Effect of the gaseous environment on the growth on meat of some food poisoning and food spoilage organisms

M. K. SHAW and D. J. NICOL

CSIRO Meat Research Laboratory,
Queensland, Australia

INTRODUCTION

Two of the most important environmental factors affecting the growth of microorganisms on meat surfaces are temperature and the gaseous environment. In a gas impermeable pack, the meat and the growth of bacteria after the gaseous environment such that the O_2 content is decreased and the CO_2 content is increased. It has been generally assumed that the low O_2 level is the controlling factor of bacterial growth in meat packs. However it has been suggested (Ingram, 1962; Gardner, Carson and Patton, 1967) that the selective action in a gas impermeable pack is not due to the lack of O_2 but to the high concentration of CO_2 built up within the pack.

The occurrence of coliforms and salmonellae on food is well recognized (Van Oye, 1964; Hall *et al*, 1967; and Weissman, 1967). *E. coli* is used as an indicator of faecal contamination and therefore its presence can indicate the possible contamination by Salmonella (Lewis and Angelotti, 1964). Little work has been done to show whether the growth on meat of *E. coli* is a satisfactory indication of the possible growth of *Salmonella*.

As some consideration has been given to ageing meat at temperatures of 15°C and above in gas impermeable packs, it is vital that a complete understanding of the ability of *Salmonella* to grow under these modified gaseous conditons should be obtained. If the growth of *E. coli* is a satisfactory indicator of the growth of *Salmonella*, then the estimation of change of *E. coli* numbers can be used to ascertain the effectiveness of storage conditions.

This paper deals with the effect of the gaseous environment, with special reference to O_2 and CO_2, on the growth of pure cultures of three psychrophilic food spoilage organisms (*Pseudomonas* str. 1482, *Microbacterium* str. 22, and lactic acid isolate, 58) on meat at 5° C, and on the growth of two enteric organisms (*Escherichia coli* type 1 and *Salmonella oranienburg*) on meat over a range of temperatures from 8° C to 37° C).

MATERIALS AND METHODS

Organisms

The food spoilage organisms used were *Pseudomonas* 1482, a psychrophilic species isolated from beef spoiled in air at O° C; *Microbacterium* 22, a psychrophilic species isolated from beef stored in N_2 at O° C (Weidemann, 1966); and a gram positive, heterofermentative, catalase negative, psychrophilic lactic acid producing isolate, 58, isolated from beef stored at 1° C in N_2 filled, foil-laminate pouches (Shaw, unpublished). The numbers used are the culture collection numbers of the Meat Research Laboratory, Cannon Hill.

In an initial survey experiment, 12 enteric organisms, 6 *Salmonella* and 6 *E. coli*, were tested for the ability to grow on meat at 20° C. The *Salmonella* serotypes, all isolated from cattle, were *S. typhimurium* (B), *S. eastbourne* (D), *S. adelaide* (O), *S. meunchen* (C2), *S. anatum* (E1), and *S. oranienburg* (C1). The *E. coli* strains were isolated from faeces, rumen and fleece of sheep. *Salmonella oranienburg*, isolated from cattle rumen, and *E. coli* type 1, isolated from sheep faeces, were used for further study.

Measurement of Growth

Growth was measured by counting the number of viable organisms on 1 cm^2 of muscle by spreading 0.1 ml of an appropriate dilution on the surface of tryptose phytone yeast extract agar (TPY) and in the case of the enteric group, on violet red bile agar (VRB) or brilliant green sulfadiazine agar (BGS). Incubation was for 4 days at 20° C (food spoilage) or 24 hrs. at 37° C (enterics).

Preparation, Inoculation and Sampling of Slices

Sterile slices (3 mm thick) were cut from post-rigor silverside muscles as previously described (Kaess 1961).

(*a*) *Food spoilage organisms*

The slices were mounted on metal supports, stored for 24 hrs. at O° C, and inoculated by spraying approximately 10^3 organisms per cm^2 uniformly on the surface (Kaess and Weidemann, 1962).

(*b*) *Enteric organisms*

Slices were prepared as for (a). Inoculation was performed by dripping a sterile velveteen pad (mounted on an aluminium block) into a suspension of the organisms and impressing the pad onto the slice. Sampling in both cases was as previously described (Kaess and Weidemann, 1962).

Storage of Slices

The inoculated slices were stored in plastic containers submerged in a refrigerated water bath with the temperature controlled to $\pm 0.1°$ C. The gas phase was humidified to 99.3 % RH by passage through 0.2 M NaCl. Oxygen concentrations were monitored with a Beckman Model 777 oxygen analyser (Beckman Instrument Inc., Calif.) and recorded on a Hitachi Model QPD 53 recorder (Hitachi Ltd., Tokyo, Japan). Traces of oxygen, in N_2 or N_2 and CO_2, were removed by passing gases through chromous salts (Marshall, 1960). More accurate determinations of O_2 and CO_2 concentrations were made using a Fisher Model 25V gas partitioner (Fisher Scientific, Pittsburg, Pa.).

RESULTS

(*a*) *Food Spoilage Organisms*

(*1*) *Effect of oxygen concentrations on growth*

Oxygen concentrations in the gas phase were varied from 0 to 100 % and the results are summarised in Table 1.

Table 1. *Effect of 0_2 concentration on generation times (hr) of selected bacteria on muscle slices at 5° C.*

Organism	*Oxygen Concentration (%)*					
	0	0.2	0.8	5.0	20.8	100
Pseudomonas 1482	NG*	16	4.8	4.4	4.4	4.0
Microbacterium 22	NSG+	8.8	9.0	8.5	8.5	8.8
Isolate 58	9.0	9.0	9.0	9.2	9.0	9.1

NG* = No growth after 2 weeks incubation.
NSG+ = No sustained growth. Population increased from 10^3 to 10^5 per cm^2 then growth ceased.

Growth of *Pseudomonas* 1482 was not inhibited until the concentration was reduced below 0.8 %.

Microbacterium 22 failed to give sustained growth in the absence of O_2, i.e. the population increased only from 10^3 to 10^5 organisms per cm^2. No inhibition of *Microbacterium* 22 occurred at 0.2 % O_2. Isolate 58 grew at the same rate in the presence and absence of O_2.

(2) *Effect of oxygen concentration in the presence of 10 % CO_2.*

Oxygen concentrations in the gas phase were varied from O to 18.7 % with the CO_2 level was fixed at 10 %. The results are summarised in Table 2.

Table 2. *Effect of oxygen concentration, in the presence of 10 % CO_2, on generation times (hr.) of selected bacteria on muscle slices at 5° C.*

Organism	*Oxygen Concentration (%)*			
	0	1.2	5	18.7
Pseudomonas 1482	NG*	8.8	8.7	8.8
Microbacterium 22	NSG+	8.4	8.6	8.8
Isolate 58	9.6	9.4	9.2	9.2

NG* = No growth after 2 weeks incubation.
NSG+ — No sustained growth, i.e. population increased from 10^3 to 10^5 per cm^2 before growth ceased.

Pseudomonas 1482 was inhibited by 50 % by 10 % CO_2. The effect of 10 % CO_2 was independent of the O_2 concentration over a range from 1.2 % to 18.7 %. *Microbacterium* 22 and isolate 58 were not effected by 10 % CO_2.

(b) *Enteric Organisms*

(1) *Survey of strains.*

Of the strains tested all reached a population of 10^8 per cm^2 after 3 days incubation in air at 20° C.

E. coli type 1 (from sheep faeces) and *S. oranienburg* were used for further study.

(2) *Effect of temperature on growth.*

Figure 1 shows the effect of temperature on the growth rates of *E. coli* and *S. oranienburg* on meat slices. Growth occurred on meat in air at 8° C (35 hr./gen.) but no growth occurred in TPY borth at 7° C within 6 weeks. There was, however, some evidence of filament formation at 7° C.

(3) *Effect of the gas phase on growth.*

The results of varying the gas phase are summarised in Table 3.

Table 3. *Effect of gaseous environment on the growth of* E. coli *and* S. oranienburg *on meat at 20° C and 10° C.*

Organism.	*Temp °C*	*% Inhibition of Rate of Growth in Air*		
		18.7 % O_2, 10 % CO_2	0.6 % O_2, 10 % CO_2	0 % O_2, 100 % N_2
E. coli	20	27	32	47
	10	34	27	50
S. oranienburg	20	27	24	41
	10	31	24	48

Anaerobic growth rates were about half that in air. The inhibition by 10 % CO_2 was not enhanced by reducing the temperature from 20° C to 10° C or by reducing the oxygen level from 18.7 % to 0.6 %.

DISCUSSION

Storage of chilled meat in gas impermeable packs restricts the growth of *Pseudomonas* and one major spoilage flora becomes *Microbacterium* or *Lactobacillus* types. It has been suggested (Ingram, 1962) that packaging in gas impermeable films is likely to produce its effects on the microflora not through restriction of O_2 but because of accumulating CO_2. Many workers (Ingram, 1962; Kitchell, 1966; Gardner and Carson, 1967; and Gardner, Carson and Patton, 1967) have stated that the oxygen concentration never falls below 1 % within the packs. This O_2 level would not limit the growth of *Pseudomonas* 1482 and thus the inhibition is probably due to CO_2.

There have been numerous studies on the inhibitory effect of CO_2 on bacterial growth (Valley & Rettger, 1927; Coyne, 1933; Haines, 1933; Scott, 1938; Ogilvy and Ayres, 1951; and King and Nagel, 1967). The organisms tested here vary in their response to CO_2. *Pseudomonas* 1482 was the only one of the spoilage organisms inhibited and this inhibition was independent of O_2 concentration above 1 % O_2. King and Nagel (1967) obtained similar results for *Pseudomonas aeruginosa* grown under controlled conditions in liquid media.

The concentration of CO_2 can reach 20 % within packs and thus if the O_2 concentration is relatively high, this CO_2 accumulation should select for the growth of *Microbacterium*. If the O_2 concentration is virtually nil then the lactic acid isolate type would be expected to dominate. Preliminary storage experiments have shown that high CO_2 selects for gram positive,

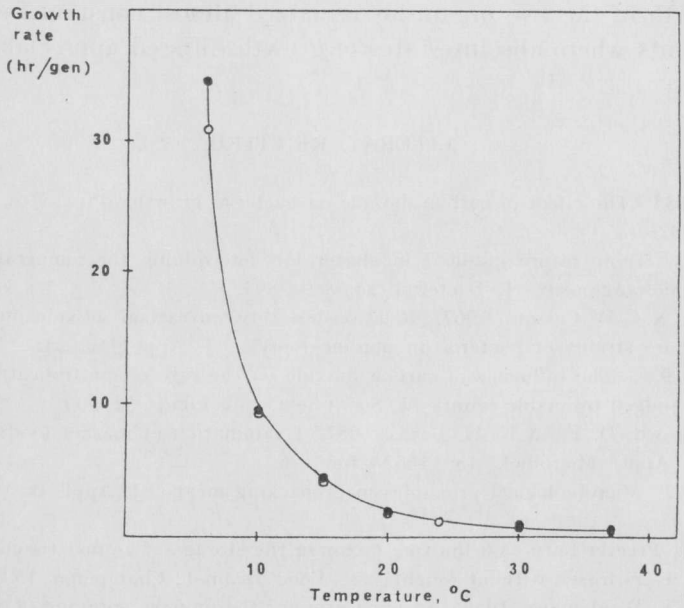

Figure 1. *Effect of temperature on the growth of* Escherichia coli *and* Salmonella oranienburg *on muscle slices.*

Symbols: — • —. S. oranienburg; and — 0 —, E. coli.

catalase positive bacteria but low O_2 (less than 0.2 %) selects for gram positive, catalase negative types (Nicol and Shaw, unpublished).

The minimum growth temperature in liquid media has been found to be about 7.5° C for *E. coli* (Elliott, 1963; Shaw, 1966) and 8.0° C for *S. typhimurium* (Elliott, 1963). On meat the minimum for both has been shown to be about 8° C. At 7° C, in liquid media, some filament formation was observed. This may still be a potential problem, as an increase in temperature causes the filaments to divide into may viable organisms (Shaw, 1968).

The growth rate of *E. coli* and *S. oranienburg* on meat under anaerobic conditions was half that in air at all temperatures tested. The presence of 10 % CO_2 in the gas phase caused only a 30 % inhibition even at reduced O_2 levels (0.6 %) and at low temperatures (10° C). Thus holding meat at elevated temperatures even in gas impermeable packs, is a potentially dangerous practice. Only by reducing the temperature to below 7° C can the growth of *Salmonella* and *E. coli* be completely inhibited on chilled meat.

E. coli is a satisfactory indicator organism for the possible presence of *Salmonella*, but even more importantly, we have shown that if this strain is typical of those contaminating meat, then the growth of *E. coli* may be a satisfactory indicator of the possible growth of *Salmonella*, since the relative

rates of growth of the two organisms remained almost constant in a number of environments where absolute rates of growth differed appreciably.

LITERATURE CITED

Coyne, F. P. 1933. The effect of carbon dioxide on bacterial growth. Proc. Roy. Soc. Ser. B, 113: 196–202.

Elliott, P. 1963. Temperature gradient incubator for determining the temperature range of growth of microorganism. J. Bacteriol. 85: 889–895.

Gardner, G. A. & A. W. Carson. 1967. Relationship between carbon dioxide production and growth of pure strains of bacteria on porcine muscle. J. Appl. Bacteriol. 30: 321–333.

Haines, R. B. 1933. The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. J. Soc. Chem. Ind, Lond., 52: 13T.

Hall, H. E., Brown, D. F., & K. H. Lewis. 1967. Examination of market foods for coliform organisms. Appl. Microbiol. 15: 1062–1069.

Ingram, M. 1962. Microbiological principles in prepacking meats. J. Appl. Bacteriol. 25: 259–281.

Kaess, G. 1961. Freezer burn as a limiting factor in the storage of animal tissues. I. Experiments with livers frozen without weight loss. Food Technol., Champaign. 15: 1–7.

Kaess, G. & J. F. Weidemann. 1962. An apparatus for the uniform spraying of solid nutrient surfaces with bacterial suspensions. J. Appl. Bacteriol. 25: 180–186.

King, A. D. Jr. & C. W. Nagel. 1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. J. Food Sci. 32: 575–579.

Kitchell, A. G. 1967. Microbiology of prepacked meats. Meat Processor & Packer 5: 6–12.

Lewis, K. H. & R. Angelotti. 1964. Examination of foods for enteropathogenic and indicator bacteria. U.S. Pub. Health Serv. Publ. 1142.

Marshall, J. H. 1960. The production of anaerobic conditions with chromous salts. J. gen. Microbiol. 22: 645–648.

Ogilvy, W. S. & J. C. Ayres. 1951. Post mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol., Champaign. 5: 97–102.

Scott, W. J. 1938. The growth of microorganisms on ox muscle. III. The influence of 10 % carbon dioxide on rates of growth at −1°. J. Coun. Scient. ind. Res., Aust. 11: 266–277.

Shaw, M. K. 1966. The growth of *Escherichia coli* at low temperature. Ph. D. Thesis, Univ. of California, Davis, California.

Shaw, M. K. 1968. Formation of filaments and synthesis of macromolecules at temperatures below the minimum for growth of *E. coli*. J. Bacteriol. 95: 221–232.

Valley, G. & L. F. Rettger. 1927. The influence of carbon dioxide on bacteria. J. Bacteriol. 14: 101–110.

Van Oye, E. 1964. »The World Problem of Salmonellosis». Monographiae Biologicae XIII. Dr. W. Junk, The Hague.

Weidemann, J. F. 1966. A note on the microflora of beef muscle scored in nitrogen at 0°. J. Appl. Bacteriol. 28: 365–367.

Weissman, M. A. 1967. A study of the occurrence and growth of *Salmonella* in meat and meat products. M.Sc. Thesis, University of Georgia, Athens, Georgia.