Storage of Spinach Under Low Oxygen Atmosphere Above the Extinction Point

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ABSTRACT

The extinction point of spinach was <0.4% but above 0.2% O_2 at 0° and 5°C. Respiration rates were ≈2.3 times greater at 5°C than at 0°C and were similar among cultivars. In 0.8% O_2 atmosphere, O_2 uptake of three cultivars was reduced by an average of 53% and CO_2 production was reduced by 35% relative to those stored in air. Deterioration of leaves was reduced by 30 to 54%, while weight loss, color and chlorophyll content were not affected by the 0.8% O_2 atmosphere. Thus O_2 could be allowed to be depleted to 0.8% in modified atmosphere packaging without quality loss due to anoxia.

Key Words: spinach, extinction point, low oxygen, modified atmosphere

INTRODUCTION

LOWERING THE O_2 in storage atmosphere can be beneficial in maintaining quality and extending the shelf life of fresh produce (Lipton, 1975; Ulrich, 1975; Robinson et al., 1975). With spinach, Platenius (1943) reported that respiration was reduced when stored at 0.8% O_2 at 20°C, and stated, without data, that appearance and taste were more acceptable than that of product stored in air. Ascorbic acid retention was increased threefold when spinach was stored in an atmosphere containing <4% O_2 (McGill et al., 1966), but the benefit of reduced O_2 was lost when the CO_2 level was elevated to 9% or greater (Burgheimer et al., 1967; Platenius and Jones, 1944).

Spinach probably would benefit from the modified atmosphere formed in film packages due to reduced O_2, but the extent of benefit at low storage temperatures is not known. If O_2 drops below the extinction point (EP), the critical O_2 level at which CO_2 production is at a minimum (Blackman, 1928), the modified atmosphere could have undesirable effects due to anaerobic respiration. The EP of vegetables has been reported to be 1–3% (Kader, 1985), but is not precisely known for spinach.

We initially determined that the EP of several spinach cultivars was <0.4%. Thus, in a subsequent study our objective was to determine the extent of benefit of low O_2 atmosphere on quality of three spinach cultivars stored at 0° and 5°C with a 0.8% O_2 atmosphere. A lower level was not used to avoid the possibility of anaerobic respiration. Quality measurements included chlorophyll content, weight loss, color, and visual deterioration.

MATERIALS & METHODS

Extinction point study

Spinach (*Spinacia oleracea* L.) cultivars grown in California and New Jersey were obtained from the Maryland Produce Distributing Center (Jessup, MD) and from a local fresh-cut processor. Spinach plants were washed with cold (13°C) chlorinated water (10 ppm chlorine, pH adjusted to 3.7 with citric acid). Medium sized leaves were cut so 0.6 cm petioles remained. The leaves were centrifuged for 2 min at 37.6 × *g* to remove surface moisture, and 200g were placed in 3.8L glass respirometer jars at 0° and 5°C. Humidified gas mixtures of reduced oxygen levels were metered through the jars at an adequate rate to keep the CO_2 accumulation <0.5%.

Air and nitrogen from a liquid nitrogen supply or from a nitrogen generator (AVIR model NA 100H5, A/G Technology, Needham, MA) were combined to provide the desired gas mixtures. Initially, gas mixtures of 0.5, 1.0, 1.5 and 2.0% O_2 levels were studied; however, the extinction point was not in that range, so lower O_2 levels of 0.1, 0.2, 0.4, and 0.6% were evaluated at 0° and 5°C.

Respiration rates, based on O_2 uptake and CO_2 production, were determined every 6 hr automatically by measuring the concentration of the gases entering and leaving the respirometer jars. Oxygen and CO_2 were measured with Model S-3A and Model CD-3A analyzers, respectively (Ametek, Pittsburgh, PA).

Data were analyzed by analysis of variance (ANOVA) using the PROC MIX procedure to identify RQ values significantly ($p < 0.05$) different from 1.0 for each level of O_2 used (SAS Institute, Inc., 1992).

Low oxygen controlled atmosphere storage

'Tyee', 'Hybrid 612' and 'Seven R' spinach were grown at the Univ. of Maryland Wye Research Farm and Education Center and freshly harvested. Spinach plants were prepared as in the extinction point study. A subsample (200g) was placed in a 3.8L respirometer jar for respiration measurements. The remainder (600g) was placed in a 19L sampling jar for periodic triplicate analysis of quality parameters. A humidified gas mixture of 0.8% O_2 from the nitrogen generator was metered through the respirometer jars and the sampling jars at a rate sufficient to keep the CO_2 <0.5%. Respiration rates were measured as described in the extinction point study.

Moisture content was determined by drying 4 to 6g of chopped spinach leaves at 65°C for 15 hr (Walsh and Beaton, 1973). Chlorophyll was extracted from 25g of leaves (with midribs removed) with acetone according to the procedure of Arnon (1949), and the concentration was based on the optical density (OD) at 645 and 663 nm measured with a Hewlett Packard Diode Array Spectrophotometer Model 8452A. Color was measured with a colorimeter (Model CR-300, Minolta, Japan). Five leaves were stacked on top of each other and the color of the top leaf was determined by making five measurements at different leaf sites. This procedure was performed 5 times. Yellowness values represented as $L^*b/|-a|$ were then calculated (Yeatman, 1969).

Visual deteriorations (decay and water soaked appearance) were determined daily on leaves used for respiration measurement. Analysis was restricted to leaves that were visible around the periphery of the jar. At the end of storage, leaves were removed from these jars and the number of deteriorated leaves was recorded. The visual condition of leaves inside sampling jars was also evaluated by removing 150g of leaves (50g of each triplicate) from each jar and recording the number of deteriorated leaves. This group of leaves was then used for color, moisture, and chlorophyll determinations. Data were analyzed by analysis of variance (ANOVA) using the GLM procedure. Least significant difference (LSD) tests at the 0.05 and 0.01 levels of significance were used to separate means when significant F-values were found (SAS Institute, Inc., 1989).

RESULTS & DISCUSSION

Extinction point

Oxygen uptake and CO_2 production rates decreased as O_2 level was lowered from 2.0% to 0.5%. The RQ was about 1 in this O_2 range, thus the EP was <0.5% (data not presented). Further reduction of O_2 to 0.1% caused a continual decrease in O_2 uptake. Carbon dioxide production increased slightly as O_2 decreased to <0.4% (data not presented). This resulted in an RQ of about 1 at 0.6% and 0.4% O_2 and a very high RQ at

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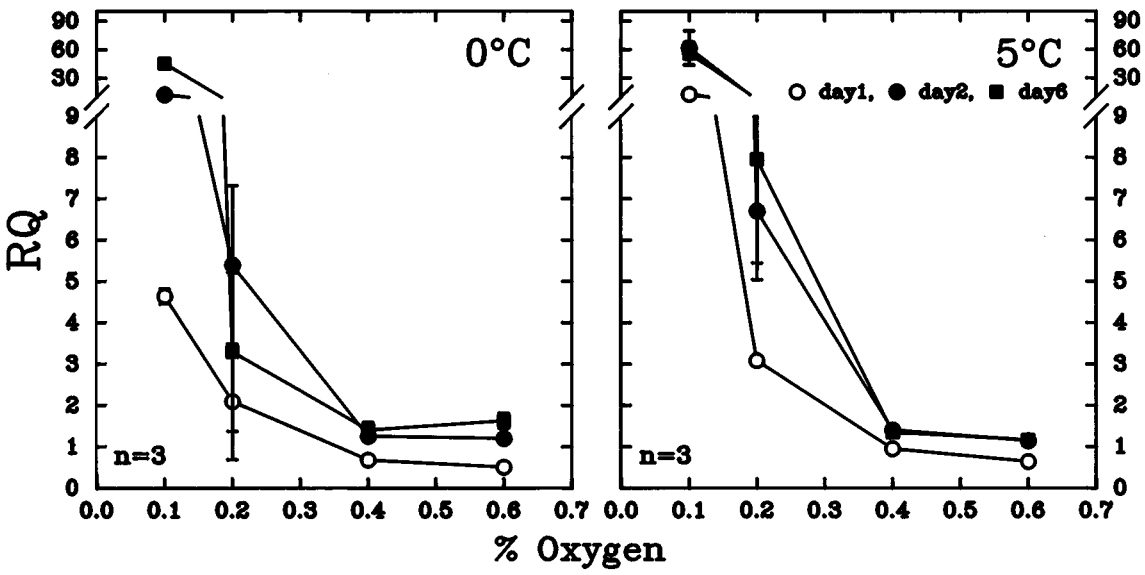

Fig. 1—Respiratory quotient (RQ) of spinach stored at 0° and 5°C in 0.1 to 0.6% O_2 atmospheres. Vertical lines represent ±SEM.

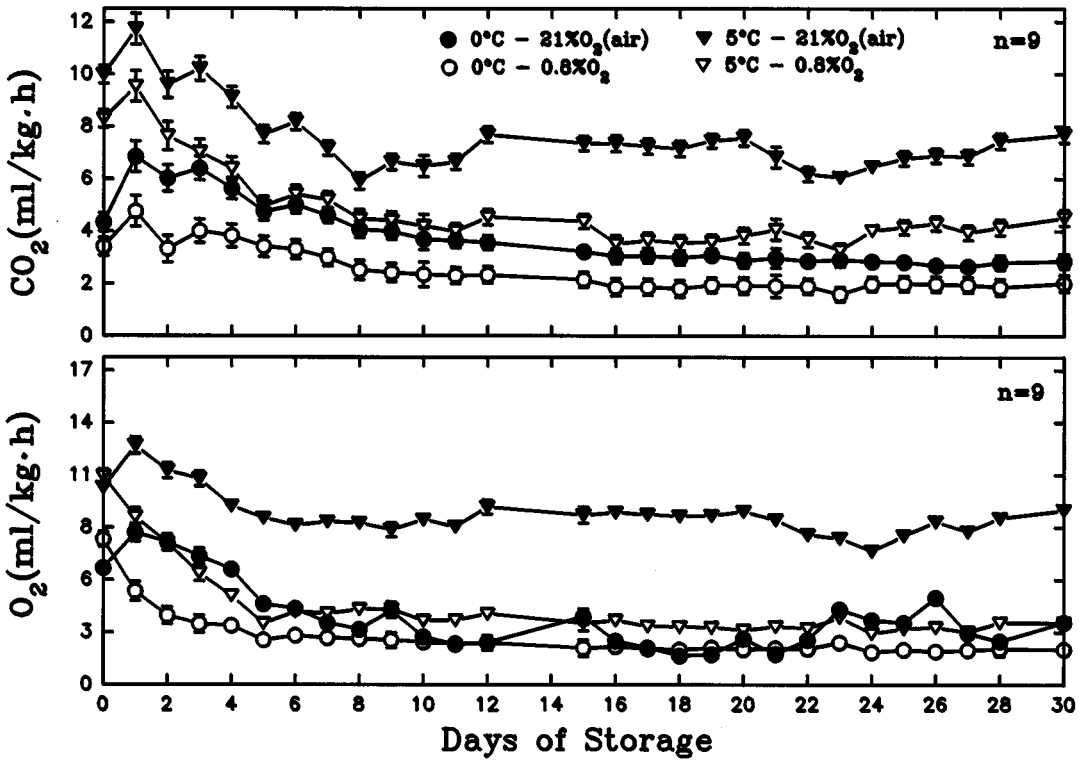

Fig. 2—Effects of temperature and O_2 level on the rate of CO_2 production and O_2 uptake of spinach leaves. Vertical lines represent ±SEM.

0.2% and 0.1% O_2, probably due to anaerobic respiration (Fig. 1). The visual consequence of anaerobic respiration was noted on day 4 to 7 by the deterioration of leaves, which was not apparent at 0.4% and 0.6% O_2. Based on these results, the EP of spinach was <0.4% but >0.2% O_2.

Low oxygen storage

The respiration rates and pattern of the three cultivars were similar; averages of the cultivars were plotted (Fig. 2). As noted with the extinction point study, O_2 consumption and CO_2 production were less in low O_2 atmosphere than under air atmosphere and less at 0°C than at 5°C. Note that the respiration rate of spinach leaves held in 0.8% O_2 at 5°C was about equivalent to the rate of leaves held in air at 0°C. Thus the use of low (0.8%) O_2 under controlled conditions could, at least partially, compensate for exposure of packaged spinach to 5°C storage temperature.

The average RQ was not affected by the 0.8% O_2 at 0°C and only slightly at 5°C (Table 1). At 0°C, 0.8% O_2 affected the RQ

Table 1—Average respiratory quotient based on steady state respiration rate of 'Tyee', 'Seven R', and 'Hybrid 612' spinach stored in air or 0.8% O_2 at 0° and 5°C.

	0°C		5°C	
	Air	0.8% O_2	Air	0.8% O_2
Tyee	1.79[a]	1.15	1.18	1.72
Seven R	1.81	2.47	1.22	1.55
Hybrid 612	1.45	1.32	1.21	2.26
Avg	1.68	1.65	1.20	1.84

[a] Average of three replicates.

of Seven R inversely to that of Tyee and Hybrid 612, which resulted in similar average RQs for air and 0.8% O_2 atmospheres. At 5°C, 0.8% O_2 increased the RQ slightly of all cultivars and most noticeably that of Hybrid 612. The increase in RQ indicated an increase in anaerobic respiration, but the amount of increase was not enough to be injurious to the leaves. At the end of the respiration study (47 days for 0°C and 33 days for 5°C), there were 30% and 54% fewer deteriorated leaves in 0.8% O_2 than in air at 0° and 5°C, respectively (Fig. 3).

The reduced deterioration of leaves in 0.8% O_2 was noted also with samples held for quality analysis. With those leaves, deterioration was first noted on day 26 and 20 at 0° and 5°C, respectively (Fig. 4). By day 31 at 0°C, an average of about 10% and 20% of leaves in 0.8% O_2 and air, respectively were deteriorated. On day 20 at 5°C, deteriorated leaves were limited to those in air except for Hybrid 612 in 0.8% O_2. By day 25, the average amounts of deteriorated leaves were about 15% and 40% in 0.8% O_2 and air, respectively. These results indicated that spinach of good quality could be maintained for about 25 days at 0°C in either air or 0.8% O_2. Also, 0.8% O_2 becomes beneficial for storage periods exceeding 30 days or at a higher storage temperature of 5°C. Low oxygen atmosphere storage had no effect on weight loss, color, or chlorophyll content of spinach stored at either temperature (data not presented).

CONCLUSION

THE O_2 ATMOSPHERE in spinach packages needs to be maintained at >0.4%, to avoid problems from anaerobiosis. A 0.8% O_2 atmosphere increased anaerobic respiration only slightly and was beneficial in maintaining quality. Therefore, an O_2 atmosphere could be allowed to decline to 0.8% in modified atmosphere packaged spinach without deleterious effects, providing the holding temperature did not exceed 5°C and CO_2 is not excessive.

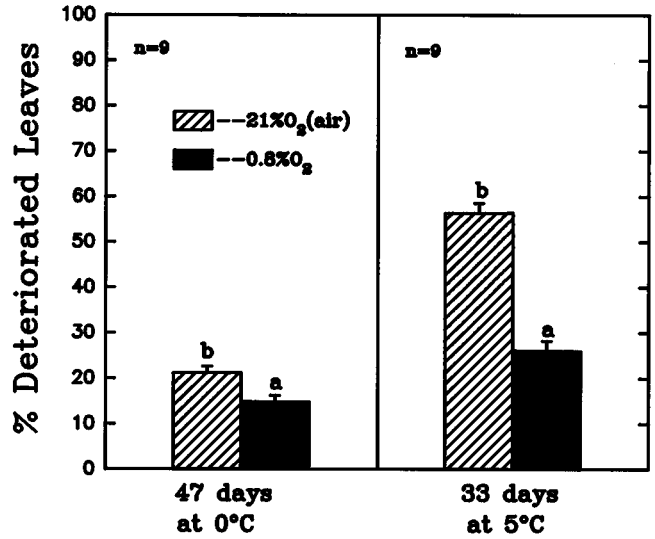

Fig. 3—Effect of O_2 levels on percent deteriorated leaves across spinach cultivars at the final day of storage at 0° and 5°C. Vertical lines represent ±SEM. [ab]Means within a single storage temperature with no superscript letter in common are highly significantly different ($p < 0.01$).

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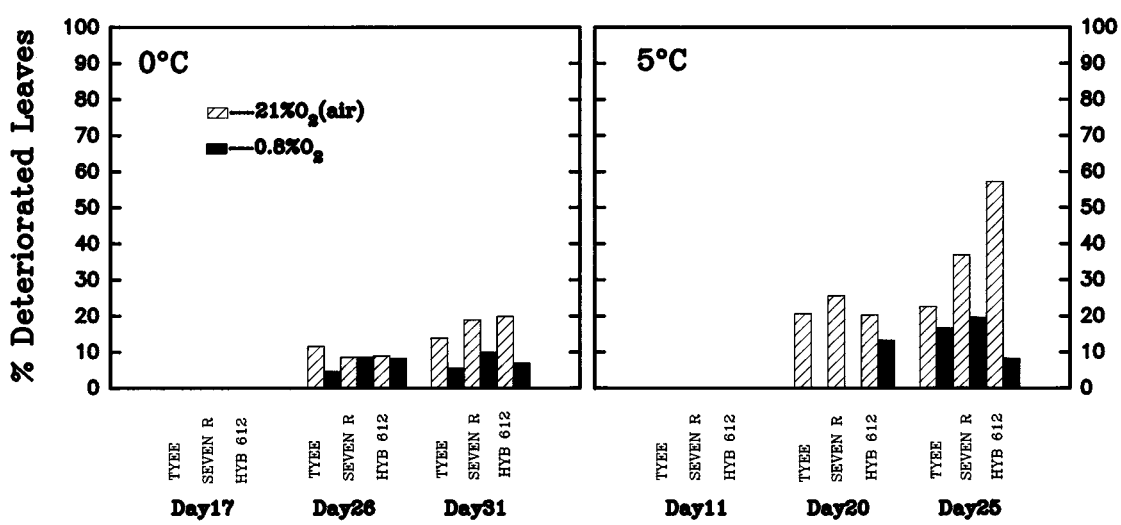

Fig. 4—Percent deteriorated leaves of spinach cultivars during storage at 0° and 5°C at each sampling day.

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