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Growth of bacteria at 1°C on beef stored in controlled atmospheres of mixtures of O_2, CO_2 and N_2 and its relevance to vacuum packaging

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INTRODUCTION

Vacuum packaging extends the storage life of meat by inhibiting the growth of spoilage bacteria. There is considerable disagreement on the cause of this inhibition. Oxygen, consumed by bacterial and meat tissue respiration, is replaced only slowly in the gas atmosphere around the meat because of the impermeability of the packaging film, and there is a belief that this restricts growth of Pseudomonas spp (Newton & Rigg 1979) and Brochothrix thermosphacta (Campbell et al. 1979). Others (Ingram 1962; Roth & Clark 1972; Enfors et al. 1979; Erichsen & Molin 1981) have argued that inhibition by CO_2 is more important.

Gas changes in vacuum packs responsible for the inhibition of spoilage bacteria must occur during the first two weeks of storage. This is evident because spoilage under conditions of high humidity existing in packages normally occurs within this period if there are no inhibitory effects from gases (as during storage in air). Examination of the few published measurements of gases in packs indicates that CO_2 accumulates quite rapidly to concentrations of approximately 20% after two weeks (Shaw et al. 1980; Seideman et al. 1980; Hall et al. 1980), though Erichsen & Molin (1981) and Seidman et al. (1979) have detected as much as 86% and 71% after one week. Oxygen concentrations detected after two weeks have been in the range 0.8 to 1.8% (Shaw et al. 1980; Seideman et al. 1980; Hall et al. 1980; Erichsen & Molin 1981).

In this study we have examined the effect of mixtures of 10, 20 and 40% CO_2 with 0, 1 or 3% O_2 on microbial growth on beef (naturally contaminated with a mixed bacterial flora), to provide information on the relative importance of these gases to the inhibition of different groups of spoilage bacteria in vacuum packs.

MATERIALS AND METHODS

Meat storage

A portion of M. semimembranosus (pH 5.4) was obtained two days post mortem from a beef carcass in a commercial slaughterhouse and cut into 100 cm^2 samples of 1 cm thickness. These were then individually wrapped in one layer of a gas-permeable plastic film (O_2 permeability at 20°C and 95% R.H., 10,000 $cm^3/m^2/day/atm\ O_2$) to contain exudate and thus prevent cross contamination during storage.

Six samples were stored for up to six weeks at 1°C in each of nine gas-impermeable plastic bags (6 litre capacity; film permeability to O_2 at 20°C and 95% R.H., 17 $cm^3/m^2/day/atm\ O_2$) containing either 0, 1 or 3% O_2 in combination with 10, 20 or 40% CO_2 plus a residue of N_2. The gas was analysed daily during the first week, every second day during the second week and every fourth day thereafter. Addition of air was made when necessary to maintain the O_2 concentration, but the CO_2 concentration remained constant (± 1%). When bags were opened to remove samples for microbiological examination, the remaining samples were immediately transferred to another bag which was filled with the appropriate gas mixture and sealed.

Microbiological examination

Ten samples were examined before storage to determine the initial microbiological condition of the meat, and two samples in each gas mixture were examined after 2, 4 and 6 weeks.

A 15 cm^2 area of 1mm thickness was cut from the sample and macerated with 100 ml sterile saline containing 0.1% peptone. Decimal dilutions were made and 0.016 ml drops delivered in duplicate on a) Plate Count Agar (PCA) + 1% NaCl incubated at 25°C for five days to obtain the total aerobic viable count b) Streptomycin Thallous Acetate Actidione Agar (STAA; Gardner 1966) incubated for two days at 20°C to enumerate B. thermosphacta c) MacConkey Agar No. 3 (Oxoid CM 115) + 1% glucose to enumerate presumptive Gram negative bacteria (colonies with a diameter of 1mm or more were counted) and d) Cavett's (1963) modification of Acetate Agar (AA; Rogosa et al. 1951) incubated at 30°C for two days to enumerate aciduric lactic acid bacteria.

Thirty isolates taken at random from the PCA + 1% NaCl and MacConkey + 1% glucose plates from one sample of meat in each gas mixture after six weeks were identified as described by Shaw & Harding (1978).

RESULTS

Total viable counts and selective counts of B. thermosphacta, aciduric lactic acid bacteria and presumptive Gram negative bacteria on samples after 2, 4 and 6 weeks in the various gas mixtures are shown in Table 1.

Total viable counts

The total viable count on samples before storage was 1.8-3.0 (Log_{10} No/cm^2) and had increased on all stored samples except those in 0% O_2 plus 20 or 40% CO_2 after two weeks. At each CO_2 concentration the total viable count increased more rapidly with the order 0, 1, 3% O_2; the effect of increasing O_2 from 0% to 1% being greater than the effect of the increase from 1% to 3%. The count increased more rapidly at each O_2 concentration with decrease in CO_2.

Brochothrix thermosphacta

Brochothrix thermosphacta was detected on seven of the ten samples examined before storage (Log_{10} No/cm^2,

1.8-2.3). The numbers had increased on all stored samples except those in 0% O_2 plus 20 or 40% CO_2 after two weeks. It was the dominant organism in nearly all the gas mixtures containing 1 or 3% O_2 after six weeks (Table 2), and at each CO_2 concentration grew much slower in 0% than in 1 or 3% O_2 (Table 1). Increase in CO_2 concentration also slowed its growth.

Presumptive Gram negative bacteria

Presumptive Gram negative bacteria were detected on six of the ten samples examined before storage (Log_{10} No/cm², 1.8-2.3). They grew on all samples except those stored in 0% O_2 plus 40% CO_2, but high numbers (> 10^6/cm²) were only detected on samples stored for six weeks in 3% O_2 plus 10 or 20% CO_2 (Table 1) on which the predominant types (Table 2) were Pseudomonas spp (3% O_2 + 10% CO_2) or Enterobacteriaceae (3% O_2 + 20% CO_2). Overall, numbers of presumptive Gram negative bacteria on stored samples increased with O_2 and decreasing CO_2.

Lactic acid bacteria

Lactic acid bacteria were not detectable before storage. After two weeks numbers were very low and no relationship between gas mixture and count was evident. Considerable growth of lactic acid bacteria had occurred in all gas mixtures by four weeks when numbers were higher in mixtures with 1 or 3% O_2 than in those with 0% O_2 (Table 1), but this effect was not evident after six weeks when they were present in high numbers (> 10^8/cm²) in all gas mixtures. No consistent relationship between CO_2 concentration and numbers of lactic acid bacteria was observed. They were the predominant group in all 0% O_2 mixtures after six weeks (Table 2).

DISCUSSION

Pseudomonas strains grow more rapidly than other types of bacteria on refrigerated meat stored in air (Gill & Newton 1977) and eventually dominate the flora, causing spoilage. Their inhibition is essential to shelf-life extension in vacuum packs. They were not detected on any samples stored in 20% CO_2, even with 3% O_2, which strongly suggests that CO_2 is the main cause of their inhibition in a vacuum pack. Nevertheless, in 10% CO_2 growth of pseudomonads was detected on a sample stored in 3% O_2 and not in 1% which shows that low concentrations of O_2 can contribute to inhibition. Clark & Burki (1972) have shown that the lag phase of Pseudomonas spp is extended in 1% O_2, but this does not appear to account for the extent of the difference between the growth in 1 and 3% O_2 with 10% CO_2. Shaw & Nicol (1969) did not observe any reduction in generation time of a Pseudomonas strain growing as a pure culture on meat in 0.8% O_2. It is possible that the growth rate is more susceptible to low atmospheric O_2 concentrations when the organisms are growing in competition with other bacteria as in this study.

Brochothrix thermosphacta becomes potentially important as a spoilage organism when pseudomonads are inhibited. Its inhibition in vacuum packs has been attributed to the growth of lactobacilli (Roth & Clark 1975), and a combination of O_2 availability and pH (Campbell et al. 1979) caused by lactate inhibition of anaerobic growth below pH 5.8 (Grau 1980). This study, using meat of pH 5.4, confirmed that O_2 availability affects the growth of B. thermosphacta, and demonstrated that this is likely to be of prime importance in controlling growth in vacuum packs containing at least 40% CO_2. Where very high concentrations of CO_2 accumulate rapidly (Erichsen & Molin 1981) more inhibition from this gas will occur, but it seems certain that O_2 limitation will still be important. The failure of lactobacilli to dominate the flora in any of the gas mixtures containing 1 or 3% O_2 suggests that their inhibition of B. thermosphacta is only likely when low O_2 availability slows its growth.

Little attention has previously been paid to the inhibition of Enterobacteriaceae in vacuum packs, probably because they are considered less important spoilage organisms than pseudomonads or B. thermosphacta. Some strains are, however, capable of producing spoilage (Patterson & Gibbs 1977; Newton & Gill 1979), and their inhibition therefore seems desirable. In the present study they were enumerated collectively with other Gram negative bacteria and their detection on samples stored in 3% O_2 + 10% CO_2 was probably masked by the high numbers of pseudomonads present. In the absence of pseudomonads they were detected in high numbers (> 10^5/cm²) only on samples stored in 3% O_2 + 20% CO_2 which demonstrates that O_2 limitation almost certainly contributes to their inhibition in vacuum packs. Evidence of an inhibitory effect of CO_2 was conflicting. In the presence of 3% O_2 numbers were higher in 20% CO_2 than in 40% but the reverse was true in 1% O_2. Nevertheless Haines (1933) and Shaw & Nicol (1969) have shown inhibitory effects of CO_2 on Enterobacteriaceae and this gas probably has some influence on the growth of these organisms, particularly when it is present at high concentrations.

The amount of O_2 available for microbial growth in a vacuum pack will depend on the volume of air left in the pack on sealing and on the O_2 permeability of the plastic film. Carbon dioxide will accumulate more rapidly in packs with a low initial gas volume and will escape less rapidly through a film of low gas permeability. The relevance of both gases to the inhibition of spoilage bacteria emphasises the importance of effective pack evacuation and low film permeability to the limitation of their growth.

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TABLE 1 Bacterial numbers on beef stored at 1°C in different gas mixtures

Gas mixtures O_2 (%)	Gas mixtures CO_2 (%)	Total aerobic viable count 2**	4	6	Brochothrix thermosphacta 2	4	6	Presumptive Gram negative bacteria 2	4	6	Aciduric lactic acid bacteria 2	4	6
0	10	3.5*	6.3	8.5	3.2	5.5	7.7	<1.8	2.6	3.0	<1.8	3.6	8.2
1	10	6.1	7.5	8.5	5.8	7.5	8.3	3.4	2.9	3.6	2.9	4.9	6.4
3	10	6.9	8.0	9.0	6.5	7.6	8.7	3.5	4.7	7.4	2.5	5.8	7.5
0	20	2.5	4.0	7.8	<1.8	3.3	5.9	1.8	<1.8	3.1	2.1	3.7	7.5
1	20	5.4	7.5	8.9	4.7	5.9	8.3	1.8	3.5	3.9	1.8	5.5	7.5
3	20	6.5	7.9	9.0	5.9	6.5	8.8	2.4	2.9	6.4	2.8	5.3	8.4
0	40	3.1	4.4	7.6	2.0	3.1	5.6	2.1	<1.8	<1.8	2.8	3.7	7.5
1	40	4.0	6.2	8.5	3.8	6.0	8.2	1.9	2.4	4.7	2.1	4.0	7.9
3	40	4.1	7.2	8.6	4.0	6.9	8.4	2.3	2.3	3.0	2.4	5.5	8.0

* Values are $\log_{10}$ value of the mean counts/cm² on 2 samples.

** Weeks storage

TABLE 2 Proportions (%) of groups of organisms isolated on PCA + 1% NaCl and MacConkey Agar + 1% glucose from beef stored for 6 weeks at 1°C.

Gas mixture O_2 (%)	Gas mixture CO_2 (%)	PCA + 1% NaCl: Brochothrix thermosphacta	PCA + 1% NaCl: Lactic acid bacteria	MacConkey + 1% glucose: Pseudomonas spp.	MacConkey + 1% glucose: Moraxella -like spp.	MacConkey + 1% glucose: Enterobacteriaceae	MacConkey + 1% glucose: Yeasts
0	10*	27	73	-	-	-	-
1	10*	100	-	-	-	-	-
3	10	100	-	83	7	-	10
0	20*	4	96	-	-	-	-
1	20	44	56	3	-	94	3
3	20	96	4	-	-	100	-
0	40*	10	90	-	-	-	-
1	40	100	-	-	-	100	-
3	40*	100	-	-	-	-	-

* No values are shown for MacConkey + 1% glucose in these gas mixtures because insufficient isolates were available for identification due to low counts.