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Microbial populations of fresh-cut spinach leaves affected by controlled atmospheres [1]

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Abstract

The effect of controlled atmospheres (CA) containing low oxygen and high carbon dioxide at low temperatures on micro-organisms on fresh-cut spinach leaves was studied. Control samples in air were compared with those kept in 0.8% O_2–N_2 or 0.8% O_2–10% CO_2–N_2 at one of two temperatures, 5 and 10°C, for 9 or 7 days, respectively. The populations of various micro-organisms on the leaf surfaces were assessed, but coliforms and lactic acid bacteria were not detected and yeast remained uniformly low. At 5°C, CA decreased micro-organisms growth by between 10- and 100-fold, except for lactic acid bacteria and yeasts. At a temperature of 10°C, populations increased regardless of the atmospheric composition to 10–100 times those at the lower temperature. Thus, low oxygen atmospheres could be used to control spoilage micro-organisms on cut spinach leaves for at least 7 days, so long as the storage does not exceed 5°C.

Keywords: Spinach; Micro-organism; Controlled atmosphere; Low temperature storage

1. Introduction

The effects of controlled atmospheres (CA) or modified atmospheres packaging (MAP) on decay and development of micro-organisms of fresh-cut fruits and vegetables have been extensively reviewed. Generally, low oxygen concentrations or high carbon dioxide concentrations decreased respiration rate, reduced the numbers of postharvest plant pathogens, and the rate of deterioration of the product (Kader, 1986; Brackett, 1987; Hotchkiss and Banco, 1992). But the beneficial effects of CA can be reversed by

[1] Use of a company or product name by the USDA does not imply approval or recommendation of the product to the exclusion of others which also may be suitable.

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too low O_2 concentrations or too high CO_2 concentrations. For instance, increasing CO_2 concentrations up to 13% caused a rapid decrease in ascorbic acid and development of off-odors in spinach that were not acceptable after 1 week of storage at 7°C (McGill et al., 1966). An atmosphere of 4.0% O_2 and 9.2% CO_2 was reported to reduce the loss of ascorbic acid by 50% compared to air (Burgheimer et al., 1967). Spinach stored in 0.8% O_2 at 20°C showed a lower respiration rate, with a superior appearance and taste than that stored in air (Platenius, 1943). Thus, CA can be beneficial in maintaining the quality of fresh-cut spinach leaves, but O_2 concentration must be kept above the extinction point to prevent quality loss due to anoxia (Ko et al., 1996). Little information is available on the effects of CA on micro-organisms of fresh-cut spinach. Babic et al. (1996) reported that spinach leaves harbored high numbers of mesophilic and psychrotrophic aerobic bacteria identified mainly as a pectinolytic species, *Pseudomonas fluorescens*. This bacterium was suspected to be the major spoilage agent of fresh-cut spinach. Consequently, a study was undertaken to determine the influence of two CA regimes at two temperatures on the evolution of different microbial populations on fresh-cut spinach leaves.

2. Materials and methods

Spinach leaves (*Spinacia oleracea* L., variety New Jersey) were obtained from a local fresh-cut produce processor and 40-g samples were placed in 2-l jars. Samples, in triplicates, were placed at 5 and 10°C with humidified air or gas mixture passed through the jar at 15 ml min^{-1}. Gas mixtures consisted of 0.8% O_2 with balance as N_2 or 0.8% O_2, 10% CO_2 and balance as N_2.

Texture was measured as the force, expressed in newtons (N), required to shear 20 g of spinach leaves using a Food Technology Corporation Texture Test System (Model TMS-90, Rockville, MD) equipped with a standard shear-compression cell (Model CS-1). Spinach leaves were placed in the cell box perpendicular to the 10 shear blades.

The pH was determined by macerating a 20-g sample of spinach leaves in 40 ml of deionized water for 1 min and measuring the pH of the macerate with an Orion Research pH meter (Model 811). Surface pH of spinach leaves was determined with Cardy twin pH B-113 after the leaf was wiped with a piece of sampling sheet soaked in deionized water.

2.1. Microbial analyses

Enumeration of micro-organisms was performed as described by Babic et al. (1996). Spinach leaves (20 g) were macerated in 40 ml of sterile peptone water (pH 7.4) with a 400 Lab Stomacher (Seward Medical, London, UK). A sample of each homogenate or appropriate dilution was spread on agar plates using a spiral plate system (Autoplate Model 3000, Spiral Biotech, Bethesda, MD). The enumeration of microbial populations was performed by using the following culture media and culture conditions: (1) Tryptic Soy Agar (TSA, Difco Laboratories, Detroit, MI) incubated at 30°C for 24 h for total mesophilic aerobic micro-organisms or incubated at 5°C for 4 days for psychrotrophic aerobic micro-organisms; (2) *Pseudomonas* Selective Isolation Agar

(PSIA) prepared according to Krueger and Sheik (1987) and incubated at 30°C for 24 h for *Pseudomonadaceae*; (3) Violet Red Bile Glucose Agar (VRBG, Difco) incubated at 37°C for 24 h for total *Enterobacteriaceae except* Erwinia; (4) Violet Red Bile Agar (VRBA, Difco) incubated at 44°C for 24 h for coliforms; (5) Chapman agar medium incubated at 30°C for 24–48 h for *Micrococcaceae*; (6) MRS agar medium with addition of bromocresol green (100 ppm) incubated under anaerobic conditions at 30°C for 3 days for lactic acid bacteria; and (7) Potato Dextrose Agar (PDA, Difco) with the addition of chloramphenicol (500 ppm) incubated at 30°C for 36 h for yeasts and molds.

2.2. Statistical analysis

The data were analyzed as completely randomized two-factor models using PROC GLM (SAS Institute). The factors were day and atmosphere. Day zero was not included in the analysis as it was the same for the 3 atmosphere treatments. The two temperatures, 5 and 10°C, were analyzed separately. Diagnostic tests were run to check that the data met the assumptions of the general linear model. For *Enterobacteriaceae* at 5°C, it was necessary to use the inverse transformation to correct for variance heterogeneity. Means separation was done with pair-wise comparison.

3. Results

3.1. Effect of CA on texture and pH

CA had no effect on shear force of fresh-cut spinach leaves, relative to air, except on day 4 at 5°C and day 2 at 10°C (Table 1). The shear force remained unchanged at 5°C but dropped sharply between day 0 and 2 at 10°C. The surface pH of spinach leaves was initially slightly acidic, ranging from 5.5 to 6.3 (Table 2). No significant difference was observed among the atmospheres during storage at 5°C. In contrast, the surface pH of samples held in CA differed significantly from those in air during storage at 10°C, but the differences were not consistent. The surface pH of samples in air was higher than those in CA on day 2 at 10°C, but reversed itself on day 4 and no differences were noted on day 7. The pH of spinach macerates, which initially ranged from 6.4 at 5°C to 6.8 at 10°C,

Table 1
Changes in texture (expressed in newtons) of fresh-cut spinach during storage in air or under controlled atmospheres at 5 or 10°C. Means were calculated for 3 replicates. Means within a line of each temperature treatment followed by the same letter are not significantly different at $P \leq 0.05$

Days of storage	5°C			10°C		
	Air	0.8% O_2	0.8% O_2 + 10% CO_2	Air	0.8% O_2	0.8% O_2 + 10% CO_2
0	1837	1837	1837	1407	1407	1407
2	1831 (a)	1787 (a)	1740 (a)	934 (a)	1097 (ab)	1213 (b)
4	1605 (a)	1503 (a)	2109 (b)	804 (a)	807 (a)	1082 (a)
7	1791 (a)	1858 (a)	1746 (a)	1184 (a)	914 (a)	1084 (a)
9	1701 (a)	1760 (a)	1930 (a)			

Table 2
Changes in surface pH of fresh-cut spinach leaves during storage in air or under controlled atmospheres at 5 or 10°C. Means were calculated for 3 replicates. Means within a line of each temperature treatment followed by the same letter are not significantly different at $P \leq 0.05$

Days of storage	5°C			10°C		
	Air	0.8% O_2	0.8% O_2 + 10% CO_2	Air	0.8% O_2	0.8% O_2 + 10% CO_2
0	6.3	6.3	6.3	5.5	5.5	5.5
2	5.2 (a)	5.2 (a)	5.2 (a)	6.8 (b)	5.9 (a)	5.8 (a)
4	5.5 (a)	5.4 (a)	5.4 (a)	7.1 (a)	8.7 (b)	8.1 (b)
7	5.4 (a)	4.9 (a)	5.1 (a)	9.1 (a)	9.4 (a)	9.0 (a)
9	4.9 (a)	5.1 (a)	5.0 (a)			

Table 3
Changes in pH of spinach macerates during storage in air or under controlled atmospheres at 5 or 10°C. Means were calculated for 3 replicates. Means within a line of each temperature treatment followed by the same letter are not significantly different at $P \leq 0.05$

Days in storage	5°C			10°C		
	Air	0.8% O_2	0.8% O_2 + 10% CO_2	Air	0.8% O_2	0.8% O_2 + 10% CO_2
0	6.4	6.4	6.4	6.8	6.8	6.8
2	6.3 (a)	6.3 (a)	6.5 (b)	7.1 (ab)	7.0 (a)	7.4 (b)
4	6.5 (b)	6.3 (a)	6.5 (b)	7.1 (a)	8.2 (b)	7.8 (b)
7	7.0 (c)	6.3 (a)	6.8 (b)	8.2 (a)	9.1 (b)	8.2 (a)
9	7.0 (c)	6.5 (a)	6.7 (b)			

in most cases increased gradually during storage at both temperatures (Table 3). There were significant differences in the pH of spinach macerates among the atmospheres at 5°C. The pHs of spinach leaves stored in 0.8% O_2 were generally higher, often significantly so, than those of samples in other atmospheres on days 4 and 7 at 10°C.

3.2. *Effect of CA on microbial populations*

The microbial populations on fresh-cut spinach leaves increased during storage in air and CA at 5°C and 10°C (Fig. 1). The initial number of micro-organisms ranged 10^7–10^8 CFU g^{-1} for mesophilics, psychrotrophics and *Pseudomonadaceae*, about 10^3 CFU g^{-1} for total *Enterobacteriaceae* cultivated at 37°C and 10^3–10^4 CFU g^{-1} for *Micrococcaceae*. At 5°C, mesophilics, psychrotrophics and *Pseudomonadaceae* increased more rapidly in air compared to CA. After 7 days of storage, numbers reached 10^{10}, 4×10^8 and 5×10^8 CFU g^{-1} in air, respectively, whereas they were 10^8, 5×10^7 and 6×10^7 CFU g^{-1} under CA, respectively (numbers significantly different at 0.05 level). Populations of *Enterobacteriaceae* and *Micrococcaceae* were not greatly affected by the type of atmosphere. At 10°C, each group of micro-organisms increased similarly regardless of the atmosphere composition and their numbers were 10–100 times higher than that at 5°C.

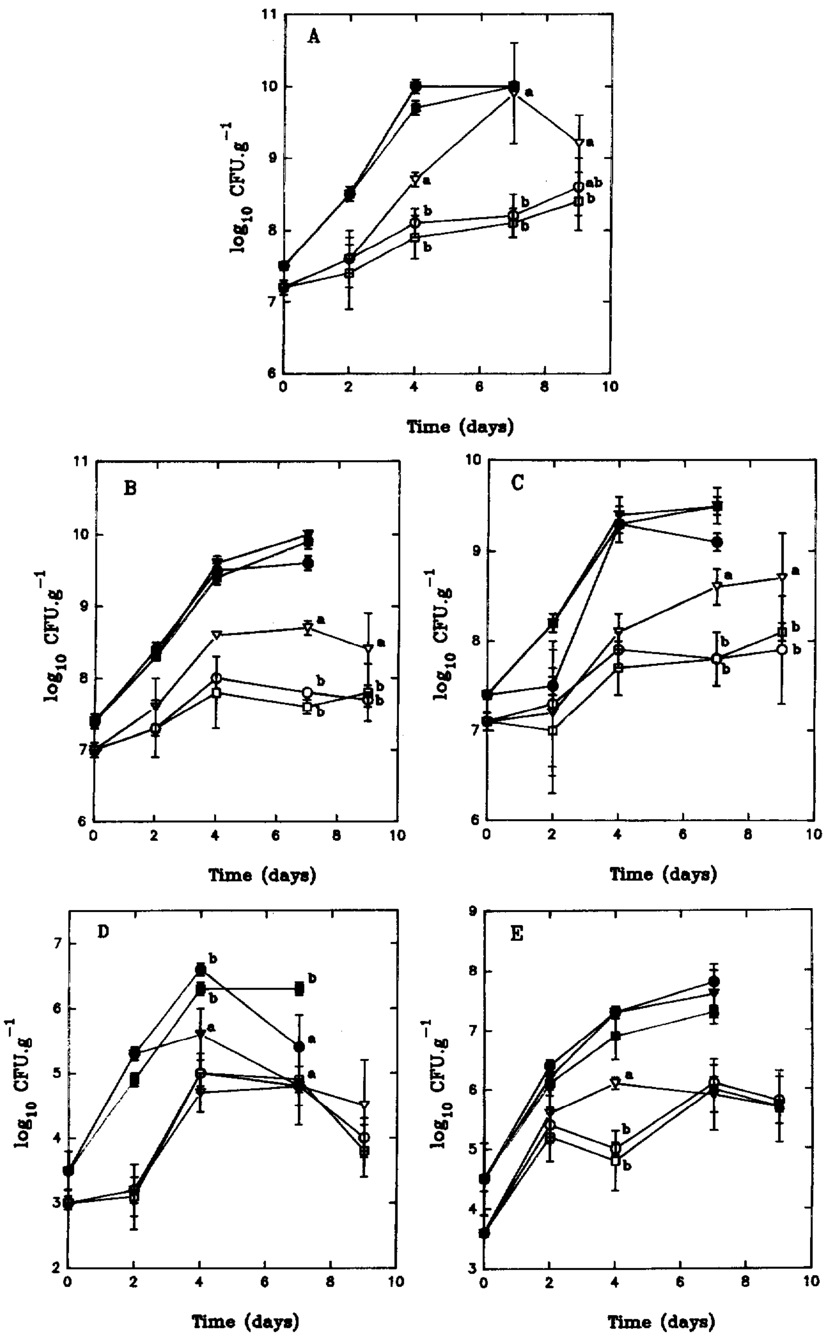

Fig. 1. Changes in microbial populations, mesophilic (A) and psychrotrophic (B) aerobic micro-organisms, *Pseudomonadaceae* (C), *Enterobacteriaceae* (D), and *Micrococcaceae* (E), on fresh-cut spinach leaves stored under air or controlled atmospheres at: 10°C, air (▼) 0.8% O_2 (•), 0.8% O_2 + 10% CO_2 (■); or 5°C, air (▽), 0.8% O_2 (○), 0.8% + 10% CO_2 (□). Means and standard deviations were calculated for 3 replicates. Comparison of means was made on the same day for each atmosphere. Means not significantly different at 0.05 level have the same letter.

Coliforms and lactic acid bacteria were not detected (numbers were below the limit of detection 60 CFU g^{-1}) and yeasts remained at low levels of 10^3–10^4 CFU g^{-1} during the entire storage period in air or under CA at both temperatures.

4. Discussion

Low O_2, or low O_2 combined with high CO_2, reduced the number of aerobic mesophilic and psychrotrophic micro-organisms on fresh-cut spinach leaves by 10- or even 100-fold compared to air at 5°C, but had no effect at 10°C. Carlin and Nguyen-the (1994) also reported that increasing CO_2 concentration significantly reduced the development of mesophilic bacteria on chicory leaves at 2 and 6°C, but had no effect at 10°C. Berrang et al. (1990) reported similarly that enriched CO_2 atmospheres had a significant inhibitory effect on the growth of aerobic micro-organisms on broccoli kept at 4°C. In our study, CA had a greater inhibitory effect on *Pseudomonadaceae* than on *Enterobacteriaceae* and *Micrococcaceae*. Babic et al. (1996) showed that *Pseudomonadaceae* were the predominant microbial population occurring on spinach leaves (1000 times more numerous than others) mainly represented by the species *Pseudomonas fluorescens*. Our results were similar to those of Ibe and Grogan (1983) who demonstrated that O_2 concentrations of 4% or less combined with CO_2 concentrations of 10% or more reduced, in vitro, the growth of *P. fluorescens* by 50% compared to air. In contrast, Hao and Brackett (1993) reported that similar modified atmosphere compositions did not affect the growth of *Pseudomonas*. The bacteriostatic effect of CO_2 is not well known, but it has been demonstrated that CO_2 could decrease the pH of the growth media (Daniels et al., 1985; Dixon and Kell, 1989). In our study conducted at 5°C, surface pH decreased and pH of spinach macerates increased during storage, regardless of the atmosphere. Therefore, the inhibitory effect of CA on micro-organisms was not due to an acidification of the microbial environment, but probably to a decreased oxygen availability. Low O_2, rather than high CO_2, seemed to be the limiting factor on the growth of aerobic micro-organisms on spinach leaves at 5°C, but not at 10°C. This observation does not support that of Wells (1974) who reported a decrease in growth rates of *P. fluorescens* by concentrations of CO_2 in excess of 10%, but not by the absence of CO_2.

Low O_2 atmosphere (0.8%) reduced the number of micro-organisms, particularly of the main spoilage agent *Pseudomonas* on fresh-cut spinach stored at 5°C. The effect of CA on micro-organisms was temperature dependent, with the inhibitory effect being less at the higher temperature. The growth of the anaerobic pathogenic bacteria *Clostridium botulinum* has been reported to occur on a few fresh-cut vegetables under low O_2 when temperature was high (Sugiyama and Yang, 1975; Solomon et al., 1990). Therefore, CA could be used to control microbial development on fresh-cut spinach only if temperature does not exceed 5°C.

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