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ARTICLE

Influence of Postharvest Treatments on Quality, Carotenoids, and Abscisic Acid Content of Stored "Spring Belle" Peach (*Prunus persica*) Fruit

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The influence of four postharvest treatments, 1-methylcyclopropene (1-MCP), carbon dioxide (CO_2), and nitrogen (N_2), followed by fruit storage at 10 °C or of hydrocooling (H_2O) at 1 °C, followed by storage at 0 °C on fruit quality, carotenoids, and abscisic acid (ABA) content as well as on ethylene and carbon dioxide production of "Spring Belle" peach fruits, has been examined. Ethylene production was reduced by all the treatments and raised after transfer the fruits at 20 °C, their ethylene production in general being lower than that of fruits continuously held at 20 °C. Nevertheless, 1-MCP removal enhanced the rise in ethylene occurring at 20 °C by the end of storage. Those changes were likely related to fruit softening but not to changes in color or in the soluble solid content (SSC). HPLC analyses showed a relative high content of xanthophylls, particularly violaxanthin. In fruits maintained in air at 20 °C, violaxanthin and β-carotene contents decreased while β-criptoxanthin increased. ABA content showed a great increase in 1-MCP and significant decrease in carbon dioxide and hydrocooling treated peaches. The results indicated hydrocooling, in combination with low temperature storage, as the best treatment maintaining fruit firmness due to the lowered respiration rate and the content of relevant carotenoids.

KEYWORDS: peach; carotenoids; abscisic acid; ethylene; carbon dioxide; nitrogen; 1-methylcyclopropene; hydrocooling; postharvest treatments.

INTRODUCTION

Carotenoids are responsible for pigmentation of fruits, flowers, and some animals, playing an important role in photoprotection and photoreception in plants. Besides their relevance for human health, carotenoids are important in fruit quality as they are responsible for color and may protect fruits against environmental cues affecting their external aspect due to their antioxidant properties (*1*). The nutritional importance of some carotenoids such as β-carotene, α-carotene, and β-cryptoxanthin has been related to their role as precursors of vitamin A, while the health benefits associated with other carotenoids appear to be derived from the antioxidant properties and stimulation of the immune and anti-inflammatory response. Furthermore, carotenoids are precursors of ABA, a plant hormone that may protect plants from stress conditions such as dehydration or oxidative stress (*2*). The synthesis of carotenoids and ABA may be affected by ethylene, an important hormone controlling fruit ripening in climacteric fruits. On the other hand, ripening of fruit is usually accompanied by enhanced carotenoids biosynthesis (*1*) and increased ABA levels (*3*).

Peach (*Prunus persica*) is a typical climacteric fruit, and "Spring Belle" is a mutant of the better known "Springcrest" peach cultivar. It ripens early during the season (June) and has a good productivity. The fruit is medium−large size, spherical, the skin is colored with a deep red, and the pulp is yellow, firm, and juicy with a good taste. In spite of its excellent quality, little is known about its qualitative and quantitative carotenoid content and how these compounds may be affected by postharvest storage conditions (*4*). Carotenoids content in ripe peaches may greatly vary among cultivars and fruit harvested from different areas (*4*, *5*), β-carotene being one of the major carotenoids in white and yellow flesh peaches, followed by β-cryptoxanthin (*5*, *6*). Ethylene promotes autocatalytic pathway of ethylene production in peaches (*7*) and is responsible for the increasing of the ripening speed rate (*8*).

Various postharvest treatments, such as low temperature and the use of other gases, may be used during storage to slow the fruit ethylene biosynthesis or to reduce ethylene action. Thus, 1-MCP, a competitive inhibitor of ethylene action, delays the physical, chemical, and biochemical changes associated with ripening of fruits (*9*, *10*). Anaerobic treatments like nitrogen and carbon dioxide may delay ethylene rise and softening in peach and nectarines (*11*). It is also well-known that elevated carbon dioxide concentrations or the use of atmospheres containing high carbon dioxide and low oxygen levels may affect the various respiratory metabolic pathways in fruits and vegetables and delay some ripening-related processes such as loss of fruit firmness. Anoxic

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treatment is a promising technology to complement or even replace refrigeration, although there is a wide variation in tolerance to anaerobic conditions among species. Thus, very low oxygen concentration or nitrogen atmosphere may delay ripening even after the transfer of fruits to air while anoxic treatments were effective in delaying ripening in peaches (*12*). It is also interesting to note that hydrocooling is the most frequent precooling technique used to shut down field temperature of peach fruits and, therefore, to maintain their quality during cold storage and shelf life period. Cold storage of peaches has an important impact in maintaining their flavor and considerably reduces postharvest fruit losses, but it can drive undesirable effects related to its sensitivity to low temperature such as wooliness and off-flavor development and to abnormal ripening symptoms (*13*, *14*). Combination of gas treatments with not too low temperatures (10 °C) instead of using 0 °C, as usually adopted for peaches, could represent an important alternative for short time storage and distribution in order to maintain better quality and save energy.

Furthermore, no data exist about the effect of postharvest treatments on ABA and carotenoids of peaches apart from those reported by Wright and Kader (*6*) and Koukounaras et al. (*15*) studying the carotenoid content modification in minimally processed peaches. Therefore, the aim of this work was to study the effect of different postharvest gas treatments such as nitrogen, carbon dioxide, and 1-MCP at 10 °C, followed by short storage at the same temperature and one day at 20 °C as well as the commercial treatment of hydrocooling and subsequent low temperature storage (0 °C) on postharvest physiology and quality parameters with special attention to carotenoids content and composition and ABA synthesis on "Spring Belle" peach.

MATERIALS AND METHODS

Plant Material and Postharvest Treatments. Peaches (*Prunus persica* L. Batsch cv. Spring Belle) manually harvested in June at commercial ripening stage (values of soluble solids content and flesh firmness reported in **Table 1**) were immediately transported to the Postharvest Laboratory of the Tuscia University (Italy). After sorting the fruits for uniform ripening and absence of defects, they were divided at random into five lots of 60 fruits each, (3 replicates of 20 fruits each), which were exposed to the following postharvest treatments:

(1) N_2: Nitrogen (99.5%) for 48 h at 10 °C + 5 days at 10 °C + 1 day at 20 °C.
(2) CO_2: Carbon dioxide (100%) for 48 h at 10 °C + 5 days at 10 °C + 1 day at 20 °C.
(3) 1-MCP: 1-Methylcyclopropene (500 nL L^{-1}) for 20 h at 10 °C + 6 days at 10 °C + 1 day at 20 °C.
(4) H_2O: Water at 1 °C for 1 h + 7 days in air at 0 °C + 1 day at 20 °C.
(5) Air: Fruits that were not exposed to any treatment and held in air at 20 °C for 8 days.

Relative humidity (RH) was maintained close to saturation during fruit treatments with nitrogen, carbon dioxide, or 1-MCP. Cold room conditions were 10 ± 0.5 °C and 85 ± 5% RH. After the maintenance at 10 °C, peaches were transferred to 20 °C and 50−60% RH to simulate retail conditions. The storage RH of fruits maintained continuously in air at 20 °C (treatment 5) was 85 ± 5% RH. The use of 10 °C temperature in combination with gas treatments (N_2, CO_2, 1-MCP) was chosen in order to delay fruit senescence and loss of peach quality without using low temperature (0 °C), which can compromise peach aroma and at the same time be more energy consuming.

The nitrogen treatment was performed by using a 99.5% nitrogen tank (Rivoira, Terni, Italy) and flushing the gas after humidification each day through a 5 L glass jar. The carbon dioxide treatment was performed by following the same procedure by using 100% liquid carbon dioxide from a tank (Rivoira, Terni, Italy), which was released by pressure. 1-MCP (Agrofresh Inc. Milan, Italy) was injected after preparation following the instructions of the manufacturer. The 1-MCP concentration was selected on the basis of the results obtained in preliminary experiments in fruits treated for 20 h at 10 °C with 100, 500, 1000, and 1500 nL L^{-1} of 1-MCP. As the lowest concentration did not affect ethylene production, whereas applying 1000 or 1500 nL L^{-1} of 1-MCP had the same effect as applying 500 nL L^{-1}, this last concentration was used in the present study. Carbon dioxide accumulation in the jars during nitrogen and 1-MCP treatments was always negligible. After the gas treatments, jars were open, flushed with air, and left in the cold room. The jars were closed with perforated Parafilm, which allowed air exchange and avoided direct air flow on the peaches. The other samples (fruits exposed to the hydrocooling treatment and fruits maintained continuously in air at 20 °C) were kept under the same experimental conditions. Film covers were removed each day and jars flushed with air.

Hydrocooling treatment was performed by immersing the fruits at 23 °C in tap water previously cooled in a cold room. The temperature of the water was 1 °C before immersing the peaches, and the peaches' flesh reached a temperature of 2 ± 1 °C at the end of the treatment. The ratio of water used per kg of fruit was 5 L kg^{-1}.

Table 1. Quality Parameters and Total Carotenoids of *Prunus persica* Spring Belle Variety before (T_0) and by 8 Days after Being Exposed to the Postharvest Treatments Assayed[a]

	treatments					
quality	T_0	N_2	CO_2	1-MCP	H_2O	air
skin color (hue angle)	86 a	75 a	84 a	86 a	76 a	78 a
flesh firmness (N)	40.8 a	11.4 c	13.3 c	1.2 d	20.9 b	2.4 d
soluble solids (°Brix)	9.9 a	10.3 a	10.5 a	10.5 a	11.3 a	11.6 a
total carotenoids (mg g^{-1}FW)	5.6 a	6.4 b	6.9 bc	7.3 c	8.4 c	13.5 d

[a] Peel colour, flesh firmness, and soluble solids are the mean of measurement performed on 20 fruits. Total carotenoids are the mean of three independent replicates. Values followed by the same letter in a row are not significantly different at $p \leq 0.05$.

Quality Analysis. Quality analyses were performed as described for apricots by Cardarelli et al. (*16*). Peel peach color was analyzed by a Minolta C2500 spectrophotometer (Konica Minolta Inc., USA) on two opposite sides of the fruit, and values are reported as hue angle. Flesh firmness was measured by using a table penetrometer (TR, Italy) on the equatorial part of the fruit after the peel removal. Soluble solids content (SSC) was measured by a digital refractometer Atago (Atago, Tokyo, Japan). The results are the mean of three replicate samples containing 20 fruits each.

Measurement of Ethylene and Carbon Dioxide Production. Ethylene production from the whole fruits was measured by withdrawing 1 mL of gas sampling from the head space of the 3 jars containing 20 fruits each that were injected in a Carlo Erba Fractovap 4200 gas chromatograph (Carlo Erba Spa Milan, Italy), equipped with a flame ionization detector (FID) and a 1 m long activated alumina column (80/100 mesh) (Alltech Associates, IL). Nitrogen was used as carrier gas, and the temperature of the column was maintained at 100 °C. To determine carbon dioxide production, 1 mL of gas sample from each jar was injected in the same gas chromatograph adapted with a FID and 1 m long Chromosorb 102 (80/100 mesh) (Varian Inc., USA) at 70 °C and a methane converter. The results are the mean of three replicates taken from 3 jars containing 20 fruits each ± SE.

Preparation of Samples for Carotenoids and Abscisic Acid Analysis. The fruit mesocarp was separated from the epicarp, frozen in liquid nitrogen, homogenized in a mortar and pestle, and kept at −20 °C until further analysis. Three biological replicates were prepared for each analysis.

Total Carotenoid Extraction and Quantification. Carotenoids were extracted as described previously (*17*). Briefly, freeze ground material of peach mesocarp (1.5 g FW) was extracted with a mixture of methanol and 50 mM Tris-HCl buffer (pH 7.5) containing 1 M NaCl and partitioned against chloroform until plant material was uncolored. Pooled organic phases were dried under vacuum and saponified overnight using a KOH: methanolic solution. The carotenoids were subsequently re-extracted with diethyl ether. An aliquot of the ethereal extract was used for quantification of total carotenoid content.

Total carotenoid content was calculated by measuring the absorbance of the saponified extracts at 450 nm, using the extinction coefficient of β-carotene, $E^{1\%} = 2500$ (*18*). Samples were dried under nitrogen and kept at −20 °C until HPLC analysis. All the process was done in almost total absence of light to avoid photodegradation, isomerization, or structural changes of carotenoids.

Carotenoid Analysis by HPLC. Prior to HPLC analysis, carotenoid extracts were dissolved in acetone and incubated overnight at −20 °C to precipitate sterols that could interfere in the carotenoid analysis and subsequently dried under nitrogen. Samples were prepared for HPLC by dissolving the dried residues in MeOH:acetone (2:1, v/v). Chromatography was carried out with a Waters liquid chromatography system equipped with a 600E pump and a model 996 photodiode array detector and Empower Software (Waters, Barcelona, Spain). A C30 carotenoid column (250 mm × 4.6 mm, 5 μm) coupled to a C30 guard column (20 mm × 4.0 mm, 5 μm) (YMC Europe GMBH, Schermbeck, Germany) were used with MeOH, water and methyl *tert*-butyl ether (MTBE). Carotenoid pigments were analyzed by HPLC using a ternary gradient elution reported in previous works (*17*, *19*). The photodiode array detector was set to scan from 250 to 540 nm throughout all the elution profile. Carotenoids were identified by comparison of the spectra and retention time with those of authentic standards when available or by matching the observed versus literature spectral data and retention time under identical chromatographic conditions (*17*, *19*, *20*). For each elution, a Maxplot chromatogram was obtained, which plots each carotenoid peak at its corresponding maximum absorbance wavelength. The carotenoid peaks were integrated at their individual maxima wavelengths, and their content were calculated using the calibration curves of lutein (Sigma), β-carotene (Sigma), β-cryptoxanthin (Extrasynthese), zeaxanthin (Extrasynthese), and phytoene. Phytoene was previously purified by thin layer chromatography from carotenoid extracts of Pinalate orange fruit, a mutant that accumulates substantial amounts of these carotenes (*17*). To quantify isomers of violaxanthin and luteoxanthin, the calibration curve of lutein was used because the absorption coefficients are very similar (*20*).

Extraction and Analysis of Abscisic Acid. ABA was extracted from representative ground samples of 0.5 g FW of mesocarp with 25 mL 80% of acetone containing 0.5 g L^{-1} citric acid and 100 mg L^{-1} of butylated hydroxytoluene (BHT) as previously described by Lafuente et al. (*3*). The ABA extract was centrifuged at 3000*g*, and the supernatant diluted in three serial dilutions in ice-cold TBS (6.05 g Tris, 8.8 g L^{-1} NaCl and 0.2 mg L^{-1} $MgCl_2$ at pH 7.8). Three samples for each dilution were analyzed by the indirect E.L.I.S.A., and the ABA-BSA-(4, conjugate) was synthesized as previously reported (*3*).

Statistics. The values are the means of three replicate samples ± SE. Data were evaluated using Minitab v14. Analysis of variance (ANOVA) was performed on the data obtained and Tukey's test was performed to identify significant differences between samples at $p \leq 0.05$.

RESULTS

Effect of Postharvest Treatments on Fruit Quality. No significant difference was found in skin color or in the SSC content among fruit exposed to the postharvest treatments, although fruit firmness was considerably affected by them (**Table 1**). Flesh firmness markedly decreased in fruits continuously maintained in air for 8 days at 20 °C and in fruit treated with 1-MCP, especially by the last day at 20 °C (data not shown). Exposing the fruit to the hydrocooling or to the carbon dioxide or nitrogen treatments significantly reduced such an effect, hydrocooling followed by fruit storage at 0 °C being the most effective treatment. Mesocarp of fruits maintained continuously in air showed a 2-fold increase in total carotenoids, while fruit exposed to the postharvest treatments assayed had lower increases. In contrast, no significant differences in SSC were found among fruits exposed to the different treatments assayed.

Effect of Postharvest Treatments on Fruit Ethylene and CO_2 Production. Hydrocooling followed by storing the fruit at 0 °C significantly reduced the respiration rate of fruits as compared to

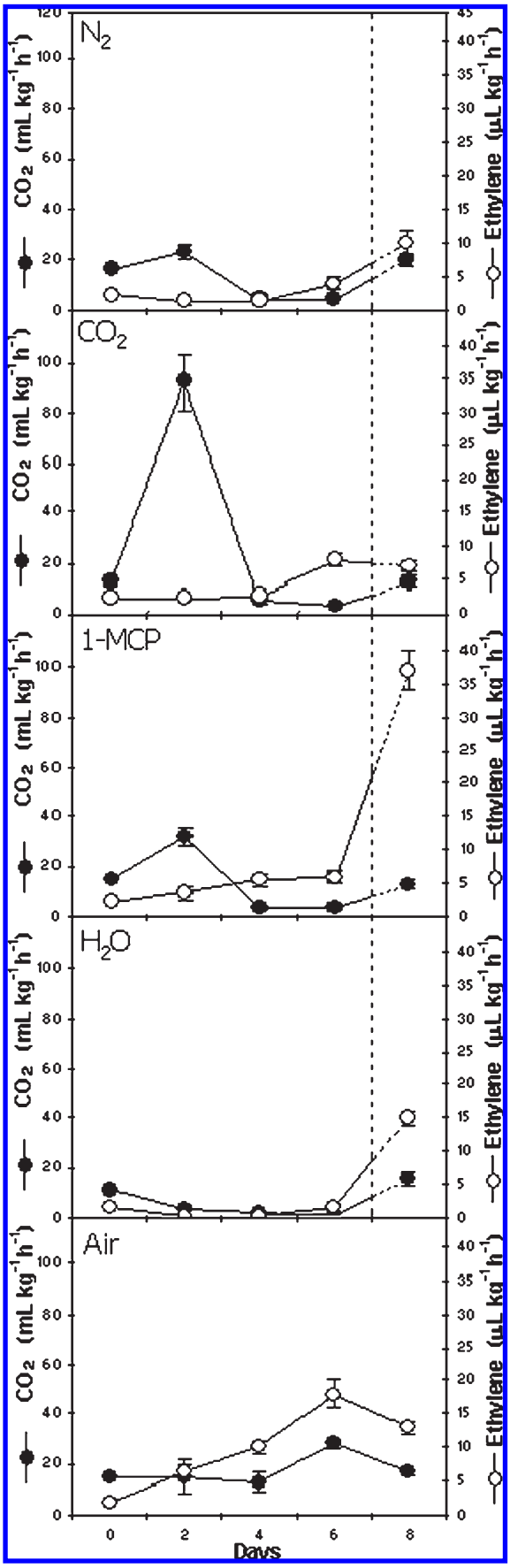

Figure 1. CO_2 (●) and ethylene production (○) of "Spring Belle" peaches after the exposure to the different postharvest treatments. Dotted lines in the plots indicate the transfer to 20 °C. Values are the mean of three replicate values at each sampling time ± SE.

Table 2. Chromatographic and Spectroscopic Characteristics of the More Relevant Carotenoids in the Mesocarp of *Prunus persica* Spring Belle Variety

observed			literature		
tentative identification[a]	λ_{max} (nm)	peak ratio[b]	λ_{max} (nm)	peak ratio[b]	ref
all-*E*-Violaxanthin	414,439,468	92	414,442,472	98	(*19*)
luteoxanthin	398,421,447	97	395,420,447	100	(*20*)
9-*Z*-violaxanthin	*cis*325,411,434,463	95	*cis*326,416,440,465	98	(*17*, *19*)
*zeaxanthin	430,451,478	30	428,450,478	26	(*20*)
*phytoene	274,285,300	10	276,286,297	10	(*17*)
*β-cryptoxanthin	425,449,475	30	428,450,478	27	(*20*)
*β-carotene	426,451,473	28	425,450,477	25	(*17*, *19*)

[a] *Identified using authentic standards. [b] Peak ratio is % III/II for carotenoids (*20*).

fruits maintained in air (**Figure 1**). Such an effect was observed by 2 days storage and was maintained until day 6, but after transferring the fruit to 20 °C, a 16-fold increase was observed. Treating the fruits with carbon dioxide, nitrogen, and 1-MCP resulted in an initial (day 2) increase in respiration, which markedly decreased thereafter at the same level of hydrocooled fruits during the storage at 10 °C. After the shift to 20 °C, fruits from all the treatments showed a similar respiration rate, ranging between 13 and 20 mL kg^{-1} h^{-1}.

Ethylene production of peaches maintained in air increased during storage at 20 °C, showing a typical climacteric rise with a peak on day 6 that was not observed in fruits treated with nitrogen or 1-MCP and stored at 10 °C or in the hydrocooled fruit stored at 0 °C (**Figure 1**). Treating peaches with carbon dioxide reduced also ethylene production, but on day 6, an upsurge of production was observed before the shift to 20 °C. In the other treatments, a great increase in ethylene was observed after the shift to 20 °C overall in the fruits treated initially with 1-MCP (**Figure 1**). In spite of this, treatment reduced the autocatalytic ethylene production for up to 6 days and it favored a burst in ethylene after 6 days storage, resulting in higher ethylene levels than those detected in fruit continuously held in air.

Identification of Carotenoids in "Spring Belle" Peach Fruit and Effect of Different Postharvest Treatments on Their Content. A total of seven carotenoids were tentatively identified among the most abundants by comparison of the spectra and retention times with those of authentic standards (**Table 2**). Among them, the epoxycarotenoid all-*E*-violaxantin was the most abundant in freshly harvested fruit (T_0), followed by luteoxanthin and the carotene phytoene (**Figure 2**). The freshly harvested fruit presented also relative high levels of β-carotene (about 200 ng g^{-1} FW) and of 9-*Z*-violaxantin, while the concentration of xantophylls zeaxanthin and β-cryptoxanthin were about 100 and 50 ng g^{-1} FW, respectively.

The concentration of carotenoids markedly increased by the end of the storage period in fruits continuously held in air at 20 °C, except luteoxanthin (**Figure 3**). This carotenoid was little affected by the hydrocooling and the 1-MCP treatments, but a marked decrease was observed in fruits pretreated with nitrogen and carbon dioxide. A similar behavior was observed when we examined the effect of the postharvest treatments on the content of β-carotene. In contrast, the levels of phytoene, zeaxanthin, and β-cryptoxanthin were reduced by all the treatments as compared to their respective levels in fruits kept continuously in air at 20 °C, being more marked in their reduction in fruits treated with nitrogen and carbon dioxide. No significant difference was observed in the content of all-*E*-violaxantin between fruits continuously maintained in air and pretreated with 1-MCP, while the levels were significantly lower in fruits exposed to the other treatments. The levels of 9-*Z*-violaxantin were reduced in the carbon dioxide treated fruits, whereas its accumulation was favored in the fruits exposed to the hydrocooling and the 1-MCP treatments.

Effect of Postharvest Treatments on Abscisic Acid Content. The content of ABA did not change either in fruits stored for 8 days at 20 °C continuously in air or in fruits treated with nitrogen (**Figure 3**). However, the carbon dioxide treatment had an important impact reducing ABA levels, whereas 1-MCP markedly increased the concentration with respect to nontreated peaches by 8 days (**Figure 3**, air). Hydrocooled fruits showed also significant higher ABA levels but were much lower than those of the 1-MCP fruits. As the cleavage of 9-*Z*-violaxantin may be a key point for the synthesis of ABA, we compared the effect of the postharvest treatments assayed in this study on the accumulation of both compounds. Data in **Figure 4** shows the relative contents of 9-*Z*-violaxantin and ABA in fruits exposed to the different treatments with respect to levels of freshly harvested fruit (T_0). Both compounds did not change in fruits treated with nitrogen, decreased in the carbon dioxide treated fruits, and increased in fruits exposed to 1-MCP. However, the increase in 9-*Z*-violaxantin was not accompanied by an increase in ABA in fruits continuously held in air at 20 °C or in the hydrocooled fruits.

DISCUSSION

The postharvest treatments used in the present study did not affect the skin color or the SSC content of mature "Spring Belle" peaches but had an important impact on fruit firmness and affected also the total carotenoid content in the mesocarp (**Table 1**). The effect of these treatments on fruit firmness appears to be related to changes in ethylene production, which has been shown to increase the ripening rate and the loss of peach firmness (*8*). Thus, the hydrocooled fruits produced very low ethylene levels during their storage (0 to 6 days) at low temperature (**Figure 1**) and maintained the highest firmness (20.9 N). The ethylene production of these fruits increased to levels higher than those of nitrogen and carbon dioxide treated fruits when transferred to 20 °C, but fruits treated with carbon dioxide produced more ethylene until the sixth day of storage. Treating the fruits with nitrogen had less residual effect than carbon dioxide on ethylene production during fruit storage at 10 °C as it was reported in apricots (*21*), and it was almost as effective as hydrocooling, followed by storage at 0 °C, in decreasing ethylene levels. Nevertheless, it has to be pointed out that ethylene action and metabolic processes associated with its increase with storage temperature were lower in the hydrocooled (0 °C) than in the nitrogen and carbon dioxide treated fruits (10 °C). It has been shown that high carbon dioxide inhibited the ethylene production by inhibiting the conversion of *S*-adenosylmethionine to 1-amino-1-cyclopropane carboxylic acid (ACC) but also ACC oxidase (ACO) activity and the accumulation of an ACO

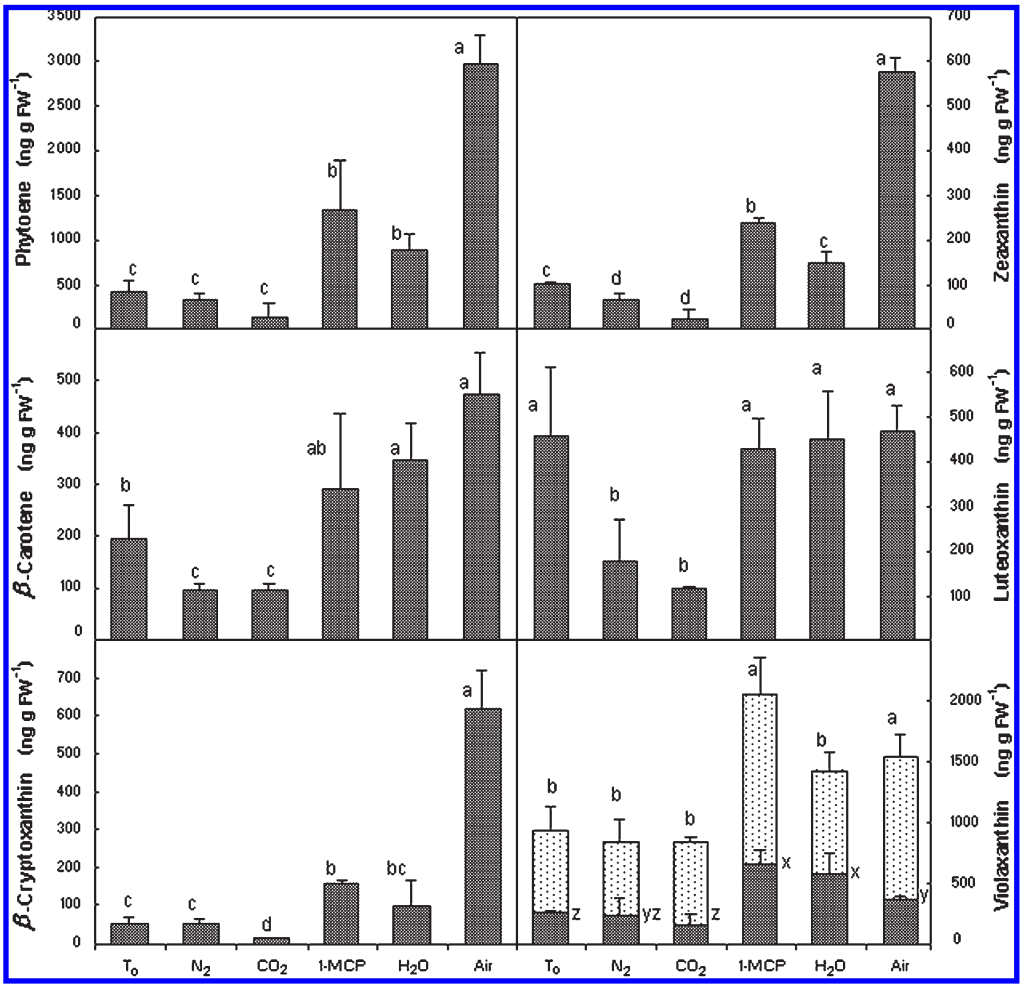

Figure 2. Concentrations of the main carotenoids identified in the mesocarp of peach fruits, phytoene, β-carotene, β-criptoxanthin, zeaxanthin, luteoxanthin, and violaxanthin, (ng g^{-1} FW) before (T_0) and by 8 days after the postharvest treatments assayed. For violaxanthin, the gray indicates 9-Z-violaxanthin and the dotted white the all-*E*-violaxanthin. Note the different scale for each carotenoid. Values are the means of three replicate samples ± SE. Mean separation was performed by applying Tukey's test. Values with different letters were significantly different ($p < 0.05$).

transcript named PP-ACO1 (*22*). Furthermore, a short carbon dioxide treatment delays the ripening process (*23*). Beyond this, hypoxic conditions (oxygen less than 2%) inhibit ethylene production and thus ripening in peaches (*23*). Although the inhibitor of ethylene action 1-MCP delays changes associated with ripening in climacteric fruits (*9*), our results showed that, by 8 days, softening of the 1-MCP-treated fruits was higher than that of fruits exposed to the other treatments because it increased markedly as in fruits held continuously in air at 20 °C. This softening occurred in the last day at 20 °C with the burst in ethylene. Our results are in agreement with those reported by Dal Cin et al. (*24*) showing the low efficacy of 1-MCP (1 μL L^{-1}) applied for 24 h at 20 °C in peaches and with those of Ziliotto et al. (*25*) in nectarines, as they found a rapid decrease of firmness associated with a rapid increase of ethylene. These results are in contrast, however, with previous papers (*26*−*28*) indicating that 1-MCP treatment delayed softening. Thus it can be concluded that experimental conditions such as peach cultivar, harvest time, temperature, time of incubation, and duration of fruit storage might affect ethylene biosynthesis as well as the synthesis of new ethylene receptors and, consequently, the firmness response.

The respiration rate of peaches was reduced immediately by the hydrocooling treatment and storage at 0 °C with respect to fruits maintained continuously in air immediately after 2 days of storage, while for the other treatments, the reduction was evident only by day 4 after a great peak of production (**Figure 1**). Such decreases in respiration rate were due to temperature effect as expected. The elevated production of carbon dioxide detected on day 2 in fruits treated with carbon dioxide is related to the release of gas from fruits following the treatment, which probably masks a physiological increase of carbon dioxide production as an effect of anaerobic conditions during the 48 h treatment. The rise in respiration rate by day 2 in the 1-MCP or the nitrogen treated peaches might be an effect of partial anaerobic respiration occurring during the 48 h treatment. In spite of the reported changes induced by the postharvest treatments, fruits from all the treatments showed the same respiration rate after simulation of shelf life period when transferred at 20 °C. Those changes in respiration were not either apparently related to changes in fruit firmness or in the total carotenoid content of fruits.

Although the synthesis of carotenoids may be affected by ethylene in climacteric and nonclimacteric fruits (*1*, *29*), changes in total carotenoids, estimated as β-carotene equivalents, occurring in the mesocarp of peaches at the end of each postharvest treatment assayed appeared to be much less affected than changes in fruit firmness by the changes in ethylene production induced by each treatment. The total carotenoid content, determined as μg of β-carotene equivalents (**Table 1**), was not coincident with the

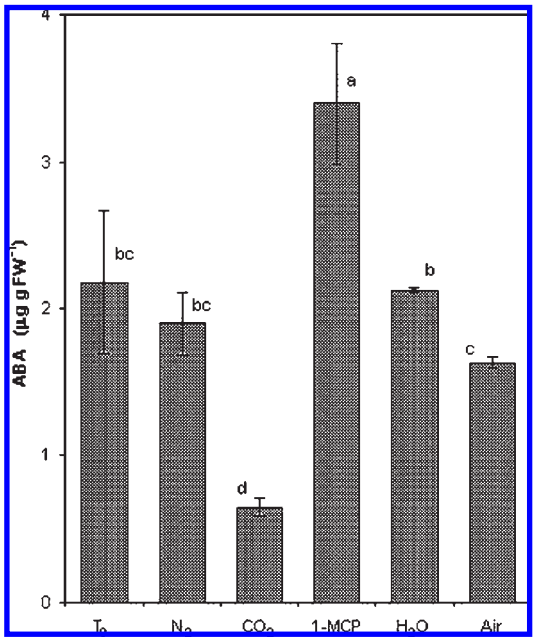

Figure 3. Concentrations of ABA (μg g^{-1} FW) before (T_0) and by 8 days after the postharvest treatments. Values are the means of three replicate samples ± SE. Mean separation was performed by applying Tukey's test. Values with different letters were significantly different ($p < 0.05$).

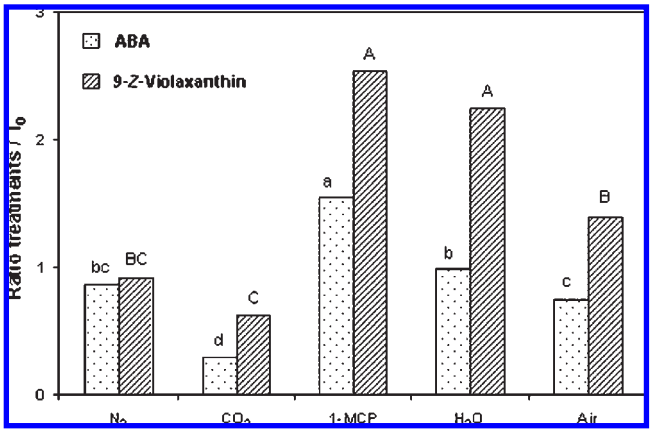

Figure 4. Relative contents of 9-Z-violaxanthin and ABA in fruits exposed to different postharvest treatments (day 8) with respect to the levels found in freshly harvested fruit (T_0). The value corresponding to T_0 was normalized to 1. Mean separation was performed by applying Tukey's test ($p < 0.05$). Values with the different letters a,b,c,d are significantly different and refer to the ABA content, while values with the different letters A,B,C refer to the 9-Z-violaxanthin content.

carotenoid content recalculated as the sum of individual carotenoids quantified by HPLC-PDA (**Figure 2**). This is due to the different maximum absorption wavelength between β-carotene and the other carotenoids present in the peach extracts. After harvest, a 2.4-fold increase in total carotenoids occurred in fruits continuously held in air at 20 °C while ethylene production continuously increased. However, total carotenoids increased to a much lower extent in fruits exposed to the other postharvest treatments, which presented similar levels without following the same pattern as ethylene. This might be related to the fact that environmental conditions, particularly temperature, have a strong effect on carotenoids biosynthesis (*1*). Therefore, the lower storage temperature in fruits pretreated with the gases or with cold water (hydrocooling) might delay synthesis of total carotenoids in harvested peaches.

A more detailed analysis of individual carotenoids revealed that they are differentially affected by the treatments. In this report, we describe changes observed in the main carotenoids identified by comparison of authentic standards or matching the spectroscopic data and retention times under identified chromatographic conditions with those present in the literature (*17*). As in the case of total carotenoids, the individual identified carotenoids (except luteoxanthin) increased during storage in fruits continuously held at 20 °C, especially phytoene, zeaxanthin, and β-cryptoxanthin, which showed at least a 6-fold increase. It is noteworthy that β-cryptoxanthin is particularly interesting because of its provitamin A activity. In spite of these increases reduced by the postharvest treatments, it is necessary to apply treatments to reduce postharvest firmness loss occurring at 20 °C. Less reduction in the synthesis of these compounds was found in fruits exposed to the 1-MCP or hydrocooling treatments than in fruits treated with nitrogen or carbon dioxide. Considering these results and that 1-MCP did not avoid the loss of fruit firmness during the simulation of shelf life period, hydrocooling, followed by storage at 0 °C, would be the more recommendable treatment to maintain external fruit quality and minimizing the reduction in carotenoids. The increase in β-carotene (2.5-fold) during fruit storage at 20 °C was lower than that of phytoene, zeaxanthin, and β-cryptoxanthin. Nevertheless, such increase was not reduced by the hydrocooling treatment or by applying 1-MCP. A similar behavior was observed when we examined the effect of these treatments in the luteoxanthin concentration, as they did not reduce its concentration with respect to fruits maintained continuously in air at 20 °C. Recently, Di Vaio et al. (*4*) have examined changes in lutein, β-carotene, and β-cryptoxanthin peaches stored at 2 °C. They did not find any significant change at this temperature, and no information was provided about the effect of transferring the fruit to room temperature. In concordance with the changes observed in phytoene, zeaxanthin, and β-cryptoxanthin, treating the fruit with nitrogen or carbon dioxide resulted in lower levels of β-carotene and luteoxanthin, probably because oxygen is necessary for their synthesis, reinforcing the idea that these treatments may be deleterious for the nutritional quality of peaches. All-*E*-violaxanthin was the most abundant identified carotenoid in freshly harvested fruits of Spring Belle as it has been reported in other cultivars (*1*). Its concentration increased to the same extent in fruits maintained in air and in fruit treated with 1-MCP and was not significantly affected by the other treatments. In contrast, 9-*Z*-violaxanthin, which was less abundant than its isomer, showed a higher increase in fruits treated with 1-MCP or exposed to the hydrocooling treatment than in fruits maintained at 20 °C. The cleavage of 9-*Z*-violaxantin is a key point for the synthesis of ABA through the indirect pathway. As far as we know, nothing is known about the effect of postharvest treatments on the synthesis of this hormone in peach fruits in spite of playing an important role against stress cues causing deleterious effects in plants. Nevertheless, it has been shown that ethephon, an ethylene releasing compound, favors its accumulation in peach bud and that ABA is involved in the process of sugar accumulation at the beginning of ripening in peaches (*30*). In addition, in mandarins, ethylene may increase ABA levels (*3*) and in peaches induces the expression of ABA-biosynthetic genes in fruits (*30*), while ABA treatment may stimulate ethylene production and participate in ripening or coloration process in strawberry (*31*). Our results showed that the levels of ABA and 9-*Z*-violaxanthin, its putative precursor, increased by the end of storage in the 1-MCP treated fruits, a feature that might be

related to the burst in ethylene production observed from 6 to 8 days in these fruits. ABA did not increase, however, in fruits continuously held in air at 20 °C in spite of the increase in ethylene or in 9-*Z*-violaxanthin, which might be related in part to the lower ethylene production found in these fruits. No relationship was either found in the synthesis of 9-*Z*-violaxanthin and ABA in the hydrocooled fruits, while both of them decreased in the carbon dioxide treated fruits and did not change in fruits treated with nitrogen. Therefore, although the content of 9-*Z*-violaxanthin might be a limiting factor for the synthesis of ABA, other steps downstream the cleavage of 9-*Z*-violaxanthin appears to be differently regulated by the postharvest treatments assayed.

In conclusion, treating peach fruits with cold water followed by storage at 0 °C was the best postharvest treatment maintaining both the content of carotenoids and the fruit firmness in Spring Belle peaches. Although the synthesis of carotenoids may be lower in fruits exposed to this treatment than in fruits continuously held in air at 20 °C, hydrocooling in combination with fruit storage at 0 °C would considerably reduce the loss of fruit firmness. Moreover, fruits exposed to hydrocooling showed also higher levels of ABA playing a protective role against environmental or oxidative processes that might be detrimental for the fruits and a lower respiration rate that is indicative of a lower rate of deterioration in perishable fruits.

ABBREVIATIONS USED

ABA, abscisic acid; ACC, 1-amino-1-cyclopropane carboxylic acid; ACO, 1-amino-1-cyclopropane carboxylic acid oxidase; β-CHX, β-carotene hydroxylase; B, breaker fruit; CO_2, carbon dioxide; FW, fresh weight; H_2O, hydrocooling; 1-MCP, 1-methylcyclopropene; N_2, nitrogen; O_2, oxygen; SSC, soluble solid content.

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