

Evaluation of different oxygen, carbon dioxide and nitrogen combinations employed to extend the shelf life of fresh-cut collard greens

Celso L. Moretti¹; Alessandra L. Araújo¹; Leonora M. Mattos²

¹Embrapa Hortaliças, C. Postal 218, 70359-970 Brasília-DF; ²Universidade Federal de Lavras, 37200-000 Lavras-MG; E-mail: celso@cnph.embrapa.br

ABSTRACT

Collard greens (*Brassica oleracea* var. *acephala*) “Manteiga” were harvested in commercial fields in Brasília, 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% O₂, 4% CO₂; 5% O₂, 5% CO₂/balance N₂), and normal air (control), at 5°C (95±2% 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% O₂, 4% CO₂ showed around 25% and 56% more vitamin C than the material stored under 5% O₂, 5% CO₂ and control, respectively. Total chlorophyll content decreased during the storage period. At the end of the experiment, fresh-cut collard greens stored under 3% O₂, 4% CO₂ showed 24% and 45% more total chlorophyll than the product stored under 5% O₂, 5% CO₂ and control, respectively. CA storage delayed organic acid degradation. On the sixth day, fresh-cut collard greens stored under 3% O₂, 4% CO₂ presented around 44% more organic acids than control. Total soluble solids content were not significantly affected.

Keywords: *Brassica oleracea* var. *acephala*, brix, chlorophyll, controlled atmosphere, quality, postharvest, vitamin C.

RESUMO

Avaliação de diferentes combinações de oxigênio, gás carbônico e nitrogênio utilizadas no aumento de vida de prateleira de couve minimamente processada

Folhas de couve (*Brassica oleracea* var. *acephala*) “Manteiga” foram obtidas em campos de produção comercial em Brasília, DF, com o objetivo de avaliar diferentes combinações de oxigênio, dióxido de carbono e nitrogênio para estender a vida de prateleira do produto minimamente processado. Após colhidas, as folhas foram levadas ao laboratório de pós-colheita, selecionadas para danos externos aparentes e minimamente processadas (3 mm) no interior de uma câmara fria (13±2°C). Depois de processado, o material foi armazenado sob duas condições de atmosfera controlada (3% O₂, 4% CO₂; 5% O₂, 5% CO₂/balanço de N₂), e ar atmosférico (controle), a 5°C (95±2% UR), por seis dias. Diariamente, foram avaliados os teores de vitamina C total, clorofila total, sólidos solúveis totais e acidez titulável. O teor de vitamina C total diminuiu para todos os tratamentos durante o período de armazenamento. A degradação da vitamina C total para as duas atmosferas estudadas foi retardada pelo armazenamento sob atmosfera controlada (AC). Ao final do período de armazenamento, a couve minimamente processada armazenada sob 3% O₂, 4% CO₂ apresentava 25% e 56% mais vitamina C total que o material armazenado sob 5% O₂, 5% CO₂ e o controle, respectivamente. O teor de clorofila total diminuiu durante o período de armazenamento. Ao final do experimento, o material minimamente processado armazenado sob 3% O₂, 4% CO₂ apresentava 24% e 45% mais clorofila total que o produto armazenado sob 5% O₂, 5% CO₂ e controle, respectivamente. O armazenamento sob AC retardou a degradação de ácidos orgânicos. No sexto dia, a couve minimamente processada armazenada com 3% O₂, 4% CO₂ possuía cerca de 44% mais ácidos orgânicos que o controle. Os teores de sólidos solúveis não foram significativamente afetados.

Palavras-chave: *Brassica oleracea* var. *acephala*, atmosfera controlada, brix, clorofila, pós-colheita, qualidade, vitamina C.

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Although commercialized at 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). In Brazil, the fresh-cut industry started at the beginning of the 1980's and is increasing considerably every year. This increase is partly due to the enormous growth of the food-

service segment (150% during the last 7 years) (Moretti & Sargent, 2002).

Fresh-cut products represent around 10% of the American fresh fruit and vegetable 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 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 to browning (Bower & Van Lelyveld, 1985; Ke & Saltveit, 1989; King & Bolin, 1989; Nicoli *et al.*, 1994; Brecht, 1995; Moretti *et al.*, 2002) are events that are commonly associated with fresh-cut tissues.

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, 2001). Another important tool to control respiration rates and deterioration is modified atmosphere (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% O₂, 5% CO₂) only slightly reduced the respiration rates of fresh-cut carrots, leek and onion, but increased slightly the rates of fresh-cut potatoes. The increase in oxygen demand caused by the increased respiratory activity dictates plastic film permeability, in order to avoid anaerobic conditions (Cantwell, 2000).

Working on fresh-cut strawberries, peaches and honeydew, Watada *et al.* (1996) observed that controlled atmospheres of 1-2% O₂ + 10% CO₂ 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 CO₂ atmospheres in air or low O₂ (Cantwell, 2000).

Despite all the interest and work done with minimally processed

products, processors still face many technological problems. Minimal processing of collard greens has shown to be a great challenge for processors basically due to its 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 days. 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 evaluated.

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

MATERIAL AND METHODS

Plant material

Collard greens (*Brassica oleracea* var. *acephala*) leaves "Manteiga" were harvested in commercial fields in Brasilia. After harvest, leaves were taken to the postharvest laboratory in plastic containers topped with crushed ice, 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 days under two controlled atmosphere (CA) conditions (3% O₂, 4% CO₂; 5% O₂, 5% CO₂/balance N₂), and normal air (control), at 5±1°C and 95±2% 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 at 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 & 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. 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 x 0.1 N x 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% O₂, 4% CO₂; 5% O₂, 5% CO₂/balance N₂), 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=0.05).

RESULTS AND DISCUSSION

Total vitamin C

Total vitamin C content decreased for all treatments during the storage

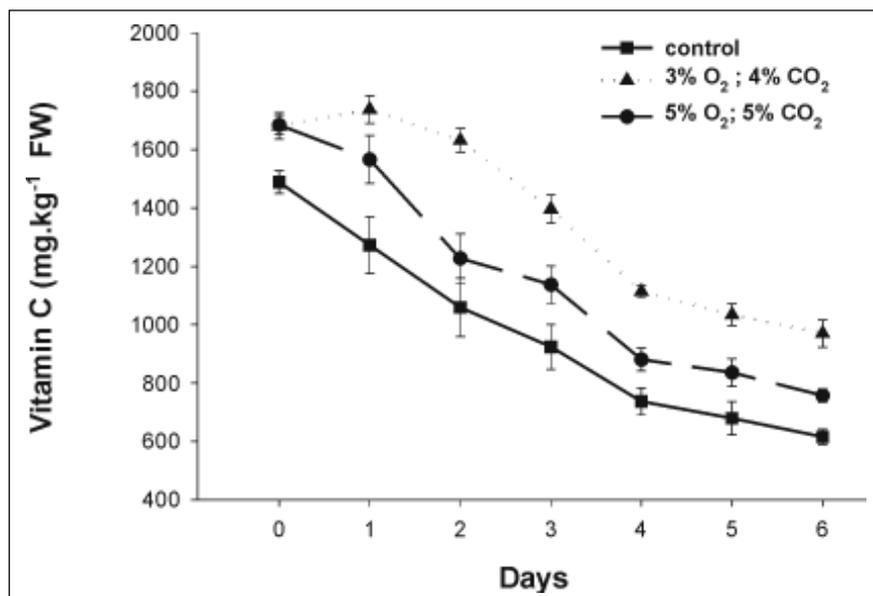


Figure 1. Total vitamin C content (mg.kg⁻¹ FW) of fresh-cut collard greens stored under controlled atmosphere (3%O₂; 4%CO₂; balance N₂ and 5%O₂; 4%CO₂; balance N₂) and control (78%N₂; 21%O₂), at 5°C for 6 days. Vertical bars indicate ± SD. Brasília, Embrapa Hortaliças, 2002.

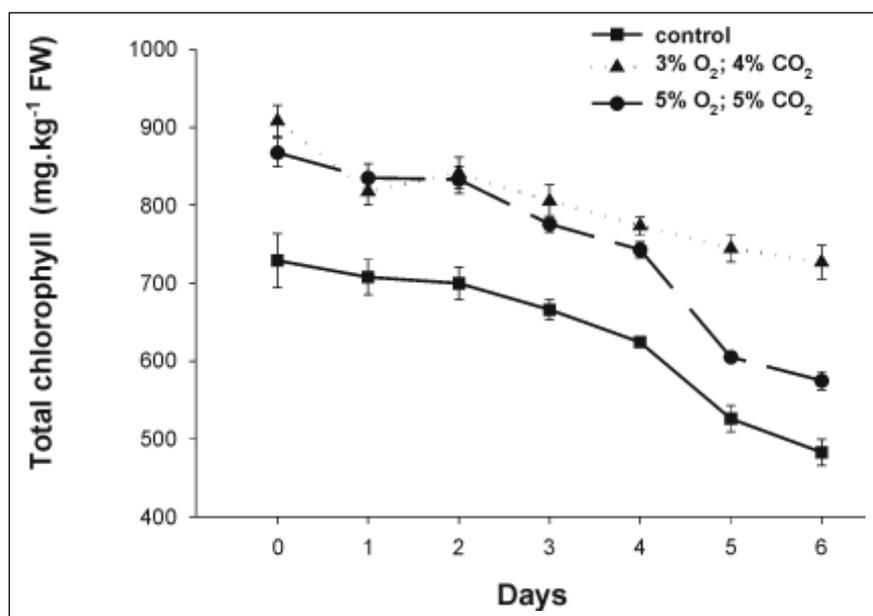


Figure 2. Total chlorophyll content (mg.kg⁻¹ FW) of fresh-cut collard greens stored under controlled atmosphere (3%O₂; 4%CO₂; balance N₂ and 5%O₂; 4%CO₂; balance N₂) and control (78%N₂; 21%O₂), at 5°C for 6 days. Vertical bars indicate ± SD. Brasília, Embrapa Hortaliças, 2002.

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% O₂, 4% CO₂ showed around 25% and 56% more vitamin C than the same material stored under 5% O₂, 5% CO₂ and control, respectively (Figure 1).

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 days. Vitamin C content and the organoleptic characteristics were acceptable at the end of the storage period.

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 (Figure 2). After the fourth day, chlorophyll degradation occurred slowly for fresh-cut collard greens stored under 3% O₂, 4% CO₂ when compared to other treatments, which 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% O₂, 4% CO₂ showed 24% and 45% more total chlorophyll than the product stored under 5% O₂, 5% CO₂ and control, respectively (Figure 2). Considering the retention of the initial total chlorophyll content, it was observed that storage under 3% O₂, 4% CO₂ allowed a retention of around 80% of the initial content (Figure 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 O₂ and 5 kPa CO₂, leading to better maintenance of the pod internal quality (chlorophyll, ascorbic acid, and sugar contents).

Titrateable acidity

Controlled atmosphere storage delayed organic acid degradation. Fresh-cut collard greens stored under 3% O₂, 4% CO₂ showed the highest titrateable acidity at the end of the storage period, having around 8 and 18% more organic

acids than the treatment stored under 5% O₂, 5% CO₂ and control, respectively (Figure 3). Lau & 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 mangoes 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 days 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% O₂, 4% CO₂ had a higher content of total soluble solids, despite the difference not being 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% O₂, 4% CO₂ at the sixth day (Figure 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.

Considering the conditions under which this work was carried out, it is recommended that fresh-cut collard greens shall be stored under 3% O₂, 4% CO₂ (balance N₂) and 5°C to maintain the quality and extend the shelf life of the processed product.

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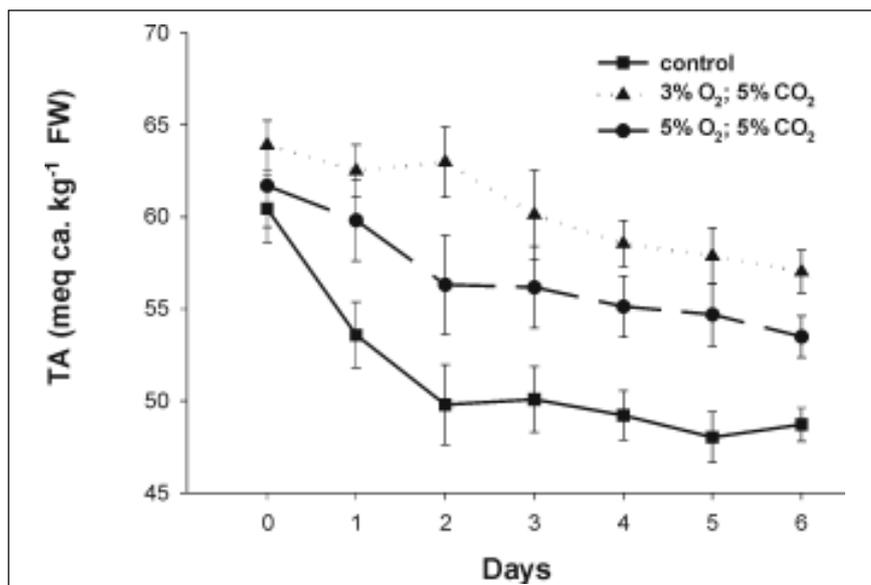


Figure 3. Titratable acidity (meq ca. kg⁻¹ FW) of fresh-cut collard greens stored under controlled atmosphere (3%O₂; 4%CO₂; balance N₂ and 5%O₂; 4%CO₂; balance N₂) and control (78%N₂; 21%O₂), at 5°C for 6 days. Vertical bars indicate ± SD. Brasília, Embrapa Hortaliças, 2002.

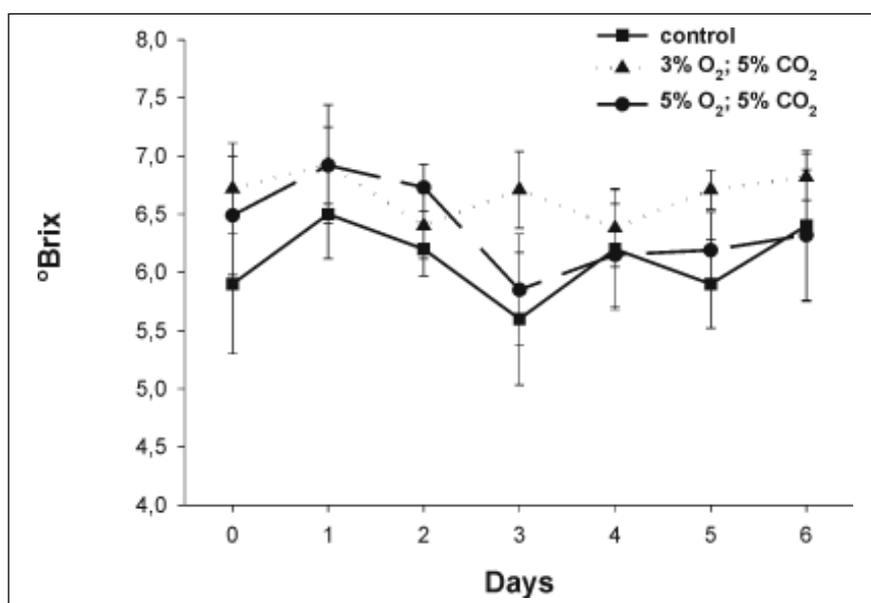


Figure 4. Total soluble solids (°Brix) of fresh-cut collard greens stored under controlled atmosphere (3%O₂; 4%CO₂; balance N₂ and 5%O₂; 4%CO₂; balance N₂) and control (78%N₂; 21%O₂), at 5°C for 6 days. Vertical bars indicate ± SD. Brasília, Embrapa Hortaliças, 2002.

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