ELSEVIER

International Journal of
Food Microbiology 28 (1995) 221–231

International Journal
of Food Microbiology

Growth/survival of psychrotrophic pathogens on meat packaged under modified atmospheres

G.D. García de Fernando [a,*], G.J.E. Nychas [b], M.W. Peck [c], J.A. Ordóñez [a]

[a] *Departamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, U.C.M., 28040 Madrid, Spain*

[b] *Agricultural University of Athens, Dept. of Agricultural Industries, Lab. of Food Microbiology and Biotechnology, Iera Odos 75, Athens 11855, Greece*

[c] *Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

Keywords: Psychrotrophic pathogens; Modified atmosphere packaging; Meat

1. Introduction

In spite of a diverse initial microbial population, bacterial spoilage of refrigerated meat aerobically stored is due to the growth of Gram-negative psychrotrophic organisms dominated by members of the genus *Pseudomonas*. As this flora is effectively inhibited by 20% or more carbon dioxide enriched atmospheres (Clark and Lentz, 1969), they have been increasingly used in the distribution of red meats, poultry and seafoods in recent years (Reddy et al., 1992; Davis, 1993). In some instances, such as red meats, the atmospheres are also enriched in oxygen (60–80%) to delay metmyoglobin formation (Ordóñez and Ledward, 1977; Asensio et al., 1988), which slows down the meat surface discoloration problem (Finne, 1982). However, several combinations of carbon dioxide, nitrogen and oxygen have also been used in modified atmosphere packaging (MAP) to sustain visual appearance and to extend the shelf-life of meat and meat products (Erichsen and Molin, 1981; Nychas and Arkoudelos, 1990).

The microbiological changes of refrigerated meat of normal pH (i.e. ~5.5) stored in MAP are reasonably well established. The final microflora is composed, in decreasing order of magnitude, by *Brochothrix thermosphacta* and/or lactic acid

* Corresponding author. Tel. +34 (1) 3943751; Fax +34 (1) 3943743.

SSDI 0168-1605(95)00058-5

bacteria, *Enterobacteriaceae*, and pseudomonas (Asensio et al., 1988; Nychas, 1994). However, the pH may play an important role in the kind of dominant flora and, therefore, in the spoilage off-odour pattern. Under low oxygen (vacuum) or high CO_2 (MAP) conditions, the spoilage of abnormal high pH meat (i.e. DFD) is faster than meat of normal pH. In this case the relatively high pH (as also occurs in turkey and chicken breast) allows the growth of the very pH sensitive (Gill and Newton, 1979) and relatively CO_2 resistant (Molin and Stenstrom, 1984) *Shewanella* (*Alteromonas*) *putrefaciens*, which produces abundant H_2S and, therefore, it may cause greening of the meat (Hood and Mead, 1993). For this reason, MAP of meat to extend shelf-life is not advised for high pH meat.

The chemical changes of refrigerated meat stored in MAP have also been studied but to a much lesser extent than microbiological changes. Although the glucose depletion in aerobic and anaerobic conditions is well established (Nychas, 1994), the patterns of the changes in the lactic acid concentration varies. Some authors (Drosinos, 1994; Nychas, 1994) have observed a decrease of L(+)lactic acid while others (Ordóñez et al., 1991) have found an increase at the end of the storage period under CO_2 enriched atmospheres, which was attributed to both muscle anaerobic glycolysis and the metabolism of glucose by *Br. thermosphacta*. However, the D(−)lactic acid isomer seems to increase during storage under different gaseous conditions (Ordóñez et al., 1991; Drosinos, 1994; Nychas, 1994). It does not come from either *Br. thermosphacta* or muscle anaerobic glycolysis because in both cases only L(+)lactic acid are produced. The increase of this compound must therefore be due to the metabolism of lactic acid bacteria, which could generate D, L or DL lactic acids (Kandler, 1984). Among the short-chain fatty acids, acetic acid shows a clear rising trend during the whole period of meat storage in MAP (Ordóñez et al., 1991; Nychas, 1994), which could be attributed to the switch off metabolism of lactic acid bacteria from homo to heterofermentative or to other predominant organisms (*Br. thermosphacta*) in this packaging system (Nychas, 1994). Other short-chain fatty acids also slightly increase (propionic, isobutyric and isovaleric) or remain stable (n-butyric) during storage (Ordóñez et al., 1991); the latter is probably not produced by microorganisms (Dainty et al., 1979).

A wide range of organic volatiles (mainly strain or branched primary, and secondary C2–C5 alcohols, C6–C8 hydrocarbons, C3–C4 ketones, and dimethylsulfides) has been detected in the head space of meat packaged under modified atmospheres (Nychas, 1994). The dairy/cheese odours mainly found in meat stored in gas mixes with CO_2 are undoubtedly produced by *Br. thermosphacta* and lactic acid bacteria, both of which can produce lactic acid, diacetyl/acetoin and alcohols (Dainty and Hibbard, 1983).

Other non-volatile compounds (amines) have also been detected in meat MAP. Among them, putrescine and cadaverine show a constant increase during storage, spermine, spermidine and tryptamine stay at a similar level and a small rise in tyramine levels is usually observed during the last half of storage (Ordóñez et al., 1991). As lactic acid bacteria (Dainty et al., 1986) and *Br. thermosphacta* (Edwards et al., 1985) are not amine producers, the formation of these compounds has been

attributed to *Enterobacteriaceae* (Ordóñez et al., 1991), although tyramine could also be formed by some strains of the genus *Lactobacillus* (Edwards et al., 1987).

The above considerations indicate that most work has been focused on studying the limiting factors of meat MAP. However, concerns have been expressed by regulatory authorities (Gill, 1988), food industry groups (Anon, 1988) and others that this practice may represent an unnecessary safety hazard. Indeed, despite increasing commercial interest in the use of MAP to extend the shelf-life of many perishable products, such as meat, the concern about the potential growth of pathogenic bacteria, which could survive and grow even at refrigeration temperatures, remain the limiting factors to further expansion of this method. Indeed, only few studies have examined the effect of MAP on the growth/survival of the foodborne psychrotrophic pathogens. This paper deals with these aspects. Nevertheless, sometimes when there is an abuse of the storage temperature, other pathogenic bacteria may come into play; their behaviour in meat MAP is exemplified in the present article with the fate of *Salmonella enteritidis* on chicken stored under different gaseous conditions.

2. *Aeromonas hydrophila*

This bacterium has a high incidence in food products. A study by Barnhart et al. (1989) detected *Aeromonas* spp. in 98% of chicken carcasses examined. Other authors found similar, although less alarming, results; Myers et al. (1982) isolated *Aeromonas* spp. in 20% of vacuum packaged pork samples examined. The data vary considerably in other meat products with mean values ranging from 28% to 35% (Hudson and DeLacy, 1991). The minimum growth temperature of *Aeromonas* strains isolated from food products is lower than that of clinical samples (Kirov et al., 1990) and varies from -0.1 to $+1.2$°C (Walker and Stringer, 1987), although recently (Hudson et al., 1994) *A. hydrophila* has been demonstrated to multiply at even lower temperatures.

Growth of *A. hydrophila* has been shown to be favoured in nitrogen atmospheres compared to in air (Varnam and Evans, 1991), although this result was not verified in turkey and pork packaged in an atmosphere of 100% N_2 and kept at 1 and 7°C. In the latter two cases in spite of microbial counts increasing during storage, the generation times (g) were longer in nitrogen than in air (Mano et al., unpublished data). *A. hydrophila* growth has been detected in a variety of vacuum packaged products stored between -2 and 10°C such as beef (Gill and Reichel, 1989), roast beef (Hudson et al., 1994) and pork kept at 1°C (Sheridan et al., 1992; van Laack et al., 1993), although results were occasionally contradictory (van Laack et al., 1993), since in one series of samples growth of the inoculated strain was not detected. In summary, the probability of bacterial growth of this microorganism in vacuum packed products is very high even at temperatures below 0°C.

It has been reported that CO_2 inhibits *A. hydrophila* (Varnam and Evans, 1991). Sheridan et al. (1992) did not detect any growth of this bacteria in lamb packaged in an atmosphere of CO_2/O_2 (20:80), CO_2/N_2 (50:50) or in 100% CO_2

at 5°C even after 21 days storage in enrichment media. This psychrotroph was also inhibited in roast beef packaged in an atmosphere of 100% CO_2 and kept at 1.5°C although growth was detected in this food product packaged in the same atmosphere at 3°C. Similar results were observed by Gill and Reichel (1989) who detected growth of this bacterium in beef (pH 6) packaged in a controlled atmosphere of CO_2 at 10°C but not at 6°C. Mano et al. (unpublished data) detected growth in pork and turkey packaged in an atmosphere of CO_2/O_2 (20:80) and kept at 7°C but not at 1°C. However, when these meat products were packaged in an atmosphere of CO_2/O_2 (40:60), growth was not even detected at 7°C. From data in the literature we can therefore conclude that growth of this organism is not completely stopped by CO_2 but it is inhibited. In general, the lag phase and generation time of *A. hydrophila* are both prolonged when packaged in CO_2-enriched atmospheres as opposed to when vacuum packed or packaged aerobically. We can thus conclude that *A. hydrophila* is not an added hazard in meat products packaged in modified atmospheres in comparison with traditional storage methods since when temperatures are kept low, *A. hydrophila* multiplication is prevented in products packaged in CO_2-enriched atmospheres and if unfavourable changes in temperature occur the rate of multiplication is prolonged in the presence of this gas.

3. *Listeria monocytogenes*

Listeria is ubiquitous. It is found in environments that range from a healthy intestine in man or animals, to household environments (refrigerators and cleaning cloths), food products and soil. Food products are often contaminated with low levels of listeria, for example approximately 8% of meat ready for consumption, 15% of cooked poultry, 2.5% of milk and milk products and 5% of vegetables are contaminated although in all cases at concentrations of less than 100 cfu/g of food (Ryser and Marth, 1991).

Meat and fish products are hazardous because this bacterium is often present in these foods and *L. monocytogenes* can survive and multiply in their normal storage conditions (Buchanan et al., 1989). At refrigeration temperatures, growth of *L. monocytogenes* is slow. Growth is considered possible at 1°C (Varnam and Evans, 1991) although it has also been reported at −1.5°C (Hudson et al., 1994).

Growth of *L. monocytogenes* in packaged meat under modified conditions has been the focus of numerous, although in some cases controversial, studies. This bacterium is not greatly inhibited by vacuum packing or packaging in CO_2 enriched atmospheres and can predominate in refrigerated meat or meat products packaged in this way (Zhao et al., 1992). This assertion is based on the fact that *L. monocytogenes* grew faster than the other flora present in raw (Wimpfhimer et al., 1990) or precooked chicken (Marshall et al., 1991) when packaged in modified atmospheres. In contrast however, other authors have shown that packaging in these atmospheres inhibits listeria growth (Gill and Reichel, 1989; Hudson et al., 1994).

The growth capacity in vacuum packaged products has been well demonstrated in beef at refrigeration temperatures (Grau and Vanderlinde, 1990), lamb at 5°C (Sheridan et al., 1992), pork at 1°C (van Laack et al., 1993) and roast beef at −1.5°C (Hudson et al., 1994). It was also present in turkey (pH 6.0) in 100% N_2 at 7°C but not at 1°C and was not detected in pork (pH 5.3) at 7°C (Mano et al., 1995). The effect of CO_2 on listeria growth and survival is not clear. A number of studies report that this organism does not develop in chicken at 1 and 6°C (Hart et al., 1991), beef at 5°C (Gill and Reichel, 1989), lamb at 5°C (Sheridan et al., 1992) or roast beef at −1.5°C (Hudson et al., 1994) packaged in atmospheres of 100% CO_2 although growth has been detected in roast beef in this atmosphere at 3°C, indicating that the growth of this organism is not prevented by the absence of oxygen. Listeria growth has been demonstrated in lamb at 5°C in an atmosphere of CO_2/N_2 (50:50) (Nychas, 1994) in 'frankfurter' type sausages packaged in atmospheres of distinct proportions of CO_2/N_2 at 4, 7, and 10°C (Krämer and Baumgart, 1992) and in pork packaged in CO_2/N_2 (40:60) at 4°C (Manu-Tawiah et al., 1993). In contrast, other authors have not detected growth in the absence of O_2 (except for the previously mentioned studies in 100% CO_2) and there was no growth in chicken packaged in CO_2/N_2 (30:70) (Hart et al., 1991) at 6°C or at 4°C in an atmosphere of CO_2/N_2 of 75:25 (Wimpfhimer et al., 1990).

The effect of including oxygen in CO_2 enriched atmospheres is also confusing. Wimpfhimer et al. (1990) showed that growth was induced when oxygen was added to atmospheres in which it was previously suppressed, i.e. addition of 5% O_2 ($CO_2/O_2/N_2$, 72.5:5:22.5) to an anaerobic atmosphere ($CO_2/O_2/N_2$, 75:0:25) induced listeria growth. In contrast, no difference was observed in listeria growth in pork at 4°C packaged in anaerobic ($CO_2/O_2/N_2$, 40:0:60) or aerobic ($CO_2/O_2/N_2$, 40:10:50) conditions. More confusing still is the absence of growth of any of the strains in unpackaged minced beef in either modified atmospheres or air at 4°C (Johnson et al., 1988; Shelef, 1989) or at 25°C (Shelef, 1989).

Other authors (Mano et al., 1995) have not detected growth in pork (pH 5.3) at either 1 or 7°C or in turkey at 1°C packaged in CO_2/O_2 atmospheres (20:80 or 40:60), whereas growth was observed in turkey in these atmospheres at 7°C. Similar results have been described above for atmospheres of 100% N_2. These results, as previously mentioned, indicate the importance of the meat pH, since growth was observed in vacuum packaged beef at 7°C at pH 5.8 but not at pH 5.6 (Kaya and Smith, 1991).

Mano et al. (1995) measured the g values of pork and turkey flora packaged in modified atmospheres and concluded that these are greater for listeria than for the other flora. Similarly, these values increase as the CO_2 concentration increases indicating therefore the inhibitory effect of CO_2.

In general, atmospheres in which *L. monocytogenes* multiplication is inhibited are not bactericidal. In cases where a decrease in listeria count was observed during storage was of little relevance (Mano et al., 1995).

These data suggest that modification of the atmosphere can not be considered as the only factor involved in listeria inhibition since changes in temperature, pH and even possibly competition of other flora also affect growth. Packaging in

modified atmospheres does therefore not necessarily have to signify additional hazard of *L. monocytogenes* growth in comparison to conventional packaging in aerobic conditions.

It has been shown that mathematical modelling techniques can be used to predict, with confidence, the effects of environmental variables, such as temperature, water activity, and pH on the growth and survival of bacteria in foods. A growth model has now been developed for *L. monocytogenes* that includes carbon dioxide as a controlling factor (Fernandez et al., 1995). This model describes the effect of carbon dioxide (balance nitrogen), NaCl, pH and temperature on growth of *L. monocytogenes*. A good agreement has been obtained between predictions of growth from the model, and the observed growth of *L. monocytogenes* in modified atmosphere packed foods, including many meat products (Fernandez et al., 1995).

4. *Yersinia enterocolitica*

Yersinia enterocolitica and related species are present in all terrestrial and fresh water ecosystems. Pork products are possibly the principal food items responsible for the presence of *Yersinia* in man.

Growth and survival of this microorganism in meat depends on the pH, temperature and environmental conditions (packaging in modified atmospheres) used in its storage (Nychas, 1994). The most accepted minimum growth temperature is 4°C (Varnam and Evans, 1991) but *Yersinia* growth has been detected in meat at 1°C (Hanna et al., 1977) and also at −1.5°C (Hudson et al., 1994) and −2°C (Gill and Reichel, 1989).

As regards vacuum packaging, *Yersinia* growth was not detected in pork at 1°C and colonies were only sporadically isolated in lamb stored for 9 days. Other authors however, detected growth of this microorganism in lamb at 0°C (Sheridan et al., 1992; Sheridan and Doherty, 1994), beef at −2°C (Gill and Reichel, 1989), and roast beef at 3°C although not at −1.5°C (Hudson et al., 1994). Growth was also detected in pork packaged in 100% N_2 and stored at 1°C (Mano et al., unpublished data).

The effect of CO_2 on *Y. enterocolitica* growth is not clear. In comparison to the other pathogens studied here, this bacterium appears to be relatively resistant to CO_2 (Rönner, 1994) thus poses a greater health risk than other organisms in food products stored in modified atmospheres. Rönner (1994) also demonstrated how this bacterium could multiply in culture media that contained 0.053 mM CO_2 per ml but not at concentrations of 0.079 mM. In contrast, other in vitro studies showed that an atmospheric CO_2 concentration greater than 40% inhibited *Yersinia* growth (Zee et al., 1984). In studies on food products growth was not detected in pork packaged in $CO_2/O_2/N_2$ (25:65:10) (Elzen et al., 1994) or in CO_2/O_2 (20:80) and stored at 7°C but there was a slight decrease in the number of bacteria surviving during storage (Mano et al., unpublished data). Other authors however (Manu-Tawiah et al., 1993) observed a quite rapid growth in vacuum packaged pork chops and in atmospheres of $CO_2/O_2/N_2$ of (20:0:80, 40:0:60 and 40:10:50)

incubated at 4°C. In contrast, when *Yersinia* cultures were incubated in air, growth was slower and finished earlier possibly because of the presence of competing flora thus the final load was significantly lower than the total microbial count (Manu-Tawiah et al., 1993). *Y. enterocolitica* has been shown in other experiments to develop in beef (pH 6.0) at 5°C in a controlled atmosphere of 100% N_2 whereas Eklund and Jarmund (1983) reported a decrease in *Y. enterocolitica* growth of 100, 98 and 43% in an atmosphere of 100% CO_2 of their growth in air at 2, 6 and 20°C respectively in an experimental model. Sheridan et al. (1992) and Sheridan and Doherty (1994), however, did not detect growth in lamb either in 100% CO_2 or in CO_2/O_2 (20:80) at 0°C but they did detect it at 5°C. Paradoxically, these authors observed multiplication of this bacterium at 1°C in an atmosphere of CO_2/N_2 of (50:50) that is theoretically more inhibiting than the latter; this seems to indicate an inhibitory effect for O_2, in accordance with observations of other authors (Manu-Tawiah et al., 1993). Moreover, Sheridan et al. (1992) and Sheridan and Doherty (1994) demonstrated that *Yersinia* did not grow in minced lamb at 0°C in an atmosphere of CO_2/N_2 of (50:50) but did grow in a whole piece of lamb under the same conditions. Mincing of the meat was also an impediment to *Yersinia* growth at 5°C and 100% CO_2, i.e. bacteria multiplied in the whole piece of meat but not in the minced meat. Similar results were also reported by Kleinlein and Untermann (1990) who found that *Y. enterocolitica* did not grow at 4°C in an atmosphere of CO_2/O_2 (20:80).

The lag phase of *Y. enterocolitica* has been observed to be the same in a vacuum as in 100% CO_2 at 3°C (Hudson et al., 1994) but the generation time is 5-times greater in the CO_2 atmosphere. Also, *Y. enterocolitica* can multiply at the same rate as the other flora in beef (pH 6.0) packaged in 100% CO_2 at 5°C (Gill and Reichel, 1989).

Although the data in the literature may seem confusing and often contradictory, Hudson et al. (1994) concluded that to ensure total inhibition of *Y. enterocolitica* growth in the absence of O_2, an atmosphere with more than 75% CO_2 was necessary. However, taking into consideration all the experiments to date on this organism, unequivocal conclusions can not yet be reached. More research is recommended into the effect of pH, temperature and packaging in modified atmospheres on growth of this bacteria with special emphasis on the possible inhibitory effect of air described by Manu-Tawiah et al. (1993).

5. *Salmonella enteritidis*

Salmonella is not considered as a psychrotrophic pathogen, but *Salmonella* survival and growth is well known to be dependent on, besides the temperature, numerous factors such as pH, atmosphere and competitive flora. Indeed, the inability of *S. enteritidis* to compete successfully with lactic acid bacteria has been reported (Gibbs, 1987). In experiments performed on poultry (breast and thigh) inoculated with *S. enteritidis* and stored in several atmospheres (vacuum, 100% carbon dioxide, 100% nitrogen and 20% carbon dioxide/80% air), this bacterium

survived, but did not grow at 3°C (Nychas, 1994). At 10°C, the numbers of *S. enteritidis* increased rapidly in samples flushed with 100% nitrogen or with CO_2/air (20:80) and to a lesser extent in vacuum-packaged samples. It is worth noting that in samples stored under 100% CO_2, *S. enteritidis* numbers decreased (about 1 log unit) after 12 days storage in breast, while in thigh (lower leg) they remained at the initial level. Similar results were obtained by Gray et al. (1984). No doubt this can be attributed to the higher pH of thigh, which could be considered as a type of DFD muscle. The type of muscle (thigh or breast) did not significantly affect survival at 3°C or the rate of growth at 10°C of *S. enteritidis* in the other atmospheres used.

This experiment demonstrates that although *S. enteritidis* can not grow in modified atmospheres at refrigeration temperatures (e.g. 3°C) it may constitute a risk when a temperature abuse is produced in the commercial chain. This situation may also occur with other non-psychrotrophic organisms.

6. Conclusions

It may be said, in conclusion, that vacuum and modified atmosphere packaging under 100% of nitrogen are atmospheres that may readily support the growth of psychrotrophic pathogen bacteria (*Y. enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes*). It also seems to be that the growth of these organisms is inhibited by the enrichment of atmosphere with carbon dioxide and, in general terms, the higher the CO_2 concentration and the lower the temperature and pH, the greater is the growth inhibition. At normal meat pH (i.e. 5.5) and at a low temperature (e.g. 1°C) the growth of psychrotrophic pathogens is stopped when the CO_2 concentration is 40%. However, when the meat pH is high (i.e. 6.0 or higher) and/or an abuse of storage temperature could occur, these organisms, as other non-psychrotrophic pathogens, may grow. Therefore, it seems to be that the use of CO_2 enriched atmospheres for meat packaging are safer than the conventional aerobic storage.

Acknowledgements

M.W.P. is grateful to the Ministry of Agriculture Fisheries and Food of the United Kingdom for funding his work. The work of J.A.O. and G.D.G.F. was supported by the 'Comisión Interministerial de Ciencia y Tecnología (CICYT)', project ALI 94-0350 of Spain.

References

Anon. (1988) Safety considerations for new generation refrigerated foods. National Food Processors Association Dairy Food Sanitation 8, 5.

Asensio, M.A., Ordóñez, J.A. and Sanz, B. (1988) Effect of carbon dioxide and oxygen enriched atmospheres on the shelf-life of refrigerated pork packed in plastic bags. J. Food Protect. 51, 356–360.

Barnhart, H.M., Pancorbo, O.C., Dresden, D.W. and Shotts, E.B. (1989) Recovery of *Aeromonas hydrophila* from carcasses and processing water in a broiler processing operation. J. Food Protect. 52, 646–649.

Buchanan, R.L., Stahl, H.G. and Whiting, R.C. (1989) Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. J. Food Protect. 52, 844–851.

Clark, D.S. and Lentz, C.P. (1969) The effect of carbon dioxide on the growth of slime producing bacteria on fresh beef. Can. Inst. Food Technol. J. 2, 72–75.

Dainty, R.H., Edwards, R.A., Hibbard, C.M. and Ramantanis, S.V. (1986) Bacterial sources of putrescine and cadaverine in chilled stored vacuum-packaged beef. J. Appl. Bacteriol. 61, 117–123.

Dainty, R.H. and Hibbard, C.M. (1983) Precursors of the major end products of aerobic metabolism of *Brochothrix thermosphacta*. J. Appl. Microbiol. 55, 127–133.

Dainty, R.H., Shaw, B.G., Harding, C.D. and Michaine, S. (1979) The spoilage of vacuum-packed beef by cold tolerant bacteria. In: A.D. Russell and R. Fuller (editors) Cold Tolerant Microbes in Spoilage and the Environment, Academic Press, London, pp. 83–100.

Davis, H.K. (1993) Fish. In: R.T. Parry (editor) Principles and Applications of Modified Atmosphere Packaging of Food, Blackie Academic and Professional, Glasgow, pp. 189–228.

Drosinos, E.H. (1994) Microbial associations of minced lamb and their ecophysiological attributes. Ph.D. Thesis, University of Bath, Bath, UK.

Edwards, R.A., Dainty, R.H. and Hibbard, C.M. (1985) Putrescine and cadaverine formation in vacuum packed beef. J. Appl. Bacteriol. 58, 13–19.

Edwards, R.A., Dainty, R.H., Hibbard, C.M. and Ramantanis, S.V. (1987) Amines in fresh beef of normal pH and the role of bacteria in changes in concentration observed during storage in vacuum packs at chill temperatures. J. Appl. Bacteriol. 63, 427–434.

Eklund, T. and Jarmund, T. (1983) Microculture model studies on the effect of various gas atmospheres on microbial growth at different temperatures. J. Appl. Bacteriol. 55, 119–125.

Elzen, A.M.G., Van den, Houben, J.H. and Snijders, J.M.A. (1994) Effect of modified atmosphere packaging on the survival of pathogens on artificially contaminated pork. Proc. 40th Int. S.IIa.11. Cong. Meat Sci. Technol., The Hague, Holland.

Erichsen, I. and Molin, G. (1981) Microbial flora of normal and high pH beef stored at 4°C in different gas environments. J. Food Protect. 44, 866–869, 873.

Fernandez, P.S., George, S.M., Sills, C.S. and Peck, M.W. (1995) The development of predictive models that describe the effect of carbon dioxide, pH, temperature, and NaCl on the growth and survival of foodborne pathogenic bacteria. Program Abstracts 64th Annual Meeting Society Applied Bacteriology (in press).

Finne, G. (1982) Modified- and controlled-atmosphere storage of muscle foods, Food Technol. 36, 128–133.

Gibbs, P.A. (1987) Novel uses for lactic acid fermentation in food preservation. In: C.S. Gutteridge and J.R. Norris (editors) Changing Perspectives in Applied Microbiology, Soc. Appl. Bacteriol. Symp. Ser. No 16, pp. 51s–58s.

Gill, C.O. (1988) Packaging meat under carbon dioxide: The Captech system. 34th Int. Cong. Meat Sci. Technol. Proceeding of Industry day, Brisbane, Australia.

Gill, C.O. and Newton, K.G. (1979) Spoilage of vacuum-packaged dark, firm, dry meat at chill temperatures. Appl. Environ. Microbiol. 37, 362–364.

Gill, C.O. and Reichel, M.P. (1989) Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. Food Microbiol. 6, 223–230.

Grau, F.H. and Vanderlinde, P.B. (1990) Growth of *Listeria monocytogenes* on vacuum-packaged beef. J. Food Protect. 53, 739–741, 746.

Gray, R.J.H., Elliot, P.H. and Tomlins, R.J. (1984) Control of two major pathogens on fresh poultry using a combination potassium sorbate/CO_2 packaging treatment. J. Food Sci. 49, 142–148.

Hanna, M.O. Stewart, J.C., Zinc, D.L., Carpenter, Z.L. and Vanderzant, C. (1977) Development of *Yersinia enterocolitica* in raw and cooked beef and pork at different temperatures. J. Food Sci. 42, 1180–1184.

Hart, C.D., Mead, G.C. and Norris, A.P. (1991) Effects of gaseous environment and temperature on the storage behaviour of *Listeria monocytogenes* on chicken breast meat. J. Appl. Bacteriol. 70, 40–46.

Hood, D.E. and Mead, G.C. (1993) Modified atmosphere storage of fresh meat and poultry. In: R.T. Parry (editor) Principles and Applications of Modified Atmosphere Packaging of Food, Blackie Academic and Professional, Glasgow, pp. 269–298.

Hudson, J.A. and DeLacy, K.M. (1991) Incidence of motile aeromonads in New Zealand retail foods. J. Food Protect. 54, 696–699.

Hudson, J.H., Mott, S.J. and Penney, N. (1994) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere-packaged sliced roast beef. J. Food Protect. 57, 204–208.

Johnson, J.L., Doyle, M.P. and Cassens, R.G. (1988) Survival of *Listeria monocytogenes* in ground beef. Int. J. Food Microbiol. 6, 243–247.

Kandler, O. (1984) Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek 49, 209–224.

Kaya, M. and Smith, U. (1991) Behaviour of *Listeria monocytogenes* on vacuum-packed beef. Fleischwirtschaft 71, 424–426.

Kirov, S.M., Anderson, M.J. and McMeekin, T.A. (1990) A note on *Aeromonas* spp. from chicken as possible foodborne pathogens. J. Appl. Bacteriol. 68, 327–334.

Kleinlein, N. and Untermann, F. (1990) Growth of pathogenic *Yersinia enterocolitica* strains in minced meat with and without protective gas with consideration of the competitive background flora. Int. J. Food Microbiol. 10, 65–71.

Krämer, K.H. and Baumgart, J. (1992) Brühwurstaufschnitt hemmung von *Listeria monocytogenes* durch eine modifizierte atmosphäre. Fleischwirtschaft 72, 666–668.

Laack, R.L.J.M. van, Johnson, J.L., Van der Palen, C.J.N.M., Smulders, F.J.M. and Snijders, J.M.A. (1993) Survival of pathogenic bacteria on pork loins as influenced by hot processing and packaging. J. Food Protect. 56, 847–851, 873.

Mano, S.B., García de Fernando, G.D., López, D., Selgas, M.D., García, M.L., Cambero, M.I. and Ordóñez, J.A. (1995) Growth/survival of *Listeria monocytogenes* on refrigerated pork and turkey packaged under modified atmospheres. J. Food Safety (in press).

Manu-Tawiah, W., Myers, D.J. Olson, D.G. and Molins, R.A. (1993) Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. J. Food Sci. 58, 475–479.

Marshall, D.L., Wiesse-Lehihg, P.L., Wells, J.H. and Farr, A.J. (1991) Comparative growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored under modified atmospheres. J. Food Protect. 54, 841–843.

Molin, G. and Stenstrom, I.M. (1984) Effect of temperature on the microbial flora of herring fillets stored in air or carbon dioxide. J. Appl. Bacteriol. 56, 275–282.

Myers, B.R., Marshall, R.T., Edmonson, J.E. and Stringer, W.C. (1982) Isolation of pectinolytic *Aeromonas hydrophila* and *Yersinia enterocolitica* from vacuum packaged pork. J. Food Protect. 45, 33–37.

Nychas, G.J.E. (1994) Modified atmosphere packaging of meats. In: R.P. Singh and F.A.R. Oliveira (editors) Minimal Processing of Foods and Process Optimization, An Interface. CRC Press Inc., London, pp. 417–436.

Nychas, G.J.E. and Arkoudelos, J.S. (1990) Microbiological and physicochemical changes in minced meat under carbon dioxide, nitrogen or air at 3°C. Int. J. Food Sci. Technol. 25, 389–398.

Ordóñez, J.A. and Ledward, D.A. (1977) Lipid and myoglobin oxidation in pork stored in oxygen- and carbon dioxide-enriched atmospheres, Meat Sci. 1, 41–48.

Ordóñez, J.A., Pablo, B. de, Pérez de Castro, B. Asensio, M.A. and Sanz, B. (1991) Selected chemical and microbiological changes in refrigerated pork stored in carbon dioxide and oxygen enriched atmospheres. J. Agric. Food Chem. 39, 668–672.

Reddy, N.R., Armstrong, D.J., Rhodehamel, E.J. and Kautter, D.A. (1992) Shelf-life extension and safety concerns about fresh fishery products packaged under modified atmospheres: A review. J. Food Safety 12, 87–118.

Rönner, U. (1994) Modified atmosphere packaging of non-respiring foods. In: L. Leistner and L.G.M. Gorris (editors), Food Preservation by Combined Processes. Final Report FLAIR Concerted Action No. 7, Subgroup B, pp. 51–58.

Ryser, E.T. and Marth, E.H. (1991) Listeria, Listeriosis and Food Safety, Marcel Dekker, New York.

Shelef, L.A. (1989) Survival of *Listeria monocytogenes* in ground beef or liver during storage at 4 and 25°C. J. Food Protect. 52, 379–383.

Sheridan, J.J. and Doherty, A. (1994) Growth of *Yersinia enterocolitica* on modified atmosphere packaging lamb. Proc. 40th Int. S.IIa.13. Cong. Meat Sci. Technol., The Hague, Holland.

Sheridan, J.J., Doherty, A. and Allen, P. (1992) Improving the safety and quality of meat and meat products by modified atmosphere and assessment by novel methods. FLAIR 89055 Iterim. 2nd year report EEC DGXII, Brussels.

Varnam, A.H. and Evans, M.G. (1991) Foodborne Pathogens. An Illustrated Text, Wolfe Publishing Ltd., London.

Walker, S.J. and Stringer, M.F. (1987) Growth of *Listeria monocytogenes* and *Aeromonas hydrophila* at chill temperatures. Tech. Memo. 462. CFPRA: Chipping Campden.

Wimpfhimer, L., Altman, N.S. and Hotchkiss, J.H. (1990) Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organism in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11, 205–214.

Zee, J.A., Bouchard, C., Simard, R.E., Pichard, B. and Holley, R.A. (1984) Effect of N_2, CO, and CO_2 on the growth of bacteria from meat products under modified atmospheres. Microbiol. Alim. Nut. 2, 354–356.

Zhao, Y., Wells, J.H. and Marshall, D.L. (1992) Description of log phase growth for selected microorganisms during modified atmosphere storage. J. Proc. Engin. 15, 299–317.