

CO₂ modified atmosphere packaging: stress condition or treatment to preserve fruit and vegetable quality?

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

Abstract: In addition to the adoption of proper temperature and relative humidity, the selection of an atmosphere surrounding packaged fresh produce with reduced O₂ and/or increased CO₂ is one of the most widely used and useful tools to prolong the shelf-life of horticultural crops. However, as O₂ and/or CO₂ values that might cause injury are strictly related to the commodity, they should be optimized for each product. Here three study cases are reported about the application of modified atmospheres (MA), with different CO₂ concentrations (0-40 kPa), to table grapes (cv. Italia) and sweet cherries (cv. Ferrovia) and, as a short-term treatment (48 h at 0°C), to fresh-cut artichokes (cv. Violet de Provence). In each trial, the effect of high CO₂ treatment on quality parameters was observed during cold storage. Concerning table grape 'Italia', our results show that low CO₂ (up to 10 kPa) MA preserved the quality and sensory parameters of the fruit, whereas high CO₂ (> 20 kPa) caused a fermentative metabolism. As for sweet cherries 'Ferrovia', 20 kPa CO₂ MA helped to maintain the quality traits during storage. On the other hand, this fruit proved to be sensitive to CO₂ accumulation (over 20 kPa) in hypoxic conditions, since it caused an increase in respiration rate and the biosynthesis of volatile fermentative metabolites. Finally, for fresh-cut artichokes, a short-term CO₂ treatment, up to 10kPa, reduced respiration rate and browning index, preserving the volatile profile, while high CO₂ (40 kPa) may have caused fermentative metabolism. In conclusion, the application of a MA enriched in CO₂ has been shown to have different effects on the quality parameters of the three products, in agreement with the fact that CO₂ sensibility depends on each specific fruit or vegetable under study.

1. Introduction

Fruits and vegetables are perishable products, and extending the keeping quality during their postharvest life represents one of the main

goals of researchers in this field. It is widely known that, together with the proper temperature and relative humidity management, the gas composition surrounding the product during storage, is one of the main factors that affect the quality of horticultural crops (Kader, 2003). In general, the decrease in oxygen, the increase in CO₂, or the association of both conditions are useful to preserve the physiological state of fruits and vegetables, reducing the rate of respiration, oxidative processes, and decay, thus prolonging their shelf-life. In contrast, inappropriate gas concentrations outside safe limits can cause stress conditions that lead to physiological disorders, development of off-odours due to fermentative metabolism, or increases in susceptibility to decay (Mangaraj and Goswami, 2009). Although low O₂ and high CO₂ have similar effects, under modified atmosphere packaging (MAP) conditions, elevated CO₂ is a major factor influencing the quality of fruits and vegetables (Watkins, 2000). In addition, the sensitivity to elevated high CO₂ and/or low O₂ levels depends on the commodity (Toivonen and DeEll, 2002). It is influenced by pre and postharvest factors, such as cultivars or stage of maturity, and by processing, since the oxygen consumption and the consequent CO₂ accumulation in fresh-cut produce is faster than in corresponding intact produce (Francis *et al.*, 2012). To obtain the beneficial effect of MAP, gas conditions should be optimized for each product. Starting from these considerations, the aim of the present work was to compare the effect of different CO₂ concentrations in MAP on the quality of table grapes, sweet cherries and fresh-cut artichokes as case studies.

2. Materials and Methods

Table grapes (*Vitis vinifera* L., cv. Italia), sweet cherries (*Prunus avium* L., cv. Ferrovia), and artichokes (*Cynara cardunculus* (L.) subsp. *scolymus* Hayek, cv. Violet de Provence) were provided by local farms located in Noicattaro and Foggia (Italy) and processed at the Postharvest Laboratory of CNR-ISPA the day of harvest. Selected bunches of table grapes (about 1 kg each) were placed in polyethylene terephthalate (PET) trays (model CL1/135 Carton Pack, Rutigliano, Italy). They were sealed with a vacuum sealer (model Boxer 50 Lavezzini Vacuum Packaging System, Fiorenzuola d'Arda, Italy) in 30 x 40 cm polyamide/polyethylene (PA/PE) bags (Orved S.p.A., Musile di Piave, Italy)

applying two modified atmosphere (MA) mixtures with different initial CO₂ concentrations plus 1 kPa of O₂: 1.0/0.03 O₂/CO₂ kPa (1 kPa-O₂), and 1.0/20.0 O₂/CO₂ kPa (1 kPa-O₂ + 20 kPa-CO₂). Unsealed bags were used as control (Air). All samples (4 replicates per treatment) were analyzed at harvest and after 20 days of storage at 5°C for respiration rate (RR), rachis browning (rB), ethanol, and acetaldehyde contents.

For sweet cherries, about 200 g of fruits, without defects or diseases, were placed in PET trays and sealed in 30 x 40 cm PA/PE bags with three MA mixtures: 1.0/0.03 O₂/CO₂ kPa (1 kPa-O₂), 16.0/20.0 O₂/CO₂ kPa (16 kPa-O₂ + 20 kPa-CO₂), and 1.0/20.0 O₂/CO₂ kPa (1 kPa-O₂ + 20 kPa-CO₂). Samples stored in unsealed bags (Air) were used as controls. All samples (3 replicates per treatment) were analyzed at harvest and after 21 days of storage at 5°C for RR, relative water content of peduncles (RWC), and volatile organic compounds (VOCs).

As for artichokes, the heads were trimmed, eliminating the external bracts and cutting the stem. The obtained artichoke hearts were then cut into quarters and dipped for 5 min in a solution of 1% ascorbic acid + 0.2% citric acid (w:v), drained, and randomly selected for different treatments. In particular, three replicates of 16 artichoke quarters were kept for the initial determinations, while the remaining quarters were closed in 50 x 50 polypropylene (PP) bags (Carton Pack® Rutigliano, Italy), about 600 g per bag, applying 4 MA mixtures with different initial CO₂ concentrations plus 10 kPa of O₂: 10.0/10.0 O₂/CO₂ kPa (CO₂-10kPa), 10.0/20.0 O₂/CO₂ kPa (CO₂-20kPa), 10.0/30.0 O₂/CO₂ kPa (CO₂-30 kPa), 10.0/40.0 O₂/CO₂ kPa (CO₂-40 kPa). Unsealed bags were used as control (Air). After 48 h of storage at 0°C, all bags were opened, artichoke quarters were placed in open polyethylene bags and analyzed after 48 h at 0°C plus 7 days of storage at 5°C for RR, browning index (BI) and VOCs profile. The headspace gas composition (O₂ and CO₂) within each MA package was monitored daily using a gas analyzer (CheckPoint, PBI Dansensor, Ringsted, Denmark). RR was measured initially (Fresh) and at the end of the storage for each product using a closed system, as reported by Kader (2002 a). Samples were put into 6 L sealed plastic jars, allowing the accumulation of CO₂ up to 0.1 kPa. For CO₂ analysis, 1 mL of gas sample was collected from the headspace of each jar and injected into a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA, USA) equipped with dual columns and a thermal conductivity detector. Carbon dioxide was analysed

with a retention time of 16 s and a total run time of 120 s on a 10 m porous polymer (PPU) column (Agilent, Santa Clara, CA, USA) at a constant temperature of 70°C. RR was expressed as mL CO₂ kg⁻¹ h⁻¹. In table grapes, rB was scored on a rating scale from 1 to 5 (1= absence, 2= light; 3= moderate; 4= severe; 5= extreme) as reported by Lichter *et al.* (2011), whereas for acetaldehyde and ethanol analysis, the procedure reported by Cefola *et al.* (2018) was used.

In sweet cherries, the RWC of peduncles was calculated in percentage, as reported by Cefola *et al.* (2018), while the VOCs analysis was carried out, as reported by Cozzolino *et al.* (2019).

In fresh-cut artichokes, BI and VOCs were evaluated, as reported by Capotorto *et al.* (2020).

3. Results and Discussion

Starting from the gas composition inside MA packages described above for each product, during storage the concentrations of O₂ and CO₂ changed due to the respiration of the products and gas permeation through packaging material, and the final gases composition were reported in Table 1.

As for table grapes, O₂ concentrations decreased from 1 kPa to about 0.2 kPa or 0.3 kPa, in 1k Pa-O₂ and in 1 kPa-O₂ + 20 kPa-CO₂ respectively, while CO₂ concentrations increased from 0.03 kPa to roughly 10 kPa in 1 kPa-O₂ packages, and from 20 kPa to about

30 kPa in 1k Pa-O₂ + 20 kPa-CO₂ MA.

For sweet cherries, in 16 kPa-O₂ + 20 kPa-CO₂ bags, the O₂ concentration gradually decreased, reaching the mean value of about 1 kPa after 21 days of storage. In 1 kPa-O₂ and 1 kPa-O₂ + 20 kPa-CO₂ samples, the initial O₂ concentration remained unchanged during the storage. On the other hand, the amount of CO₂ increased during conservation, reaching the final mean values of 25.7 kPa, 45.3 kPa and 42.4 kPa in 1 kPa-O₂, 16 kPa-O₂ + 20 kPa-CO₂ and 1 kPa-O₂ + 20 kPa-CO₂ packages, respectively. Finally, as for fresh-cut artichokes, no significant changes in gas composition inside bags were observed.

Results on table grapes are reported in Table 2. In the Fresh samples the RR measured was equal to 4.2 (± 0.4) mL CO₂ kg⁻¹ h⁻¹; after 20 days of the storage, a reduction in RR was measured in air samples (3.0 ± 0.2 mL CO₂ kg⁻¹ h⁻¹), while it remained almost constant in table grapes samples treated with 1 kPa O₂ (4.7 ± 0.6 mL CO₂ kg⁻¹ h⁻¹). On the contrary the use of high CO₂ concentrations (>20 kPa) in the MA mixture (1 kPa-O₂ + 20 kPa-CO₂) increased the value of RR resulting more than a 2-fold higher than Fresh sample. Significant differences were observed, for sample stored in air and 1 kPa-O₂ + 20 kPa-CO₂ (Table 2). As shown in Table 1, browning of the table grapes rachis was found in all treatments after 20 days of storage. However, higher browning was observed in air and in 1 kPa-O₂ + 20 kPa-CO₂ samples, whereas the use of 1 kPa-O₂ was able to keep a light to moderate browning of the rachis (mean value of 2.5). After storage ethanol and acetaldehyde concentrations did not change from their initial values (4.2 and 0.6 mg L⁻¹, respectively) in the Air samples, whereas they significantly increased in table grapes exposed to low (<10 kPa) or high CO₂ (>20 kPa) concentrations (Table 2). Moreover, samples exposed to 1 kPa-O₂ + 20 kPa-CO₂ showed higher accumulations of ethanol and acetaldehyde than table grapes packed in 1 kPa-O₂.

Table 1 - Initial and final concentration of O₂ and CO₂ for each treatment on table grape

Treatment	Initial kPa		Final kPa	
	O ₂	CO ₂	O ₂	CO ₂
1 kPa-O ₂	1.0	0.04	0.2	10.0
1 kPa-O ₂ + 20 kPa-CO ₂	1.0	20.0	0.3	30.0
Air	20.0	0.03	20.0	0.03

Table 2 - Effect of CO₂ treatments on respiration rate, rachis browning, ethanol and acetaldehyde contents of table grapes (*Vitis vinifera* cv. Italia) after 20 days of storage at 5°C

Treatment	Respiration rate (mL CO ₂ kg ⁻¹ h ⁻¹)	Rachis browning (1-5)	Ethanol (mg L ⁻¹)	Acetaldhyde
Fresh	4.2 b	1.0 c	4.2 c	0.6 c
1 kPa-O ₂	4.7 b	2.5 b	2142 b	8.9 b
1 kPa-O ₂ + 20kPa-CO ₂	9.0 a	3.6 a	3606 a	17.6 a
Air	3.0 c	4.4 a	5.8 c	0.6 c

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 20, respectively, according to LSD test (P≤0.05).

Sweet cherries (Table 3) showed an initial respiration rate of $8.2 (\pm 0.3) \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ which increased 1.5 fold in air and more than 5 times in the other MA treatments. The highest RR was observed in $1 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$ ($48.9 \pm 0.7 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) followed by $16 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$ ($44.4 \pm 0.6 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and 1 kPa-O_2 ($43.2 \pm 0.1 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$).

The RWC % of peduncle increased in all MA treatments, maybe due to the high relative humidity inside the packages. The highest RWC % values were observed in low O_2 treatments, probably thanks to the lower respiration rate of these samples.

Among VOCs analysed, 1-pentanol, marker of sensory alteration, was closely associated with negative aroma intensity which resulted directly described as pungent, and fermented flavour. Whereas the reduction of hexenal and 2-hexenal were indicators of lost in freshness (Cozzolino *et al.*, 2019).

As reported in Table 2, 1-pentanol was not detected in Fresh and Air samples, while a significant increase of this alcohol was observed in the other

MA treatments. Samples treated with $1 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$ had the highest value of 1-pentanol, while 1 kPa-O_2 and $16 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$ MA treatments had similar values (Table 2). As for hexenal, (Table 3) statistical analysis did not show significant changes after 21 days at 5°C excepted for the treatment, $1 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$ which showed a reduction with respect to the fresh sample. In contrast, 2-hexenal decreased significantly during storage, but no differences in its concentration were observed when comparing MA treatments (Table 3).

Results of fresh-cut artichokes are reported in Table 4. RR of the fresh sample was $120.8 (\pm 0.2) \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$. After the short-term CO_2 treatments (48 h, 0°C) and 7 days of storage at 5°C , RR decreased significantly in all samples, except in artichokes treated with 40 kPa of CO_2 . The lowest RR was detected in fresh-cut artichokes treated with CO_2 -10 kPa ($44.5 \pm 4.3 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$), followed by CO_2 -20 kPa and CO_2 -30 kPa, which reported similar values (68.1 ± 1.1 and $63.9 \pm 5.2 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, respectively), and Air (93.6

Table 3 - Effect of CO_2 treatments on respiration rate, relative water content (RWC) of peduncles, 1-pentanol, hexenal and 2-hexenal relative peak area (RPA) contents of sweet cherries (*Prunus avium* cv. Ferrovia) after 21 days of storage at 5°C

Treatment	Respiration rate ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$)	Relative water content of peduncles (%)	1-Pentanol relative peak area (%)	Hexenal	2-Hexenal
Fresh	8.2 B	53.9 B	0.0 B	112.3 NS	366.6 A
1 kPa-O_2	43.2 Ac	65.4 Aa	6.0 Ab	47.3 NS a	118.0 B NS
$16 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$	44.4 Ab	58 Ab	7.6 Ab	26.4 NS ab	99.9 B NS
$1 \text{ kPa-O}_2 + 20 \text{ kPa-CO}_2$	48.9 Aa	60.5 Ab	18.6 Aa	14.3 Bb	55.7 B NS
Air	12.1 Ad	53.6 NS c	0.0 NS c	40.5 NS ab	125.2 B NS

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 21, respectively, according to LSD test ($P \leq 0.05$).

Table 4 - Effect of short-term CO_2 treatments on respiration rate, browning index, ethanol and hexenal relative peak area (RPA) contents of fresh-cut artichokes (*Cynara cardunculus* cv. Violet de Provence) after 7 days of storage at 5°C

Treatment	Respiration rate ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$)	Browning index	Ethanol relative peak area (%)	Hexenal
Fresh	120.8 A	0.0 B	34.5 NS	41.7 NS
CO_2 -10 kPa	44.5 Bd	131 Ad	114.0 NS c	5.4 NS a
CO_2 -20 kPa	68.1 Bc	137 Abc	379.1 Ab	5.0 NS a
CO_2 -30 kPa	63.9 Bc	135 Acd	482.5 Ab	4.3 NS a
CO_2 -40 kPa	121.5 Aa	140 Ab	363.5 Ab	2.1 Bb
Air	93.6 Bb	152 Aa	886.3 Aa	3.8 NS a

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 7, respectively, according to LSD test ($P \leq 0.05$).

$\pm 1.7 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$), while the highest RR was observed in CO₂-40 kPa ($121.5 \pm 0.2 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$). As expected, all samples developed browning after 7 days of storage, regardless of treatment. However, differences in the severity of browning were observed when comparing treatments at the end of the storage: the application of short-term CO₂ treatments (from 10 kPa to 40 kPa) significantly reduced the incidence of browning compared to Air samples and, among the CO₂ treatments, CO₂-10 kPa had the lowest browning index (Table 4). Considering all the VOCs identified by HS-SPME/GC-MS analysis, ethanol and hexenal were, respectively, the most representative compounds of negative and positive aspects of fresh-cut artichokes (Capotorto *et al.*, 2020).

As shown in Table 4, ethanol significantly increased during storage, except for the CO₂-10kPa treatment, where its concentration was similar to that of fresh samples. The highest ethanol concentration was found in Air samples, followed by treatments added with CO₂ at 20, 30, and 40 kPa that showed similar values. As for hexenal, it was significantly lower only in fresh-cut artichokes treated with CO₂-40 kPa (Table 4).

For table grapes, data related to ethanol and acetaldehyde, together with the RR results, indicate that high CO₂ concentrations (>20 kPa) on this commodity may cause physiological injury and the induction of anaerobic metabolism.

The present results are supported by similar findings on the effect of high CO₂ concentrations (>20 kPa) on table grapes by Cefola and Pace (2016). High CO₂ concentrations also negatively influence the acceptability of table grapes by consumers: rachis browning, is, in fact, the main issue that limits the acceptability of table grapes by consumers (Cefola *et al.*, 2018). A similar effect of high CO₂ concentrations (>20 kPa) on the acceleration of rachis browning was previously observed on table grapes (Crisosto *et al.*, 2002; Deng *et al.*, 2006) and is a consequence of the stress induced by exposure to high CO₂ concentrations (Crisosto *et al.*, 2002; Liguori *et al.*, 2015).

For sweet cherries, considering that the highest RR was observed when MA with 20kPa CO₂ was applied, these results indicate that this CO₂ concentration, especially when associated with low oxygen, can cause stress, as confirmed by VOC analysis. Similar behaviour in the production of C5 volatiles, such as 1-pentanol, was previously observed (Contreras *et al.*, 2017; Mastrandrea *et al.*, 2017),

and it seems to be favoured under low O₂ and high CO₂ atmospheres. The present results on the cv. Ferrovia are in contrast with previous results on other sweet cherries cultivars (Kader *et al.*, 1989; Esturk *et al.*, 2012), but those cultivars have better tolerance to high CO₂.

It has been stated that the physiological susceptibility of commodities to high CO₂ can be cultivar-dependent, and is generally seen with vegetables and other fruit (Watkins, 2000).

Results on fresh-cut artichokes indicate that the application of high CO₂ concentrations (around 40 kPa) has a negative effect on the shelf-life.

Similar results on the detrimental effect of high CO₂ were previously observed on fresh-cut artichokes during storage (La Zazzera *et al.*, 2012, 2015). As observed for table grapes, sweet cherries, and fresh-cut artichokes, the exposure to elevated CO₂ atmospheres can stimulate respiration and ethylene production rates, indicating a stress response (Kader, 2002 a). These increases in respiration might be related to the inhibition by high CO₂ of several enzymes of the Krebs cycle, including succinate dehydrogenase, which triggers anaerobic respiration or causes the accumulation of succinic acid, which is potentially toxic to cells (Kays, 1991; Varoquaux, 1991; Kader, 2002 b). Furthermore, for fresh-cut artichokes, the intolerance to high CO₂ concentration and mechanical wounding enhances a different array of enzymatic pathways, many of which are associated with volatile accumulation, which lead to development of off-flavors (Salunkhe *et al.*, 1976; La Zazzera *et al.*, 2015).

4. Conclusions

For table grapes, the storage in high CO₂ (>20 kPa) caused a severe increase in respiration rate, ethanol and acetaldehyde accumulation, and a decline in sensory quality due to the rachis browning, all probably consequences of the induction of the anaerobic metabolism. The application of CO₂ up to 10kPa was able, instead, to keep the good quality table grapes during storage.

Sweet cherry (cv. Ferrovia) is very sensitive to high CO₂ when it is applied together with low oxygen in MA, as indicated by responses in respiration rate, relative water content of the peduncles, and VOC emissions, with some of these responses being considered positive and some negative in relation to quality.

Short-term treatment with high CO₂ (around 40 kPa) caused an increase in respiration rate and the induction of fermentative metabolism in fresh-cut artichoke. The application of CO₂ concentrations up to 10 kPa reduced respiration rate and tissue browning during storage in air at 5°C and preserved the fresh VOC profile. Application of short-term CO₂ might be a promising postharvest treatment to preserve the quality and the volatile profile of fresh-cut artichokes during storage.

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