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Impact of Innovative Controlled Atmosphere Storage Technologies and Postharvest Treatments on Volatile Compound Production in Cv. Pinova Apples

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Organically grown apples cv. Pinova harvested at two different dates were stored at 1.3 °C for up to 4 months in air, up to 7 months in ULO (1.5 kPa of O_2 and 1.3 kPa of CO_2) and in dynamic controlled atmosphere (DCA) conditions (0.4−0.6 kPa of O_2 and 0.6−0.8 kPa of CO_2); the DCA storage method involved the use of a chlorophyll fluorescence monitoring system in order to detect low-O_2 stress in apples and to allow for the dynamic adaptation of storage atmosphere to O_2 levels that were lower than in ULO but still tolerated by fruits. A postharvest 1-MCP treatment (for 24 h at 1.3 °C) and a hot water treatment (for 180 s at 50 °C) were also tested on apples stored afterward in ULO and air, respectively. Volatile compounds isolated from the pulp of fruits were measured after 4 and 7 months, just upon removal from storage and after 11 days at 22 °C. Total amount of aroma compounds detected in apples stored in DCA was markedly higher (from 2- to 4-fold) than in fruits exposed to 1-MCP + ULO but, at most sampling times, significantly lower than in ULO fruits. Moderate differences in storage atmosphere composition between ULO and DCA significantly affected both total amount and profile of volatile esters. Analogous effects were observed on the alcohol precursors of the main esters. Exposure to 1-MCP inhibited biosynthesis of straight-chain esters more than that of branched-chain esters. The hot water treatment did not seem to produce marked changes in volatile composition after four months of air storage, except for a sharp accumulation of aldehydes during the shelf-life time. DCA storage technology, besides avoiding any chemical treatment, can preserve apple aroma compounds better than 1-MCP + ULO during long-term storage.

KEYWORDS: Apple (*Malus* × *domestica* Borkh.); aroma compounds; stir bar sorptive extraction (SBSE); dynamic controlled atmosphere; 1-methylcyclopropene

INTRODUCTION

In the commercial storage of apples the well-established controlled atmosphere (CA) storage technology is widely used in order to decrease the incidence of physiological disorders during long-term storage, and to retain fruit quality, in particular by delaying loss of firmness, acidity and chlorophyll (*1, 2*). At the same time among innovative approaches treatments with 1-methylcyclopropene (1-MCP) have attracted increasing use for postharvest management of several apple cultivars (*3*). However, one drawback of both CA storage with ultralow oxygen (ULO) concentrations and exposure to 1-MCP is the partial suppression of production of aroma compounds, a key contributor to apple flavor (*4*−*6*). Inhibition of ethylene action has been suggested as the mechanism by which volatile compound biosynthesis is decreased by both technologies (*6*). The suppression of volatiles induced by CA depends particularly on the composition of storage atmosphere and on the length of storage time, lower O_2 and higher CO_2 levels as well as longer durations resulting in a greater reduction (*4*). Similarly, the extent of inhibitory effect on the volatiles production caused by exposure to 1-MCP greatly varies with its concentration during treatment (*7*) and with duration of storage time (*6*). Treatments and storage conditions significantly influence not only total amount of formed volatiles but also their profile (*6, 8, 9*). Moreover, fruit maturity at harvest is another important factor affecting fruit response to postharvest handling and poststorage flavor development (*9, 10*).

Increasing concern on the use of chemicals in the food chain has recently encouraged efforts to improve and develop physical treatments also in postharvest handling of apples, in order to replace those chemical treatments that are still needed. As an example, postharvest treatments with the antioxidant diphenylamine (DPA) are still needed to control superficial scald in many apple cultivars susceptible to this major physiological

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disorder, which cannot be fully controlled by conventional CA-ULO storage alone (*11*). Among physical approaches an innovative dynamic controlled atmosphere (DCA) storage technique, which uses a fluorescence-based technology to monitor the responses of fruit to low oxygen, has been shown to be an effective alternative to DPA treatment for complete control of scald and preservation of apple inner quality (*12, 13*). This technique involves the use of a nondestructive monitoring system of fruit chlorophyll fluorescence, which allows the detection of low-O_2 stress before development of associated disorders in apple fruit (*14, 15*). This system, by indicating when the fruit is under low-O_2 stress during storage, allows for the dynamic adaptation of the atmosphere composition in the CA room to O_2 levels lower than those set in ULO storage, but still tolerated by fruits; in this way it becomes possible to optimize the greatest benefits of ULO without producing detrimental effects due to anaerobic conditions (*15, 16*). The interest on this technology is also related to its potential application to the increasing organic production of apples, on which the use of postharvest chemicals to control decay, disorders and quality loss is generally prohibited, whereas changes of O_2, CO_2 and N_2 gas levels in storerooms are permissible (*17*).

Another physical treatment, based on a hot water dipping, has been shown to be effective for the control of postharvest microbiological decay in organically grown apples, resulting in extended shelf life and higher consumer quality standard (*18*).

In previous studies on these innovative approaches quality evaluation of fruit after storage has focused on firmness, acidity and soluble solids; in particular, no information about the effects of the DCA storage method and hot water treatments on fruit aroma compounds has been reported to date. The aim of this work was to compare the effect of the DCA storage technology on fruit volatile profile in organically grown Pinova apples after four and seven months of storage with that produced by air and conventional ULO-CA storage and by 1-MCP postharvest treatment, followed by ULO-CA storage. The impact of a postharvest hot water dipping treatment on biosynthesis of volatile compounds after four months of air storage was also investigated.

MATERIALS AND METHODS

Plant Material, Treatments and Storage Experiment. Apple fruits [*Malus* × *domestica* Borkh.] cv. Pinova, grown in an organically managed orchard by the Research Centre for Agriculture and Forestry "Laimburg" at S. Leonardo in Passiria (690 m asl, South-Tyrol, Italy), were harvested at two different dates: the first date (fruit starch index equal to 6.1 and firmness to 61 N) was within the optimal harvest window for long-term CA storage (defined by the starch index range 5.5−6.7 and firmness range 59−69 N), whereas the second date was 15 days later, the starch index (9.7) being clearly out of the harvest window and firmness reduced (58 N). Total soluble solids and titratable acidity in malic acid equivalents were equal to 12.4, 12.0 °Brix and 4.7, 4.9 g L^{-1}, respectively. Firmness, content of total soluble solids and titratable acidity were assessed on samples of 45 fruits each by means of the automated instrument "Pimprenelle" (Setop Giraud Technologie, Cavaillon, France). Starch index was determined according to CTIFL (Paris, France) starch conversion chart (Index 1−10) on equatorial halves of apples soaked in iodine−potassium iodide solution (iodine, 1%; potassium iodide, 4%) for 10 s. A batch consisting of approximately 150 fruits from each harvest date was stored in air at 1.3 °C and 95% relative humidity (R.H.) for four months. A batch of apples, from the second harvest date, was subjected to a postharvest hot water dipping treatment (at 50 °C for 180 s) and then stored in the same conditions as above (*19*). This treatment was applied only on fruits from the second harvest date on the basis of previous observations in our laboratory, which showed that apples harvested after the optimal window were particularly susceptible to rot development. Furthermore, not treated fruits from both harvest dates were stored in the following two different CA conditions for seven months, after precooling for 7 days at 2.5 °C: fruits were stored at 1.3 °C in gastight stainless steel containers (volume 0.77 or 0.58 m^3; apples ∼235 kg m^{-3}) (i) in the optimal recommended ULO-CA conditions for this cultivar (1.5 kPa O_2, 1.3 kPa CO_2 and 99% R.H.); (ii) in DCA (*12*) (0.4−0.6 kPa O_2, 0.6−0.8 kPa CO_2 and 99% R.H.). As for the determination of DCA set points, a chlorophyll fluorescence (F) nondestructive monitoring system (HarvestWatch, Satlantic Inc., Halifax, Canada) was used, which measures F at low irradiance and can produce a theoretical estimate of F at zero irradiance, F_α: this parameter is able to indicate the occurrence of fruit low-O_2 stress (*15*). The monitoring system, by indicating when the fruit is under low-O_2 stress during storage, enabled an operator to adjust the O_2 levels in response to any fluctuations in the fluorescence signal. In practice, the critical oxygen concentration (anaerobic compensation point) was assessed at the beginning of storage and monitored during the entire storage period. In our experiment the O_2 level was set 0.2 unit above the critical oxygen limit within the range 0.4−0.6 kPa of O_2, in order to provide a safety margin against injury. Previous tests in our laboratory showed that a parallel decrease in CO_2 level (within the range 0.6−0.8 kPa) gave place to optimal atmosphere composition for DCA storage. Finally, a group of fruits from each harvest date, after 6 days of precooling at 2.5 °C, was treated with 7.326 mmol m^3 (0.625 μL L^{-1}) 1-methylcyclopropene (1-MCP, SmartFresh, AgroFresh Inc., Philadelphia, PA) for 24 h at 1.3 °C in the above-mentioned chambers and then stored for seven months in the ULO-CA conditions described above.

Determination of Volatile Compounds. Volatile compounds were analyzed at harvest and after 4 and 7 (only for CA storage) months, just upon removal from cold storage and after 11 days at 22 °C in air. For the homogenization of fruits we followed the procedure used by Lurie et al. (*7*): 200 g of apple pulp (slices cut from fifteen fruits, devoid of peel and seeds) was homogenized with a Waring blender for 2 min after addition of 400 mL of deionized water containing 20% NaCl in order to inhibit enzymatic reactions. The homogenate was then filtered under vacuum with Whatman filter paper n.113 and glass wool, and 50 μL of the internal standard solution (1-octanol 1 μL mL^{-1} in methanol) was added to 100 mL of the obtained juice. Isolation of volatile compounds from this working solution was performed by the stir bar sorptive extraction (SBSE) technique (*20, 21*): 15 mL of the solution was stirred at 800 rpm with a PDMS-coated stir bar (1.0 mm thickness, 10 mm length, Gerstel GmbH, Mülheim an der Ruhr, Germany) for 90 min, at room temperature, in hermetically closed recipients. The stir bar was then removed from the solution, rinsed with distilled water, dried with filter paper and immediately transferred into a thermal desorption tube, which was inserted into the thermal desorption unit (TDU Gerstel GmbH) mounted onto the GC injector, where the analytes were thermally desorbed. All analyses were performed in triplicate.

Sorption conditions used in this work gave place to acceptable repeatability of the isolation method: the whole method (SBSE plus GC−MS) provided coefficients of variation below 5% for all the identified compounds (close to 3% for the three main esters) in a repeatability test carried out by extractions on the same apple juice sample with five different stir bars. Similar coefficients have been obtained on the application of SBSE to the analysis of volatile constituents on analogous aqueous matrices (*21−23*).

The thermal desorption unit was installed on an Agilent 6890 GC 5973N MS system (Agilent Technologies Inc., Palo Alto, CA). Desorption was carried out at 250 °C for 5 min, with a flow rate of the carrier gas (He) of 50 mL min^{-1}. A Gerstel CIS-4 PTV injector was used for cryogenic focusing of the analytes thermally desorbed from the stir bar. The PTV was cooled at −30 °C using liquid CO_2, and injection temperature raised to 250 °C (3 min) at 12 °C s^{-1}. Capillary GC−MS analyses were performed by using a HP-5MS (Agilent Technologies Inc.) column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Chromatographic conditions were as follows: split injection (by setting the solvent vent mode, and purge flow to split vent at 20 mL min^{-1} after 0.01 min); temperature program, from 40 °C (2 min) to 250 °C (5 min) at 4 °C min^{-1}; the linear velocity of the He carrier

gas was 36 cm s^{-1}. A mass spectrometer with a quadrupole mass filter was used for detection. Mass spectra were recorded in electronic impact ionization mode at 70 eV. Transfer line, source, and quadrupole temperatures were set, respectively, at 280, 230 and 150 °C. Mass spectra were scanned in the range m/z 40−600 amu. Identification of compounds was carried out by two methods: by comparing spectra of sample compounds with those of authentic standards (all purchased from Sigma-Aldrich, Milwaukee, WI) and by comparing linear retention indices (Kovats indices, calculated in relation to a homologous series of *n*-hydrocarbons) of sample compounds with those of authentic standards. A pure standard was not available for α-farnesene, but only a mixture of farnesene isomers (from Sigma-Aldrich): in this mixture α-farnesene was tentatively identified by comparison with a spectrum reported in the literature (*24*), and on the basis of the spectrum and Kovats index of the corresponding chromatographic peak we tentatively identified α-farnesene in sample chromatograms. Two chromatographic peaks were partially identified as acetate and hexanoate on account of the presence of diagnostic fragments in the mass spectrum. Quantification was performed on the basis of chromatograms obtained in the scan mode, and levels of volatile compounds were expressed as microgram equivalents of internal standard per kilogram of fruit fresh weight. Concentration data are to be considered as relative data because recovery after isolation and response factors related to the internal standard were not determined.

Statistical Analysis. Analytical data were subjected to a one-way analysis of variance (ANOVA) to evaluate the significance of differences between treatment-storage combinations: for each sampling time (after 4 and 7 months, upon removal from storage and after 11 days at 22 °C in air) and for samples from the first and the second harvest date, separately, the analysis of variance was carried out to evaluate significance of differences between samples stored in air, ULO, DCA, treated by 1-MCP (+ ULO storage) or by hot water (+ air storage). The mean values for each considered compositional parameter were compared by a multiple comparison Duncan test to look for grouping (at $p = 0.05$ level).

RESULTS

Apple aroma perception is the result of a complex mixture of several volatile compounds, which include, in particular, esters, aldehydes and alcohols (*5*, *25*). GC−MS analysis of the volatile fraction isolated by SBSE from the pulp of apples cv. Pinova at harvest allowed to identify as main compounds 19 esters (10 acetates, 5 butanoates, 2 propanoates, 2 hexanoates), 2 aldehydes (1-hexanal, *tr*-2-hexenal), 3 alcohols (1-butanol, 2-methyl-1-butanol, 1-hexanol), 2 allylbenzenes and 1 sesquiterpene (α-farnesene) (**Table 1**). The three compounds detected at the highest level, butyl acetate, 2-methylbutyl acetate, and hexyl acetate, are considered major contributors to the characteristic apple-like aroma and flavor in most apple cultivars (*4*). In all samples that we analyzed butyl acetate plus hexyl acetate and 2-methylbutyl acetate formed more than 90% of total straight-chain and branched chain esters, respectively. Moreover, among all quantified compounds, butyl acetate, 2-methylbutyl acetate, 2-methylpropyl acetate, hexyl acetate, butyl propanoate, butyl butanoate, 2-methylbutyl butanoate, 1-hexanol and 1-hexanal have been recently recognized as principal odorant compounds also in cv. Golden Delicious, Fuji and Braeburn apples by GC−olfactometry analysis (*26*).

Postharvest production of volatile compounds was strongly affected by the different combinations of treatments and storage conditions examined in our experiment (**Figure 1**); on the other hand similar effects were observed comparing samples from the two harvest dates. By considering total level of detected aroma compounds in fruits sampled just upon removal from storage, 1-MCP treated apples showed a markedly lower level than all others after both four and seven months. At the end of the shelf life period apples stored in air (only four months of storage), both hot water treated and not treated, showed the highest production of volatiles, fruits stored in ULO or DCA produced intermediate amounts, whereas 1-MCP-treated fruits were characterized again by the lowest levels throughout the whole storage period. Interestingly, all fruits stored in air, and particularly those from the first harvest date, showed an increase in the sum of volatiles during the shelf life period, whereas CA fruits displayed a marked depletion, with the only exception of fruits after seven months in ULO.

Table 1. Volatile Compounds Isolated by SBSE from the Pulp of Cv. Pinova Apples at Harvest (Second Harvest Date)

RI[a]	compound	concn (μg/kg of f.w.) mean value (std dev[b])
708	propyl acetate	67 (3)
749	2-methylpropyl acetate	12.6 (0.6)
786	butyl acetate	1943 (90)
852	2-methylbutyl acetate	592 (18)
893	pentyl acetate	79 (4)
1005	*cis*-hexenyl acetate	23 (1)
1017	hexyl acetate	3285 (92)
1122	unidentified acetate	11.4 (0.6)
1163	benzyl acetate	1.8 (0.2)
1255	2-phenylethyl acetate	13.0 (0.3)
	total acetates	6029 (210)
886	butyl propanoate	49 (2)
1105	hexyl propanoate	7.9 (0.5)
	total propanoates	57 (2)
873	propyl butanoate	9.9 (1.0)
997	butyl butanoate	40 (2)
1041	butyl 2-methylbutanoate	18.1 (0.5)
1191	hexyl butanoate	16.0 (0.8)
1236	hexyl 2-methylbutanoate	17.3 (0.4)
	total butanoates	101 (5)
1372	unidentified hexanoate	27 (4)
1384	hexyl hexanoate	3.1 (0.3)
	total hexanoates	30 (4)
	total esters	6217 (221)
656	1-butanol	42 (3)
722	2-methyl-1-butanol	14.8 (1.0)
839	1-hexanol	166 (5)
	total alcohols	222 (9)
769	1-hexanal	26 (2)
821	*tr*-2-hexenal	42 (2)
	total aldehydes	68 (5)
1196	1-methoxy-4-(2-propenyl)benzene	34 (2)
1402	1,2-dimethoxy-4-(2-propenyl)benzene	14.4 (1.1)
1506	α-farnesene	1.3 (0.2)

[a] Retention index in a HP5-MS column (a 5% phenyl column based on diphenylmethylsiloxane). [b] Analyses performed in triplicate (triplicate SBSE extraction on the same homogenate).

Total amount of detected volatiles in fruits after storage in DCA was significantly (at $p = 0.05$ level) lower than in ULO, except for fruits upon removal from storage after seven months. On the other hand, at all sampling times it was markedly higher (from 2- to 4-fold) than in fruits exposed to 1-MCP + ULO.

Changes observed on total volatile levels were mainly due to those produced on the amount of butyl and hexyl acetate (**Figures 2**, **3**), so that changes in the level of these compounds reflected the trends of total detected volatiles. Their amount was reduced to extremely low levels in 1-MCP + ULO fruits, compared to apples stored in ULO or DCA. In 1-MCP + ULO butyl acetate amount was on average 5.7% and 8.6% of that in ULO and DCA fruits, respectively, whereas hexyl acetate level was 13.4% and 20.1%. Fruits stored in ULO showed significantly ($p = 0.05$ level) higher amounts of butyl acetate than fruits in DCA, with only one exception, in which there were no significant differences (upon removal from storage after seven months). Similarly, hexyl acetate levels were generally higher

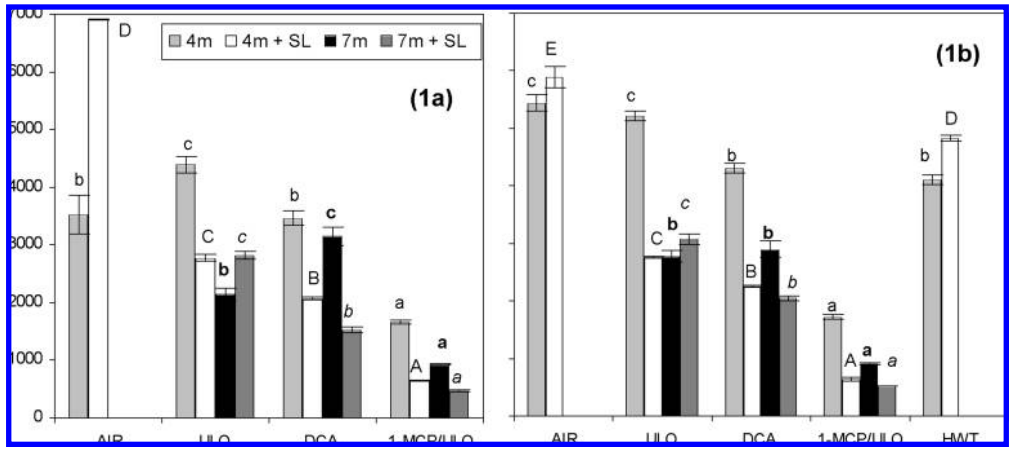

Figure 1. Total level of detected volatiles (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO (ULO storage after 1-MCP tretament). Volatiles were determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**1a**) and the second (**1b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and following four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). For each sampling time (4m, regular font style; 4m + SL, uppercase letters; 7m, boldface; 7m + SL, italics) different letters denote significant differences at $p = 0.05$ (Duncan test).

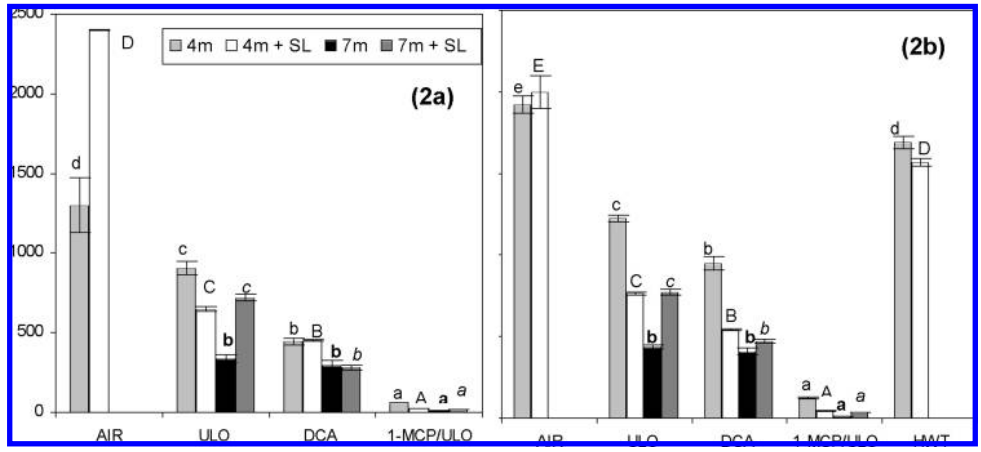

Figure 2. Butyl acetate levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**2a**) and the second (**2b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

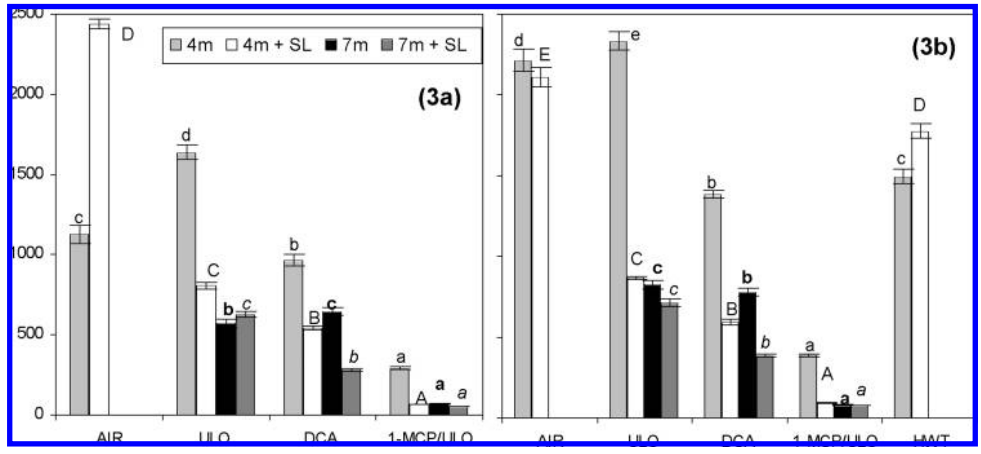

Figure 3. Hexyl acetate levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**3a**) and the second (**3b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

in ULO than in DCA fruits (differences significant at $p = 0.05$ level). A quite different picture was shown by 2-methylbutyl acetate, by far the main branched-chain ester (**Figure 4**). The inhibitory effect due to 1-MCP treatment was less pronounced

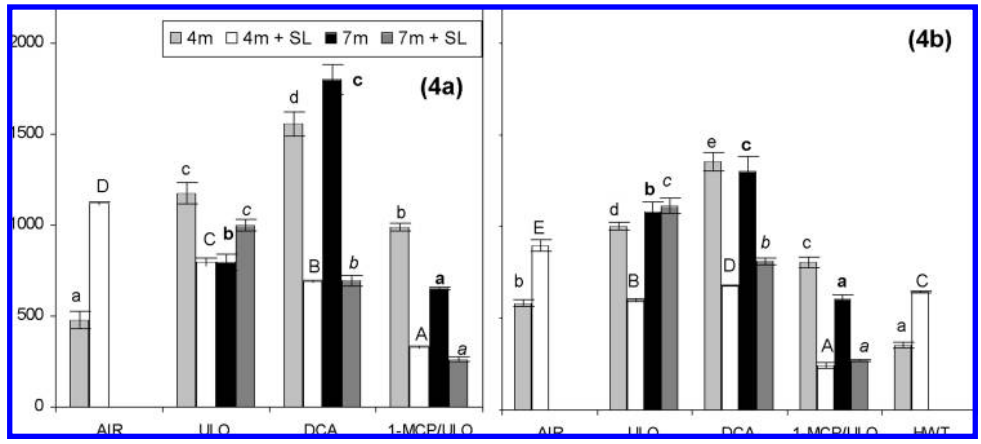

Figure 4. 2-Methylbutyl acetate levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**4a**) and the second (**4b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

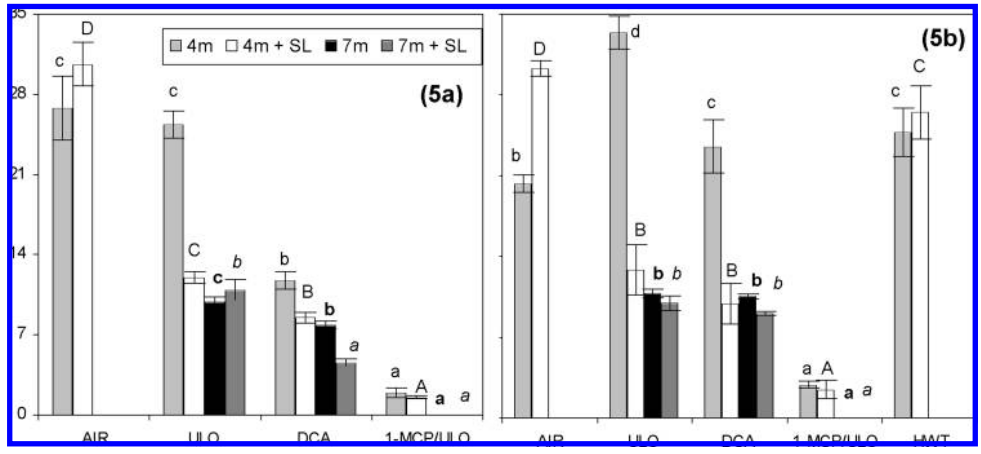

Figure 5. 1-Butanol levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**5a**) and the second (**5b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

on this compound than on the biosynthesis of the above considered straight-chain esters: in fruits exposed to 1-MCP its amount was on average 55.0% and 46.8% of that in ULO and DCA fruits, respectively. By comparing ULO with DCA storage conditions, contrary to what was observed on the main straight-chain esters, a higher production of 2-methylbutyl acetate was observed on DCA samples in most cases (differences significant at $p = 0.05$ level). Moreover, at removal from storage all CA fruits showed higher levels of this compound than fruits stored in air; during the following shelf life period CA fruits generally showed a sharp reduction whereas fruits stored in air displayed a marked increase.

The alcohols detected, 1-butanol, 1-hexanol, and 2-methyl-1-butanol, are the immediate precursors in the biosynthesis of the three main esters, which are formed by condensation of the alcohol with acetic acid. The effects of treatments and storage conditions on alcohol production (**Figure 5**, **6**, **7**) were analogous to those on the corresponding esters, though in some cases differences between apple samples were not significant. Differently from what was observed on fruits stored in air, in all CA conditions fruit alcohol levels clearly decreased during the shelf life period, marked reductions in alcohol corresponding to sharp decreases in the related ester (particularly for 2-methyl-1-butanol and 2-methylbutyl acetate in DCA and 1-MCP samples).

Regarding aldehydes, differently from esters and alcohols, ULO and DCA, but not all 1-MCP-treated fruits, showed an increase of their levels during the shelf life period similar to that observed on fruits stored in air (**Figure 8**). Interestingly, a sharp increase was observed on hot water treated apples after removal from cold storage.

1-methoxy-4-(2-propenyl)benzene is a volatile phenylpropanoid found in some apple cultivars, which contributes to apple flavor with a spicy note (*27*); CA conditions, both ULO and DCA, produced a significant reduction of its level, whereas an additional lowering effect seemed to be associated with exposure to 1-MCP (**Figure 9**).

DISCUSSION

At present, few data are available in the literature on the effects of the considered DCA storage method on apple internal quality. In a comparative analysis of the impact of DCA and 1-MCP + ULO on some quality parameters of cv.

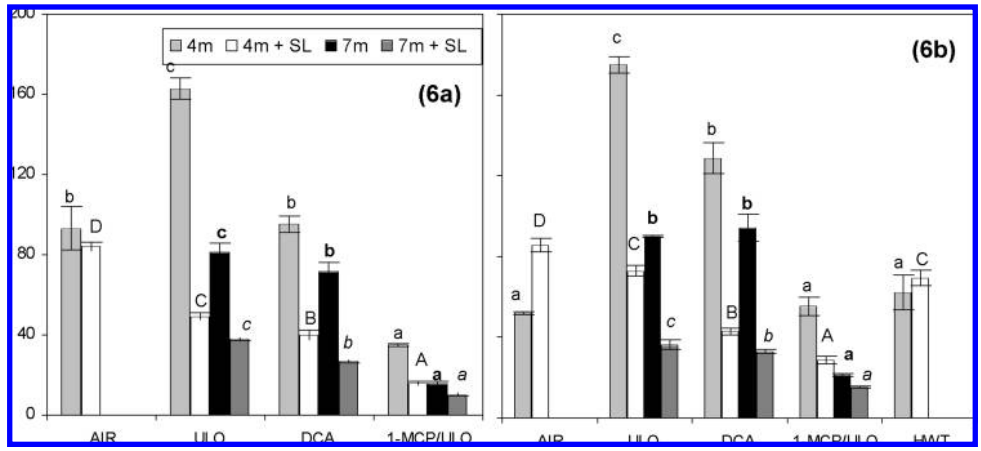

Figure 6. 1-Hexanol levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**6a**) and the second (**6b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

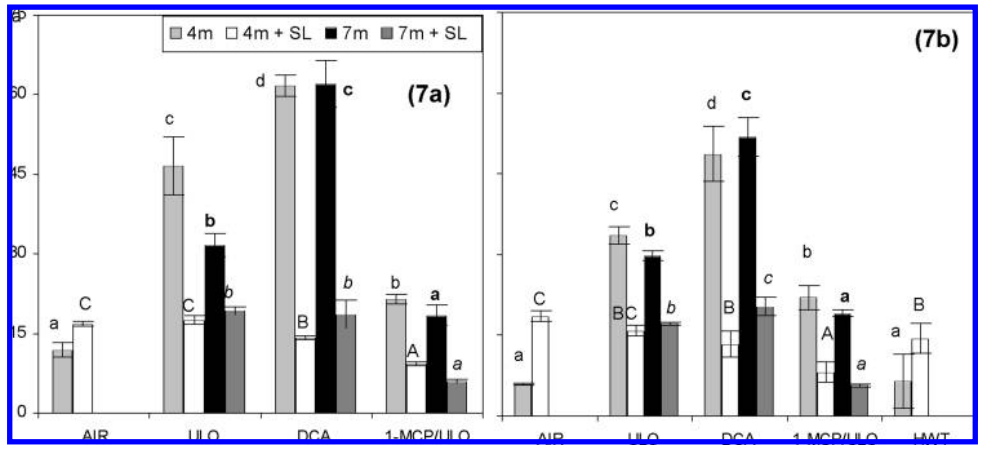

Figure 7. 2-Methyl-1-butanol levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**7a**) and the second (**7b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

Granny Smith apples, a similar effectiveness in firmness retention was observed, whereas fruits exposed to 1-MCP maintained higher levels of titratable acidity during storage and following shelf life (*12*). Results obtained in the present study on cv. Pinova apples highlighted a quite better performance of DCA than 1-MCP + ULO in terms of preservation of aroma compounds during seven months of storage and a following period of shelf life, due to a reduced inhibitory effect on production of volatile compounds. In a previous study on the application of the 1-MCP + ULO combination (*6*) the inhibitory effect due to the action of 1-MCP added up to that one associated to the change of atmosphere (from air to ULO conditions). Results obtained in the present work show that in comparison with the simple ULO storage the additional inhibitory effect due to 1-MCP treatment was more pronounced than the effect due to the further atmosphere modification from ULO to DCA conditions. Moreover, even though O_2 concentration in DCA was set to values close to the limit of anaerobic conditions, we did not detect any of the volatiles formed by fermentative metabolism, such as ethanol, ethyl acetate, acetaldehyde, confirming previous observations that indicated the absence of off-flavors (*12*).

A common effect associated with all the examined CA conditions was a reduced fruit ability to produce volatiles during the poststorage shelf life period, when compared to fruits stored in air. This residual effect on apple volatile biosynthesis has been observed on various cultivars (*4*, *9*, *10*, *28*). In the present experiment it was observed on the main esters and also on their alcohol precursors: this suggested that the decreased ability of esters biosynthesis during shelf life was attributable to a reduced availability of substrate (the alcohol used in the final step of ester synthesis) rather than to a lowered activity or degradation of alcohol acetyl-CoA transferase, the key enzyme involved in the final step of ester biosynthesis. A similar conclusion has been already drawn in previous works on other apple cultivars (*8*, *29*−*32*), whereas various studies have clearly demonstrated the general importance of alcohols level as limiting factor of volatile ester production (*33*−*35*). Moreover, in ULO and DCA apples alcohol decrease was accompanied by a significant accumulation of aldehydes during the shelf life period, indicating that CA storage was associated to a reduced conversion rate of

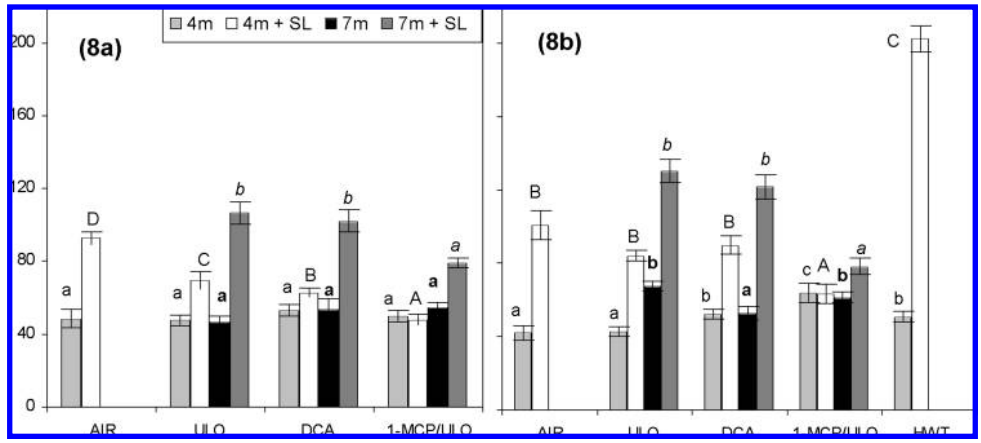

Figure 8. Total aldehydes levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**8a**) and the second (**8b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

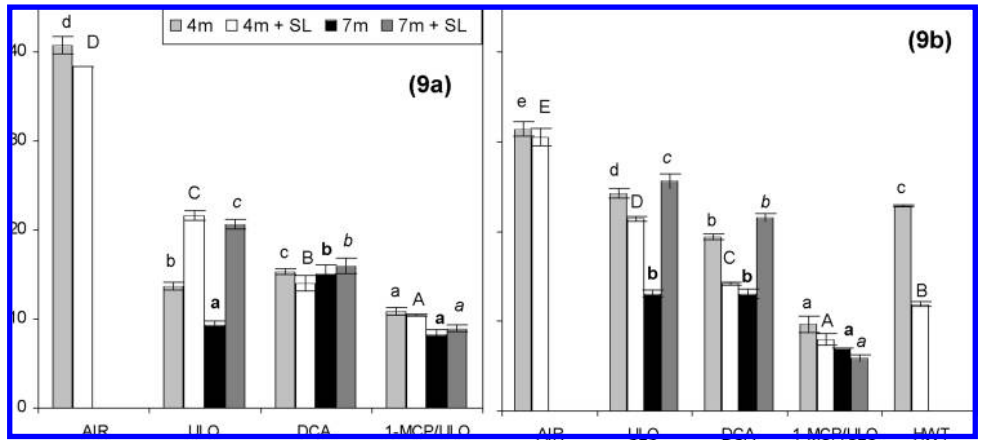

Figure 9. 1-Methoxy-4-(2-propenyl)benzene levels (μg/kg of f.w.) in Pinova apples after four and seven months of storage in AIR, ULO, DCA and 1-MCP/ULO, determined upon removal from cold storage (4m and 7m) and after subsequent 11 days of shelf life (4m + SL and 7m + SL) at 22 °C. Apples from the first (**9a**) and the second (**9b**) harvest dates. Volatile level in fruits subjected to hot water treatment (HWT) and four months (4m and 4m + SL) of storage in air was also reported (only for the second harvest date). See caption to **Figure 1** for the meaning of letters (Duncan test).

aldehydes to alcohols when fruits were exposed again to ambient temperature after cold storage.

By comparing the impact of the two CA technologies ULO and DCA we found that even moderate differences in O_2 and CO_2 concentrations in storage atmosphere produced significant differences in total amounts of quantified volatiles as well as differential effects on straight- and branched-chain esters. Inhibition of straight-chain ester biosynthesis was higher in DCA conditions, with lower O_2 levels, whereas branched-chain ester formation was more strongly inhibited in ULO, with higher CO_2 concentrations. Differential effects of low O_2 and high CO_2 on emission of straight- and branched-chain esters have been previously reported (*8*, *31*, *32*) and explained by relating the different CA conditions to the distinct biosynthetic pathway of the two groups of compounds. β-Oxidation of fatty acids and lipoxygenase activity, by which straight-chain organic acid precursors are known to be formed, are both O_2-requiring processes and are presumably slowed down by storage atmospheres with lowered O_2. On the other hand, branched-chain organic acid precursors are formed from the metabolism of amino acids, whose precursors in plant cell are mainly produced by the tricarboxylic acid cycle, which in turn is known to be inhibited by elevated CO_2 concentrations (*36*). The described differential effects on esters were also observed on alcohols, confirming that these effects were expressed on stages upstream of the final step of condensation of an alcohol with a carboxylic acid.

Also exposure to 1-MCP produced differential effects on the two groups of esters, the straight-chain esters being reduced much more strongly by the action of 1-MCP than the branched-chain one. This confirmed a previous observation on cv. Gala apples (*6*), supporting the idea that the two biosynthetic pathways responded differently to inhibition of ethylene action.

Another effect, previously observed on Gala apples, was that on 1-methoxy-4-(2-propenyl)benzene, whose production is lowered by both CA storage and exposure to 1-MCP (*6*); this response suggested that its formation is at least partly regulated by ethylene action.

The only previous study on the effect of a heat treatment on apple volatile production was carried out by using quite different treatment conditions (in a thermostatically controlled chamber at 38 °C for four days) and showed that heat treatment temporarily inhibited, but did not destroy, the ability of fruit to synthesize volatile compounds (*37*). In our experiment a short dipping treatment at a higher temperature had a limited adverse

effect on volatile biosynthesis, and after four months of air storage and following shelf life period, treated fruits preserved aroma compounds much more effectively than not treated fruits stored in CA.

In conclusion, previous studies have shown that both DCA storage and exposure to 1-MCP, followed by ULO storage, may be effective alternatives to DPA treatments on those apple cultivars that are susceptible to scald symptoms during long-term storage (*12*, *13*). The present work shows that these two storage strategies can have a differential impact on the internal quality of fruits: DCA has the potential to better preserve aroma compounds during long-term storage, whereas in a previous experiment 1 MCP + ULO was found to be more effective in delaying organic acid degradation (*12*). This information could be useful for the selection of the most suitable storage method for individual apple cultivars, taking into account the impact of different technologies on specific quality characteristics of each cultivar. Moreover DCA, differently from 1-MCP + ULO, does not involve any chemical treatment and, by virtue of this, can find a specific application field in long-term storage of organic apples.

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