8.18	6.77
100% CO_2	7.58	5.03

Standard error of difference between means = 0.44.
Degrees of freedom = 36.

Table 7
Mean aerobic plate counts ($\log_{10}$cfu/g) at 42 days on minced lamb of high pH packaged in different gas atmospheres at 5 or 0°C

Gas atmosphere	Storage temperature (°C)	
	5	0
Air	8.82	9.05
Vacuum pack	8.38	8.16
80% O_2/20% CO_2	9.25	8.70
50% CO_2/50% N_2	8.19	7.68
100% CO_2	8.10	6.83

Standard error of difference between means = 0.44.
Degrees of freedom = 36.

On minced lamb of normal pH at 5°C, significant differences in aerobic plate counts were observed between 80% O_2/20% CO_2 and the other atmospheres ($p < 0.01$) (Table 5). At 0°C, significant differences occurred between some of the atmospheres ($p < 0.05$). The aerobic plate counts at 5°C were higher than at O°C in 80% O_2/20% CO_2 and 100% CO_2 ($p < 0.05$).

On lamb pieces of high pH stored at 5 or 0°C, counts in 50% CO_2/50% N_2 and 100% CO_2 were generally lower than under the other atmospheres ($p < 0.05$) (Table 6). Significant differences were noted between counts at 5 and 0°C in all atmospheres except air and vacuum packs ($p < 0.01$).

Small, but significant differences in aerobic plate counts were noted between atmospheres on minced lamb of high pH at 5°C ($p < 0.05$) (Table 7). At 0°C, highly significant differences were recorded between the two high CO_2 atmospheres and some of the other atmospheres ($p < 0.005$). Small differences were noted between the other atmospheres at 0°C ($p < 0.05$). Total counts at 0°C were significantly lower than at 5°C in 100% CO_2 only ($p < 0.005$).

The mean pH values and standard deviations over 42 days, for lamb pieces of normal pH, at 5 or 0°C are presented in Table 8. Similar values were recorded for minced lamb of normal pH and lamb pieces and mince of high pH (not shown). In

Table 8
Mean pH and standard deviation values over 42 days on lamb pieces of normal pH packaged in air and a range of modified atmospheres at 5 or 0°C

Gas atmosphere	Storage temperature (°C)			
	5		0	
	Mean pH	S.D.	Mean pH	S.D.
Air	5.77	0.20	5.77	0.15
Vacuum pack	5.46	0.12	5.60	0.04
80% O_2/20% CO_2	5.85	0.23	5.59	0.03
50% CO_2/50% N_2	5.51	0.04	5.61	0.02
100% CO_2	5.52	0.08	5.59	0.03

S.D. = standard deviation.

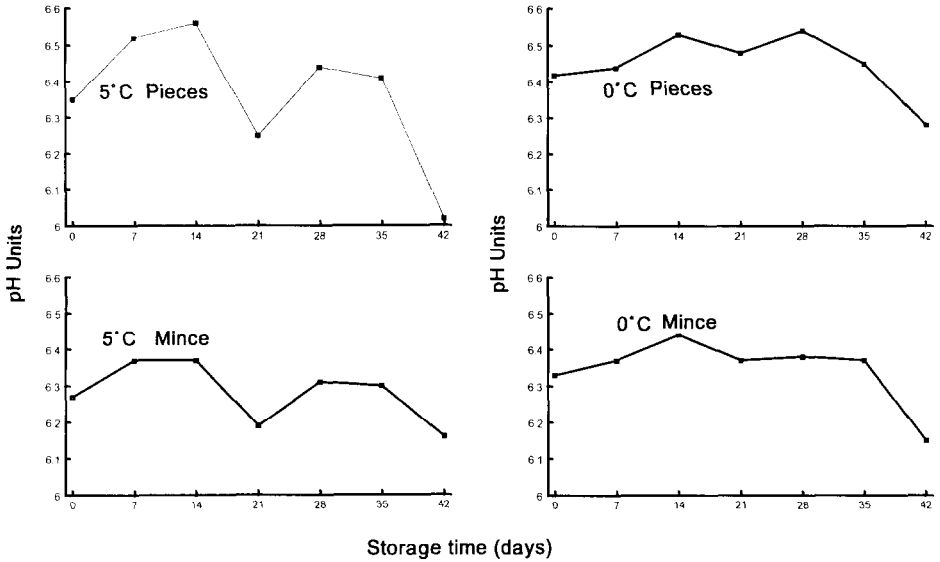

Fig. 1. Mean pH values over 42 days on lamb pieces and mince of high pH packaged in 50% CO_2 /50% N_2 and stored at 5 or 0°C.

general, pH did not increase or decrease in a uniform manner on lamb pieces and mince of normal or high pH in any of the atmospheres at 5 or 0°C. A typical example of the pH variation observed is shown in Fig. 1.

In lamb held at 5°C, higher mean pH values were observed after storage in air or in an atmosphere containing 80% O_2/20% CO_2, irrespective of product format or initial pH, i.e., normal or high pH lamb. In lamb held at 0°C, the highest mean pH values were noted in air. The range of pH values noted on normal and high pH lamb stored at 5°C was wider than the range on lamb stored at 0°C.

4. Discussion

The most noteworthy observation of the present study was the considerable variations in rates of growth and/or survival of *A. hydrophila* on MAP lamb within and between replicates. Although a similar finding has been noted by van Laack et al. (1993), other workers have not reported such variation (Palumbo, 1988; Gill and Reichel, 1989). van Laack et al. (1993) examined the growth of *A. hydrophila* on pork loins stored at 1 ± 1°C in three packaging treatments. In the first experiment, growth of *A. hydrophila* occurred on all the pork loins examined. However, in a subsequent, repeat experiment, *A. hydrophila* numbers remained nearly constant or decreased during refrigerated storage. In both experiments, the inoculum included samples of pigd faeces. The authors suggested that differences between the natural competitive microfloras of the two samples of pig faeces used may have

been responsible for the observed differences in *A. hydrophila* growth and survival rates. They also proposed that the natural microflora on the pork loins used in the second experiment was more antagonistic towards *A. hydrophila* than the microorganisms present on the loins used in the first experiment.

Earlier studies by Palumbo (1988) indicated that the growth of *A. hydrophila* may be affected by the background flora. When the normal microflora of ground pork was reduced by irradiation, more rapid growth of *A. hydrophila* K144 occurred than in non-irradiated samples. Palumbo and Buchanan (1988) stated that, while not completely inhibiting the growth of *A. hydrophila*, the background flora does limit the maximum attainable population. Inhibitory effects of the background flora have been also been reported for *Y. enterocolitica*, another psychrotrophic pathogen (Schiemann and Olson, 1984; Nielsen and Zeuthen, 1985; Fukushima and Gomyoda, 1986; Kleinlein and Untermann, 1990).

In the present study there was no evidence to indicate that the background flora was antagonistic to the growth of *A. hydrophila*. For example, on minced lamb of high pH stored at 0°C, more growth occurred in vacuum packs (replicates I and III) than in air (replicate I). This suggests that the background flora of the minced lamb packaged in air was more antagonistic to the growth of *A. hydrophila* than in vacuum packs. However, examination of the aerobic plate counts of minced lamb of high pH stored at 0°C, shows significantly higher counts in air than in vacuum packs. This suggestion that the background flora is not antagonistic to *A. hydrophila* is in agreement with Ingham and Potter (1988). These authors showed that *A. hydrophila* was able to compete with *Pseudomonas fragi* on mince and low-salt surimi.

It appears from the present study that the *A. hydrophila* counts recorded on lamb pieces and mince at 5°C were similar. As the counts were not analysed statistically, this could not be confirmed. The observed pattern is different from that noted in the case of *Y. enterocolitica* (Doherty et al., 1995). These authors reported significant differences between the counts obtained from lamb pieces and mince. Specifically they found that *Y. enterocolitica* counts from minced lamb were significantly lower than the counts on lamb pieces, under the same set of modified atmospheres as used in this study.

Growth of *A. hydrophila* occurred on lamb pieces and mince of high pH packaged in air or under vacuum in the present study. Growth of this organism in air has been observed by other workers (Palumbo et al., 1985; Palumbo, 1988). Similarly, growth of *A. hydrophila* on vacuum packaged pork (Palumbo, 1988; van Laack et al., 1993) and vacuum packaged beef (Gill and Reichel, 1989) at refrigeration temperatures has also been recorded.

On lamb pieces and mince of high pH stored at 5 or 0°C in the present study, growth of *A. hydrophila* was inhibited in an atmosphere containing 100% CO_2. Inhibition of growth has also been noted on high pH beef (> 6.0) packaged under CO_2 at 5 and 0°C (Gill and Reichel, 1989). In contrast to these results, growth of *A. hydrophila* was observed at 3°C on sliced roast beef packaged in a saturated CO_2 controlled atmosphere (Hudson et al., 1994).

Although *A. hydrophila* has been widely reported as a psychrotroph (Palumbo,

1986; Gill and Reichel, 1989; van Laack et al., 1993; Hudson et al., 1994), very limited growth of the organism was observed at 0°C in this study. In fact, while the organism survived for 42 days under all of the storage atmospheres, the only increases in pathogen numbers were noted on some replicates of minced lamb of high pH packaged in air or under vacuum.

In the present study, there was no increase in *A. hydrophila* numbers on lamb pieces or mince of normal pH stored at 5 or 0°C. On lamb pieces and mince of high pH, increases in *A. hydrophila* numbers were observed in some of the packaging treatments at 5 and 0°C. Similar results have been reported for *A. hydrophila* K144 on ground pork held at 5°C (Palumbo, 1988). The author reported that the organism was sensitive to pH values below 6.0 in the form of either a low starting pH in the pork itself, or induced by the action of lactic acid bacteria on the addition of 1% glucose. At pH 5.9 *A. hydrophila* grew more slowly and to a lower population than at pH 6.1.

A. hydrophila did not grow on MAP lamb of normal pH, thus this storage format does not appear to represent a safety hazard for the normal pH lamb products. However, the organism did grow on high pH lamb under a number of modified atmospheres. In this study the only consistent suppression of the growth of *A. hydrophila* on chilled lamb products of high pH was observed in high CO_2 storage. The ability of this species to grow on high pH products under a range of commercially applied modified atmospheres could be of clinical concern. The development of multiple hurdle mechanisms for the control of this ubiquitous organism is therefore necessary.

Growth and/or survival of *A. hydrophila* on MAP lamb is extremely variable and as yet the factors and interactions influecing such variability are unclear. A fuller understanding of these factors may allow the development and application of better methods for suppressing the survival and/or growth of *A. hydrophila* in susceptible foods.

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