*Proc. Fla. State Hort. Soc.* 115:64-67. 2002.

# EVALUATION OF DIFFERENT OXYGEN, CARBON DIOXIDE AND NITROGEN COMBINATIONS EMPLOYED TO EXTEND THE SHELF LIFE OF FRESH-CUT COLLARD GREENS

CELSO L. MORETTI[1], ALESSANDRA LIMA ARAUJO, WALDIR APARECIDO MAROUELLI
AND WASHINGTON LUIZ CARVALHO SILVA
*Embrapa Vegetables, CP 218, 70.359-970*
*Brasilia, DF, Brazil*

*Additional index words. Brassica oleracea,* Brix, chlorophyll, modified atmosphere, quality, postharvest, vitamin C

*Abstract.* **Collard greens (*Brassica oleracea* var. *acephala*) "Manteiga" were harvested in commercial fields in Brasilia, Brazil, aiming to evaluate different oxygen, carbon dioxide and nitrogen combinations to extend the shelf life of the fresh cut product. After harvest, leaves were taken to the postharvest laboratory, selected for external blemishes and minimally processed (3 mm thick) inside a cold room (13 ± 2 °C). After processing, fresh cut collard greens were stored under two controlled atmosphere (CA) conditions (3% $O_2$, 4% $CO_2$; 5% $O_2$, 5% $CO_2$ - balance $N_2$), and normal air (control), at 5 °C (95% RH), for six days. Daily, minimally processed collard greens were evaluated for total vitamin C, total chlorophyll, total soluble solids content, and titratable acidity. Total vitamin C content decreased for all treatments during the storage period. Storage under CA conditions delayed total vitamin C degradation for both atmospheres studied. At the end of the storage period, fresh cut collard greens stored under 3% $O_2$, 4% $CO_2$ showed around 25% and 56% more vitamin C than the material stored under 5% $O_2$, 5% $CO_2$ and control, respectively. Total chlorophyll content decreased during the storage period. At the end of the experiment, fresh cut collard greens stored under 3% $O_2$,**

[1]CNPq fellow, corresponding author; e-mail: celso@cnph.embrapa.br.

**4% $CO_2$ showed 24% and 45% more total chlorophyll than the product stored under 5% $O_2$, 5% $CO_2$ and control, respectively. CA storage delayed organic acid degradation. At the sixth day, fresh cut collard greens stored under 3% $O_2$, 4% $CO_2$ had around 44% more organic acids than control. Total soluble solids content were not significantly affected.**

Although commercialized with higher prices when compared to fresh products, fresh-cut fruits and vegetables have the advantage of being perceived by customers to be fresher than other products, such as frozen or canned food. Since 1994, "fresh" has remained the most desirable food label claim for 79% of american customers, followed by low fat (62%), and natural (41%). Besides that, freshness is a serious issue for shoppers and retailers, being the third reason for selecting a primary supermarket, just behind high quality and a "clean and neat store" (Sloan, 2000).

Fresh-cut products represent around 10% of the American fresh fruit and vegetables market, resulting in a volume of 8 to 10 billion dollars per year (Cantwell, 2000). The consumption of fresh-cut products is increasing year after year, showing many advantages when compared to fresh products.

While most of food technologies available are designed to extend the shelf life of perishable products, minimal processing abbreviates the conservation of fruits and vegetables. This is basically due to the mechanical injuries occurred during the different steps involved in the obtention of a fresh-cut product. Thus, changes in carbon dioxide evolution (Moretti et al., 2000) and increase in the activity of many enzymes related with browning (Bower and Van Lelyveld, Brecht, 1995; 1985; Ke and Saltveit, 1989; King and Bolin, 1989; Moretti et al., 2001b; Nicoli et al., 1994) are events that are commonly associated in fresh-cut tissues.

Fresh-cut products have, in general, higher respiratory activity than intact products, indicating a more active metabolism and, generally, higher perishability. The increase in oxygen demand caused by the increased respiratory activity dictates plastic film permeability, in order to avoid anaerobic conditions (Cantwell, 2000).

The best way to circumvent the alterations caused by mechanical damages is a strict temperature control. Respiration and deterioration rates can be rapidly decreased by storage under refrigerated conditions (Moretti et al., 2001a). Another important tool to control respiration rates and deterioration is modified atmospheres (MA). Unfortunately, limited information is available regarding respiration rates of fresh-cut products under controlled or modified atmosphere conditions. Matilla et al. (1995) verified that storage under modified atmosphere (5% $O_2$; 5% $CO_2$) only slightly reduced the respiration rates of fresh-cut carrots, leek and onion, but increased slightly the rates of cut potatoes.

Working on fresh-cut strawberries, peaches and honeydew, Watada et al. (1996) observed that controlled atmospheres of 1-2% $O_2$ + 10% $CO_2$ reduced respiration rates by 25 to 50% at 5 °C. These same atmospheres also substantially reduced ethylene production rates and softening of fruit tissues. MA also had a positive effect in retarding microbial growth, softening, color change, and off-odors in fresh-cut cantaloupe stored under high $CO_2$ atmospheres in air or low $O_2$ (Cantwell, 2000).

Despite all the interest and work done with minimally processed products, processors still face many technological problems. Minimal processing of collard greens have shown to be a great challenge for processors basically due to their high respiratory activity after processing. One of the strategies carried out to reduce respiration rates and to extend the shelf life is the utilization of modified atmosphere packaging in conjunction with low temperatures. Different plastic films have already been tested and the shelf life achieved is only around 6 d. Teles (2001) verified that the utilization of plastic films (multi-layer nylon) in association with active atmosphere modification can be a way to extend fresh-cut collard greens shelf life. However, different oxygen, carbon dioxide and nitrogen combinations still need to be tested.

The present work was carried out to aiming to evaluate different oxygen, carbon dioxide and nitrogen combinations to extend the shelf life of the fresh-cut product.

## Material and Methods

*Plant material.* Collard greens (*Brassica oleracea* var. *acephala*) leaves "Manteiga" were harvested in commercial fields in Brasilia, Brazil. After harvest, leaves were taken to the postharvest laboratory, selected for external blemishes and minimally processed (3 mm thick) inside a cold room (13 ± 2 °C). After processing, fresh-cut collard greens were stored for 6 d under two controlled atmosphere (CA) conditions (3% $O_2$, 4% $CO_2$; 5% $O_2$, 5% $CO_2$ - balance $N_2$), and normal air (control), at 5 ± 1°C and 95 to 98% RH, and evaluated daily.

*Chemical and physical analysis.* Fresh-cut collard greens were evaluated for total vitamin C, total chlorophyll, total soluble solids content, and titratable acidity.

*Total vitamin C.* For total vitamin C analysis, 2 g of homogenized tissue were combined with 20 mL of 6% metaphosphoric acid in 2 N acetic acid and centrifuged for 20 min at 18,000 $g_n$ 4 °C. The analysis was performed by the dinitrophenylhydrazine method of Terada et al. (1978). The concentration of total vitamin C was calculated in mg·kg$^{-1}$ of fresh weight from absorbance measured at 540 nm using pure ascorbic acid as a standard.

*Total chlorophyll.* Total chlorophyll was determined according to Inskeep and Bloom (1985). Two g of tissue were added to aluminum foil-covered vials containing 5 mL of N,N dimethylformamide (DMF). After 10 days at 4 °C, solutions were filtered through Miracloth and absorbance read at 647 nm and 664.5 nm. Pigments were expressed as mg·kg$^{-1}$ of fresh tissue.

*Titratable acidity and soluble solids.* Determination of titratable acidity and soluble solids content was performed according to Moretti et al. (1998). Forty grams of the fresh tissue were homogenized in a commercial blender at high speed and centrifuged for 15 min at 18,000 $g_n$. Aliquots of the supernatant were diluted with 50 mL of deionized water and titrated with 0.1 N NaOH to an end point of pH 8.2 using an automatic titrimeter. The amount of NaOH was converted to miliequivalents of citric acid per kg of fresh weight (mL NaOH × 0.1 N × 0.064). Soluble solids content was determined using a benchtop digital refractometer.

*Experimental design and statistical analysis.* Analysis were performed using a completely randomized design, with 21 treatments arranged in a factorial scheme (two controlled atmosphere conditions - 3% $O_2$, 4% $CO_2$; 5% $O_2$, 5% $CO_2$/balance $N_2$), and normal air - (control), 7 sampling times, and 3 replicates. Data were subjected to analysis of variance and the least significant difference procedure was carried out. Differences between any two treatments larger than the sum of two standard deviations were always significant ($P \leq 0.05$).

## Results and Discussion

*Total vitamin C.* Total vitamin C content decreased for all treatments during the storage period. Storage under CA conditions delayed total vitamin C degradation for both atmospheres studied. At the end of the storage period, fresh-cut collard greens stored under 3% $O_2$, 4% $CO_2$ showed around 25% and 56% more vitamin C than the material stored under 5% $O_2$, 5% $CO_2$ and control, respectively.

Senesi et al. (2000) studied the effect of modified atmosphere packaging on color, firmness, vitamin C (ascorbic acid), microbial quality and sensory characteristics of fresh-cut green bell peppers stored at 8 ± 1 °C for up to 11 d. Vitamin C content and the organoleptic characteristics were acceptable until the end of the storage time.

The effect of modified atmosphere packaging (MAP) on vitamin C content (ascorbic + dehydroascorbic acid; AA + DHAA) of fresh-cut spinach was evaluated by Gil et al. (1999). They verified that vitamin C was better preserved in MAP-stored spinach when compared to normal air.

Watada (1987) observed that vitamin C degradation is product, temperature and atmosphere dependent. He also verified that reduction in oxygen concentration in the storage ambient delayed ascorbic acid degradation. However, different species responded differently to distinct atmospheres combinations.

*Total chlorophyll.* Total chlorophyll content decreased during the storage period. After the fourth day, chlorophyll degradation occurred slowly for fresh-cut collard greens stored under 3% $O_2$, 4% $CO_2$ when compared to other treatments. That was a clear sign that controlled atmosphere storage was an efficient method to delay pigment degradation. At the end of the experiment, fresh-cut collard greens stored under 3% $O_2$, 4% $CO_2$ showed 24% and 45% more total chlorophyll than the product stored under 5% $O_2$, 5% $CO_2$ and control, respectively (Fig. 2). Considering the retention of the initial total chlorophyll content, it was observed that storage under 3% $O_2$, 4% $CO_2$ allowed a retention of around 80% of the initial content (Fig. 2).

The effects of modified atmosphere packaging and controlled atmosphere storage on the storability of snow pea pods (*Pisum sativum* var. *saccharatum*) at 5 °C were determined by Pariasca et al (2001). Bagging pods with polymethyl pentene polymeric films modified the in-bag atmosphere concentration to approximately 5 kPa $O_2$ and 5 kPa $CO_2$, leading to better maintenance of the pod internal quality (chlorophyll, ascorbic acid, and sugar contents).

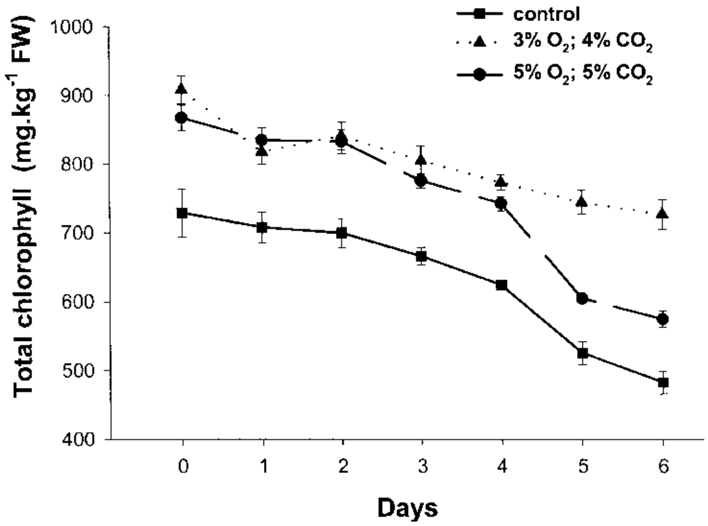


Fig. 2. Total chlorophyll content (mg·kg$^{-1}$ FW) of fresh-cut collard greens stored under controlled atmosphere (3%$O_2$; 4%$CO_2$; balance $N_2$ and 5%$O_2$; 4%$CO_2$; balance $N_2$), and normal air (78%$N_2$; 21%$O_2$), at 5°C for 6 d. Vertical bars indicate ±SD.

*Titratable acidity.* CA storage delayed organic acid degradation. Fresh-cut collard greens stored under 3% $O_2$, 4% $CO_2$ showed the highest titratable acidity at the end of the storage period, having around 8 and 18% more organic acids than the treatment stored under 5% $O_2$, 5% $CO_2$ and control, respectively (Fig. 3). Lau and Looney (1982) also verified that storage under controlled atmosphere delayed organic acid degradation in intact apples. Gonzalez et al. (2000) studied the combination of antibrowning agents and modified atmosphere packaging to extend the shelf life of fresh-cut man-

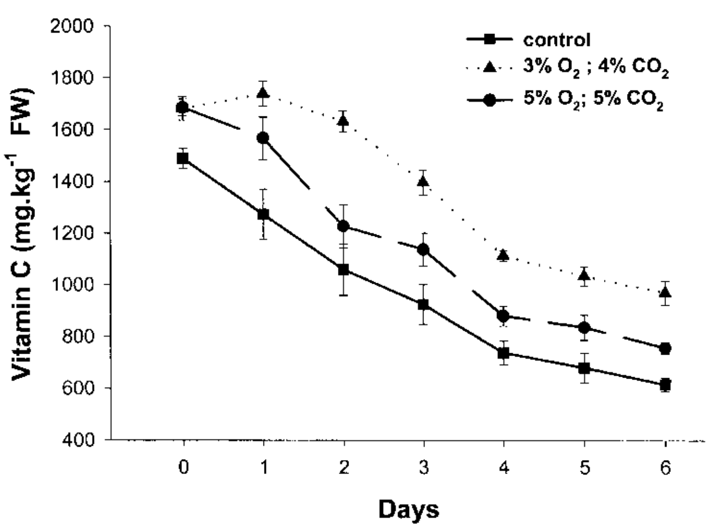


Fig. 1. Total vitamin C content (mg·kg$^{-1}$ FW) of fresh-cut collard greens stored under controlled atmosphere (3%$O_2$; 4%$CO_2$; balance $N_2$ and 5%$O_2$; 4%$CO_2$; balance $N_2$), and normal air (78%$N_2$; 21%$O_2$), at 5°C for 6 d. Vertical bars indicate ±SD.

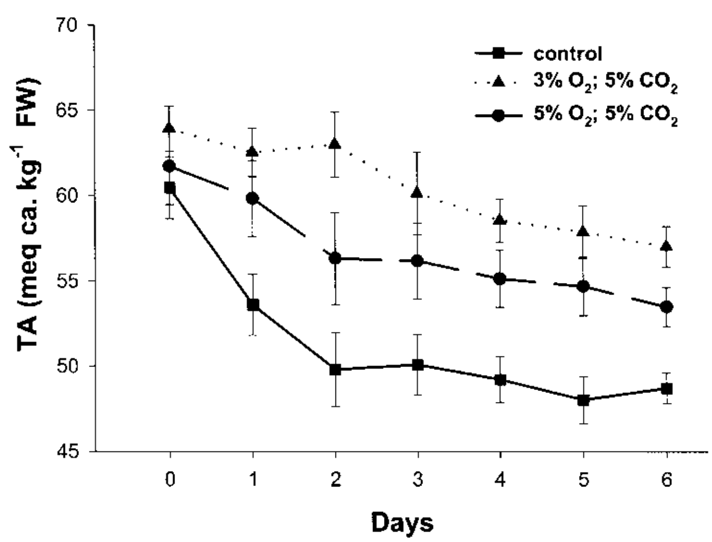


Fig. 3. Titratable acidity (meq·kg$^{-1}$ FW) of fresh-cut collard greens stored under controlled atmosphere (3%$O_2$; 4%$CO_2$; balance $N_2$ and 5%$O_2$; 4%$CO_2$; balance $N_2$), and normal air (78%$N_2$; 21%$O_2$), at 5°C for 6 d. Vertical bars indicate ±SD.

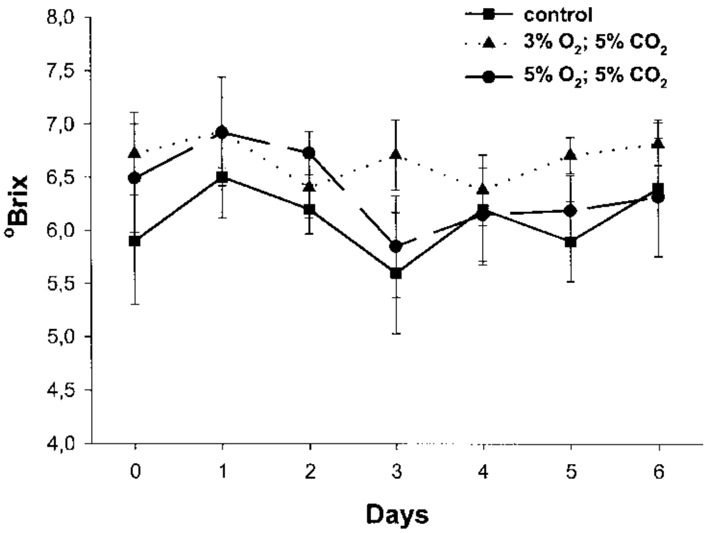


Fig. 4. Total soluble solids (°Brix) of fresh-cut collard greens stored under controlled atmosphere (3%$O_2$; 4%$CO_2$; balance $N_2$ and 5%$O_2$; 4%$CO_2$; balance $N_2$), and normal air (78%$N_2$; 21%$O_2$), at 5°C for 6 d. Vertical bars indicate ±SD.

goes stored at 10 °C. Combinations of several browning inhibitors were more effective than those applied individually and did not affect significantly the changes in organic acids and sugar content of slices during 14 d of storage at 10 °C.

*Total soluble solids.* Total soluble solids content were not significantly affected. It was verified that fresh-cut collard greens stored under 3% $O_2$, 4% $CO_2$ had a higher content of total soluble solids, despite the difference was statistically significant. On the other hand, it was verified that the fresh-cut product stored under normal atmosphere showed the lowest content of total soluble solids during the storage period, being 6% lower than the treatment stored under 3% $O_2$, 4% $CO_2$ at the sixth day (Fig. 4).

Teixeira et al. (2001) studied the effect of cutting size, storage temperature, atmosphere modification rate and chemical characteristics of 'Formosa' fresh-cut papaya packed in plastic cups (500 mL). They verified that total soluble solids content did not vary among treatments.

From this work, it is recommended that fresh-cut collard greens should be stored under 3% $O_2$, 4% $CO_2$ (balance $N_2$) and 5 °C to maintain the quality and extend the shelf life of the processed product.

## Literature Cited


Bower, J. P. and L. J. Van Lelyveld. 1985. The effects of stress history and container ventilation on avocado fruits polyphenol oxidase activity. J. Hort. Sci. 60:545-547.

Brecht, J. K. 1995. Physiology of lightly processed fruits and vegetables. Hortscience, 30:18-24.

Cantwell, M. I. 2000. Preparation and quality of fresh-cut produce. In II Encontro Nacional sobre Processamento Mínimo de Frutas e Hortaliças. Viçosa, MG, Brazil, 1:156-182.

Gil, M. I., F. Ferreres, and F. A. Tomas-Barberan. 1999. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. J. Ag. Food Chem. 47:2213-2217.

Gonzalez-Aguilar, G. A., C. Y. Wang, and J. G. Buta. 2000. Maintaining quality of fresh-cut mangoes using antibrowning agents and modified atmosphere packaging. J. Ag. Food Chem. 48:4204-4208.

Inskeep, W. P. and P. R. Bloom. 1985. Extinction coefficients of chlorophyll *a* and *b* in N,N-Dimethylformamide and 80% acetone. Plant Physiol. 77:483-485.

Ke, D. and M. E. Saltveit. 1989. Wound induced ethylene production, phenolic metabolism, and susceptibility to russet spotting in iceberg lettuce. Plant Physiol. 76:412-418.

King, A. D. and H. R. Bolin. 1989. Physiological and microbiological storage stability of minimally processed fruits and vegetables. Food Technol. 43:132-139.

Lau, O. L. and N. E. Looney. 1982. Improvement of fruit firmness and acidity in controlled-atmospheres-stored "Golden Delicious" apples by a rapid $O_2$ reduction procedure. J. Amer. Soc. Hort. Sci. 107:531-535.

Matilla, M., R. Ahvenainen, E. Hurme, and L. Hyvonen. 1995. Respiration rates of some minimally processed vegetables. In J. DeBaerdemaeker et al. (eds.). Proc. Workshop Postharvest Treatment of Fruits and Vegetables, Commission European Communities, p. 135-145.

Moretti, C. L., S. A. Sargent, D. J. Huber, A. G. Calbo, and R. Puschman. 1998. Chemical composition and physical properties of pericarp, locule and placental tissues of tomatoes with internal bruising. J. Amer. Soc. Hort. Sci. 123:656-660.

Moretti, C. L. 2001a. Technology of fresh-cut products. XXX Brazilian Congress of Agricultural Engineering Agrícola, Foz do Iguaçu, PR, Brazil, 5 p. (In Portuguese)

Moretti, C. L., W. A. Marouelli, and W. L. C. Silva. 2001b. Respiratory activity and browning of minimally processed sweet potatoes. Proc. Fla State Hort.Soc. 114:110-113.

Moretti, C. L., W. L. Silva, and A. L. Araujo. 2000. Quality attributes and carbon dioxide evolution of bell peppers as affected by minimal processing and storage temperature. Proc. Fla. State. Hort. Soc. 113:156-159.

Pariasca, J. A. T., T. Miyazaki, H. Hisaka, H. Nakagawa, and T. Sato. 2001. Effect of modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. var. *saccharatum*). Postharvest Biol. Technol. 21:213-223.

Senesei, E., C. Prizivalli, M. Sala, and M. Gannari. 2000. Physicochemical and microbiological changes in fresh-cut green bell peppers as affected by packaging and storage. Ital. J. Food Sci. 12:55-64.

Sloan, E. A. 2000. A taste of tomorrow. In Fresh-cut products: maintaining quality and safety. Univ. California, Davis. Postharvest technology and information center, September, 2000. p. 23.

Teixeira, G. H. A, J. F. Durigan, B. H. Mattiuz, and O. D. Rossi-Junior. 2001. Fresh-cut of 'Formosa' papaya. Ciência e Tecnologia de Alimentos. 21:47-50

Teles, C. S. Utilization of modified atmosphere storage to extend the shelf life of minimally processed collard greens (*Brassica oleraceae* var. *acephala*). Federal University of Viçosa, Viçosa, MG, Brazil. MSc. dissertation. 115 p. (In Portuguese)

Terada, M., Y. Watanabe, M. Kunitoma, and E. Hayashi. 1978. Differential rapid analysis of ascorbic acid and ascorbic acid 2-sulfate by dinitrophenilhydrazine method. Ann. Biochem. 84:604-608.

Watada, A. E. 1987. Vitamins. In J. Weichmman (ed.). Postharvest physiology of vegetables. New York, Marcel Dekker, Inc., p. 455-468.

Watada, A. E., N. P. Ko, and D. A. Minott. 1996. Factors affecting quality of fresh-cut horticultural products. Postharvest Biol. Technol. 9:115-125.