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Postharvest Biology and Technology 26 (2002) 113–116

Research Note

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**Postharvest  
Biology and  
Technology**

# Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen

Pilar Jamie <sup>1</sup>, Mikal E. Saltveit \*

*Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616-8631, USA*

Received 9 November 2001; accepted 20 December 2001

## Abstract

Controlled atmospheres containing 90% argon and 2% oxygen did not delay the accumulation of phenolics in fresh-cut lettuce, or the loss of chlorophyll from broccoli florets beyond that of low oxygen atmospheres made with helium or nitrogen. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Broccoli; Chlorophyll; Controlled atmosphere; Fresh-cut; Lettuce; MAP; Modified atmosphere; Phenols

## 1. Introduction

Low O<sub>2</sub> CA, MA, and MAP made with Ar instead of N<sub>2</sub> have been reported to improve the storage life of fresh fruits and vegetables. A US patent was issued for the use of Ar in the preservation of cut and segmented fresh fruit (Powrie et al., 1990). Argon used as a major component of the atmosphere in MAP has been reported to reduce microbial growth and improve product quality retention (Berne, 1994; Day, 1996, 1998). The ripening of mature-green tomatoes was delayed and their rate of CO<sub>2</sub> and C<sub>2</sub>H<sub>4</sub> production reduced in

3% O<sub>2</sub> CA made with Ar compared with the same CA made with N<sub>2</sub> (Lougheed and Lee, 1991). However, there was no difference in the viability of cells, the uptake of sucrose, or the density of tobacco suspension cultures grown for 8 days in 21% O<sub>2</sub> and 79% of either Ar or N<sub>2</sub> (Sidorkin et al., 1989). Likewise, Ar has no effect on selected plant enzyme systems (Gorny and Agar, 1998). However, atmospheres rich in Ar or He have diffusivity characteristics that might modify the diffusion of O<sub>2</sub>, CO<sub>2</sub>, and C<sub>2</sub>H<sub>4</sub> in some commodities (Burg and Burg, 1965). The latter work showed that replacing the N<sub>2</sub> in air with He enhanced gas diffusion and reduced the concentration gradient of O<sub>2</sub> between the inside and outside of a commodity. These changes should allow the storage of commodities that experience internal low O<sub>2</sub> deficiencies at lower O<sub>2</sub> concentrations than they could tolerate in N<sub>2</sub> atmospheres.

\* Corresponding author. Tel.: +1-530-752-1815; fax: +1-530-752-4554

E-mail address: mesaltveit@ucdavis.edu (M.E. Saltveit).

<sup>1</sup> Permanent address: Tecnologia de los Alimentos, Universidad de Zaragoza, Miguel Servet, 177, E-50013 Zaragoza, Spain.

This paper reports some postharvest changes that occur during storage of broccoli and lettuce in Ar, He, and N<sub>2</sub> atmospheres containing 2% O<sub>2</sub>. Fresh-cut broccoli and lettuce tissue were used because there has been commercial interest in using Ar in place of N<sub>2</sub> in MAP of lettuce and broccoli, and because of conflicting reports on the effectiveness of atmospheres in which N<sub>2</sub> was replaced with Ar.

## 2. Materials and methods

Horticulturally mature broccoli (*Brassica oleracea* L. var *botris*), and crisphead (Iceberg) and green leaf lettuce (*Lactuca sativa* L.) were obtained from commercial sources and stored at 0° C until used within a few days. Broccoli florets (ca. 5 cm long) were cut from the stalks. Fully expanded leaves were chosen from the middle of the heads of lettuce, eliminating the damaged outer leaves and immature inner leaves. The leaves were cut into 2 × 2 cm pieces, washed twice in tap water, and spun dry. About 200 g of broccoli florets or lettuce leaf pieces were put into 4-l glass jars, and held at 15° C for 3 days (green leaf lettuce), 4 days (crisphead lettuce), or 7 or 9 days (broccoli). The higher than recommended storage temperature (i.e. 0° C) was used to shorten the storage period.

The jars were ventilated with ethylene-free, humidified gas mixtures made by combining 1 l h<sup>-1</sup> of air with 9 l h<sup>-1</sup> air, Ar, He, or N<sub>2</sub>. The resulting mixtures were: air (20.9% O<sub>2</sub>, 0.9% Ar, and 78.1% N<sub>2</sub>), Ar CA (2% O<sub>2</sub>, 8% N<sub>2</sub>, and 90% argon), He CA (2% O<sub>2</sub>, 8% N<sub>2</sub>, and 90% He), and N<sub>2</sub> CA (2% O<sub>2</sub> and 98% N<sub>2</sub>).

Phenolic compounds were extracted in methanol (Ke and Saltveit, 1988) and the absorbance read at 320 nm to determine total phenolics, and at 437 nm to determine soluble o-quinones as previously described (Loaiza-Velarde and Saltveit, 2001).

Color values (*a*\*, *b*\*, *L*\*) were measured on broccoli flowers that ranged from dark green to yellow, with a chromameter (CR-200; Minolta Camera Co., Palo Alto, CA) after calibrating with a white plate (*L*\* = 97.63; *a*\* = -0.53; and *b*\* =

2.38). Chlorophyll was then extracted from 5 g of macerated broccoli by homogenizing it in 20 ml of 80% acetone with a Ultra-Turrax tissue homogenizer (Takmar, Cincinnati, OH) at a moderate speed for 30 s. The homogenate was filtered through four layers of cheesecloth, centrifuged at 15 000 × *g* for 15 min, and absorbance read at 647 and 664.5 nm with an UV-VIS recording spectrophotometer (UV-160A; Shimadzu Scientific Instrument, Columbia, MD) and the total chlorophyll content calculated [chlorophyll (mg l<sup>-1</sup>) = 17.95 × Abs<sub>647</sub> + 7.9 × Abs<sub>664.5</sub>; Inskeep and Bloom, 1985]. Chlorophyll content was regressed against the color values, and was found to be the best described by the equation: chlorophyll (mg l<sup>-1</sup>) = 79.4 - (1.33 × *L*\*) with an *R*<sup>2</sup> of 0.89.

Each experiment had two replicates and all experiments were run three times with similar results. Measurements from all the replicates were combined and treatment effects analyzed.

## 3. Results and discussion

A 2% O<sub>2</sub> CA reduced the wound-induced accumulation of phenolic compounds and o-quinones in fresh-cut crisphead lettuce (Fig. 1A) and in green leaf lettuce (Fig. 1B) over that of similar tissue stored in air. Wounding stimulated phenolic accumulation 1.8- and 2.0-fold during the 4 and 3 days storage of crisphead and green leaf lettuce, respectively. The 2% O<sub>2</sub> CA reduced the accumulation of phenolics in both types of lettuce by about 35%, irrespective of the residual gas (i.e. Ar, He, or N<sub>2</sub>). In contrast, the content of o-quinones in fresh-cut crisphead lettuce was higher in tissue stored in the CA made with Ar than in that with He or N<sub>2</sub>. This was not the case in fresh-cut green leaf lettuce where there were no detectable differences in o-quinone content of tissue stored in any of the CAs.

A 2% O<sub>2</sub> CA reduced the decline in chlorophyll in broccoli florets (Fig. 2). The chlorophyll content declined about 75% from an initial value of 0.11 ± 0.01 mg g<sup>-1</sup> fw to around 0.029 ± 0.003 mg g<sup>-1</sup> fw during either 7 or 9 days of storage at 15° C (Fig. 2). The decline after 7 and 9 days was

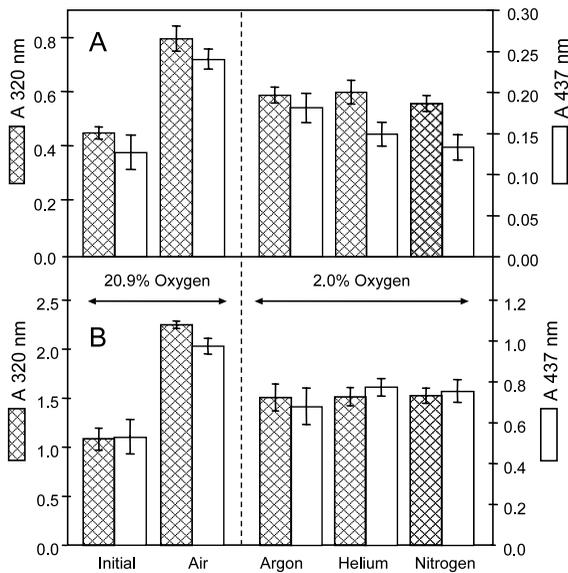


Fig. 1. Content of phenolics (A 320 nm) and o-quinones (A 437 nm) in fresh-cut crisphead (Iceberg) lettuce (A) and green-leaf lettuce (B) stored at 7° C for 4 days in 2% O<sub>2</sub> CA made up in Ar, He, N<sub>2</sub>. The lines atop the bars represent the SE of the means ( $n = 6$ ).

reduced to 25 and 50%, respectively, when the 2% O<sub>2</sub> CA was made with either He or N<sub>2</sub>. In contrast, the decline in chlorophyll content showed a significant increase up to 40 and 58% for broccoli

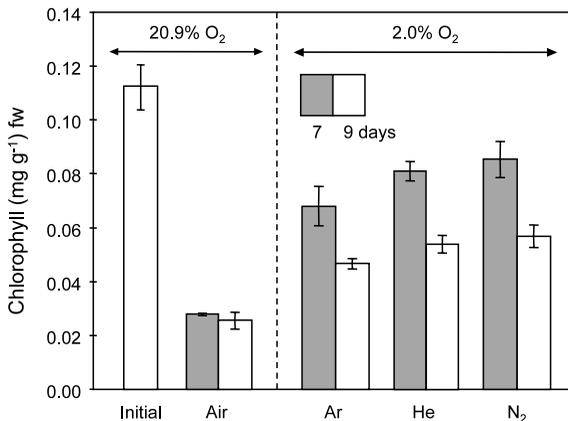


Fig. 2. Chlorophyll content of broccoli florets held for 7 or 9 days at 15° C in air (21% O<sub>2</sub>) or in 2% O<sub>2</sub> CA made up in Ar, He, or N<sub>2</sub>. The lines atop the bars represent the SE of the means ( $n = 6$ ).

florets stored for 7 and 9 days, respectively, at 15° C in 2% O<sub>2</sub> CA made with Ar.

Treatments or storage conditions that improve gas exchange in fruits and vegetables would reduce the gradient in gas concentration between internal tissue and the ambient atmosphere and would improve the overall storage life of the commodity under CA because more of the tissue would be exposed to the optimal concentration of gases (Burg and Burg, 1965). Low O<sub>2</sub> storage atmospheres made with a preponderance of Ar, He, or N<sub>2</sub> have different diffusive characteristics. Both Ar and He are monatomic gases and are smaller in size than the diatomic gas N<sub>2</sub>. The small 5.9% increase in the diffusivity of O<sub>2</sub> in Ar over N<sub>2</sub> atmospheres (Hodgman, 1961), and the small size of lettuce leaf pieces and broccoli florets probably precluded any beneficial effect that would be associated with diffusivity differences. At high concentrations and/or under pressure, He and N<sub>2</sub> have an anesthetic effect in mammals and reduce the chilling injury of sensitive plants (Saltveit, 1993). They may be chemically inert, but they have some physiological effects, although it does not appear to be through modification of enzyme activity (Gorny and Agar, 1998).

Data presented in this paper show that atmospheres containing 90% Ar and 2% O<sub>2</sub> did not delay the accumulation of phenolics in fresh-cut lettuce, or the loss of chlorophyll from broccoli florets.

## Acknowledgements

Pilar Jaime is indebted to the Spanish Ministry of Science and Technology for a pre-doctoral scholarship.

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