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Effects of carbon dioxide on the fate of *Listeria monocytogenes*, of aerobic bacteria and on the development of spoilage in minimally processed fresh endive

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Abstract

Minimally processed fresh broad-leaved endive (*Cichorium endivia* L.) were stored at 3 and 10°C in modified atmospheres containing air, 10% CO_2/10% O_2, 30% CO_2/10% O_2, and 50% CO_2/10% O_2. The effects of these modified atmospheres on the fate of both aerobic bacteria and three strains of *Listeria monocytogenes*, was investigated. Increases in CO_2 concentrations significantly reduced the growth of the aerobic microflora. The best preservation of the visual quality occurred on endive leaves stored in 10% CO_2/10% O_2, whereas leaves stored in 30% CO_2/10% O_2 and 50% CO_2/10% O_2, and to a lesser extent in air, showed extensive spoilage after storage. *Listeria monocytogenes* was slightly affected at 3°C by the modified atmospheres, as compared to air. At 10°C, results varied between replicate experiments, but *L. monocytogenes* generally grew better as the CO_2 concentration was increased. The three test strains behaved in a similar way. In conclusion, among the modified atmospheres tested, a modified atmosphere containing 10% CO_2/10% O_2 resulted in improved visual quality of minimally processed fresh endive, without a marked effect on the growth of the aerobic microflora or of *L. monocytogenes*.

Keywords: Listeria monocytogenes; Aerobic bacteria; Spoilage; Minimally processed vegetables; Modified atmosphere; Refrigeration

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1. Introduction

Modified atmosphere packaging combined with storage at refrigeration temperatures is now a technology widely used to control the quality of foods. Modified atmospheres can reduce or inhibit the growth of foodborne spoilage or pathogenic microorganisms (Daniels et al., 1985; Dixon and Kell, 1989; El-Goorani and Sommer, 1981; Farber, 1991) in many foods. In fruits and vegetables, exposure of the commodity to lowered oxygen concentrations above tolerance limits or to enhanced carbon dioxide concentrations below tolerance limits also reduce their post-harvest biochemical and physiological activities (Kader et al., 1989). This technology has been widely applied to the packaging of minimally processed fresh vegetables (Zagory and Kader, 1988). Modified atmospheres in minimally processed vegetables are achieved through the respiration of the commodity within the package ('passive atmosphere modification') and/or through flushing of a gas mixture immediately after packaging ('active atmosphere modification') (Kader et al., 1989; Zagory and Kader, 1988).

Many authors have reported the beneficial effects of modified atmospheres, resulting in an increased shelf-life or in an improved preservation of the organoleptic qualities of minimally processed fresh vegetables, such as shredded iceberg lettuce (Ballantyne et al., 1988; Barriga et al., 1991) or shredded carrots (Carlin et al., 1990). However there have been few works devoted to the effects of modified atmospheres on the microbial safety of minimally processed vegetables, or on the possible control of the growth of psychrotrophic foodborne pathogens on these foods.

Listeria monocytogenes is generally considered as a potential vehicle of foodborne outbreaks caused by the consumption of contaminated minimally processed fresh vegetables (Ryser and Marth, 1991). Previous major outbreaks gave evidence of this risk: one in Canada in 1981 caused by the consumption of contaminated coleslaw (Schlech et al., 1983) and one in Boston in 1979 possibly associated with the consumption of raw vegetables (Ho et al., 1986). *Listeria monocytogenes* has been isolated from minimally processed fresh vegetables at frequencies that varied from 0 to 19% of samples (Beaufort et al., 1992; Breer and Baumgartner, 1992; Lainé and Michard, 1988; Sizmur and Walker, 1988; Velani and Roberts, 1991). Previous work has shown that *L. monocytogenes* survives or grows at refrigeration temperatures on many raw or processed vegetables, such as iceberg lettuce (Steinbruegge et al., 1988; Beuchat and Brackett, 1990), cabbage (Beuchat et al., 1986), shredded cabbage (Kallander et al., 1991), asparagus, broccoli and cauliflower (Berrang et al., 1989).

Previous work has shown that *L. monocytogenes* can grow on cut leaves of broad-leaved endive (Carlin and Nguyen-the, 1994; Carlin et al., 1995). The aim of this work was to study the effects of a wide range of CO_2 concentrations (0–50%) on the fate of *L. monocytogenes* on cut leaves of broad-leaved endive (*Cichorium endivia* L.) at refrigeration temperatures. Broad-leaved endive is a green leafy vegetable widely consumed in France and Europe and present in ready-to-use salads. The effects of modified atmospheres on aerobic bacteria and development of

spoilage were also followed. Experiments were done at two storage temperatures, with strains of different origin, and with leaves from different harvests to take account of the influence of uncontrolled factors linked to salad origin.

2. Materials and methods

2.1. Plant material

Fresh unprocessed broad-leaved endive (synonymous with escarole, *Cichorium endivia* L.) were obtained from a local company producing 'ready-to-use' salads. Outer or damaged leaves, as well as the core of endives were removed and discarded, as in commercial practice. Leaves were washed with distilled water, disinfected, rinsed, drained on absorbent paper, and cut with a surgical blade in pieces of approximately 5 cm × 5 cm, as previously described (Carlin et al., 1995).

The extent of spoilage was expressed as the percentage of the surface of the leaves exhibiting soft-rot, necroses or other decay.

Measurements of the pH of leaf macerates were done within the 15 min following removal from the modified atmospheres on non-inoculated leaves blended separately for 1 min with a homogenizer (Polytron, Lucerne, Switzerland) in 30 ml of freshly distilled water.

2.2. Bacteria

The strains used were *Listeria monocytogenes* strains F4642 (Scott A), isolated from a patient affected in the Massachussetts outbreak associated with pasteurized milk (Fleming et al., 1985), *L. monocytogenes* strains LCDC 81-861 and LCDC 81-1087 isolated from the coleslaw implicated in the outbreak of listeriosis in Canada (Schlech et al., 1983). The three strains were provided by Dr. B.M. Lund, Institute of Food research, Norwich, UK.

2.3. Inoculation of salad leaves with Listeria monocytogenes

Strains were subcultured twice for 4 days at 10°C in tryptose soya broth (Carlin et al., 1995), to a level between 10^8 and 10^9 viable bacteria/ml.

The culture was then diluted and dispersed in 2 l (for experiments with separate inoculation of three strains of *L. monocytogenes*) or 5 l of sterile distilled water (for experiments with a single strain of *L. monocytogenes*) to obtain the final desired concentration (a suspension of 10^5 CFU/ml to obtain about 10^4 CFU/g on the leaves). Leaves were dipped into the suspension for 10 min, drained twice on absorbent paper, and then placed in a 90-mm diameter petri dish. Non-inoculated endive leaves dipped into sterile distilled water were used as a pH control.

2.4. Storage of leaves in modified atmospheres

Petri dishes containing endive leaves were placed in plastic boxes (L × W × H (cm), 36 × 24 × 14). Boxes also contained an Erlenmeyer filled with wet absorbent paper and were covered with a perforated polymeric film, diameter of perforations: 0.5 mm; 1.25 perforations/cm^2 (Film SM60D, Grace Cryovac, Epernon, France). Each box was placed in a 160-l gas tight container (one box per atmosphere and per sampling date). The modified atmospheres were created and adjusted every 8 h to the desired concentration by a flushing of carbon dioxide, oxygen or nitrogen into the containers, using the device previously described by Lopes-Briones et al. (1992). The gaseous composition of the atmospheres was analyzed by measuring the IR absorbance of CO_2 and paramagnetic resonance of O_2, using a Servomex gas analyzer apparatus (Servomex, La Plaine Saint-Denis, France). The compositions of the four modified atmospheres tested were 10 ± 1% CO_2/10 ± 1% O_2, 30 ± 2% CO_2/10 ± 1% O_2, 50 ± 2% CO_2/10 ± 1% O_2 (balance nitrogen), and air as control.

2.5. Bacterial counts

Leaf pieces were placed in a Stomacher bag (Seward medical, London, England) with 6 ml peptone phosphate buffer (Carlin et al., 1995) and were homogenized with a mortar and pestle. Samples were aseptically filtered on a sterile nylon tissue and spread on agar plates of appropriate medium using a spiral plate maker (Spiral meter, Interscience, Saint-Nom-la-Bretèche, France).

Colonies of *L. monocytogenes* were enumerated on *Listeria* selective agar (Oxford formulation, Oxoid, Unipath, Dardilly, France) after incubation for 24 and 48 h at 30°C (Curtis et al., 1989). Ten colonies per experiment were isolated on Oxford medium plates for confirmation of their identity as *L. monocytogenes*, with tests done as described by Carlin et al. (1995). All strains were confirmed as *L. monocytogenes*. Before inoculation, detection of *L. monocytogenes* in each salad sample was done in *Listeria* Enrichment Broth (Lovett and Hitchins, 1988) followed by plating on Oxford agar (Curtis et al., 1989). *L. monocytogenes* was not detected in any of the non-inoculated leaves.

Numbers of aerobic bacteria were determined on Trypticase Agar medium (Carlin et al., 1995) after 48 h incubation at 30°C. Whenever necessary, *Listeria* spp. were distinguished by colony morphology from the other aerobic bacteria and excluded from the counts. Lactic acid bacteria (LAB) were enumerated on MRS medium (De Man et al., 1960) containing 100 mg/l bromocresol green, which was incubated for 5 days at room temperature in candle jars.

Bacterial counts were expressed as log CFU/g of leaf.

2.6. Experimental design

Three replicate experiments were done at both temperatures. Experiments done at 3°C were numbered 1, 2 and 3. Leaves were stored for 4, 7 and 10 days in

experiment 1, and for 2, 4, 7, 10 and 14 days in experiments 2 and 3. Experiments done at 10°C, where leaves were stored for 2, 4 and 7 days were numbered 4, 5 and 6. *L. monocytogenes* Scott A strain was used in all experiments. Experiments 1 and 4 were designed to compare the growth of Scott A strain relative to that of strains LCDC 81-861 and LCDC 81-1087. In experiments 2, 3, 5 and 6, the pH of leaf macerates was measured and LAB were enumerated.

2.7. Statistical analysis

Statistical analyses was done using SAS software (SAS Institute Inc., Cary, NC, USA). Statistical analyses of bacterial populations were done using logarithm (base 10) of the bacterial counts. Means were separated using the Tukey's Honest Significant Difference Test at the 5% level.

3. Results

3.1. Effects of modified atmospheres on the growth of the aerobic microflora and of LAB on endive leaves

There was an increase in populations of aerobic bacteria on endive leaves stored in air and in the modified atmospheres containing 10% CO_2/10% O_2, 30% CO_2/10% O_2 and 50% CO_2/10% O_2 (Table 1). The populations increased faster at 10 than at 3°C. The highest increase occurred in air, then in 10% CO_2/10% O_2 and the lowest increase in 50% CO_2/10% O_2. At both 10 and 3°C, numbers of aerobic bacteria at the end of storage were markedly different between the three replicate experiments. CO_2 concentrations of 30 or 50% significantly delayed growth of aerobic bacteria in all experiments.

Populations of LAB were always below the minimum level of detection, i.e. about 200 CFU/g (results not presented).

3.2. Effects of modified atmospheres on the growth of L. monocytogenes on endive leaves. Influence of the type of strain

At 3°C, the increase in population of *L. monocytogenes* strains Scott A and LCDC 81-861 inoculated onto endive leaves were higher than those of strain LCDC 81-1087, in the different modified atmospheres tested (Fig. 1). Nevertheless, the increase in numbers of *L. monocytogenes* on endive leaves after 10 days of storage at 3°C was low (i.e. between 0.3 and 1.5 log CFU/g; Fig. 1, Table 2) and was slightly higher during storage in air in experiments 1 and 2. The modified atmospheres affected the three strains in a similar way (Fig. 1). In experiment 3, there was no significant difference ($P < 0.05$) in the populations of *L. monocytogenes* measured on the endive leaves stored under the four modified atmospheres tested (Table 2).

Table 1
Effects of CO_2 on the populations of aerobic bacteria on minimally processed broad-leaved endive at 3 and 10°C

Storage temperature (°C)	Experiment no.	CO_2 (%)	Aerobic bacteria (log CFU/g)					
			Time (days)					
			0	2	4	7	10	14
3	2	0.04 (a)	3.85	4.59 a	5.09 a	5.81 a	6.78 a	6.84 a
		10 (b)		3.94 a	4.20 b	4.87 ab	5.79 b	6.42 ab
		30 (b)		4.29 a	4.11 b	4.75 ab	5.34 bc	5.77 b
		50 (b)		4.03 a	4.05 b	3.99 b	4.67 c	5.82 ab
	3	0.04	4.37	5.05 a	5.75 a	6.69 a	7.04 a	8.00 a
		10		4.98 a	5.28 ab	6.64 a	6.72 a	7.75 a
		30		4.41 a	4.59 bc	5.92 b	5.75 b	6.75 b
		50		4.98 a	4.25 c	5.17 c	5.48 b	5.88 c
10	5	0.04	4.82	5.36 a	6.51 a	6.54 a		
		10		5.00 a	5.48 b	6.47 a		
		30		5.02 a	5.06 bc	5.86 a		
		50		5.05 a	4.78 c	6.22 a		
	6	0.04	3.83	6.06 a	7.28 a	8.13 a		
		10		5.29 ab	6.93 ab	7.77 ab		
		30		4.80 bc	4.55 c	7.39 b		
		50		4.06 c	6.26 b	7.37 b		

For each experiment, means in columns followed by the same letter are not significantly different at the 5% level (Tukey's Honest Significant Difference Test).
$n = 10$, except on unstored products where $n = 5$.
(a): air.
(b): with 10% O_2 (balance N_2).

At 10°C, the influence of the modified atmospheres on the changes in the populations of *L. monocytogenes* was the same for the three strains tested (Fig. 1). In experiments 4 and 5, the higher the CO_2 concentration in the modified atmosphere, the higher was the increase in populations of *L. monocytogenes* on endive leaves (Fig. 1, Table 2). In contrast, in experiment 6 the highest increase in numbers of *L. monocytogenes* occurred in air, and in 50% CO_2/10% O_2, and the lowest increases occurred in 10% CO_2/10% O_2 and 30% CO_2/10% O_2.

3.3. Effects of modified atmospheres on the extent of spoilage on endive leaves

At 3°C as well as at 10°C, the lowest extent of spoilage of endive leaves occurred generally in the modified atmospheres containing 10% CO_2/10% O_2 (Table 3). Spoilage of leaves stored in air or in 30% CO_2/10% O_2 generally occurred to a greater extent than that of leaves stored in 10% CO_2/10% O_2. The most extensive spoilage occurred on leaves stored in 50% CO_2/10% O_2 at 3°C.

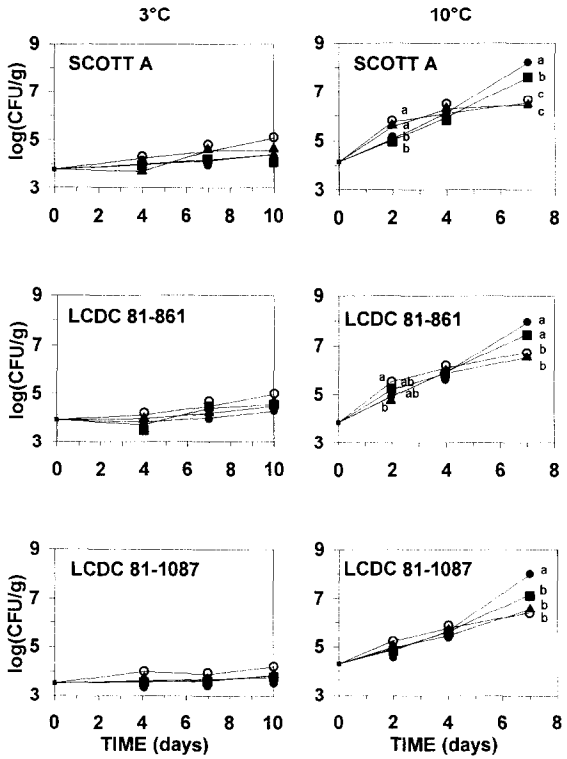

Fig. 1. Effects of modified atmospheres containing air (○), 10% CO_2/10% O_2 (▲), 30% CO_2/10% O_2 (■), and 50% CO_2/10% O_2 (●) on the fate of three strains of *L. monocytogenes* on minimally processed fresh broad-leaved endive. $n = 6$, except on unstored products where $n = 5$. At 10°C and at each sampling date, means followed by the same letters or by no letter are not significantly different at the 5% level (Tukey's Honest Significant Difference Test).

3.4. Effects of modified atmospheres on the pH of endive leaf macerates

The initial pH of endive leaves was about 6.0 and did not change after storage in air for 7 days at 10°C or 14 days at 3°C (results not shown). The pH of endive leaves increased as the CO_2 concentration in the modified atmosphere increased, and the highest pH (about 7.0) occurred in blended endive leaves stored for 7 days at 10°C or 14 days at 3°C in 50% CO_2/10% O_2 (results not shown).

4. Discussion

The growth of the aerobic microflora on endive leaves at temperatures of refrigeration was reduced or delayed by storage in CO_2-enriched modified atmospheres. The visual quality of the leaves was improved by storage in 10% CO_2/10% O_2, although this gaseous composition had only a slight effect on the aerobic microflora as compared to air. This effect could be due to a reduction in the physiological activity and to a delayed senescence of endive tissue. Other work has demonstrated the improvement of the visual quality of minimally processed green salad vegetables after storage in CO_2-enriched modified atmospheres. For instance, the visual quality of shredded endive was improved after storage at 10°C by packaging in 20% CO_2 in air as initial headspace, as compared with air as initial headspace (Carlin and Nguyen-the, 1989). A similar effect also occurred on shredded butterhead lettuce packed in 10% CO_2/90% N_2 as initial headspace (Mazollier et al., 1989), on salad vegetables packed in 10.5% CO_2/2.25% O_2 as initial headspace (Priepke et al., 1976) and on shredded iceberg lettuce stored in a controlled atmosphere containing 10% CO_2/3% O_2 (Barriga et al., 1991). The modified atmospheres used in the works reported above had no significant effect on the growth of the aerobic microflora. Nevertheless, King et al. (1991) reported both a reduction in the growth of the aerobic microflora and an improvement in the visual quality of shredded iceberg lettuce stored in sealed bags (in which CO_2 increased to 19% after 14 day-storage at 2.8°C), as compared to unsealed bags. In contrast, in the present study, endive leaves stored in 30% CO_2/10% O_2, 50% CO_2/10% O_2, and, in a lesser extent, in air, showed extensive spoilage after storage. CO_2 concentrations above 30% were probably higher than those tolerated by endive and could have induced physiological injuries of endive leaves. Our work did not show any increase in the populations of lactic acid bacteria, although MRS medium has proven to be an appropriate medium to count LAB in vegetables (Carlin and Nguyen-the, 1989; Carlin et al., 1990). In the latter studies increased CO_2 concentrations resulted in higher populations of lactic acid bacteria. The increase in pH of leaf macerates after storage of leaves in CO_2-enriched modified atmospheres was previously observed on iceberg lettuce by Siriphanich and Kader (1986).

Concerning *L. monocytogenes*, our work shows that at 3°C an increase in CO_2 concentration slightly reduced or did not affect the growth of the bacterium on endive leaves. At 10°C, CO_2 significantly affected the fate of *L. monocytogenes*, but in ways that were different between the replicate experiments. In two experiments,

Table 2
Effects of CO_2 on the populations of *Listeria monocytogenes* on minimally processed broad-leaved endive at 3 and 10°C

Storage temperature (°C)	Experiment no.	CO_2 (%)	*L. monocytogenes* (log CFU/g)					
			Time (days)					
			0	2	4	7	10	14
3	2	0.04 (a)	4.66	4.48 a	4.74 a	4.94 a	5.81 a	4.86 a
		10 (b)		3.88 b	4.18 b	4.03 b	4.46 c	4.98 a
		30 (b)		3.91 b	4.22 b	4.27 b	4.73 bc	4.95 a
		50 (b)		4.34 ab	4.40 b	4.61 ab	4.89 b	5.24 a
	3	0.04	3.30	4.03 a	3.69 a	4.56 a	4.48 a	4.71 a
		10		3.79 a	3.10 a	4.57 a	4.57 a	4.95 a
		30		3.97 a	3.39 a	4.57 a	4.54 a	4.95 a
		50		3.72 a	3.25 a	4.17 a	4.77 a	5.14 a
10	5	0.04	4.42	4.69 a	5.14 a	5.05 a		
		10		4.29 a	5.00 a	5.51 ab		
		30		4.51 a	5.08 a	6.22 b		
		50		4.70 a	5.77 a	7.07 c		
	6	0.04	4.06	4.65 a	6.24 a	6.30 a		
		10		3.82 b	5.45 bc	5.52 bc		
		30		4.38 ab	5.01 c	5.25 c		
		50		4.20 ab	5.81 ab	6.02 ab		

For each experiment, means in columns followed by the same letter are not significantly different at the 5% level (Tukey's Honest Significant Difference Test).
$n = 10$, except on unstored products where $n = 5$.
(a): air.
(b): with 10% O_2 (balance N_2).

Table 3
Effects of CO_2 on the extent of spoilage on minimally processed broad-leaved endive at 3 and 10°C

Storage temperature (°C)	Experiment no.	CO_2 (%)	Extent of spoilage (%)					
			Time (days)					
			0	2	4	7	10	14
3	2	0.04 (a)	0	3.8 a	2.3 a	3.9 ab	8.7 a	5.9 a
		10 (b)		2.4 a	1.6 a	1.2 b	1.4 a	2.3 a
		30 (b)		1.6 a	2.1 a	5.9 ab	45.0 b	32.0 b
		50 (b)		1.1 a	2.7 a	12.9 a	48.0 b	75.5 c
	3	0.04	0	24.0 a	17.8 a	19.0 a	14.5 a	22.4 ab
		10		24.0 a	16.4 a	23.8 a	19.4 a	14.4 b
		30		24.8 a	28.0 a	21.9 a	30.3 ab	25.1 ab
		50		30.1 a	22.6 a	37.0 a	50.0 b	43.3 a
10	5	0.04	0	2.0 a	4.3 a	9.3 a		
		10		2.4 a	2.5 a	1.3 a		
		30		2.2 a	2.8 a	6.7 a		
		50		3.7 a	5.4 a	8.6 a		
	6	0.04	0	1.6 a	5.2 a	17.8 a		
		10		4.2 a	9.7 ab	6.9 a		
		30		4.7 a	6.2 ab	8.5 a		
		50		2.9 a	13.5 b	12.8 a		

For each experiment, means in columns followed by the same letter are not significantly different at the 5% level (Tukey's Honest Significant Difference Test).
$n = 10$, except on unstored products where $n = 5$.
(a): air.
(b): with 10% O_2 (balance N_2).

L. monocytogenes tended to grow better as the CO_2 concentration increased, but in one experiment the highest populations of *L. monocytogenes* were observed with air and 50% CO_2. Despite slight differences in growth at 3°C, the three strains inoculated onto endive leaves behaved in a similar way, as previously reported by Beuchat and Brackett (1990) on iceberg lettuce, Breer and Baumgartner (1992) in a range of fresh vegetables and Carlin et al. (1995) on endive leaves. Growth of *L. monocytogenes* on asparagus, broccoli, and cauliflower stored in modified atmospheres respectively containing 6% CO_2/15% O_2, 10% CO_2/11% O_2, and 3% CO_2/18% O_2 was not affected after storage for 21 days at 4°C or 10 days at 15°C (Berrang et al., 1989). Growth of *L. monocytogenes* at 5°C on shredded cabbage stored in 70% CO_2/30% N_2 was similar to that occurring in air (Kallander et al., 1991). On raw chicken there was no difference in the growth of *L. monocytogenes* in air and in 75% CO_2/5% O_2 (Wimpfheimer et al., 1990). In cooked chicken incubated at 3 and 7°C, increase in populations of *L. monocytogenes* was lower in modified atmospheres containing 80% CO_2/20% N_2 or 50% CO_2/10% O_2 than in air, whereas at 11°C only the modified atmosphere containing 80% CO_2/20% N_2 had an effect on the growth of *L. monocytogenes* (Ingham et al., 1990). Increases in populations of *L. monocytogenes* on sliced frankfurter-type sausage stored in 50% CO_2/15% O_2 were lower than those measured in air, 20% CO_2/12% O_2, and 30% CO_2/13% O_2 at 4 and 7°C, but they were not different at 10°C (Krämer and Baumgart, 1993). Manu-Tawiah et al. (1993) reported a slightly higher growth of *L. monocytogenes* on pork chops in modified atmospheres containing 20% CO_2/80% N_2, 40% CO_2/60% N_2 and 40% CO_2/10% O_2 than in air. In turkey rolls, growth of *L. moncytogenes* at 10 and 4°C was inhibited with a modified atmosphere containing 70% CO_2/30% N_2, and delayed with 30% CO_2/70% N_2 and 50% CO_2/50% N_2 as compared to air (Farber and Daley, 1994). In vitro studies in both showed that at 0 and 5°C, only CO_2 concentrations above 30% significantly reduced the growth of *L. monocytogenes*, and, at 10°C, only concentrations above 60% (Brunskill et al., 1991). From the research reported on above, it can be concluded that (i) the fate of *L. monocytogenes* on foods in CO_2-enriched atmospheres depend on the food, but CO_2 is inhibitory in laboratory media, (ii) CO_2 effects are more pronounced at low temperature, (iii) CO_2 concentrations below 30% are generally ineffective, (iv) even concentrations close to 70% CO_2 can in some instances be ineffective.

Carbon dioxide probably affected *L. monocytogenes* indirectly through modification of the salad leaves and of their microflora. Better growth of *L. monocytogenes* at high CO_2 concentrations could be due to a reduction in levels of competing aerobic bacteria; however, this does not explain the results obtained in experiment 6. If one assumes that the microflora of leaves and their physiological response to CO_2 varied among the experiments performed, then this could explain why we observed different effects on *L. monocytogenes* as the origin of the salads varied. CO_2-enriched modified atmospheres are not a reliable way to control the fate of *L. monocytogenes* on endive leaves, as we have observed that they can actually promote the growth of the bacterium. Farber (1991) and Doyle (1990) for fruits and vegetables suggested that modified atmosphere packaging of foods can result in a prolonged shelf-life and increased time for foodborne pathogens to grow, thereby

increasing the potential risk of foodborne illness. In this respect, an atmosphere containing 10% CO_2 may increase the risk of *L. monocytogenes* on minimally processed fresh endive leaves, although it did not directly increase growth of the bacterium in any of the experiments performed.

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