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The Effect of Vacuum and Modified Atmosphere Packaging on the Shelf-life of Lamb Primals, Stored at Different Temperatures

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ABSTRACT

Lamb primals (shoulders) were vacuum packaged or packaged in modified atmospheres containing 80% O_2/20% CO_2, 50% CO_2/50% N_2 or 100% CO_2, and stored at 5 or 0°C. They were examined microbiologically at 7 day intervals for total counts obtained under (1) aerobic, (2) CO_2 enriched or (3) anaerobic conditions; B. thermosphacta; *pseudomonad and* Enterobacteriaceae *counts. Off-odour assessments were also carried out at these times. In general, there were no significant differences between the total counts obtained from the different incubation conditions in any of the atmospheres. The only exception was noted in 80% O_2/20% CO_2 at 5°C. Significant differences between atmospheres for the total counts were observed at 0°C only. In the case of* B. thermosphacta, *the pseudomonads and the* Enterobacteriaceae, *differences between atmospheres were noted at 5 and 0°C. In general, vacuum packs and 80% O_2/20% CO_2, and the two high CO_2 atmospheres fell into distinct groups. Storage temperature had a significant effect on all three counts. The relationship between bacterial counts and time was modelled using regression analysis. Data from total counts gave the equations of best fit. Significant differences between atmospheres in terms of off-odour production were observed at 5°C only. The effect of temperature on off-odour production was significant in all atmospheres except 100% CO_2. A scheme was devised based on the growth of different groups of organisms which facilitated comparisons between studies on packaged meats. The results of the present work and that of others are discussed in relation to the different growth patterns which developed with packaging treatments and storage temperature. Copyright © 1996 Elsevier Science Ltd*

INTRODUCTION

Modified atmosphere packaging (MAP) alters the environment surrounding foodstuffs, suppressing or slowing the growth of undesirable species and selecting desirable microflora which may extend the shelf-life of meat. Of the currently available packaging

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systems, vacuum packaging is most frequently used, although the use of mixed gas systems has increased. The exact nature and scale of the microflora selected in such systems is strongly dependent on the mix of gases employed and on the temperature of storage. The interaction of the packaging environment and temperature will thus have a profound effect on product shelf-life.

MAP of lamb has been investigated in carcass and primal format. Results from carcass investigations are not directly comparable to results obtained from primal examination. It is not possible to completely evacuate the carcass packaging prior to the injection of gas, resulting in an ill-defined packaging atmosphere. Limited work on lamb primals has been published (Shaw *et al.*, 1980; Gill, 1984) and in particular, information on the effect of different packaging atmospheres on the microbial flora and shelf-life has not been previously reported.

The present study examined the effect of vacuum packaging and storage in a number of different gaseous mixtures on the shelf-life of lamb primals. The primals were stored in a range of modified atmospheres at 5 and 0°C to investigate the role of temperature on shelf-life extension and microfloral selection and development.

MATERIALS AND METHODS

Animals

Lambs were purchased in local markets (mean live weight 34.0 kg; range 29.0–54.0 kg). They 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 (mean carcass weight 16.9 kg; range 14.5–27.0 kg) were not washed and were chilled for 16 h at 4°C at an air speed of $<0.2\ m\,s^{-1}$ and at a relative humidity of 85–90% until the temperature of the deep round reached 4°C. The internal and surface temperature of the deep leg were recorded hourly using copper constantan thermocouples attached to a Hewlett Packard 3054 DL data logger (Amsteleveen, The Netherlands). After chilling the pH of the striploin was measured using a spear glass electrode (Type EC-2010, Amagruss, Unit 4, Industrial Estate, Castlebar, Co. Mayo, Ireland) and an Orion portable meter (Model 201, ATI Orion Europe, York Street, Cambridge, England). Only carcasses with a pH below 5.8 were accepted for packaging.

Packaging

Primals with a mean weight of 2871 g (range 2424–3812 g) were removed from carcasses at the sixth rib, divided into left and right sides and randomly assigned to one of four packaging treatments namely: vacuum packaging, 80% O_2/20% CO_2; 50% CO_2/50% N_2 or 100% CO_2. Before packaging all exposed bone was completely covered with waxed bone guard to prevent bag puncture. Vacuum packaging was carried out using BB6 bags [W.R. Grace (Ireland) Ltd., Co. Dublin, Ireland] which have an oxygen transmission rate (OTR) of 48 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$, and a Swissvac vacuum packaging machine [Model 380, Swissvac (GB) Ltd., Langley, Berkshire, England]. After vacuum packaging the packs were dipped in a water bath at 90°C for 5 s to shrink the packaging.

A KM 100–3M (Witt Gasetechnik, Postfach, Germany) mixer was used to obtain the three gas atmospheres. Lamb primals were packaged in bags 700 mm wide × 450 mm deep made from CVP AMB 130 material using a CVP-200 gas flushing machine [CVP Systems (UK) Ltd., Unit 22 Sarum Complex, Uxbridge]. The bags had an OTR of 3.5 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$. The volume of gas in the bags was measured by immersion and displacement. Individual primals were placed in bags 700 mm wide × 450 mm deep.

The flushing cycle was: evacuation (28 bar, 15 s), gassing (5.5 s), evacuation (15 s), and final gassing (0.6 s). The first gassing introduced a larger volume into the bag (30 l) to aid in the removal of residual O_2 from the packs. The final gas volume in these packs was about 10 l, giving a gas to meat ratio of at least 2:1.

Storage

All packs were stored at 5 or 0°C for up to 28 days. At 7 day intervals, packs ($n=8$) were removed and examined. Six replicates were set up for each atmosphere/temperature combination.

Microbiology

A 10 g sample was removed from the lean surface of the lamb primal and homogenised in a Colworth Stomacher (Model BA6021, A.J. Seward & Company Ltd., London, UK) for 1 min with 90 ml of maximal recovery diluent [MRD; Becton Dickinson Microbiology Systems (BBL), Cockeysville, MD USA]. Bacterial numbers were estimated from plates surface inoculated with 0.1 ml of the neat solution or successive 10-fold dilutions in MRD. All plates were inoculated in duplicate and the inoculum spread on the surface using a sterile glass rod. The following media were used to provide presumptive counts of the lamb microflora:

Nonspecific counts

All Purpose Tween agar (APT; BBL) was inoculated and incubated at 25°C for 3 days to determine:

1. The flora present under aerobic conditions.
2. The flora present in aerobic conditions with an increased CO_2 level. The APT plates were placed in an anaerobic jar to which a CO_2 generator envelope was added (BBL 70308). The envelope was activated by the addition of 10 ml of water. This resulted in an aerobic atmosphere with 5–10% CO_2 present inside the jar.
3. The flora present under anaerobic conditions. The APT plates were placed in an anaerobic jar to which a H_2 and CO_2 generator envelope (BBL 71040) and a disposable anaerobic indicator (BBL 70504) were added. The envelope was activated by the addition of 10 ml of water. Immediately the lid was closed the jar was evacuated for 20 s using a vacuum pump.

Specific counts

Brochothrix thermosphacta and pseudomonads were isolated on STAA medium (Oxoid, Unipath Ltd., UK) and pseudomonas agar (Oxoid), respectively, incubated at 25°C for 3 days. The *Enterobacteriaceae* were enumerated using pour plates of violet red bile glucose agar (Oxoid) incubated at 30°C for 24 h.

Odour

Odour was measured by a panel of three men and three women at least 1 h after the packs were opened. The panellists were asked to assess odour and classify the meat as acceptable or unacceptable if available for sale. The assessments were carried out in well ventilated areas. Packs with very strong off-odours were not presented to the panel.

Statistical analysis

The data were analysed as a split-plot design in which pack type and storage temperature were examined as main plot effects and time, a repeated measure, as a sub-plot effect. Differences between means were determined using least significant differences or, one and two-tailed *t*-tests, as appropriate. The linear and quadratic effects of time and their interactions with the main plot effects were obtained and the most appropriate model for each atmosphere/temperature was obtained. The odour data were analysed using analysis of variance (ANOVA) to identify any significant differences between gas atmospheres and temperatures.

RESULTS

The mean total bacterial counts after 28 days storage under the different packaging treatments are shown in Table 1. In an atmosphere containing 80% O_2/20% CO_2 at 5°C, there was a significant difference between the aerobic and anaerobic counts ($P<0.05$). Apart from this, there were no significant differences in counts from APT plates incubated under aerobic, CO_2 enriched or anaerobic conditions.

The total counts obtained on primals at 28 days were not significantly different at 5°C in any of the packaging treatments. At 0°C, there were no significant differences at 28 days between the total counts in vacuum packs and 80% O_2/20% CO_2. A similar result was observed for the high CO_2 atmospheres (50% CO_2/50% N_2 and 100% CO_2). However, at this temperature, the total counts in the high CO_2 atmospheres were significantly lower than those in vacuum packs and 80% O_2/20% CO_2 ($P<0.001$).

Overall, the data shows that a combination of high CO_2 and low temperature storage (0°C) caused a significant reduction in the total bacterial counts. In contrast, high CO_2 levels had no effect on the total counts at 5°C.

In general, at 28 days, the mean presumptive *B. thermosphacta* counts at 5 and 0°C, were significantly reduced in the high CO_2 atmospheres compared to vacuum packs and 80% O_2/20% CO_2 ($P<0.05$) (Table 2a). The effect of temperature for the individual atmospheres was significant in vacuum packs and 100% CO_2 ($P<0.05$).

TABLE 1

Mean Total Bacterial Counts ($\log_{10}$ cfu g^{-1}) at 28 Days, from APT Plates Incubated (1) Aerobically, (2) in a CO_2-enriched, Aerobic Atmosphere and (3) Anaerobically, on Lamb Primals Packaged in Different Gas Atmospheres at 5 or 0°C

Storage temperature (°C)	*Vacuum pack*			*80% O_2/20% CO_2*			*50% CO_2/50% N_2*			*100% CO_2*		
	1[a]	*2*[b]	*3*[c]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
5	7.8	7.7	7.8	8.5	8.5	7.6	8.1	7.8	7.3	7.2	7.3	7.5
0	6.3	6.5	6.6	7.4	7.2	7.1	4.2	4.0	3.9	3.9	3.9	3.8

Standard error of differences between means = 0.76, except when comparing means with the same level of atmosphere/temperature = 0.30.

Degrees of freedom = 61.

[a]APT plates incubated aerobically.

[b]APT plates incubated in an aerobic CO_2 enriched atmosphere.

[c]APT plates incubated anaerobically.

At 5°C, there was a significant difference between the pseudomonad counts recorded in vacuum packs and 80% O_2/20% CO_2 ($P < 0.05$) (Table 2b). Apart from this, the atmospheres could again be considered as two groups, namely vacuum packs and 80% O_2/20% CO_2, 50% CO_2/50% N_2 and 100% CO_2. These groups were significantly different from each other at 5 and 0°C. Within atmospheres, significant differences in growth were observed between temperatures, the exception being 100% CO_2 ($P < 0.05$).

Examination of the data for the *Enterobacteriaceae* showed that the same grouping of atmospheres could usually be made as for *B. thermosphacta* and the pseudomonads (Table 2c). In general, *Enterobacteriaceae* counts in the high CO_2 atmospheres were significantly reduced compared to vacuum packs and 80% O_2/20% CO_2. The effect of temperature was significant in vacuum packs and 50% CO_2/50% N_2 ($P < 0.05$).

TABLE 2

Mean Presumptive (a) *Brochothrix thermosphacta*, (b) Pseudomonad and (c) *Enterobacteriaceae* Counts ($\log_{10}$ cfu g^{-1}) at 28 Days from Lamb Primals Packaged in Different Modified Atmospheres at 5 or 0°C

(a)

	Storage temperature (°C)	
Gas atmosphere	5	0
Vacuum pack	6.1	4.2
80 O_2/20% CO_2	6.9	5.8
50% CO_2/50% N_2	3.9	2.7
100% CO_2	2.8	1.0

Standard error of difference between means = 1.07
Degrees of freedom = 31

(b)

	Storage temperature (°C)	
Gas atmosphere	5	0
Vacuum pack	5.2	3.4
80 O_2/20% CO_2	7.5	4.8
50% CO_2/50% N_2	2.9	0.0
100% CO_2	2.3	1.4

Standard error of difference between means = 0.86
Degrees of freedom = 34

(c)

	Storage temperature (°C)	
Gas atmosphere	5	0
Vacuum pack	6.0	3.1
80 O_2/20% CO_2	4.8	3.5
50% CO_2/50% N_2	3.4	0.5
100% CO_2	2.2	0.5

Standard error of difference between means = 1.31
Degrees of freedom = 34

Relationship between bacterial growth and time

In the present study, the relationship between bacterial counts and time was represented using mathematical equations. The equations of best fit were obtained by regressing mean bacterial numbers ($\log_{10}$ cfu g^{-1}) against time. Although analysis based on means takes no account of the variation between individual replicates, the trends in bacterial growth are well represented by the mean data.

In all cases, where a relationship existed, a single equation was adequate to represent the data from APT plates incubated under (1) aerobic, (2) CO_2 enriched and (3) anaerobic conditions, in each packaging treatment. Highly significant relationships were observed between total counts and time in all atmospheres at 5°C ($R^2 \geq 0.98$) (Table 3). At 0°C, relationships between bacterial counts and time were observed in vacuum packs and in an atmosphere containing 80% O_2/20% CO_2. There were no significant growth relationships in the high CO_2 atmospheres at this temperature.

At 5°C, 100% CO_2 was the only atmosphere where there was no relationship between *B. thermosphacta* counts and time (Table 4). There were good relationships in all the other atmospheres at this temperature as indicated by the high R^2 values ($R^2 = 0.98$). At 0°C, a relationship between the growth of *B. thermosphacta* and time was evident in vacuum packs and 80% O_2/20% CO_2. There were no significant relationships between counts and time in the high CO_2 atmospheres at 0°C.

The data for presumptive pseudomonad and *Enterobacteriaceae* counts are shown in Table 5. There were no relationships between pseudomonad counts and time in the high

TABLE 3
The Relationship Between Total Counts and Time ($\log_{10}$ cfu g^{-1}) from APT Plates on Lamb Primals Packaged in Different Modified Atmospheres at 5 or 0°C

Gas atmosphere	*Storage temperature (°C)*	*Intercept*	*Linear coefficient*	*Quadratic coefficient*	R^2	*RSD*
Vacuum pack	5	3.81	+0.43	−0.009	0.99	0.35
	0	4.13	−0.001	+0.004	0.99	0.11
80% O_2/20% CO_2	5	3.76	+0.38	−0.007	0.98	0.41
	0	4.40	+0.11		0.94	0.37
50% CO_2/50% N_2	5	3.73	+0.18		0.99	0.19
100% CO_2	5	3.88	+0.18		0.98	0.33

TABLE 4
The Relationship Between Presumptive *Brochothrix thermosphacta* Counts ($\log_{10}$ cfu g^{-1}) and Time on Lamb Primals Packaged in Different Modified Atmospheres at 5 or 0°C

Gas atmosphere	*Storage temperature (°C)*	*Intercept*	*Linear coefficient*	*Quadratic coefficient*	R^2	*RSD*
Vacuum pack	5	0.17	+0.46	−0.008	0.98	0.53
	0	0.46	+0.17		0.92	0.65
80% O_2/20% CO_2	5	0.63	+0.64	−0.01	0.98	0.64
	0	0.22	+0.21		0.97	0.51
50% CO_2/50% N_2	5	0.14	+0.32	−0.006	0.98	0.33

CO_2 atmospheres at 5 or 0°C. The best relationships for the other two atmospheres were observed at 5°C. The data for the *Enterobacteriaceae* showed that there were good relationships for vacuum packs and 80% O_2/20% CO_2 at 5 and 0°C ($R^2 \geq 0.98$). The only relationship in the high CO_2 atmospheres was in 100% CO_2 at 0°C.

Odour

The results of the assessment of packs for off-odours are shown in Table 6. Significant differences in off-odour production between atmospheres were observed at 5°C ($P < 0.01$). Two distinct groups emerged, i.e., vacuum packs and 80% O_2/20% CO_2, 50% CO_2/50% N_2 and 100% CO_2. At 0°C, there were no significant differences between atmospheres in terms of off-odour production. Temperature had a significant effect on off-odour production in all atmospheres except 100% CO_2 ($P < 0.05$).

TABLE 5

The Relationship Between Growth and Time for Presumptive Pseudomonad and *Enterobacteriaceae* Counts ($\log_{10}$ cfu g^{-1}) on Lamb Primals Packaged in Different Modified Atmospheres and Stored at 5 and 0°C

Gas atmosphere	*Storage temperature (°C)*	*Intercept*	*Linear coefficient*	*Quadratic coefficient*	R^2	*RSD*
			Pseudomonad counts			
Vacuum pack	5	1.53	+0.36	−0.007	0.99	0.25
	0	1.56	+0.08		0.90	0.31
80% O_2/20% CO_2	5	1.76	+0.25		0.95	0.74
			Enterobacteriaceae counts			
Vacuum pack	5	0.54	+0.52	−0.01	0.99	0.38
	0	0.62	+0.28	−0.007	0.98	0.25
80% O_2/20% CO_2	5	0.49	+0.49	−0.01	0.99	0.39
	0	0.62	+0.01	+0.005	0.99	0.16
100% CO_2	0	0.58	+0.21	−0.008	0.98	0.16

TABLE 6

Mean Percentage (%) of the Total Number of Packs Containing Lamb Primals, Packaged in Different Modified Atmospheres at 5 or 0°C, which were Considered Acceptable on Examination at 28 Days

	Storage temperature (°C)	
	5	*0*
Gas atmosphere		
Vacuum pack	11.0	72.0
80 O_2/20% CO_2	32.0	71.0
50% CO_2/50% N_2	63.0	94.0
100% CO_2	70.0	97.0

Standard error of difference between means = 0.17.
Degrees of freedom = 34.

DISCUSSION

It is extremely difficult to obtain an agreed picture of the patterns of bacterial growth and the changes involved in the development of spoilage in complex biological materials, such as meat and meat products. Many workers have investigated the ability of various organisms to cause spoilage, in particular the lactic acid bacteria, *B. thermosphacta*, pseudomonads and *Enterobacteriaceae*, on fresh beef, pork and lamb packaged under vacuum or different gas atmospheres. In order to define patterns of bacterial growth and spoilage, the present authors examined the growth of these organisms on packaged meats.

It has been reported that meat spoilage does not occur until the total bacterial count reaches a level of 10^{6-8} organisms g^{-1} or cm^{-2} (Gill, 1983). This count is composed of combinations of each of the different groups of organisms referred to above, at levels generally below that of the total count. In order to play a role in the spoilage process, bacteria must grow and produce metabolites that contribute to the development of off-odours. In the present discussion, growth was defined in terms of the difference between the initial and final bacterial counts on completion of the storage period, for each group of organisms present. An arbitrary increase of 10^3 organisms g^{-1} or cm^{-2} for each group, was used to define growth in the current and other studies as 'high growth'. For example, growth from an initial level of log 2 to a final level of log 5 indicated 'high growth'. Increases of less than 10^3 organisms g^{-1} or cm^{-2} were considered to represent 'low growth'. It seems reasonable to assume that 'high growth' organisms will have a major effect on the development of off-odours, while those showing 'low growth' will not. It would however, be unwise to assume that the dominant microflora will be necessarily responsible for dictating the course of spoilage development in every case (Ingram & Dainty, 1971). In one study by Gill and Harrison (1989), 'high growth' of the lactic acid bacteria was observed on pork packaged in 100% CO_2 at −1.5°C. The authors however, attributed spoilage to 'low growth' of *Enterobacteriaceae* (log 3.08 cm^{-2} at 12 weeks), although it has been shown that menbers of the lactic acid bacteria can also produce putrid off-odours (Borch & Agerhem, 1992)

Using the above criteria, the growth patterns of a range of organisms, from this study or derived from the literature, were obtained for the temperature ranges −1.5–2°C and 3–7°C, for packaging films of low O_2 permeabilities (< 73 cm^3 m^{-2} 24 h^{-1} atm^{-1}). In some instances spoilage may not have occurred, especially at low temperatures (−1.5–0°C). It is assumed however, that the pattern of spoilage can still be determined from the growth of the organisms present. Thus, in the present study, the spoilage pattern of vacuum packaged lamb stored at 5°C, was attributed to 'high growth' of lactic acid bacteria, *B. thermosphacta*, pseudomonads and *Enterobacteriaceae*. A similar pattern has been observed for this product stored at 7°C, except that the lactic acid bacteria were not detected (Patterson & Gibbs, 1978).

In further experiments not reported in this study, lamb primals were stored for 42 days in vacuum packs at 0°C. The observed spoilage pattern was attributed to 'high growth' of lactics, *B. thermosphacta* and *Enterobacteriaceae*. This spoilage pattern is broadly in accordance with the results of Gill (1984), although this author suggested that spoilage was mainly due to 'high growth' of lactics and *Enterobacteriaceae*, and in some instances, *B. thermosphacta*. Analysis of data in the literature showed two spoilage patterns. One pattern consisted of 'high growth' of lactic acid bacteria, *B. thermosphacta* and pseudomonads (Patterson & Gibbs, 1978). An alternative pattern was observed due to 'high growth' of *B. thermosphacta* only (Shaw *et al.*, 1980).

Three spoilage patterns were identified in vacuum packaged beef. One pattern observed within the two temperature ranges consisted of 'high growth' of the lactics and low growth of the other organisms (Pierson *et al.*, 1970; Erichsen & Molin, 1981; Taylor *et*

al., 1990). The remaining two spoilage patterns were recorded for meat stored at 0–2°C. Applying the criteria outlined above, the data of Sutherland *et al.* (1975) could be interpreted as 'high growth' of lactics, *B. thermosphacta* and pseudomonads, while the results of Rousset and Renerre (1991) would be recorded as 'high growth' of lactics and *Enterobacteriaceae*.

On vacuum-packaged pork stored at 3°C, spoilage was attributed to 'high growth' of the lactic acid bacteria and *B. thermosphacta* (Gill & Harrison, 1989). At −1.5–1°C, the spoilage patterns were due to 'high growth' of lactics only (Gill & Harrison, 1989) or a combination of 'high growth' of lactics and *Enterobacteriaceae* (Taylor *et al.*, 1990).

In the present study, the spoilage pattern of lamb primals packaged in an atmosphere containing 80% O_2/20% CO_2 at 0°C, was due to 'high growth' of lactic acid bacteria, *B. thermosphacta*, pseudomonads and *Enterobacteriaceae*. This pattern is at variance with the results obtained by Newton *et al.* (1977) where the spoilage of lamb chops stored at −1°C was attributed entirely to 'high growth' of *B. thermosphacta*.

The results of this study showed 'high growth' of the lactic acid bacteria and 'low growth' of the other organisms on lamb primals packaged in 100% CO_2 at 5°C. A similar pattern emerged for lamb primals packaged in 100% CO_2 at 0°C, but this only became evident when the storage period was extended from 28 to 42 days.

Three patterns of bacterial development could be derived for pork packaged in an atmosphere containing 100% CO_2 at 3–4°C. In one study, 'high growth' of the lactic acid bacteria and 'low growth' of the other organisms was noted (Enfors *et al.*, 1979). The second pattern, obtained from the results of Gill and Harrison (1989), showed 'high growth' of the lactic acid bacteria and *B. thermosphacta*. The third pattern consisted of 'high growth' of lactics and *Enterobacteriaceae* (Blickstad *et al.*, 1981; Blickstad & Molin, 1983; Grant & Patterson, 1991).

The results of studies involving storage of meat in high CO_2 atmospheres at −1.5–2°C, indicated 'high growth' of lactic acid bacteria only, in the case of beef (Rousset & Renerre, 1991) and of pork (Blickstad & Molin, 1983; Gill & Harrison, 1989).

The above discussion clearly demonstrates that different patterns of bacterial growth exist on packaged meats. These have been observed to vary within and between meat species, primarily depending on the packaging environment and storage temperature. Other extrinsic factors may be involved including initial counts and the composition of the bacterial flora (Gill & Penney, 1988), drip (Khan *et al.*, 1975) and the physical form of the meat (Doherty *et al.*, 1995; Sheridan *et al.*, 1995).

Intrinsic factors within the meat may also contribute to the spoilage pattern which develops. These include such variables as lean to fat ratio (Grau, 1983; Vanderzant *et al.*, 1986), and pH. Although the effects of pH extremes on bacterial growth are well documented (Erichsen & Molin, 1981; Rousset & Renerre, 1991), the influence of small incremental change is unknown.

Levels of muscle metabolites, such as glucose and glucose-6-phosphate, may also be important as they are utilised during the aerobic and anaerobic growth of bacteria on meat (Gill & Newton, 1977; Newton & Gill, 1978). Since the rate at which such metabolites become available, varies within (Crouse & Smith, 1986) and between meat species (Lawrie, 1974), their presence may greatly influence the emerging and final microflora.

The exact mechanisms of off-odour development resulting from microbial metabolism are unknown, although the rate and extent of development is influenced by storage conditions and temperature. The recognition of a wide range of different spoilage patterns makes it extremely difficult to assign off-odour production to specific organisms or groups of organisms.

The results of the present study showed that regression analysis could be used to model the relationship between bacterial counts and time; with the equations of best fit being

either linear or quadratic. The use of a predictive mathematical model to describe the relationship between the growth of lactic acid bacteria on vacuum packaged meat has been carried out previously (Nicolai *et al.*, 1993). These authors concluded that the data was best represented by a sigmoidal log-linear growth curve. Although lag time was taken into account in their model, it was not calculated in the present study using the linear or curvilinear equations.

In the present study, with the exception of the high CO_2 atmospheres at 0°C, a single equation was adequate to represent the total counts obtained under aerobic, CO_2 enriched and anaerobic conditions. Examination of the colonies present on APT plates incubated under the three conditions, indicated that the lactic acid bacteria were the principal organisms on plates incubated under (i) aerobic conditions with increased CO_2 levels and under (ii) anaerobic conditions.

Fewer significant relationships were observed between bacterial counts and time for *B. thermosphacta*, the pseudomonads and the *Enterobacteriaceae*. This could be attributed to the inability of the media to effectively suppress the background microflora. For example, examination of the pseudomonas agar plates from the present study indicated that lactobacilli, *B. thermosphacta* and pseudomonads were present. Similar variation in colony types was reported in another study by Stanbridge and Board (1991) from pseudomonas agar plates supplied from our experiments. This lack of uniformity of colony types on the selective plates made it difficult to establish a relationship between a specific organism and time.

The analysis also provided valuable information on the growth characteristics of the different groups of organisms. It indicated whether bacterial growth was increasing, as demonstrated by a linear equation, or had reached its maximum level and was either static or in decline, as shown by quadratic equations. If organism populations are declining, their influence in the environment is difficult to interpret, and this is manifested by the problems already referred to in respect of off-odour development (Ingram & Dainty, 1971).

In conclusion, the relationship between bacterial growth metabolism and the production of off-odours on MAP meat is complex. Considering the enormous variability within and between meat species, caution should be exercised when comparing microbial growth on beef, pork, lamb and other meats. Similarly the development of off-odours cannot be correlated to observed growth patterns.

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REFERENCES

Blickstad, E. & Molin, G. (1983). *J. Food Prot.*, **46**, 756.
Blickstad, E., Enfors, S.-O. & Molin, G. (1981). *J. Appl. Bacteriol.*, **50**, 493.
Borch, E. & Agerhem, H. (1992). *Int. J. Food Microbiol.*, **15**, 99.
Crouse, J. D. & Smith, S. B. (1986). *Am. J. Vet. Res.*, **47**, 939.
Doherty, A., Sheridan, J. J., Allen, P., McDowell, D. A., Blair, I. S. & Harrington, D. (1995). *Food Microbiol.*, **12**, 251.
Enfors, S.-O., Molin, G. & Ternström, A. (1979). *J. Appl. Bacteriol.*, **47**, 197.
Erichsen, I. & Molin, G. (1981). *J. Food Prot.*, **44**, 866.

Gill, C. O. (1983). *J. Food Prot.*, **46**, 444.
Gill, C. O. (1984). *23rd Res. Conf. of the Meat Ind. of NZ*, **135**.
Gill, C. O. & Harrison, J. C. L. (1989). *Meat Sci.*, **26**, 313.
Gill, C. O. & Newton, K. G. (1977). *J. Appl. Bacteriol.*, **43**, 189.
Gill, C. O. & Penney, N. (1988). *Meat Sci.*, **22**, 53.
Grant, I. R. & Patterson, M. F. (1991). *Int. J. Food Sci. and Technol.*, **26**, 507.
Grau, F. H. (1983). *J. Food Sci.*, **48**, 326.
Ingram, M. & Dainty, R. H. (1971). *J. Appl. Bacteriol.*, **34**, 21.
Khan, M. A., Newton, I. A., Seaman, A. & Woodbine, M. (1975). In: *Problems of Listeriosis* (ed. M. Woodbine), Leicester University Press, UK, p. 75.
Lawrie, R. A. (1974). In: *Meat* (ed. D. J. A. Cole & R. A. Lawrie.) Butterworths, UK, p. 249.
Newton, K. G. & Gill, C. O. (1978). *J. Appl. Bacteriol.*, **44**, 91.
Newton, K. G., Harrison, J. C. L. & Smith, K. M. (1977). *J. Appl. Bacteriol.*, **43**, 53.
Nicolai, B. M., Van Impe, J. F., Verlinden, B., Martens, T., Vandewalle, J. & De Baerdemaeker, J. (1993). *Food Microbiol.*, **10**, 229.
Patterson, J. T. & Gibbs, P. A. (1978). *J. Food Technol.*, **13**, 1.
Pierson, M. D., Collins-Thompson, D. L. & Ordal, Z. J. (1970). *Food Technol.*, **24**, 1171.
Rousset, S. & Renerre, M. (1991). *Int. J. Food Sci. and Technol.*, **26**, 641.
Shaw, B. G., Harding, C. D. & Taylor, A. A. (1980). *J. Food Technol.*, **15**, 397.
Sheridan, J. J., Doherty, A., Allen, P., McDowell, D. A., Blair, I. S. & Harrington, D. (1995). *Food Microbiol.*, **12**, 259.
Stanbridge, L. & Board, R. G. (1991). *EEC Food-linked Agro-Industrial Research (FLAIR) project No. 89055. 1st year report.*
Sutherland, J. P., Patterson, J. T. & Murray, J. G. (1975). *J. Appl. Bacteriol.*, **39**, 227.
Taylor, A. A., Down, N. F. & Shaw, B. G. (1990). *Int. J. Food Sci. and Technol.*, **25**, 98.
Vanderzant, C., Savell, J. W., Hanna, M. O. & Potluri, V. (1986). *J. Food Sci.*, **51**, 5.