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Comparison of the Volatile Profile and Sensory Analysis of 'Golden Reinders' Apples after the Application of a Cold Air Period after Ultralow Oxygen (ULO) Storage

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ABSTRACT: All efforts to improve fruit quality are rewarded when consumers are satisfied after tasting the fruit. Apples are often stored under controlled atmosphere conditions to preserve them over time, but this frequently results in a loss of flavor. The aim of this work, which was based on two seasons, was to evaluate the influence of a period of short-term air storage (periods of 2 and 4 weeks) after removal from ultralow oxygen (ULO) storage (1 kPa of O_2/1 kPa of CO_2) with respect to increases in volatile compound emissions and the effect on standard and sensory quality in 'Golden Reinders' apples. The results showed that emissions of 26 volatile compounds increased as a result of ULO + 2 weeks or ULO + 4 weeks of storage. However, the results of tastings involving a panel of consumers and trained experts revealed that this increase was not matched by corresponding increases in either the degree of consumer preference or flavor attributes.

KEYWORDS: degree of consumer preference, flavor, volatile compound emissions, standard quality, sensory attributes, ULO storage

INTRODUCTION

Flavor is becoming one of the most important quality attributes of horticultural products and one that producers seek to optimize through the breeding, cultivation, and postharvest processes that they apply to fruit and vegetable crops.[1] It is significant to highlight that consumers are often dissatisfied with the flavor of fruits that have been subjected to cold storage, regardless of whether this involves air or controlled atmosphere (CA). Low-oxygen conditions are considered beneficial for apple storage as fruits tend to maintain their epidermis color and flesh firmness longer than when stored in cold air. However, it has been suggested that a certain antagonism exists between key attributes such as appearance and flavor quality during shelf life.[2] Apple flavor is a complex combination of taste and odor sensations. It has been suggested[3] that flavor (taste, odor, and mouthfeel) has a greater influence on quality impressions than optical or acoustic signals. Furthermore, some of the compounds that emanate from apples have been shown to have a decisive impact on sensory quality and have therefore been designated "impact compounds".[4]

Subjecting fruit to modified atmospheres, as in CA storage, can induce metabolic changes to flavor compounds in fresh produce either during storage or in subsequent shelf life. For various apple varieties, long-term storage under ultralow oxygen (ULO) conditions reduces volatile production, resulting in poor flavor and aroma compared to fruits stored in air.[5−11] In the absence of oxygen, the esterification reactions that take place in fruit tend to stop, whereas concentrations of free alcohol tend to increase. When these fruits are returned to aerobic conditions, these alcohols are metabolized to either esters or to shorter chain compounds before esterification[12] or they evaporate from the tissue.[6] After removal of fruit from hypoxic conditions, concentrations of a wide range of esters related with apple aroma may therefore increase.[13−15] Various techniques have been tested to enhance the aroma of apples after storage under ULO storage conditions. Previous work conducted in our laboratories with 'Fuji' apples showed that short-term air storage after removal from ULO storage resulted in an increase in some volatile compounds and particularly in those that are most characteristic for this variety.[16] Fruit aroma is cultivar-specific[17] and, accordingly, differences in the respective contributions of individual compounds to overall flavor and consumer acceptance have been observed between different apple cultivars.[11,18−21] In the European Union, the most cultivated apple group is 'Golden', with 24.6% of total production, followed by 'Gala' at 10.5%.[22] CA technology is usually applied when 'Golden' apples are stored. It therefore seems relevant to conduct an in-depth study into how to enhance the aroma of these apples after ULO storage and to see whether or not this influences the sensory perception of consumers. The 'Golden Reinders' variety, on which we have mainly based our study, is a mutation of 'Golden Delicious'. It should be noted that several new mutant varieties have now emerged which offer improvements in both aspects of production and sensory attributes.

The present study evaluates the effect of short-term air storage on the flavor of 'Golden Reinders' apples following storage under ULO atmosphere conditions; it also assesses the consequences of this storage regimen for their sensory profile. The study was carried out over two years to establish whether there could be a degree of seasonal variability. From a commercial point of view, these findings should enable us to make recommendations to the apple industry that will help it to optimize the storage of fruit under ULO conditions and to improve its flavor.

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MATERIALS AND METHODS

Plant Material and Storage Conditions. 'Golden Reinders' apples were harvested in 2006 (2006; first season) and 2007 (2007; second season) at commercial maturity: 147 and 139 days after full bloom (dafb), from 6- and 7-year-old trees, respectively. The trees were grown on M-9 EMLA rootstock at the IRTA-Experimental Station, Lleida (northeastern Spain). Immediately after harvest, three lots of 100 kg of apples per season were selected for uniformity and absence of defects and stored at 1 °C and 92% relative humidity in an ULO atmosphere (1 kPa of O_2/1 kPa of CO_2). One lot remained under ULO conditions for the whole 19 or 30 week period (ULO). A second lot was kept under ULO conditions for either 17 or 28 weeks and then stored for 2 weeks under cold-air (ULO+2w). The third lot was kept for either 15 or 26 weeks under ULO conditions followed by a further 4 weeks under cold air (ULO+4w). Volatile compound emissions, quality parameters, and degree of consumer preference were measured at harvest and after removal from storage plus 7 days at 20 °C. In addition, sensory attributes were evaluated by a trained panel after storage plus 7 days at 20 °C.

Quality Parameter Analyses. Fifteen fruits per treatment (season × storage condition × storage period) were individually assessed for flesh firmness, soluble solids content (SSC), titratable acidity (TA), starch index, and skin color. Flesh firmness, SSC, and TA were analyzed both at harvest and after removal from cold storage, whereas the starch index and color were measured only at harvest. Flesh firmness was measured on two opposite surfaces at the equatorial zone with an Effegi penetrometer (FT 327; Effegi, Alfonsine, Italy) fitted with an 11 mm plunger; the results were expressed in newtons (N). SSC and TA were measured in juice pressed from a whole fruit. SSC was determined with a hand-refractometer (Atago, Tokyo, Japan), and the results were expressed as °Brix in an equivalent solution. To determine TA, 10 mL of pulp juice was diluted with 10 mL of water and titrated with a 0.1 N NaOH solution to pH 8.2, and the results were expressed as grams of malic acid per liter. Epidermis color was determined with a portable tristimulus colorimeter (Chroma Meter CR-200, Minolta Corp., Osaka, Japan) using CIE illuminant D_{65} and an 8 mm diameter measuring aperture; color was measured at the equator of each fruit, and the hue parameter (arc tg (b^*a^{*-1})) was calculated. The starch index was determined by dipping cross-sectional fruit halves in an iodide solution (15 g of KI + 6 g of I_2 per liter) for 30 s; starch hydrolysis was rated using a 1−10 scale (1 = full, 10 = no starch).

Chemicals. All of the compounds reported in this paper were identified in our laboratory using reference compounds. The chemicals were of the highest quality available and, unless otherwise indicated, were supplied by Sigma-Aldrich (Steinheim, Germany). Ethyl acetate, propyl acetate, 1-propanol, ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, 2-methyl-1-propanol, 1-butanol, pentyl acetate, 2-methyl-1-butanol, hexyl acetate, 1-hexanol, hexyl 2-methylbutanoate, and 2-ethyl-1-hexanol were obtained from Fluka (Buchs, Switzerland). Ethanol was purchased from Panreac Química, S.A. (Castellar del Vallès, Spain). 2-Methylpropyl acetate was obtained from Avocado Research Chemicals Ltd. (Madrid, Spain).

Analysis of Volatile Compounds. Eight kilograms of apples (2 kg per replicate × 4) per treatment (season × storage condition × storage period) was selected for volatile compound analysis both at harvest and after removal from storage. Intact fruits were placed in an 8 L Pyrex container through which an air stream (900 mL min^{-1}) was passed for 4 h. The resulting effluent was then passed through an adsorption tube (ORBO-32; Supelco, Bellefonte, PA) filled with 100 mg of activated charcoal (20/40 mesh), from which volatile compounds were desorbed by agitation for 40 min with 0.5 mL of diethyl ether. Identification and quantification of the volatile compounds was performed on a HP 5890 series II gas chromatograph (Hewlet-Packard Co., Barcelona, Spain) equipped with a flame ionization detector (GC-FID), using a cross-linked free fatty acid as the stationary phase (FFAP; 50 m × 0.2 mm i.d. × 0.33 μm), into which a volume of 1 μL of the extract was injected in all analyses. The oven program was set at 70 °C (1 min), and the temperature was first raised by 3 °C min^{-1} to 142 °C and later by 5 °C min^{-1} to 225 °C. It was then kept at this second temperature for a further 10 min. Helium was used as the carrier gas, at a flow rate of 0.8 mL min^{-1} (42 cm s^{-1}), with a split ratio of 40:1. The injector and detector temperatures were held at 220 and 240 °C, respectively. A second capillary column (SGE, Milton Keynes, U.K.) with 5% phenyl polysilylphenylene−siloxane as the stationary phase (BPX5, 30 m × 0.25 mm i.d. × 0.25 μm) was used for compound identification under the same operating conditions as described above. Compounds were identified by comparing their respective retention indices with those of standards and by enriching the apple extract with authentic samples. Quantification was carried out by adding 25 μL of a 0.2% solution of butylbenzene (assay > 99.5%, Fluka) as an internal standard. A GC-MS system was used for compound confirmation, using the same capillary column as in the GC analyses. Analysis was carried out using an Agilent 6890N gas chromatograph interfaced to a 5973N mass selective detector. Mass spectrometric data were collected in full scan. The scan ranged from 30 to 500 amu, and the scan rate was 3.1 scans s^{-1}. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as the carrier gas (42 cm s^{-1}), following the same temperature gradient program as previously described. Spectrometric data were recorded (Hewlett-Packard 3398 GC Chemstation) and compared with those from the NIST HP59943C original library mass spectra and thereafter were compared with reference compounds. Results were expressed as micrograms per kilogram.

Sensory Assessment. Fruit samples removed from each storage condition and corresponding to each storage period were kept at 20 °C for 7 days. Fifteen apples per treatment (season × storage conditions × storage period) were used for consumer evaluation. Prior to the evaluation of the degree of consumer preference, after flesh firmness had been measured, two longitudinal wedges were cut from each fruit and instrumentally analyzed, as explained under Quality Parameter Analyses; the rest of the fruit was divided into pieces and used for consumer evaluation. Three fruit samples (one per storage condition) were placed on white plates and immediately presented to a tasting panel of 50 consumers. The tasting panel was the same for all the tests. The fruit tasters were volunteers from the staff working at the UdL-IRTA research institute and students from the University of Lleida (UdL). All of the participants were regular apple consumers. Each piece was identified by a random three-digit code. The order of presentation of the three pieces of fruit was randomized for each taster. Mineral water was used as a palate cleanser between tastings. All evaluations were conducted in individual booths under white illumination and at room temperature. Each taster assessed all three samples and was asked to indicate his/her degree of liking/disliking using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely). The samples could be retasted as often as desired.

Three apples per treatment were used for the description of the sensory profiles by a trained panel. The intensities of the following attributes were evaluated by the panel: sweetness, sourness, crispness, firmness, juiciness, mealiness, and apple flavor. The intensity of each attribute was recorded on 150 mm unstructured line scales, anchored at 0 = absent and 150 = extreme, with the exception of firmness, which was anchored at 10 = low and 140 = high. The attributes were defined according to the definitions given in Harker et al.[23,24] During testing, cubes of outer cortical flesh (approximately 3.4 cm^3; 1.5 × 1.5 × 1.5 cm) were also presented to the panelists. Nine panelists (trained according to ISO 1993, no. 8586-1) scored the sensory attributes of the samples. Sensory assessment took place at the Sensory Laboratory of the Food Technology Department (UdL). All evaluations were conducted in individual booths under white illumination and at room temperature. Mineral water and crackers were provided as palate cleansers. Data were

Table 1. Quality Parameters of 'Golden Reinders' Apples at Harvest for the First (2006) and Second Seasons (2007)

	2006[a]	2007[a]
starch index (1−10)	4.5 a	4.6 a
firmness (N)	73.1 a	72.7 a
soluble solids content (°Brix)	13.3 b	14.1 a
titratable acidity (g L^{-1})	5.4 b	6.0 a
skin color (hue)	108.3 a	110.7 a

[a] Means followed by different letters for each quality parameter are significantly different at $p \leq 0.05$ (LSD test).

collected on paper ballots. All of the panel members assessed all of the samples. The allocation of fruit for assessment by each panel member was conducted according to a complete balanced block design. Peeled fruit samples were presented to panel members in white plastic cups. Samples were coded using three-digit, randomly generated numbers.

Statistical Analyses. A multifactorial design was used to statistically analyze the data. The factors considered were season, storage period, and storage condition. All data were tested by analysis of variance (GLM-ANOVA procedure) using the SAS program package.[25] Means were separated by the LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physicochemical Parameters. Table 1 shows the quality parameters at harvest for both seasons. Starch index, firmness, and skin color values were similar, whereas there were significant differences in SSC and TA. Within the 'Golden' apple group, the best maturity indicator is the starch index:[26] from our results, it can therefore be assumed that the fruits corresponding to the two seasons presented similar maturity indices at harvest.

The physicochemical parameters (SSC, TA, and firmness) were generally well preserved throughout all 19 and 30 weeks of storage (Figure 1). Only minimal changes were observed in SSC, which gives an estimation of the sugar concentration in fruit. SSC was not affected by storage conditions for short storage periods, but after 30 weeks of storage, the lower SSC values associated with 4 week air-stored fruit (ULO+4w) could have been caused by higher respiration rates in fruits subjected to the air atmosphere. Starch breaks down and is converted into sugars as fruit ripens, but sugars are readily consumed as respiratory substrates. These results were in accordance with previous works on 'Fuji' apples.[27] The balance between starch breakdown and respiration rates therefore determines SSC. Overall, the values observed remained higher than the 12 °Brix minimum value recommended for maximizing consumer acceptance of 'Golden' apples in Europe.[28] Harker et al.[29] showed that acceptability increased with greater firmness and that, in firm fruit, increasing the SSC could increase acceptability. From the results obtained, it was evident that values of TA remained above the recommended minimum of 3.2 g of malic acid L^{-1} for acceptable eating quality in 'Golden' apples.[28] With respect to the effect of the season, in Figure 1 it is possible to observe that fruits from 2006 exhibited higher TA values than those from 2007, especially after 30 weeks of storage. In relation to the influence of short-term air storage on TA, for the 2006 fruit, storage under air resulted in a decrease in TA values, whereas for the 2007 fruit, the pattern was not clear. In the case of fruit firmness, the maximum value was observed for fruit stored under ULO in 2007 (around 70 N). Overall, short-term air storage after removal from ULO caused a decline in

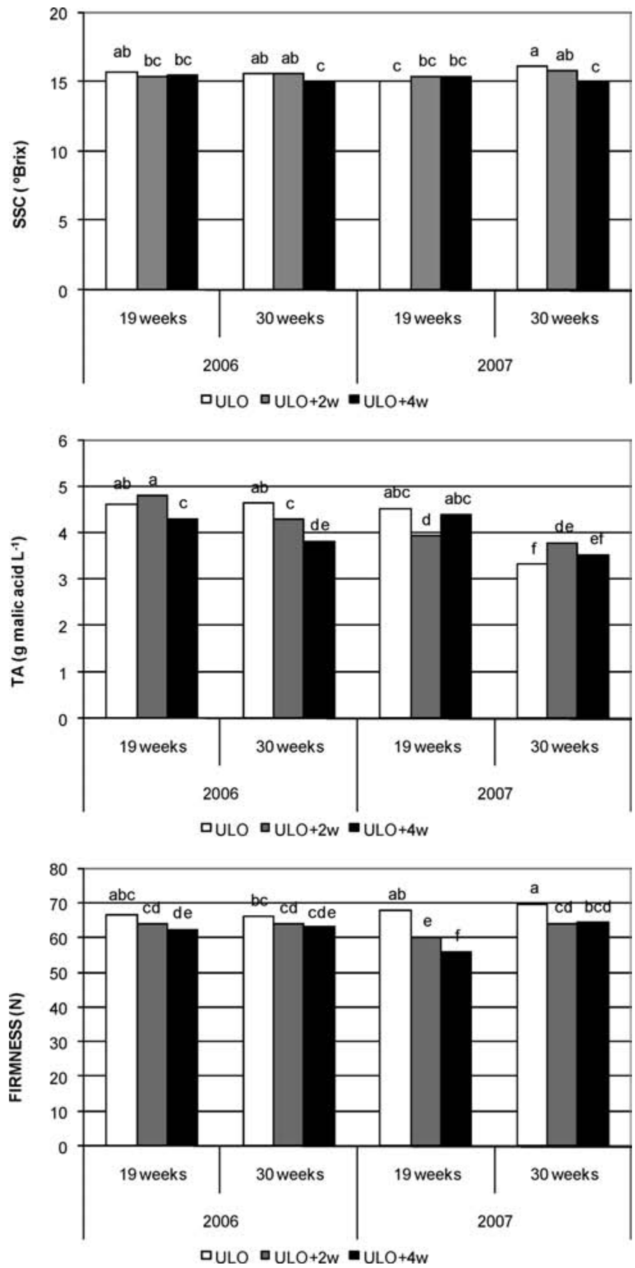

Figure 1. Physicochemical parameters of 'Golden Reinders' apples under different storage conditions (ULO, ultralow oxygen; ULO+2w; ULO+4w) after 19 and 30 weeks of storage plus 7 days at 20 °C for two seasons (2006, 2007). Mean comparisons are across treatments (season × storage condition × storage period). Values followed by different letters for each quality parameter are significantly different at $p \leq 0.05$ (LSD test).

firmness, particularly in 2007 fruit. These values were >44 N, the value recommended by other authors for 'Golden Delicious'.[28] Firmness also decreased after short-term air storage following controlled atmosphere storage of 'Spartan' and 'Delicious' apples.[30] A previous study on several different varieties 'Red Delicious', 'Gala', 'Fuji', 'Golden Delicious' and 'Braeburn' revealed that firmness was the most important edible quality factor that contributed to consumer acceptance and preferences in the United States and that 62 N was considered to be the minimum threshold to achieve success in the marketplace.[29]

Table 2. Volatile Compounds (Micrograms per Kilogram) Emitted by 'Golden Reinders' Apples at Harvest for Two Successive Seasons (2006 and 2007)

volatile compound[a]	RI_1[b]	RI_2[c]	2006[d]	2007[d]
methyl acetate	854		6.0 b	13.5 a
ethyl acetate	882	609	27.4 a	29.2 a
ethanol	912		14.6 a	34.0 a
propyl acetate	945	649	5.4 a	12.4 a
methyl butanoate	955	656	1.6 a	4.0 a
2-methylpropyl acetate	976	691	0.8	tr
1-propanol	992		1.1 b	7.7 a
ethyl butanoate	1002	803	tr	0.8 a
propyl propanoate	1008	809	nd	tr
ethyl 2-methylbutanoate	1015	845	42.1 b	125.8 a
butyl acetate	1040	813	3.6 a	3.7 a
2-methyl-1-propanol	1054	996	1.8 a	1.9 a
2-methylbutyl acetate	1096	876	12.8 a	14.0 a
1-butanol	1119	626	1.6 b	16.8 a
butyl propanoate	1123	910	1.1 a	0.8 a
butyl 2-methylpropanoate	1129	1009	tr	nd
2-methylpropyl butanoate	1140	954	5.5 a	6.2 a
pentyl acetate	1161	914	5.9 a	3.8 a
2-methylbutyl propanoate	1180	950	0.7 b	2.8 a
2-methyl-1-butanol	1199	667	1.4 a	1.1 a
butyl butanoate	1218	1000	2.1 a	1.5 a
butyl 2-methylbutanoate	1235	1042	1.4 b	20.2 a
ethyl hexanoate	1239	1002	0.7 a	4.0 a
pentyl propanoate	1247	969	nd	tr
1-pentanol	1262	688	2.0 a	1.2 a
hexyl acetate	1292	1015	30.3 a	48.5 a
2-methylbutyl 2-methylbutanoate	1300	1106	nd	11.8
propyl hexanoate	1353	1099	0.6 a	7.1 a
hexyl propanoate	1379	1109	3.3 b	36.8 a
1-hexanol	1392	869	13.2 a	5.7 b
2-methylpropyl hexanoate	1399	1153	2.0	nd
butyl hexanoate	1473	1196	0.9 b	62.9 a
hexyl butanoate	1477	1197	9.8 b	86.1 a
hexyl 2-methylbutanoate	1488	1239	15.3 b	135.4 a
ethyl octanoate	1499	1201	5.5 a	5.4 a
2-ethyl-1-hexanol	1565	1031	13.5 b	44.7 a
pentyl hexanoate	1590	1293	1.3 a	3.2 a
hexyl hexanoate	1687	1392	5.8 b	38.4 a
butyl octanoate	1690	1394	2.2 a	3.3 a
hexyl octanoate	1840	1707	5.7	nd

[a] All compounds were identified on the basis of reference volatiles. [b] Kovats retention index in cross-linked FFAP column.[44] [c] Kovats retention index in cross-linked BPX5 column. [d] Means followed by different letters for each volatile compound are significantly different at $p \leq 0.05$ (LSD test). nd, not detected; tr, traces (≤ 0.5 $\mu g\ kg^{-1}$).

Volatile Compound Production at Harvest. Volatile compound emissions at harvest and the retention index for each individual compound are presented in Table 2. Total volatile compound emissions for 2007 fruit exhibited higher concentrations than 2006 fruit (2006, 256.0 $\mu g\ kg^{-1}$; 2007, 797.8 $\mu g\ kg^{-1}$; LSD = 107.1). Despite the volatile profiles being similar for the two seasons, analysis of variance showed higher concentrations of some volatile compounds in the second season (Table 2). To evaluate the possible effect of climate, we collected data on maximum and minimum temperatures for up to 3 months before harvest (data not shown). From the results obtained, we observed that the preharvest period of 2007 was colder than that of 2006. Other authors have reported how fruits that had experienced colder preharvest periods showed greater increases in the quantity of their volatile esters at harvest than those from warmer areas. Even so, emissions from the latter increased upon their removal from CA storage.[31,32] Our results corroborate these previous works: the 2007 fruit presented higher emissions of volatile compounds at harvest than the 2006 fruit.

Volatile esters are the most important contributors to apple aroma for whole fruits, in both quantitative and qualitative terms.[9,13−15,17,31] Quantitatively speaking, the most important esters registered in the present study were ethyl 2-methylbutanoate, 2-methylbutyl acetate, hexyl acetate, and hexyl 2-methylbutanoate; these esters contributed 40% of total volatile compound emissions in both seasons. For 2007 fruit, esters butyl 2-methylbutanoate, butyl hexanoate, and hexyl hexanoate also made important contributions to the volatile compound profile. Alcohol-related emissions, ethanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 1-pentanol, remained at the same level in both seasons. In contrast, levels of 1-propanol, 1-butanol, and 2-ethyl-1-hexanol were higher in the 2007 fruit.

Previous works on 'Golden Delicious' apples reported that ethyl 2-methylbutanoate and hexyl acetate were the main contributors to flavor at harvest.[5]

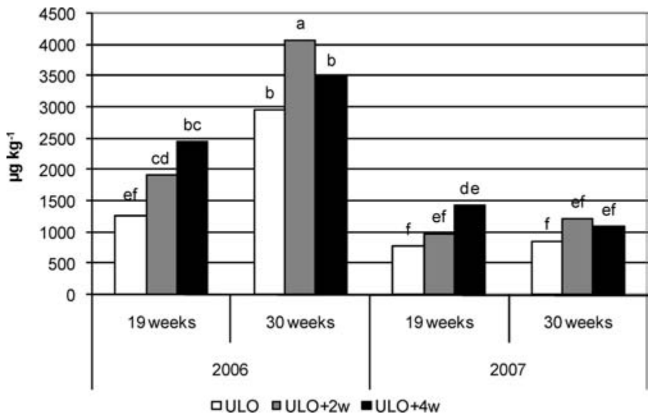

Figure 2. Volatile compound total emissions from 'Golden Reinders' apples under different storage conditions (ULO, ultralow oxygen; ULO+2w; ULO+4w) after 19 and 30 weeks plus 7 days at 20 °C in two seasons (2006, 2007). Mean comparisons are across treatments (season × storage condition × storage period). Values followed by different letters are significantly different at $p \leq 0.05$ (LSD test).

Volatile Compound Production after Cold Storage in ULO or ULO plus AIR. To provide an overview of the impact of short-term air storage after ULO storage on total volatile compound emissions, Figure 2 shows these values for each storage condition and period. From the results obtained, we can observe significant differences in total volatile emissions between seasons. Fruits from 2006 presented higher concentrations of volatile compounds than those from 2007. As mentioned in the previous section, this was possibly due to warmer weather conditions during the preharvest period as the same pre- and postharvest management operations were applied to all

Table 3. Enhanced Esters and Alcohols (Micrograms per Kilogram) after Short-Term Air Storage Compared to Ultralow Oxygen Atmosphere after 19 Weeks of Storage plus 7 Days at 20 °C in the 2006 Season

	storage conditions		
volatile compound	ULO[a]	ULO+2w[a]	ULO+4w[a]
propyl acetate	2.6 b	5.4 ab	6.7 a
2-methylpropyl acetate	5.7	7.6	11.1
butyl acetate	84.3 b	236.7 a	207.3 a
2-methylpropyl propanoate	nd	tr	tr
2-methyl-1-propanol	2.1	2.3	3.0
2-methylbutyl acetate	128.6 b	263.4 a	317.9 a
1-butanol	13.9 b	33.0 a	31.3 a
butyl propanoate	8.0 b	19.8 a	17.1 a
butyl 2-methylpropanoate	5.3 b	13.2 a	11.4 ab
pentyl acetate	13.5 b	26.1 a	26.9 a
2-methylbutyl propanoate	1.6	2.5	3.7
butyl butanoate	27.6 b	55.4 a	51.6 a
butyl 2-methylbutanoate	56.1 b	98.8 a	119.5 a
ethyl hexanoate	0.8	1.5	1.5
hexyl acetate	158.7 b	338.8 a	328.0 a
2-methylbutyl 2-methylbutanoate	5.6	9.0	12.4
propyl hexanoate	4.0 b	3.6 b	14.3 a
hexyl propanoate	22.9 b	41.3 a	40.9 a
1-hexanol	14.2	17.2	15.9
2-methylpropyl hexanoate	2.4	4.8	3.7
butyl hexanoate	111.9 c	168.0 b	235.4 a
hexyl butanoate	123.7 b	182.7 ab	213.7 a
hexyl 2-methylbutanoate	259.8	336.5	402.5
pentyl hexanoate	10.8	16.1	17.9
hexyl hexanoate	102.5 b	140.9 ab	160.2 a
butyl octanoate	5.4 b	15.2 a	15.1 a

[a] Means followed by different letters for each volatile compound are significantly different at $p \leq 0.05$ (LSD test). nd, not detected; tr, traces (<0.5 μg kg^{-1}).

Table 4. Enhanced Esters and Alcohols (Micrograms per Kilogram) after Short-Term Air Storage Compared to Ultralow Oxygen Atmosphere after 30 Weeks of Storage plus 7 Days at 20 °C in the 2006 Season

	storage conditions		
volatile compound	ULO[a]	ULO+2w[a]	ULO+4w[a]
propyl acetate	6.5 b	11.6 a	8.9 ab
2-methylpropyl acetate	15.0 b	23.2 a	20.7 a
butyl acetate	153.4 b	231.2 a	202.0 a
2-methylpropyl propanoate	0.9 ab	1.3 a	0.8 b
2-methyl-1-propanol	7.6 b	11.4 a	7.7 b
2-methylbutyl acetate	300.8 b	487.9 a	441.6 a
1-butanol	47.74	63.73	52.4
butyl propanoate	24.8 b	40.3 a	23.8 b
butyl 2-methylpropanoate	16.5 b	26.9 a	15.9 b
pentyl acetate	42.2	45.6	42.4
2-methylbutyl propanoate	8.6 b	14.9 a	9.2 b
butyl butanoate	87.0	93.6	84.4
butyl 2-methylbutanoate	147.7 b	183.9 a	179.9 ab
ethyl hexanoate	4.2 b	4.5 b	5.4 a
hexyl acetate	386.2	451.6	400.6
2-methylbutyl 2-methylbutanoate	26.4 b	33.8 a	27.9 ab
propyl hexanoate	23.2 b	32.0 a	28.8 a
hexyl propanoate	97.2 ab	103.7 a	84.1 b
1-hexanol	31.4 b	37.2 a	21.4 b
2-methylpropyl hexanoate	10.5 ab	12.6 a	9.0 b
butyl hexanoate	290.7 b	364.0 a	337.2 ab
hexyl butanoate	426.0 a	417.2 a	296.3 b
hexyl 2-methylbutanoate	677.0	793.4	731.0
pentyl hexanoate	38.0 ab	43.7 a	32.4 b
hexyl hexanoate	250.6	267.8	223.2
butyl octanoate	21.0 b	23.4 ab	29.6 a

[a] Means followed by different letters for each volatile compound are significantly different at $p \leq 0.05$ (LSD test).

fruits. Despite the seasonal influence on total volatile emissions, the effects of the air storage period after ULO storage were similar in both seasons and behaviors were similar for the analyzed storage periods. After 19 weeks of storage, clear storage condition effects were observed in both seasons; ULO+4w proved to be a more efficient storage condition for enhancing total volatile compound emissions than ULO. However, after 30 weeks of storage, fruit behavior was different: ULO+2w storage was the more efficient condition, although significant differences were observed only for 2006 fruit. Other authors have detected season-to-season variations in total aroma emissions from apples.[10,31]

Although short-term air storage has been reported to have had a positive effect on total volatile compound emissions, not all of the compounds followed the same pattern. We would therefore like to focus our study on the specific volatile compounds that were boosted after the extra period under cold-air after ULO storage. These compounds are presented in Tables 3−6. In general, the volatile compounds for which quantities increased as a result of the period under air after ULO storage were similar for the two seasons; the effects of the storage conditions were also similar to those described above for total volatile compound emissions (Figure 2), although the first season presented higher quantities of the volatile compounds that were present. A total of 26 volatile compounds were enhanced (23 esters and 3 alcohols); 14 of the 23 esters were straight-chain esters. According to Young et al.,[9] these results show that the concentration of low molecular weight esters increased more quickly after withdrawal from CA conditions than that of high molecular weight esters. The importance of some of these esters has been highlighted by previous works,[33] with ethyl and hexyl 2-methylbutanoate and hexyl- and 2-methylbutyl acetate making important contributions to 'Golden' variety flavor.

A previous study of three different apple varieties ('Golden Delicious', 'Fuji', and 'Braeburn') revealed that 15 compounds appeared to be responsible for the same principal odorants in all of these varieties.[20] In our results, 8 of these 15 principal odorants were boosted by short-term air storage after removal from the ULO atmosphere, and this may have had an effect on the flavor perceived by the tasters. More specifically, 2-methylpropyl acetate, butyl acetate, 2-methyl-1-propanol, 2-methylbutyl acetate, butyl propanoate, and hexyl acetate were reported to have contributed to fruity, sweet, apple, pear, and strawberry odors, but butyl

Table 5. Enhanced Esters and Alcohols (Micrograms per Kilogram) after Short-Term Air Storage Compared to Ultra-low Oxygen Atmosphere after 19 Weeks of Storage plus 7 Days at 20 °C in the 2007 Season

	storage conditions		
volatile compound	ULO[a]	ULO+2w[a]	ULO+4w[a]
propyl acetate	1.3	1.7	2.3
2-methylpropyl acetate	5.0	4.0	7.0
butyl acetate	39.2 b	41.2 b	68.6 a
2-methylpropyl propanoate	tr	tr	0.6
2-methyl-1-propanol	0.7 b	0.6 b	1.5 a
2-methylbutyl acetate	79.5 b	106.4 ab	138.9 a
1-butanol	4.7 b	5.8 b	10.2 a
butyl propanoate	4.3 b	5.6 b	8.2 a
butyl 2-methylpropanoate	nd	nd	0.6
pentyl acetate	6.3 b	7.2 b	12.6 a
2-methylbutyl propanoate	nd	0.6 b	10.3 a
butyl butanoate	26.9 b	18.0 b	38.3 a
butyl 2-methylbutanoate	23.3 b	22.6 b	39.5 a
ethyl hexanoate	nd	0.5 a	nd
hexyl acetate	95.6 b	78.7 b	142.9 a
2-methylbutyl 2-methylbutanoate	5.5	5.4	6.9
propyl hexanoate	3.3	3.2	4.8
hexyl propanoate	40.2 b	32.3 b	66.4 a
1-hexanol	13.0	8.4	18.8
2-methylpropyl hexanoate	18.0 b	16.9 b	38.6 a
butyl hexanoate	92.8 b	68.9 b	154.7 a
hexyl butanoate	118.0 b	88.2 b	177.7 a
hexyl 2-methylbutanoate	131.7 b	91.8 c	221.9 a
pentyl hexanoate	1.7	nd	1.4
hexyl hexanoate	6.7 b	5.7 b	11.3 a
butyl octanoate	58.7 b	68.1 b	117.2 a

[a] Means followed by different letters for each volatile compound are significantly different at $p \leq 0.05$ (LSD test). nd, not detected; tr, traces (<0.5 $\mu g\ kg^{-1}$).

Table 6. Enhanced Esters and Alcohols (Micrograms per Kilogram) after Short-Term Air Storage Compared to Ultra-low Oxygen Atmosphere after 30 Weeks of Storage plus 7 Days at 20 °C in the 2007 Season

	storage conditions		
volatile compound	ULO[a]	ULO+2w[a]	ULO+4w[a]
propyl acetate	2.2 b	21.3 a	1.9 b
2-methylpropyl acetate	5.5 b	25.7 a	9.4 b
butyl acetate	45.3 b	92.7 a	58.1 b
2-methylpropyl propanoate	0.6 c	1.0 b	1.3 a
2-methyl-1-propanol	1.5 c	2.3 b	2.9 a
2-methylbutyl acetate	111.9	116.3	106.2
1-butanol	10.0	13.3	11.4
butyl propanoate	5.9 b	9.0 a	7.8 a
butyl 2-methylpropanoate	0.5 b	0.7 ab	0.8 a
pentyl acetate	7.7 b	12.0 a	10.3 ab
2-methylbutyl propanoate	1.3	1.0	1.9
butyl butanoate	26.0 b	44.0 a	39.5 a
butyl 2-methylbutanoate	27.5 b	44.9 a	36.0 ab
ethyl hexanoate	nd	25.2 a	nd
hexyl acetate	89.1 b	156.4 a	113.0 b
2-methylbutyl 2-methylbutanoate	7.0 b	21.2 a	7.3 b
propyl hexanoate	4.4 b	22.6 a	5.8 b
hexyl propanoate	27.8 b	55.4 a	35.6 b
1-hexanol	14.7 b	30.9 a	15.9 b
2-methylpropyl hexanoate	0.6 b	5.1 a	2.2 ab
butyl hexanoate	76.8 b	124.8 a	91.9 b
hexyl butanoate	123.6 b	162.1 a	160.8 a
hexyl 2-methylbutanoate	118.2 b	151.0 ab	168.2 a
pentyl hexanoate	0.9	2.8	nd
hexyl hexanoate	9.4	12.9	10.4
butyl octanoate	83.5	83.1	93.3

[a] Means followed by different letters for each volatile compound are significantly different at $p \leq 0.05$ (LSD test). nd, not detected.

butanoate was described as contributing the smell of rotten fruit.[20] In the case of the alcohols, 1-hexanol had a pleasant odor, which was described as fresh and with green notes, whereas 1-methyl-1-propanol was described as contributing to a plastic odor.

Other works also reported that butyl acetate, hexyl acetate, butanol, 2-methybutyl acetate, butyl butanoate, hexyl butanoate, and butyl hexanoate were the most abundant substances found and accounted for >80% of the total volatiles produced by several apple varieties.[34]

Fifteen of the 26 volatile esters regenerated by 'Golden Reinders' apples in the present study had also been increased by the same storage conditions in a previous study on 'Fuji Kiku 8' apples.[16] This would seem to indicate that the regeneration potential of the conditions tested behaved in a similar way for different varieties; even so, it is important to emphasize that, being a bicolor cultivar, 'Fuji Kiku 8' has a different volatile profile from 'Golden Reinders'. Several studies have shown that red and bicolor apples tend to exhibit higher total ester values than green apples.[9,35] Previous works revealed that lipoxygenase (LOX) enzyme activity, which catalyzes the hydroperoxidation of polyunsaturated fatty acids, is essential if 'Golden Reinders' and 'Fuji' apples are to recover their ability to synthesize volatile esters after ULO storage.[36,37]

Sensory Analysis after Cold Storage in ULO and ULO plus AIR. The results from the consumer test showed that the degree of consumer preference remained similar for the different storage conditions, periods, and seasons. Despite that, an increase in some volatile compounds was observed and particularly in ones that may have contributed to fruit aroma. The average degree of consumer preference did not, however, seem to have been affected by differences in storage conditions, except for the case of ULO+4w stored fruit from the second season (2007) after 19 weeks of storage compared to ULO stored fruit. The values obtained remained between 6 and 7 on the 9-point hedonic scale, which is a value considered to be acceptable for commercial quality (data not shown). It is interesting to note that other acceptability studies relating to 'Fuji' apples also produced similar scores when fruits stored under different CA conditions were compared after 3−7 months of storage.[27,38] These results seem to suggest that the conditions utilized did not cause any significant differences in the instrumentally measured parameters (volatile compound emissions and quality parameters) that could

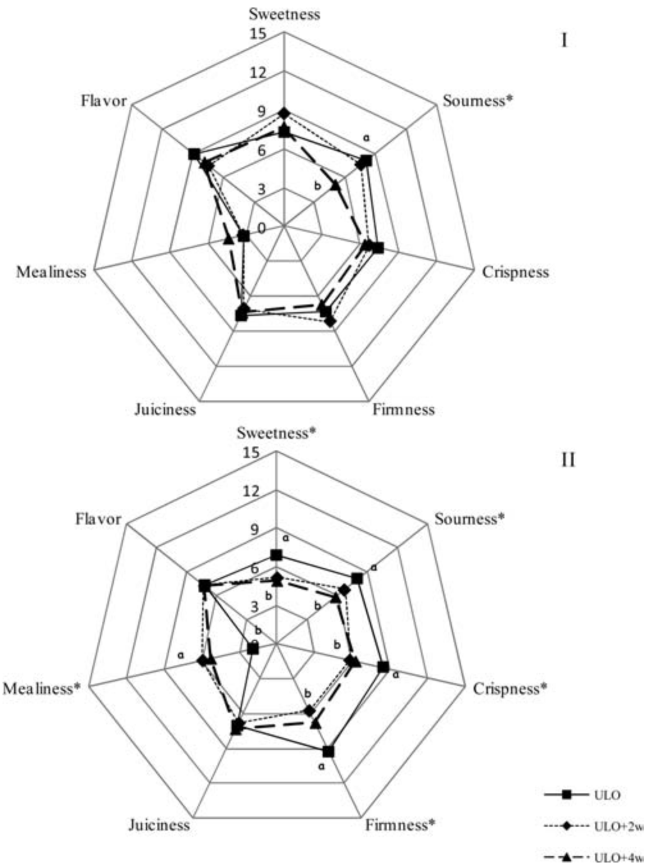

Figure 3. Sensory attributes for 'Golden Reinders' apples stored for 19 (I) and 30 weeks (II) under ULO, ULO+2w, and ULO+4w conditions in the first season (2006). ∗, means for different storage conditions for each sensory attribute are significantly different at $p \leq 0.05$ (LSD test).

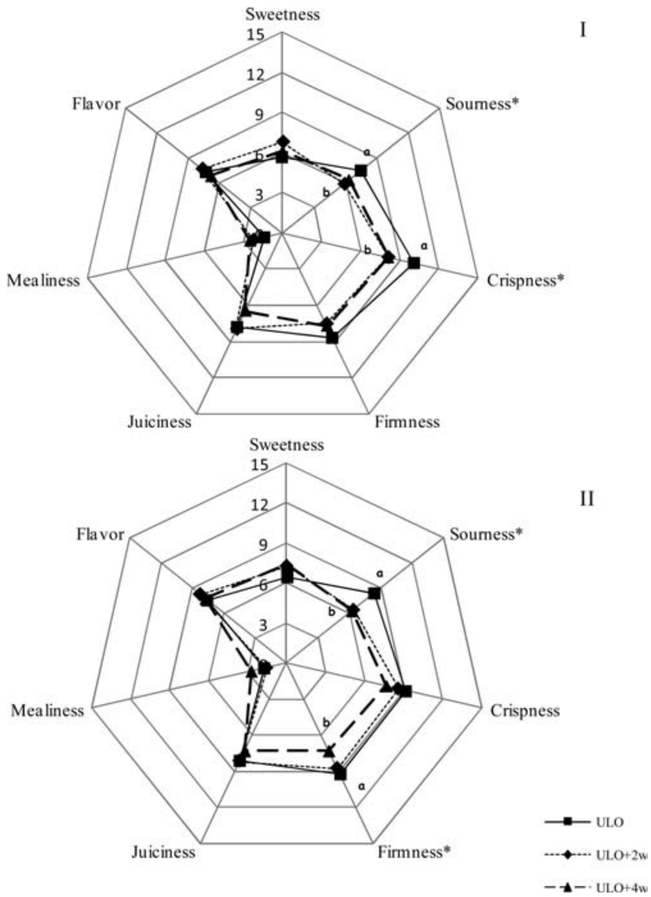

Figure 4. Sensory attributes for 'Golden Reinders' apples stored for 19 (I) and 30 weeks (II) under ULO, ULO+2w, and ULO+4w conditions in the second season (2007). ∗, means for different storage conditions for each sensory attribute are significantly different at $p \leq 0.05$ (LSD test).

be appreciated by consumers, nor did they change the average degree of consumer preference.

To focus on the differences between fruits stored under ULO, ULO+2w, and ULO+4w conditions and to try to better understand consumer preferences, we decided to evaluate fruits using a trained panel and to obtain a sensory descriptive profile of the fruit. The panel assessed different attributes such as sweetness, sourness, crispness, firmness, juiciness, mealiness, and flavor. The results are presented in Figures 3 (2006 fruit) and 4 (2007 fruit). The results for 2006 fruit after 19 weeks of storage (Figure 3, I) showed no significant differences between attributes except for sourness. Storage under ULO+4w conditions was associated with a decline in the acidity perceived by the judges. This was in line with the corresponding loss of TA (instrumentally assessed). However, no significant differences were observed between storage under ULO and ULO+2w conditions. After 30 weeks of storage (Figure 3, II), the effects of cold air storage were highly significant compared to ULO storage, particularly in terms of texture attributes such as firmness, crispness, and mealiness. ULO+4w and ULO+2w stored fruits were also perceived as being less acidic. Other authors[39] have suggested that texture is an attribute that has an important influence on consumer acceptability. In fact, according to Harker and Johnston,[39] texture is as important as flavor in influencing consumer preferences and other choices relating to many different fruits. It is therefore important to emphasize that although instrumental values of firmness remained at acceptable levels under all storage conditions tested, differences were perceived by the trained panel. These results are in line with those previously reported by Harker et al.,[23,24] who reported that differences in firmness of 4.9 N or more can be detected by the human senses.

For 2007 fruit (Figure 4), both ULO+2w and ULO+4w storage conditions had a negative effect on sourness and on texture attributes such as firmness and crispness. Previous works have demonstrated that the texture characteristics that are most valued by consumers in fresh apples are crispness and firmness.[40] A study by Peneau et al.[41] involving 5778 consumers suggested that optimal sensory quality was the most important factor consumers used to judge the freshness of apples. They also found that the sensory texture attributes of juiciness, crunchiness, and mealiness were correlated with freshness. On the basis of our results, these storage conditions could therefore negatively influence the freshness perceived by consumers.

Other authors have also demonstrated the influence of controlled atmospheres on optimal values and the determination of highly acceptable ranges of firmness.[42] A specific study on 'Golden Delicious' apples stored for 4 and 6 months revealed that decreases in total pectins and hemicelluloses and increases in free pectins were lowest under low oxygen−CA conditions, were high under standard CA conditions, and were highest under normal atmosphere conditions.[43] Our results showed that an extra 4 week period under cold air led to the predicted degradation. Our research confirmed observations by other researchers[40] that, in general, mealy apples are associated with lower levels of consumer acceptance than nonmealy ones.

From our results relating to volatile analysis, we can conclude that the tested conditions caused an increase in some volatile compounds. Even so, those that are known to be impact compounds for the variety did not cause a significant increase in the flavor perceived by the trained panel. Although aroma volatile emission tests were carried out for whole fruits and sensory analysis was based on individual pieces of fruit, this is common practice and we point out that the increase in total volatile compound emissions and in the characteristic compounds for certain varieties were probably not sufficiently great to be perceived by the judges. Alternatively, the judges should perhaps have been asked more specific questions about aroma without necessarily relating this parameter to taste (or flavor). Thus, in our opinion, further studies will be needed and should focus on a more specific sensory evaluation of the fruit and examine more specific traits such as 'green notes', 'fruity notes', 'alcoholic notes', and 'fermentative notes'. Even so, the values of the instrumental quality parameters for fruits remained above the recommended levels and the degree of consumer preference was >6; packing houses should therefore consider the economic benefits of removing fruit from CA storage for up to 1 month before commercialization to reduce the energy costs associated with operating CA equipment.

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