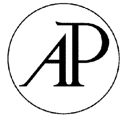
Food Microbiology, 2000, **17**, 657–669 doi:10.1006/fmic.2000.0358
Available online at http://www.idealibrary.com on IDEAL®



ORIGINAL ARTICLE

Growth/survival of natural flora and *Aeromonas hydrophila* on refrigerated uncooked pork and turkey packaged in modified atmospheres

S. B. Mano*, J. A. Ordoñez and G. D. Garcia de Fernando

To evaluate the growth/survival of natural flora and Aeromonas hydrophila *on refrigerated normal low (pork) and high (turkey) pH meats packaged in modified atmospheres,* A. hydrophila *was inoculated onto fresh pork and turkey meat slices. Inoculated and control samples were packaged in modified atmospheres (100% N_2, 20/80 and 40/60 CO_2/O_2) or in air in plastic bags and kept at 1 and 7°C. Samples packaged in air showed a similar microbiological pattern to that usually observed in fresh meat stored aerobically. Packaging in modified atmosphere produced a strong inhibition of bacterial growth at 1°C, particularly in samples stored in CO_2/O_2 enriched atmospheres.* Aeromonas hydrophila *grew on turkey and pork meat stored in 100% N_2 at 1 and 7°C. Likewise, growth of this bacterium was detected on turkey stored in 20/80 CO_2/O_2 at 7°C. No growth was observed in 40/60 CO_2/O_2 in any meat at both temperatures assayed.*
© 2000 Academic Press

Introduction

The shelf-life of meat may be extended by packaging it under either vacuum or different gas mixtures in combination with refrigeration. The ability of Modified Atmosphere Packaging (MAP) to prolong the shelf-life of foods has been well recognized for many years (Luiten et al. 1982, Gray et al. 1983) and this preservation system is becoming increasingly common. According to Brody (1993), nearly a third of all fresh poultry in North America is master-packaged in bulk under modified atmospheres for distribution to retail grocery and hotel, restaurant and institutional outlets. This author reports that dozens of meat packers and thousands of retail stores employ modified atmosphere packaging to distribute retail cuts of red meat to retail stores and consumers in Europe.

The great vulnerability of MAP of foods, from the safety standpoint, is that it inhibits the aerobic spoilage organisms, which usually warn consumers of spoilage, while the growth of psychrotrophic pathogens (e.g. *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila*) might be allowed or even stimulated (Farber 1991). A number of claims have been made in this respect, because the shelf-life extension might increase the microbiological hazard (Enfors et al. 1979, Silliker and Wolfe 1980, Palumbo 1986, Berrang et al. 1989, Day 1993).

*Corresponding author: Departamento de Tecnologia de los Alimentos, Facultad de Veterinária, U.F.F. 24230-340, Niterói/RJ, Brazil. Fax: +55-21-714-4041. E-mail: mtasbm@vm.uff.br

Received:
22 February 2000

Departamento de Nutrición y Bromatología III (Higiene y Tecnología de los Alimentos) Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain

0740-0020/00/060657 + 13 $35.00/0 © 2000 Academic Press

The role of *Aeromonas* spp. in human disease is controversial. Some strains may be capable of causing enteric disease in man; however, there have been few instances in which a causal link has only occasionally been established between the presence of aeromonads in a food and human enteritis (Fries et al. 1996). Several investigations indicate that some species possess virulence factors (Davis et al. 1978, Kaper et al. 1981, Gracey et al. 1982, Le Chevallier et al. 1982, Burker et al. 1984, Havelaar et al. 1992, Kirov and Brodribb 1993), which could be involved in human infections, including acute diarrhoea disease, which occurs mainly in children, elderly and immunocompromised individuals (Fries et al. 1996). On the other hand, despite the essentially negative result obtained in a single human trial experiment, there is strong evidence that some strains can be enteropathogenic (Kirov 1997). The same author states that at present it is not possible to identify the disease-causing strains because of our lack of knowledge about *Aeromonas* virulence mechanisms.

Aeromonas spp. are common in raw foods. For instance, Myers et al. (1982) isolated *Aeromonas* spp. in 20% of vacuum-packaged pork samples examined. The data varies 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 varies from 1 to 2°C (Fries et al. 1996), although Gill and Reichel (1989) demonstrated that *A. hydrophila* might grow in foods at even lower temperatures (−2°C). *Aeromonas hydrophila* growth has been detected in a variety of vacuum-packaged products stored between −2 and 10°C such as beef (Gill and Reichell 1989), roast beef (Hudson et al. 1994) and pork (Sheridan et al. 1992, van Laack et al. 1993). Doherty et al. (1996) showed that growth is influenced by the meat pH. These authors detected growth in lamb at pH 6·0 but not in lamb at a lower pH (5·5–5·8), both stored under vacuum and maintained at 5°C. These authors reported that CO_2 inhibits *A. hydrophila* growth. Sheridan et al. (1992) did not detect any growth of this bacterium in lamb packaged in either an atmosphere of CO_2/O_2 (20/80) or CO_2/N_2 (50/50) or 100% CO_2 at 5°C after 21 days of storage, although Doherty et al. (1996) observed growth in lamb (pH 6·0) at 5°C, except under 100% CO_2. However, Blickstad and Molin (1983) reported *A. hydrophila* growth on pork in this atmosphere. From the data presented in the above mentioned articles, it may be concluded that the behavior of this pathogen in different conditions (pH, kind of meat, stored temperature and MAP environment) still remains uncertain. This work was, therefore, undertaken to clarify this matter and to know the effect of carbon dioxide/oxygen enriched atmospheres on the growth of this bacterium since these atmospheres are widely used to maintain a bright red colour of raw meat (Ordóñez and Ledward 1977, Brody 1993). The aim of the present study was to determine the growth/survival of natural microbiota and *A. hydrophila* in refrigerated pork (pH ~ 5·4) and turkey (pH ~ 6·0) slices packaged under different commercial modified atmospheres (air, 100% N_2, and 20/80 CO_2/O_2 and 40/60 CO_2/O_2) stored at 1°C and at 7°C. Comparison of the growth rates of the natural flora and *A. hydrophila* allows the hazard of MAP to be assessed in relation with traditional packaging and distribution systems.

Materials and Methods

Preparation of meat samples

Pork (*Longissimus dorsi*) was purchased in a local market and cut aseptically into *c.* 0·5-cm-thick slices, the surface being about 18 cm^2. Two turkeys were purchased in a local market and after removing their breasts, these were sliced aseptically to the size mentioned for pork. Slices from one breast were stored at 1°C and those from the other at 7°C (see below).

Experimental procedure

The samples were divided into two batches. One was untreated (control) and slices of the other were immersed in a suspension of *Aeromonas hydrophila* containing 10^3–10^5 cfu ml^{-1}. The strain was isolated from a human diarrheic faeces by staff of Son Dureta Hospital

(Palma de Mallorca, Spain) and was maintained at -80°C until use. Once unfrozen, the strain was revitalized by culturing three times at 28°C for 24 h in triptic soy broth. The suspension was made by mixing an aliquot of a culture of *A. hydrophila* with 2 l sterile saline solution (0·85% NaCl). Fillets, inoculated and non-inoculated, were packaged in laminated bags (one fillet per bag) of 20 × 22 cm Cryovac BB4L, with diffusion coefficients, according to the supplier, of 150 cm^3/24 h·m^2·bar to CO_2, 35 cm^3/24 h·m^2·bar to O_2 and 1·4 cm^3/24 h·m^2·bar to N_2. However, these parameters were measured by the Insituto Nacional de Engenharia e Tecnologia Industrial (INET, Lisboa, Portugal), who established the following diffusion coefficients: 77 cm^3/24 h·m^2·bar to CO_2, 24 cm^3/24 h·m^2·bar to O_2 and 1·4 cm^3/24 h·m^2·bar to N_2. Samples of each species were subdivided into four batches. Air was removed from the bags which were then filled with either 1 l of air (100%), nitrogen (100%), CO_2/O_2 (20/80) or CO_2/O_2 (40/60) in an 'Euvac 65' packaging machine, which, finally, heat-sealed the bags. 'Carburos de España S.A' supplied the gases. A portion of the samples of each atmosphere was stored in walk-in cold rooms at 1°C and the rest at 7°C until sampling. Samples were periodically taken and analysed.

Analyses

Before microbiological sampling, the composition of the bag atmosphere was analysed for CO_2 and O_2 using a combined gas analyser 'Abiss' mod. GT12. When the packages were open, objectionable changes in the appearance and odour of the slices were recorded. Microbiological analyses were performed on duplicate samples, i.e. two slices from different bags. After opening the bags, slices were immersed in 25 ml sterile physiological saline solution (0·85% NaCl) and homogenized in a Stomacher for 15 s after which decimal dilutions were prepared in saline solution. After taking the inoculum for microbiological analysis, the homogenized sample was used for pH measurement by introducing an electrode of a 'Crison' mod. Micro pH 2001 pH meter.

Microbial analyses

Total viable counts (TVC) were determined on PCA (plate count agar; Oxoid) at 32°C for 48 h. Lactic acid bacteria (LAB) on double layer MRS agar base (Oxoid) pH 5·6 (de Man et al. 1960), incubating at 32°C for 72 h. *Brochothrix thermosphacta* was counted on STAA (streptomycin, thallous acetate, actidione) agar base CM881 with selective supplement SR151E (streptomycin sulfate, cyclohexamide and thallous acetate, Oxoid), at 24°C for 72 h. Enterobacteriaceae were enumerated on double layer VRBG agar base (Oxoid) at 32°C for 48 h. *Aeromonas hydrophila* were counted on aeromonas agar base enriched with 0·5% ampicilin (selective supplement SR136E, Oxoid) at 28°C for 48 h. Counts were expressed as cfu cm^{-2}.

Bacterial growth parameters (lag phase and doubling time) were assessed using Gompertz equations and the shelf-life of meat was defined as days to reach 10^7 cfu cm^{-2}.

Results and Discussion

Changes in atmospheric composition

The atmospheric composition remained fairly stable during meat storage. There is little information about changes in gas composition during meat storage in modified atmospheres. Furthermore, contradictory results have been presented in some occasions without clear data about the gas permeability of the packaging materials. Assuming that the gas permeability is adequate for this kind of packaging, the controversy in the literature about the changes in the atmospheric composition during storage are probably related with the gas/product ratio, which is not always indicated. If this ratio is high, no important changes must be expected and *vice versa*. While Seideman et al. (1980) indicated that CO_2 concentration diminished during the storage of pork (at 2°C) packaged in atmospheres composed initially by 20 and 40% of CO_2 with 5 and 10% of O_2, Spahl et al. (1981) assured that CO_2 levels increased during storage of the same meat in atmospheres enriched in the same gases. Fang and Lin (1994) observed an O_2 decrease and CO_2 increase during storage of cooked pork

packaged in air at 4°C, indicating that the microflora grew with a classic aerobic metabolism. Ingham and Potter (1988) in surimi and McMullen and Stiles (1991) and Sørheim et al. (1995) in uncooked pork observed a similar behavior. Nissen et al. (1996) observed a CO_2 increase in beef packaged in 100% N_2 at 2°C for 5 weeks.

pH changes

The initial and final pH values of pork and turkey in different atmospheres at 1 and 7°C, as well as the time of storage of the samples, are reported in Table 1. Pork samples presented an initial pH of 5·3. This value showed a tendency to increase. The variations were smaller in CO_2 enriched atmospheres, since the increases hardly amounted to 0·5 pH units. In samples of turkey meat, the pH values varied to 5·7 for samples stored at 7°C and 6·0 for those maintained at 1°C. A remarkable increase in pH was only observed in samples packed in air, coinciding with food spoilage. In the other atmospheres the pH remained stable throughout the experiment. CO_2 enrichment of the atmosphere prevented aerobic spoilage of meat, and hence, meat pH was unchanged. Other authors have studied the effect of atmosphere enriched in CO_2 on food pH. Clark and Lentz (1973), Daniels et al. (1985) and McMullen and Stiles (1991) observed pH decreases as a consequence of the CO_2 solubility in foods, whereas pH modifications were not observed in salmon (Fey and Regenstein 1982, López-Gálvez et al. 1995), lamb (Doherty et al. 1995, Sheridan et al. 1995, Doherty et al. 1996) and beef (Avery et al. 1995) packaged in atmospheres enriched in CO_2. This may be due to the buffer capacity of food components that is sufficient to counteract the effect of the CO_2 solution.

Shelf-life

Tables 2 and 3 show the effect of the gas atmosphere on pork meat shelf-life (controls and inoculated) packaged in modified atmospheres. The bacterial load value of 10^7 cfu cm^{-2} was not reached after 16 days of modified atmosphere storage at 1°C. In turkey meat (Tables 4 and 5), in spite of the difference between initial counts, shelf-life of control and inoculated samples was similar from a microbiological point of view. The shelf-life was longer at 1°C (Table 4) than that at 7°C (Table 5), reaching approximately 1 week in air, 8 days in 100% N_2, 26 days in 20/80 CO_2/O_2 and more than 1 month in 40/60 CO_2/O_2. The extension of the meat shelf-life observed in this work (Tables 2–5) is along the levels of that shown by other authors (Parry 1993, Church 1994). The conservative effect of the CO_2-enriched atmospheres was as evident as the synergistic effect of combined temperature and modified atmosphere packaging, as other authors (Ordóñez and Ledward 1977, Enfors et al. 1979, Blickstad and Molin 1983, Farber 1991, Marshall et al. 1991, Nissen et al. 1996) previously showed. This synergistic effect impeded, in some cases, samples reaching 10^7 cfu cm^{-2} (Tables 2 and 4). Of course, meat packaged in CO_2-enriched MAP may be not stored indefinitely at refrigeration temperatures because the limiting factor of the meat shelf-life has not always been microbial development. For example, either the enzymatic changes may lead to an excessive meat softening, or the meat may become brown because of myoglobin oxidation. Therefore, in order to slow down this phenomenon, the atmospheres

Table 1. Initial and final pH of slices of pork and turkey meat in different atmospheres at 1 and 7°C and time (days) of storage of the samples

	Pork meat						Turkey meat					
	1°C			7°C			1°C			7°C		
	Days	Initial	Final	Days	Initial	Final	Days	Initial	Final	Days	Initial	Final
Air 100 %	13	5·3	6·9	8	5·3	6·8	16	6·0	7·0	12	5·7	7·0
N_2 100 %	16	5·3	5·7	11	5·3	6·1	23	6·0	6·3	14	5·7	5·6
20/80 CO_2/O_2	16	5·3	5·8	13	5·3	5·4	34	6·0	6·0	20	5·7	5·7
40/60 CO_2/O_2	16	5·3	5·6	13	5·3	5·5	34	6·0	6·0	20	5·7	5·6

Table 2. Shelf-life and growth parameters of micro-organisms on pork *Longissimus dorsi* slices packaged in plastics bags (Cryovac BB4L) filled with several gas mixtures and stored at 1°C for 13 (air), or 16 days (100% N_2, 20/80 CO_2/O_2, 40/60 CO_2/O_2)

	Atmospheres	SL	Parameter	TVC	LAB	*Enter.*	*Br. th.*	*A. h.*
Control			IC	4·7	3·8	3·4	3·0	ND
	Air	7	LP	3·0	2·2	4·5	1·7	-
			DT	0·5	1·3	0·3	0·5	-
			NC	9·0	4·9	7·0	7·5	-
	N_2 (100%)	>16*	LP	4·5	2·5	7·0	3·0	-
			DT	1·3	1·5	0·5	0·7	-
			NC	6·5	5·6	4·0	6·0	-
	20/80 CO_2/O_2	>16*	LP	7·2	5·3	GND	5·8	-
			DT	2·4	1·7	-	1·0	-
			NC	5·4	5·0	1·0	4·7	-
	40/60 CO_2/O_2	>16*	LP	8·0	6·9	GND	7·0	-
			DT	3·4	1·6	-	1·4	-
			NC	5·5	4·9	0·8	4·5	-
Inoculated			IC	4·7	3·8	3·4	3·0	2·5
	Air	10	LP	3·5	3·4	7·0	1·9	4·5
			DT	0·4	1·4	0·3	0·5	0·5
			NC	8·8	4·7	6·5	7·0	4·2
	N_2 (100%)	>16*	LP	4·5	3·0	7·1	3·0	5·5
			DT	1·8	1·4	0·6	1·1	1·9
			NC	6·4	5·7	4·2	6·0	3·6
	20/80 CO_2/O_2	>16*	LP	7·4	4·1	GND	5·1	GND
			DT	2·1	1·5	-	1·3	-
			NC	6·0	4·9	2·6	5·9	1·8
	40/60 CO_2/O_2	>16*	LP	8·6	7·3	GND	6·5	GND
			DT	3·0	1·5	-	1·7	-
			NC	6·1	5·2	1·0	4·7	0·9

SL — Shelf-life (days to reach 10^7 cfu cm^{-2}).
TVC —Total viable count (log cfu cm^{-2}).
LAB —Lactic acid bacteria (log cfu cm^{-2}).
Enter.—*Enterobacteriaceae* (log cfu cm^{-2}).
Br. th.—*Brochothrix thermosphacta* (log cfu cm^{-2}).
A. h. —*Aeromonas hydrophila.*
IC — Initial charge (log cfu cm^{-2}).
ND — Not detected.
LP— Lag phase (days).
DT— Doubling time (days).
NC — Number of cells at stationary phase or, when GND, at the end of storage (log cfu cm^{-2}).
*10^7 cfu cm^{-2} level was not reached after 16 days.
GND — Growth not detected.

used to extend the red meat shelf-life must include O_2 (Ordoñez and Ledward 1977).

Natural microbiota

Tables 2–5 summarize the growth parameters of the micro-organisms studied and the initial and final counts in pork and turkey fillets packaged in modified atmospheres at 1 and 7°C. The initial count in samples of pork (control and inoculated, Tables 2 and 3) was practically not modified by inoculation with the pathogen, since the load of *A. hydrophila* was lower than 1% of the total flora. The lag phase and doubling time were higher at 1 than at 7°C (Tables 2 and 3). In turkey meat fillets, the initial

Table 3. Shelf-life and growth parameters of microorganisms on pork *Longissimus dorsi* slices packaged in plastics bags (Cryovac BB4L) filled with several gas mixtures and stored at 7°C for 8 (air), 11 (100% N_2), or 13 days (20/80 CO_2/O_2, 40/60 CO_2/O_2)

	Atmospheres	SL	Parameter	TVC	LAB	*Enter.*	*Br. th.*	*A. h.*
Control			IC	4·7	3·8	3·4	3·0	ND
	Air	4	LP	1·5	1·0	1·9	0·9	-
			DT	0·2	0·3	0·3	0·2	-
			NC	9·1	6·2	7·3	7·8	-
	N_2 (100%)	6	LP	2·0	1·0	1·9	0·8	-
			DT	0·5	0·4	0·5	0·4	-
			NC	7·3	6·3	5·5	6·2	-
	20/80 CO_2/O_2	10	LP	2·2	1·3	GND	0·8	-
			DT	0·7	0·3	-	0·5	-
			NC	7·1	6·2	2·0	5·8	-
	40/60 CO_2/O_2	>13*	LP	4·0	2·0	GND	3·0	-
			DT	0·5	0·5	-	0·5	-
			NC	6·6	6·1	2·0	5·2	-
Inoculated			IC	4·7	3·8	3·4	3·0	2·5
	Air	4	LP	1·7	1·2	2·6	0·9	2·0
			DT	0·2	0·3	0·3	0·3	0·2
			NC	9·3	6·4	7·3	7·4	7·4
	N_2 (100%)	6	LP	2·0	1·0	2·6	1·0	3·5
			DT	0·5	0·3	0·5	0·4	0·4
			NC	7·5	6·9	6·2	6·3	5·4
	20/80 CO_2/O_2	9	LP	2·4	1·3	GND	0·8	GND
			DT	0·5	0·3	-	0·5	-
			NC	7·1	6·4	3·8	6·2	2·2
	40/60 CO_2/O_2	>13*	LP	3·8	1·9	GND	2·7	GND
			DT	0·7	0·5	-	0·4	-
			NC	6·9	6·4	2·0	5·3	2·2

SL — Shelf-life (days to reach 10^7 cfu cm^{-2}).
TVC —Total viable count (log cfu cm^{-2}).
LAB —Lactic acid bacteria (log cfu cm^{-2}).
Enter.—*Enterobacteriaceae* (log cfu cm^{-2}).
Br. th.—*Brochothrix thermosphacta* (log cfu cm^{-2}).
A. h. —*Aeromonas hydrophila.*
IC — Initial charge (log cfu cm^{-2}).
ND — Not detected.
LP— Lag phase (days).
DT— Doubling time (days).
NC — Number of cells at stationary phase or, when GND, at the end of storage (log cfu cm^{-2}).
*10^7 cfu cm^{-2} level was not reached after 16 days.
GND — Growth not detected.

number of micro-organisms in control samples was slightly lower, between 10^3 and 10^4 cfu cm^{-2} (Tables 4 and 5). However, the inoculation of *A. hydrophila* elevated the count up to 5·1 log_{10} cfu cm^{-2} (Tables 4 and 5). At 7°C, doubling times were prolonged as the atmosphere became more selective, from about 0·2 days in air up to 2·7 in control fillets packaged in 40/60 CO_2/O_2 (Table 5). At 1°C (Table 4), the behavior of the microflora was similar to that described for 7°C. Few works have determined doubling times of total microbiota in modified atmospheres. Manu-Tawiah et al. (1993) affirmed that the aerobic microflora developed in pork chops at 4°C presented, in an experiment, duplication times of 0·8 days in air and

Table 4. Shelf-life and growth parameters of microorganisms on turkey breast slices packaged in plastics bags (Cryovac BB4L) filled with several gas mixtures and stored at 1°C for 16 (air), 23 (100% N_2), or 34 days (20/80 CO_2/O_2, 40/6 CO_2/O_2)

	Atmospheres	SL	Parameter	TVC	LAB	*Enter.*	*Br. th.*	*A. h.*
Control			IC	3·9	0·8	3·5	0·8	ND
	Air	6	LP	2·1	8·0	4·6	4·0	-
			DT	0·6	0·4	0·5	0·5	-
			NC	9·4	1·7	8·0	7·6	-
	N_2 (100%)	8	LP	1·9	12·0	4·5	6·4	-
			DT	1·2	0·7	0·8	0·9	-
			NC	7·8	3·5	6·4	5·0	-
	20/80 CO_2/O_2	> 34*	LP	15·8	16·1	18·3	4·8	-
			DT	4·0	2·5	4·5	1·4	-
			NC	6·5	3·4	4·0	5·1	-
	40/60 CO_2/O_2	> 34*	LP	18·3	16·7	GND	6·1	-
			DT	8·5	5·4	-	1·0	-
			NC	5·1	2·9	2·1	3·6	-
Inoculated			IC	5·1	0·8	3·5	0·8	5·1
	Air	7	LP	1·8	6·3	1·3	4·5	6·9
			DT	0·5	0·3	0·8	0·5	0·5
			NC	9·3	2·4	8·4	7·7	9·3
	N_2 (100%)	8	LP	1·2	12·2	4·0	6·0	7·0
			DT	1·1	0·5	1·0	0·9	1·0
			NC	8·0	3·7	6·1	5·4	7·1
	20/80 CO_2/O_2	26	LP	15·4	15·2	7·9	4·5	GND
			DT	6·4	2·3	4·3	1·3	-
			NC	7·6	3·6	4·6	7·6	5·1
	40/60 CO_2/O_2	> 34*	LP	GND	17·1	GND	5·9	GND
			DT	-	4·5	-	1·1	-
			NC	5·2	3·0	2·1	4·8	4·1

SL — Shelf-life (days to reach 10^7 cfu cm^{-2}).
TVC —Total viable count (log cfu cm^{-2}).
LAB —Lactic acid bacteria (log cfu cm^{-2}).
Enter.—*Enterobacteriaceae* (log cfu cm^{-2}).
Br. th.—*Brochothrix thermosphacta* (log cfu cm^{-2}).
A. h. —*Aeromonas hydrophila.*
IC — Initial charge (log cfu cm^{-2}).
ND — Not detected.
LP— Lag phase (days).
DT— Doubling time (days).
NC — Number of cells at stationary phase or, when GND, at the end of storage (log cfu cm^{-2}).
*10^7 cfu cm^{-2} level was not reached after 16 days.
GND — Growth not detected.

of more than 6 days in all studied atmospheres: vacuum, 20/80 CO_2/N_2, 40/60 CO_2/N_2 and 40/10/50 $CO_2/O_2/N_2$, while in other experiments the results were 1·6 days in air and between 4·6 and 4·9 in the other atmospheres. These data are in general agreement with those recorded in the present work (Tables 2–5).

Lag phases of lactic acid microflora at 1°C were more than 2 days in air and 100% N_2 and longer in enriched CO_2-atmospheres (Table 2). On the other hand, no differences were observed in the doubling times (1·5–1·7 days) between atmospheres (Table 2). Increasing the storage temperature from 1°C to 7°C decreased

Table 5. Shelf-life and growth parameters of microorganisms on turkey breast slices packaged in plastics bags (Cryovac BB4L) filled with several gas mixtures and stored at 7°C for 12 (air), 14 (100% N_2), or 20 days (20/80 CO_2/O_2, 40/6 CO_2/O_2)

	Atmospheres	SL	Parameter	TVC	LAB	*Enter.*	*Br. th.*	*A. h.*
Control			IC	3·0	1·8	1·6	1·1	ND
	Air	5	LP	2·1	1·8	1·0	1·7	-
			DT	0·2	0·8	0·3	0·3	-
			NC	9·5	4·0	8·5	6·9	-
	N_2 (100%)	11	LP	3·2	2·2	3·7	8·4	-
			DT	0·7	0·7	0·4	2·7	-
			NC	7·7	2·7	7·4	2·3	-
	20/80 CO_2/O_2	11	LP	2·5	3·8	1·3	3·7	-
			DT	0·9	0·8	0·7	3·9	-
			NC	8·5	4·0	7·9	2·4	-
	40/60 CO_2/O_2	> 20*	LP	3·5	6·2	6·7	GND	-
			DT	2·7	0·8	1·2	-	-
			NC	4·4	3·1	4·2	1·5	-
Inoculated			IC	5·1	1·8	1·6	1·1	4·4
	Air	4	LP	1·5	1·7	0·8	0·8	2·9
			DT	0·5	0·6	0·3	0·3	0·3
			NC	9·5	4·0	8·5	6·8	9·4
	N_2 (100%)	7	LP	3·8	2·0	1·4	9·0	4·3
			DT	0·7	0·6	0·2	3·1	0·8
			NC	7·9	2·9	7·9	1·8	7·1
	20/80 CO_2/O_2	11	LP	5·0	3·6	1·0	3·5	6·8
			DT	0·9	0·9	0·6	4·6	0·8
			NC	8·6	4·0	8·0	2·3	8·6
	40/60 CO_2/O_2	> 20*	LP	GND	6·2	1·0	GND	GND
			DT	-	0·8	1·9	-	-
			NC	4·8	3·2	4·4	1·7	3·5

SL — Shelf-life (days to reach 10^7 cfu cm^{-2}).
TVC —Total viable count (log cfu cm^{-2}).
LAB —Lactic acid bacteria (log cfu cm^{-2}).
Enter.—*Enterobacteriaceae* (log cfu cm^{-2}).
Br. th.—*Brochothrix thermosphacta* (log cfu cm^{-2}).
A. h. —*Aeromonas hydrophila.*
IC — Initial charge (log cfu cm^{-2}).
ND — Not detected.
LP— Lag phase (days).
DT— Doubling time (days).
NC — Number of cells at stationary phase or, when GND, at the end of storage (log cfu cm^{-2}).
*10^7 cfu cm^{-2} level was not reached after 16 days.
GND — Growth not detected.

the lag phase and reduced by *c.* four-fold the doubling times (Table 3). At both temperatures assayed, final counts were similar in all atmospheres (Tables 2 and 3). In turkey meat, the initial numbers of lactic acid bacteria were lower than 10^2 cfu cm^{-2} (Tables 4 and 5). At both 1 and 7°C, lag phases were longer as the atmosphere became more selective (Tables 4 and 5). At 7°C, doubling times were similar (about 0·7 days) in all atmospheres, while they increased in CO_2/O_2 enriched atmosphere at 1°C (Table 4). Final counts of lactic acid bacteria were low, always less than 10^4 cfu cm^{-2} (Tables 4 and 5). From these data, it may be concluded that

lactic acid microflora was not significantly affected by the studied atmospheres because the doubling times were similar in almost all cases, although the lag phases were usually longer in CO_2 enriched atmospheres. These observations confirm that lactic acid bacteria are quite resistant to CO_2, as other authors (Farber 1991, Parry 1993, Sørheim et al. 1995, Brody 1996) have previously reported.

The initial load of Enterobacteriaceae in pork meat only increased in samples packed in air and N_2 at 1 and 7°C, while it decreased in CO_2-enriched atmospheres (Tables 2 and 3). Lag phases at 7°C were *c.* 2·3–2·7 times shorter than at 1°C, while doubling times were similar at both temperatures. Doubling times in turkey breast increased as the atmosphere became more selective. The counts in stationary phase were always smaller at 1°C, and growth was not observed at this temperature in 40/60 CO_2/O_2 (Table 4). All these results indicate that enterobacteria are sensitive to CO_2, as other authors have previously stated (McMullen and Stiles 1991, Fu et al. 1992, Sawaya et al. 1995). Nevertheless, growth was not completely stopped and numbers of this microbiota may become noticeable as McMullen and Stiles (1991) observed in pork packaged in 40/60 CO_2/N_2 at 10°C, where enterobacteria were the dominant organisms. This behaviour was previously explained by Grau (1981), who pointed out that the enterobacteria could grow in refrigeration temperatures and in an anaerobic environment. Data from the present work confirm these findings.

Brocthothrix thermosphacta grew in all studied atmospheres and temperatures, except in turkey meat at 7°C in 40/60 CO_2/O_2 (Table 5). At 1°C, the lag phases were longer as the atmosphere became more selective. A similar behavior was observed in the doubling times, which increased from 0·5 days in air to approximately 1·7 days (pork meat, Table 2) and 1·1 days (turkey meat, Table 4) in 40/60 CO_2/O_2. As expected, a lower level of *B. thermosphacta* was detected in the stationary phase as the atmosphere became more selective. On the other hand, the behavioral trends of this bacterium in pork meat at 7°C (Tables 3) were not as clear as at 1°C. The lag phases in air, 100% N_2 and 20/80 CO_2/O_2 were very similar and a considerable increase was only observed in 40% CO_2 atmosphere. Regarding the doubling times, an increase was observed in the organisms growing on turkey meat when the atmosphere became more selective and growth was not detected under 40/60 CO_2/O_2 atmosphere (Table 5). The final counts followed the pattern observed at 1°C and more than 100 times *B. thermosphacta* was detected in air than that in the most selective atmosphere. An interesting parallelism was found between the development of *B. thermosphacta* and lactic acid bacteria (Tables 2–5). This fact, beside the psychrotrophic condition of these bacteria, indicates that these microorganisms may be dominant in modified atmosphere packaged foods (López-Gálvez et al. 1995) because of their relative resistance to CO_2 and ability to grow in a wide pH range. In this respect, Gill and Harrison (1989) observed that *B. thermosphacta* grew similarly in pork of quite different pH values (DFD and normal).

Aeromonas hydrophila *growth*

Tables 2 and 3 show the results with this microorganism in pork. *Aeromonas hydrophila* was not detected in any control sample. This fact confirmed the effectiveness of the selective medium used for counting *A. hydrophila* organisms.

The bacterium showed a high sensitivity to CO_2 (more marked at 1°C). The initial load of inoculated samples was $2{\cdot}5 \log_{10}$ cfu cm^{-2}, which meant less than 1% of the total microbiota. The organism grew in samples maintained in air and N_2, while it could not grow in CO_2 enriched atmospheres, in which, even a decrease of the viable count was observed over the storage period. As expected, this decrease was more noticeable at 1°C. At this temperature, the lag phases were 4·5 days in air and 5·5 in N_2, and doubling times were 0·5 and 1·9 days respectively. The final numbers were 4·2 $\log_{10}$ cfu cm^{-2} in air and 3·6 $\log_{10}$ cfu/cm^{-2} in N_2, which indicates a modest growth over the 16 days (Table 2). On the other hand, at 7°C the growth began, was faster and higher numbers were reached in the stationary phase (Table 3).

Tables 4 and 5 show the data of the growth of this pathogen in turkey meat. *Aeromonas*

hydrophila was not detected in any control sample. The initial load of the pathogen was 5·1 $\log_{10}$ cfu cm^{-2} in inoculated samples stored at 1°C (Table 4) and 4·4 $\log_{10}$ cfu cm^{-2} in those maintained at 7°C (Table 5). Therefore, the pathogen was dominant at the beginning of the experiment in both cases. At 1°C, these bacteria grew in samples maintained in air and nitrogen and not in CO_2 and O_2 enriched atmospheres. The lag phase was similar in air and N_2 (1 week), while doubling times were 0·5 days in air and 1·0 day in N_2. The organism level reached in stationary phase was higher in air (more than 10^9 cfu cm^{-2}) than in the other atmosphere. At 7°C, the pathogen grew in the same atmospheres as at 1°C and also in 20/80 CO_2/O_2. The lag phases lengthened as atmospheres were more selective from almost 3 days in air until almost 1 week in 20/80 CO_2/O_2. Doubling times were 0·3 days in samples packaged in air and less than 1 day in those packaged in N_2 and in 20/80 CO_2/O_2. The *A. hydrophila* number in the stationary phase was 9·4 $\log_{10}$ cfu cm^{-2} in slices maintained in air, more than 7·1 $\log_{10}$ cfu cm^{-2} in N_2 and 8·6 $\log_{10}$ cfu cm^{-2} in 20/80 CO_2/O_2. On the other hand, the viable count diminished logarithmically in samples packed in 40/60 CO_2/O_2 over the 20 days of the experiment (Table 5).

The best environment for *A. hydrophila* growth was the aerobic atmosphere (Tables 2–5). According to Golden et al. (1989) and Varnam and Evans (1991), the growth of this pathogen is favored in nitrogen atmospheres, although this result was not verified in this study either in turkey or in pork packaged in 100% N_2 and kept at 1 and 7°C. It was observed that the doubling times were always longer in nitrogen than in air. Several authors (Golden et al. 1989, Varnam and Evans 1991) stated that CO_2 has an inhibitory effect in *A. hydrophila* growth. The data of the present study confirm these results, since growth was only detected on turkey packaged in CO_2/O_2 (20/80) atmosphere stored at 7°C but not at 1°C (Table 6), while the pathogen failed to grow under higher CO_2 concentration (CO_2/O_2, 40/60) at 7°C. Similar findings have been reported by Sheridan et al. (1992) and Doherty et al. (1996). These authors did not detect any growth on lamb packaged in 20/80 CO_2/O_2, 50/50 CO_2/N_2 and 100% of CO_2 at 5°C.

Table 6. Growth of *Aeromonas hydrophila* on pork (pH ~ 5·5) and turkey meat (pH ~ 6·0) packaged in modified atmospheres

	Pork meat		Turkey meat	
	1°C	7°C	1°C	7°C
Air 100%	+	+	+	+
N_2 100%	+	+	+	+
20/80 CO_2/O_2	–	–	–	+
40/60 CO_2/O_2	–	–	–	–

+ Growth; – No growth.

Hudson et al. (1994) also confirmed the failure of this psychotroph in roast beef packaged in CO_2 saturated atmosphere at 1·5°C, but growth was detected at 3°C in the same food and atmosphere. Growth was also detected in beef under CO_2 controlled atmosphere at 10°C (Gill and Reichel 1989), in crab thermally treated and stored in 80% CO_2 atmosphere at 8°C (Ingham 1990), and surimi packaged in 36/13/51 $CO_2/O_2/N_2$ at 4°C (Ingham and Potter 1988). Nevertheless, no growth of this bacterium was observed in cod packaged either in 40/30/30 $CO_2/O_2/N_2$ at 5°C (Davies and Slade 1995) or in CO_2 saturated atmosphere at −1·5 (Bell et al. 1995), although the last authors detected growth in the same atmosphere at 3°C.

Table 6 summarizes the behavior of *A. hydrophila* on turkey and pork meat stored under modified atmospheres. It must be taken into consideration that the pathogen survived in samples that did not support growth and tended to decline slightly in pork meat under CO_2/O_2 enriched atmospheres (Tables 2 and 3) and only in the most inhibitory atmosphere (40/60 CO_2/O_2) in turkey meat (Tables 4 and 5).

From the data in the literature, confirmed by the observations of this study, it can be concluded that *A. hydrophila* growth is not completely stopped by CO_2 but is strongly inhibited. In general, lag phase and doubling time of *A. hydrophila* are prolonged when packaged in CO_2-enriched atmospheres in comparison to aerobic packaging. Therefore, MAP does not increase the hazard of growing *A. hydrophila* in refrigerated foods. Mano et al. (1995) concluded the same for *Listeria monocytogenes*. Both pathogens may grow in CO_2, but this gas restrains their growth rates, at least in a similar way to the inhibition of spoilage microbiota. On the

other hand, N_2 atmospheres should be avoided because they are neither as protective for foods, nor as safer for the consumers, as CO_2 enriched ones.

It would be very interesting to know the possible interactive effect between O_2 and CO_2 on *A. hydrophila* growth. Unfortunately, with the data obtained in the present work this would only be highly speculative. A research project is currently in progress of which one of the objectives is to determine the effect of different combinations of $CO_2/O_2/N_2$ atmospheres on the parameter *A. hydrophila* growth. The first data obtained seems to indicate that O_2 is inhibitory, although the effect of CO_2 is much more intense. A comparison of the effect of atmospheres containing the same percentage of CO_2 with and without O_2 seems to indicate that the interactive effect between both gases is negligible (unpublished data).

Acknowledgements

This work was supported by the 'Comisión Interministerial de Ciencia y Tecnología (CICYT)', project ALI94-0350 and ALI94-0405. Likewise, it is included in the AAIR Concerted Action PL 920630 'Physiology Food Poisoning Microorganisms'. The CAPES DAFA of the Brazil Government grants author Sergio Mano.

References

Avery, S. M., Rogers, A. R. and Bell, G. (1995) Continued inhibitory effect of carbon dioxide packaging on *Listeria monocytogenes* and other microorganisms on normal pH beef during abusive retail display. *Int. J. Food Sci. Technol.* **30,** 725–735.

Bell, R. G., Penney, N. and Moorehead, S. M. (1995) Growth of the psychrotrophic pathogens *Aeromonas hydrophila*, *Listeria monocytogenes* and *Yersinia enterocolitica* on smoked blue cod (*Parapercis colias*) packed under vacuum or carbon dioxide. *Int. J. Food Sci. Technol.* **30,** 515–521.

Berrang, M., Brackett, R. and Beuchat, L (1989) Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. *Appl. Environ. Microbiol.* **55,** 2167–2171.

Blickstad, E. and Molin, G. (1983) Carbon dioxide as a controlled atmosphere of spoilage flora of pork with special reference to temperature and sodium chloride. *J. Food Protect.* **46,** 756–763.

Brody, A. L. (1993) The market. In *Principles and Applications of Modified Atmosphere Packaging of Food.* (Ed. R. T. Parry) pp. 19–40. London, Blackie Academic & Professional.

Brody, A. L. (1996) *Envasado de alimentos en atmósferas controladas, modificadas y a vacío.* Zaragoza/ España Acribia.

Burker, V., Robinson, J, Cooper, M., Beaman, J., Partridge, K., Peterson, D. and Gracey, M. (1984) Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. *Appl. Environ. Microbiol.* **47,** 1146–1149.

le Chevallier, M. W., Evans, T. M., Seidler, R. J., Daily, O. P., Merrell, B. R., Rollins, D. M. and Joseph, S. W. (1982) *Aeromonas sobria* in chlorinated drinking water supplies. *Microb. Ecol.* **8,** 325–333.

Church, N. (1994) Developments in modified-atmosphere packaging and related technologies—review. *Trends Food Sci. Technol.* **5,** 345–352.

Clark, D. S. and Lentz, C. P. (1973) Use of mixtures of carbon dioxide and oxygen for extending shelf-life of pre-packaged fresh beef. *Can. Inst. Food Sci. Technol. J.* **6,** 194–196.

Daniels, J. A., Krishnamurthi, R. and Rizvi, S. S. H. (1985) A review of effects of carbon dioxide on microbial growth and food quality. *J. Food Prot.* **48,** 532–537.

Davies, A. R. and Slade, A. (1995) Fate of *Aeromonas* and *Yersinia* on modified atmosphere packaged (MAP) cod and trout. *Lett Appl. Microbiol.* **21,** 354–358.

Davis, W. A., Kane, J. G. and Garagusi, V. P. (1978) Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. *Medicine* **57,** 267–277.

Day, B. P. (1993) Fruit and vegetables. In *Principles and Applications of Modified Atmosphere Packaging of Food* (Ed. R. T. Parry) pp. 114–133. London, Blackie Academic & Professional.

Doherty, A., Sheridan, J. J., Allen, P., Mcdowell, D. A., Blair, I. S. and Harrington, D. (1995) Growth of *Yersinia enterocolitica* O:3 on modified atmosphere packaged lamb. *Food Microbiol.* **12,** 251–157.

Doherty, A., Sheridan, J. J., Allen, P., Mcdowell, D. A., Blair, I. S. and Harrington, D. (1996) Survival and growth of *Aeromonas hydrophila* on modified atmosphere packaged normal and high pH lamb. *Int. J. Food Microbiol.* **28,** 379–392.

Enfors, S., Molin, G. and Ternström, A (1979) Effect of packaging under carbon dioxide, nitrogen on air on the microbial flora of pork at 4°C. *J. Appl. Bacteriol.* **47,** 197–208.

Fang, T. J. and Lin, L. W. (1994) Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination system. *J. Food Prot.* **57,** 479–485.

Farber, J. M. (1991) Microbiological aspects of modified-atmosphere packaging technology—a review. *J. Food Prot.* **54,** 58–70.

Fey, M. S. and Regenstein, J. M. (1982) Extending shelf-life of fresh, wet, red hake and salmon using CO_2–O_2 modified atmosphere and potassium sorbate ice at 1°C. *J. Food Sci.* **47,** 1048–1054.

Fries, R., Hildebrandt, G., Humphrey, T. J., Nesbakken, T., Mulder, R. W. A. W., Schott, W. and Skovgaard, N. (1996) *Bacterial Pathogens on Raw Meat and their Properties* (Eds G. C. Mead and M. H. Hinton) Bristol UK, University of Bristol Press.

Fu, A. H., Molins, R. A. and Sebraneck, J. G. (1992) Storage quality characteristics of beef rib eye steaks packaged in modified atmospheres. *J. Food Sci.* **57,** 283–287.

Gill, C. O. and Harrison, C. L. (1989) The storage life of chilled pork packaged under carbon dioxide. *Meat Sci.* **24,** 313–324.

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.

Golden, D. A., Eyles, M. J. and Beuchat, L. R. (1989) Influence of modified-atmosphere storage on the growth of uninjured and heat-injures *Aeromonas hydrophila. Appl. Environ. Microbiol.* **55,** 3012–3015.

Gracey, M., Burke, V. and Robinson, J. (1982) Aeromonas-associated gastroenteritis. *Lancet* **ii,** 1304–1306.

Grau, F. H. (1981) Role of pH, lactate, and anaerobiosis in controlling the growth of some fermentative gram-negative bacteria on beef. *Appl. Environ. Microbiol.* **42,** 1043–1050.

Gray, R. J. H., Hoover, D. G. and Muir, A. M. (1983) Attenuation of microbial growth on modified-atmosphere packed fish. *J. Food Prot.* **47,** 610–618.

Havelaar, A., Schets, F., van Silfhout, A., Jansen, W., Wieten, G. and van der Kooij, D. (1992) Typing of *Aeromonas* strains from patients with diarrhoea and from drinking water. *J. Appl. Bacteriol.* **72,** 435–444.

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

Hudson, J. A., 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 Prot.* **57,** 204–208.

Ingham, S. C. (1990) Growth of *Aeromonas hydrophila* and *Plesiomonas shigelloides* on cooked crayfish tails during cold storage under air, vacuum, and modified atmosphere. *J. Food Prot.* **53,** 665–667.

Ingham, S. C. and Potter, N. N. (1988) Growth of *Aeromonas hydrophila* and *Pseudomonas fragi* on mince and surimis made from Atlantic pollock and stored under air or modified atmosphere. *J. Food Prot.* **51,** 966–970.

Kaper, J. B., Lockman, H., Colwell, R. R. and Joseph, S. W. (1981) *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. *J. Appl. Bacteriol.* **50,** 359–377.

Kirov, S. M. (1997) *Aeromonas* and *Plesiomonas* species. In *Food Microbiology. Fundamentals and frontiers*. (Eds M. P. Doyle, L. R. Beuchat and T. J. Montville) pp. 265–287, Washington D. C. ASM Press.

Kirov, S. M. and Brodribb, F. (1993) Exotoxin production by *Aeromonas* spp. in foods. *Lett. Appl. Microbiol.* **17,** 208–211.

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

López-Gálvez, D., Hoz, L. and Ordóñez, J. A. (1995) Effect of carbon dioxide and oxygen enriched atmospheres on microbiological and chemical changes in refrigerated tuna (*Thunnus alalunga*) steaks. *J. Agric. Food Chem.* **43,** 483–490.

Luiten, L. S., Marcello, J. A. and Dryden, F. D. (1982) Growth of *Salmonella typhimurium* and mesophilic organisms on beef steaks as influenced by type of packaging. *J. Food Prot.* **45,** 263–269.

McMullen, L. M. and Stiles, M. E. (1991) Changes in microbial parameters and gas composition during modified atmosphere storage of fresh pork loin chops. *J. Food Prot.* **54,** 778–783.

de Man, J. C. Rogosa, M. and Sharpe, M. E. (1960) A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **23,** 130–135.

Mano, S. B., García de Fernando, G. D., López-Gálvez, D., Selgas, M. D., García, M. L., Cambero, M. I. and Ordóñez, J. A. (1995). Growth/survival of natural flora and *Listeria monocytogenes* on refrigerated uncooked pork and turkey packaged under modified atmospheres. *J. Food Safety* **15,** 305–319.

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., Wiese-Lehigh, 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 Prot.* **54,** 841–843, 851.

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 Prot.* **45,** 33–37.

Nissen, H., Sørheim, O. and Dainty, R. (1996) Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. *Food Microbiol.* **13,** 183–191.

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.

Palumbo, S. A. (1986) Is refrigeration enough to restrain foodborne pathogens? *J. Food Prot.* **49,** 1003–1009.

Parry, (1993) Introduction. In *Principles and Applications of Modified Atmosphere Packaging of Food* (Ed. R. T. Parry) pp. 1–18, London, Blackie Academic & Professional.

Sawaya, W. N., Elnawawy, A. S., Abu-Ruwaida, A. S., Khalafawi, S. and Dashti, B. (1995) Influence of modified atmosphere on shelf-life of chicken carcasses under refrigerated conditions. *J. Food Safety,* **15,** 35–51.

Seideman, S. C., Vanderzant, C., Smith, G. C., Dill, C. W. and Carpenter, Z. L. (1980) Appearance of beef, pork and lamb stored in vacuum or modified gas atmospheres. *J. Food Prot.* **43,** 252–258.

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. In *FLAIR* 89055 *Iterim. 2nd year report*. EEC DGXII, Brussels.

Sheridan, J. J., Doherty, A., Allen, P., Mcdowell, D. A., Blair, Y. S. and Harrington, D. (1995) Investigations on the growth of *Listeria monocytogenes* on lamb packaged under modified atmospheres. *Food Microbiol.* **12,** 259–266.

Silliker, J. H. and Wolfe, S. K. (1980) Microbiological safety consideration in controlled atmosphere storage of meats. *Food Technol.* **34,** 59–64.

Sørheim, O., Grini, J. A., Nissen, H., Andersen, H. J. and Lea, P. (1995) Pork loins stored in carbon dioxide. Colour and microbiological shelf-life. *Fleischwirtschaft* (English part) **75,** 679–681.

Spahl, A., Reineccius, G. and Tatini, S. (1981) Storage life of pork chops in CO_2-containing atmospheres. *J. Food Prot.* **44,** 670–673.

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