

# Influence of Different Oxygen and Carbon Dioxide Concentrations during Storage on Production of Volatile Compounds by Starking Delicious Apples

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Starking Delicious apples were cold-stored in semicommercial chambers in normal atmosphere and in different controlled atmospheres in which oxygen and carbon dioxide were held at 1%, 2%, and 3% for 3, 5, and 7 months. After storage, fruit was kept at ambient temperature (20 °C) for 1, 5, and 10 days. The volatile emission was collected and analyzed. After all storage periods and conditions, the total aromatic volatile emission was higher than at commercial harvest. The highest emission of volatile components was obtained after 5 months of storage, in which controlled-atmosphere conditions gave a lower concentration than normal cold storage. Esters were the main volatiles detected (>98%). Acetate ester concentrations after all storage periods assayed were higher at 3% and 2% O<sub>2</sub>/CO<sub>2</sub>. The components that contributed most to the characteristic aroma of Starking Delicious were ethyl 2-methylbutyrate, ethyl butyrate, and ethyl hexanoate. Their concentrations were higher after 5 months of storage and also during poststorage at ambient temperature (20 °C).

**Keywords:** *Controlled atmosphere; esters; aromatic volatiles; Starking Delicious*

## INTRODUCTION

In the area of Lleida, Spain, the controlled-atmosphere (CA) storage of apples with low (2%) or ultralow (1%) oxygen concentration, combined with similar CO<sub>2</sub> levels, is increasingly used compared with "standard" CA (3% O<sub>2</sub> and 3% CO<sub>2</sub>) in an attempt to extend the storage period beyond 7 months without a decrease in apple quality and sensory characteristics.

However, such conditions may decrease the volatile production responsible for the aroma in apple varieties such as Cox Orange (Knee and Sharples, 1981), McIntosh (Yahia et al., 1985), or Golden Delicious (Streif and Bangerth, 1988; Brackmann et al., 1993). This reduction is considered responsible for the decrease in flavor (Willaert et al., 1983; Streif, 1985).

The action of CA increases with lower O<sub>2</sub> and higher CO<sub>2</sub> concentrations and longer storage (Lidster et al., 1983; Smith, 1984; Streif and Bangerth, 1988; Yahia et al., 1990). Besides, it is considered that the production capacity of certain aromatic compounds can decrease when apples are placed under normal ambient conditions owing to the residual effect (Bangerth and Streif, 1987; Willaert et al., 1983; Yahia, 1991). Production of straight-chain esters originating from lipid metabolism decreases (Brackmann et al., 1993); however, no residual effect is detected in branched-chain esters coming from amino acids as a result of CA storage (Hansen et al., 1992; Mattheis et al., 1995).

The present study shows the effect of three different combinations of oxygen and carbon dioxide (3%, 2%, and 1%) at 1 °C on the production of volatile aromatic compounds in Starking Delicious apples during storage and poststorage ripening in air at 20 °C or shelf life.

## MATERIALS AND METHODS.

**Material.** Starking Delicious (*Malus communis* Borth) apples were harvested 170 days after full bloom in 1993 in Raimat de Lleida, Spain, at commercial harvest determined by flesh firmness, titratable acidity, soluble solids content, starch hydrolysis, fruit size, and ethylene production.

**Fruit Maturity Analysis.** Flesh firmness was measured on two pared surfaces per fruit using a penetrometer with an 11 mm tip (Effegi, Milan, Italy). Soluble solids content (SSC) and titratable acidity (TA) were determined using freshly prepared juice. The SSC was determined using a hand refractometer (Atago, Tokyo, Japan), and TA was determined by titrating 10 mL of juice to pH 8 using phenolphthaleine. Starch hydrolysis was rated visually using a 1–6 scale (1, full starch; 6, no starch) after an equatorial section was stained with a 0.5% I<sub>2</sub>–KI solution. Ethylene production was measured by taking samples from the effluent air from respiration jars where the fruit was placed and continuously aerated with humidified air at a rate of ~2 L/h at 20 °C. A 1 mL gas sample was injected into a gas chromatograph (Hewlett-Packard 5890 Series II, Barcelona, Spain) equipped with a flame ionization detector and a 1.5 m × 3 mm column packed with activated aluminum oxide. Gas analyses were conducted isothermally at 100 °C. N<sub>2</sub> carrier, air, and H<sub>2</sub> flows were 45, 400, and 45 mL/min, respectively. The injector and detector were held at 120 and 180 °C, respectively.

**Storage Conditions.** Semicommercial cool-storage rooms (22 m<sup>3</sup>) were used. Immediately after picking, apples were selected for uniformity and stored at 1 °C at different conditions: normal cold storage (NCS) and controlled atmosphere with different concentrations of oxygen and carbon dioxide. NCS contained 21% O<sub>2</sub>/0.03% CO<sub>2</sub> at 92% relative humidity (RH); standard CA contained 2.8–3% O<sub>2</sub>/2.8–3% CO<sub>2</sub> at 95%

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RH; CA with low oxygen (LO) contained 1.8–2% O<sub>2</sub>/1.8–2% CO<sub>2</sub> at 92–94% RH; and CA with ultralow oxygen (ULO) contained 0.8–1% O<sub>2</sub>/0.8–1% CO<sub>2</sub> at 92–94% RH. Oxygen concentrations of ~5% were obtained within 36–48 h by flushing with nitrogen. CO<sub>2</sub> concentrations were obtained by normal respiration of fruits and CO<sub>2</sub> injection if necessary. Final O<sub>2</sub> and CO<sub>2</sub> concentrations were established within 8 days of harvest. O<sub>2</sub> and CO<sub>2</sub> concentrations in the rooms were measured by an electrochemical O<sub>2</sub> analyzer and an infrared gas analyzer for CO<sub>2</sub> and maintained automatically (Isoscell, Milan, Italy).

**Experimental Assay.** Lots of 15 kg of fruits were placed in each of the four storage conditions. Samples of 5 kg were taken after 3, 5, and 7 months. From each sample, four 1 kg replicates were analyzed after remaining 1, 5, and 10 days at room temperature (20 °C) and 85% RH. The replicates analyzed at different days (1, 5, and 10) were the same samples.

**Volatile Compound Analysis.** For the extraction of volatile components from the intact apples, the method of dynamic headspace described by Kakiuchi et al. (1986) and Lavilla et al. (1993) was used. Each fruit sample (1 kg) was placed in a 10 L Pyrex glass container, and a nitrogen (N-50, assay >99.999%, Air Liquide, Barcelona, Spain) stream of 50–60 mL/min was passed through for 24 h; the effluent was then passed through a glass column 15 cm × 1.5 cm (i.d.) with 1 g of Tenax-GC 60–80 mesh (Tecknokroma, Barcelona, Spain). The extraction procedure was performed in the dark in a temperature-conditioned room (20 °C). The volatiles were recovered from the adsorbent with 20 mL of diethyl ether (ACS > 99.8% GC, which contained 0.0005% 2,6-di-*tert*-butyl-*p*-cresol; Fluka, Barcelona, Spain). The solid-ether phases were treated by ultrasonic waves for 5 min. Phases were then separated by centrifugation for 2 min at 1000 rpm and 0 °C (Beckmann, J2–21, Barcelona, Spain). Two further extractions were performed with 20 and 10 mL of diethyl ether, respectively, in the conditions described above. Previously, it had been ascertained that additional extractions did not increase the yield of volatile extract. The organic phase were collected together and evaporated to a volume of 0.1 mL by bubbling a nitrogen (N-50, assay > 99.999%, Air Liquide) stream of 20–30 mL/min through a capillary tube. After this, 10 μL of *n*-hexane (assay > 99.7%, Fluka) with 5% of butylbenzene (assay > 99.5%, Fluka) as internal standard was added to this volume.

After the concentration of 50 mL of ethyl ether to 0.1 mL and its injection to the gas chromatograph, no product was detected that could interfere in the chromatogram of the aromatic fraction.

The adsorbent Tenax-GC used was recovered by washing in 20 mL of diethyl ether and activated in an oven (Büchi GKR-51, Barcelona, Spain) at 150 °C for 6 h with a nitrogen stream (N-50, assay > 99.999%, Air Liquide) passing through it at 150 mL/min. The adsorbent phase can be used five or six times without loss of effectiveness.

The identification and quantification of the volatile compounds were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector. The capillary column was a cross-linked FFAP [50 m × 0.2 mm (i.d.) × 0.33 mm]. Nitrogen was used as the carrier gas at a flow rate of 0.8 mL/min, and the carrier linear velocity was 17.4 cm/s, with a split ratio of 1:200. The injector and detector were held at 240 °C. The analysis was conducted with the following program: 70 °C for 1 min, from 70 to 142 °C at 3 °C/min, 142 °C for 2 min, from 142 to 230 °C at 25 °C/min, and at 230 °C for 5 min. A volume of 1 μL was injected in all of the analyses.

For the identification and confirmation of the different compounds, a mass spectrometer/gas chromatograph Hewlett-Packard 5890 coupled to a gas chromatograph (GC/MS) was used. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as carrier gas at a flow rate of 0.6 mL/min (20.4 cm/s) in splitless mode, and a program of 40 °C for 15 min, 40–210 °C at 5 °C/min, and 210 °C for 15 min was used in the GC. Two capillary columns with station-

**Table 1. Quality Parameters<sup>a</sup> of Analyzed Apples That Define the Commercial Harvest Date in Lleida**

	sample apples at harvest	commercial recommendation at harvest
texture (kg)	6.8 ± 0.6	8.3–7.3
acidity (g/L of malic acid)	2.8 ± 0.3	3.9–3.1
soluble solids (g/100 g of fresh wt)	13.5 ± 0.5	12–13
starch index (1–6)	2.3 ± 0.2	2
fruit size (mm)	70–80	70–80
ethylene production (μL/kg·h)	20.0 ± 3.0	

<sup>a</sup> Values are means and standard deviations calculated from 10 apples.

ary phases were used in these analyses: the above-mentioned cross-linked FFAP and a Carbowax 20 M [20 m × 0.2 mm (i.d.) × 0.33 mm]. The spectrometric data were compared with those from the NIST HP59943C original library mass spectra (Hewlett-Packard).

**Statistical Treatment.** Results were analyzed as a factorial (3 storage periods × 4 O<sub>2</sub>–CO<sub>2</sub> concentrations × 3 ripening periods) using the ANOVA procedure of Statistical Analyses System (SAS Institute Inc., 1988 Cary, NC). Tukey's LSD (*p* > 0.05) was used to separate treatment means.

## RESULTS AND DISCUSSION

Fruits were harvested at the usual maturity stage for our area, although somewhat advanced according to the recommendations and at the early climacteric stage. Ethylene production was ~20 μL/kg·h. Quality parameters that define the commercial harvest date and our results are shown in Table 1. The apples showed normal behavior during cold storage. Ripening proceeded at a lower rate in all CA conditions as observed in the measurement of parameters such as texture, acidity, and SSC (Table 2).

The volatile fraction emitted by Starking Delicious was collected and analyzed before the fruit was placed under storage conditions. The aromatic compounds identified and quantified at harvest date are shown in Table 3. A total of 24 volatile compounds were identified. Esters constitute more than 98% of the total volatile compounds emitted. The main compound was ethyl 2-methylbutyrate (30.8%), which is the most important in Delicious varieties (Flath et al., 1967; Belitz and Grosch, 1988) and which has an olfactory threshold of only 0.006 ppb (Takeoka, 1992). It is followed by ethyl acetate (21.7%), ethyl butyrate (16.6%), *tert*-butyl propionate (12.8%), ethyl hexanoate (3.9%), ethyl propionate (3.2%), and 2-methylbutyl acetate (2.5%). These seven esters represent 91.5% of the total volatile fraction.

2-Methylbutyl 2-methylbutyrate was not detected after 3, 5, or 7 months of cold storage. 4-Methyl-2-pentanol and the three terpenes (linalool, terpeniol, and nerol) were irregularly detected during cold storage. They are not taken into account in the statistical analysis.

During the 10 days storage at room temperature (20 °C) after 3, 5, or 7 months of cold storage there was an increase in the total aromatic volatile components of fruit in all CA conditions in relation to the harvest day (Figure 1). The highest increase in aroma was obtained after 5 months of storage. The four storage conditions used give the maximum production of aromatic volatile components. The reduction of volatiles in CA compared with normal cold storage shown in different papers (Lidster et al., 1983; Smith, 1984; Streif and Bangerth,

**Table 2. Fruit Quality Values<sup>a</sup> for Starking Delicious Apples after 3, 5, and 7 Months of Cold Storage<sup>b</sup> and 1 and 10 Days of Ripening at 20 °C**

	texture (kg)	acidity (g/L of malic acid)	SSC (g/100 g of fresh wt)
3 months, NSC, 1 day	5.5 ± 0.1	2.1 ± 0.1	12.7 ± 0.2
3 months, NCS, 10 days	4.7 ± 0.2	1.7 ± 0.1	12.4 ± 0.3
3 months, AC, 1 day	5.5 ± 0.2	2.4 ± 0.2	13.4 ± 0.3
3 months, AC, 10 days	4.6 ± 0.1	2.3 ± 0.1	13.1 ± 0.3
3 months, LO, 1 day	5.5 ± 0.1	2.5 ± 0.2	13.9 ± 0.2
3 months, LO, 10 days	4.5 ± 0.2	2.3 ± 0.2	13.5 ± 0.4
3 months, ULO, 1 day	5.4 ± 0.3	2.6 ± 0.3	13.9 ± 0.2
3 months, ULO, 10 days	4.5 ± 0.1	2.3 ± 0.1	13.3 ± 0.2
5 months, NSC, 1 day	4.5 ± 0.2	1.7 ± 0.1	12.2 ± 0.3
5 months, NCS, 10 days	4.1 ± 0.2	1.3 ± 0.1	12.8 ± 0.4
5 months, AC, 1 day	4.6 ± 0.1	2.0 ± 0.2	12.4 ± 0.4
5 months, AC, 10 days	4.0 ± 0.1	1.7 ± 0.2	12.8 ± 0.2
5 months, LO, 1 day	4.6 ± 0.2	2.1 ± 0.3	13.7 ± 0.2
5 months, LO, 10 days	4.3 ± 0.1	2.1 ± 0.4	13.6 ± 0.4
5 months, ULO, 1 day	4.7 ± 0.2	2.3 ± 0.4	14.0 ± 0.3
5 months, ULO, 10 days	4.4 ± 0.3	2.2 ± 0.1	14.0 ± 0.2
7 months, NSC, 1 day	3.5 ± 0.1	1.6 ± 0.2	12.3 ± 0.3
7 months, NCS, 10 days	3.0 ± 0.1	1.3 ± 0.3	12.5 ± 0.3
7 months, AC, 1 day	4.5 ± 0.2	2.1 ± 0.2	13.8 ± 0.4
7 months, AC, 10 days	4.0 ± 0.3	1.7 ± 0.2	13.5 ± 0.4
7 months, LO, 1 day	4.5 ± 0.2	2.1 ± 0.1	13.6 ± 0.3
7 months, LO, 10 days	4.0 ± 0.2	2.0 ± 0.1	13.6 ± 0.3
7 months, ULO, 1 day	4.5 ± 0.1	2.3 ± 0.3	13.5 ± 0.2
7 months, ULO, 10 days	4.0 ± 0.1	2.0 ± 0.3	13.6 ± 0.2

<sup>a</sup> Values are means and standard deviations calculated from 30 apples. <sup>b</sup> NSC, normal cold storage (21% O<sub>2</sub>, 0.03% CO<sub>2</sub>); AC, standard controlled atmosphere (2.8–3% O<sub>2</sub>, 2.8–3% CO<sub>2</sub>); LO, low oxygen (1.8–2% O<sub>2</sub>, 1.8–2% CO<sub>2</sub>); ULO, ultralow oxygen (0.8–1% O<sub>2</sub>, 0.8–1% CO<sub>2</sub>).

1988; Mattheis et al., 1995) was found to be significant ( $p > 0.05$ ) only after 5 months in CA storage. After 3 and 7 months there were no significant differences among the different storage conditions.

**Table 3. Quantification of Aromatic Volatile Compounds in Apple Extracts at Harvest Date of Starking Delicious Variety by Gas Chromatography and Their Odor Thresholds in Water**

compound	RI <sup>a</sup>	amount <sup>b</sup> (μg)	odor threshold in water (μg/L)
ethyl acetate	895	655.8 ± 183.7	5000 (Flath et al., 1967)
ethyl propionate	925	97.9 ± 11.4	10 (Takeoka et al., 1992)
ethanol	936	14.6 ± 0.8	100000 (Flath et al., 1967)
<i>tert</i> -butyl propionate	967	385.9 ± 81.6	
propyl acetate	990	36.6 ± 2.9	
2-methylpropyl acetate	1023	23.6 ± 0.5	65 (Takeoka et al., 1992)
1-propanol	1042	10.2 ± 1.6	9000 (Flath et al., 1967)
ethyl butyrate	1047	500.7 ± 64.1	1 (Flath et al., 1967)
ethyl 2-methylbutyrate	1061	928.9 ± 65.3	0.006 (Takeoka et al., 1992)
ethyl 3-methylbutyrate	1068	ND	
butyl acetate	1086	39.8 ± 4.2	66 (Flath et al., 1967)
2-methyl-1-propanol	1095	6.5 ± 0.6	
2-methylbutyl acetate	1134	76.3 ± 6.3	11 (Takeoka et al., 1992)
1-butanol	1148	N.D.	
4-methyl-2-pentanol	1165	8.1 ± 0.1	
pentyl acetate	1194	N. D.	
2-methyl-1-butanol	1213	5.2 ± 1.0	
butyl butyrate	1229	7.2 ± 0.3	
ethyl hexanoate	1245	116.6 ± 4.5	1 (Takeoka et al., 1992)
hexyl acetate	1283	50.0 ± 0.7	2 (Buttery et al., 1982)
2-methylbutyl 2-methylbutyrate	1292	8.5 ± 0.3	
butylbenzene (IS)	1338	ND	
hexyl propionate	1350	ND	
1-hexanol	1363	8.3 ± 0.3	2500 (Takeoka et al., 1990)
hexyl butyrate	1433	3.8 ± 0.4	250 (Takeoka et al., 1992)
butyl 3-methylbutyrate	1442	5.1 ± 0.	60 (Jakob et al., 1969)
linalool	1563	4.9 ± 1.0	
terpeniol	1723	6.7 ± 1.0	
nerol	1819	10.6 ± 1.8	

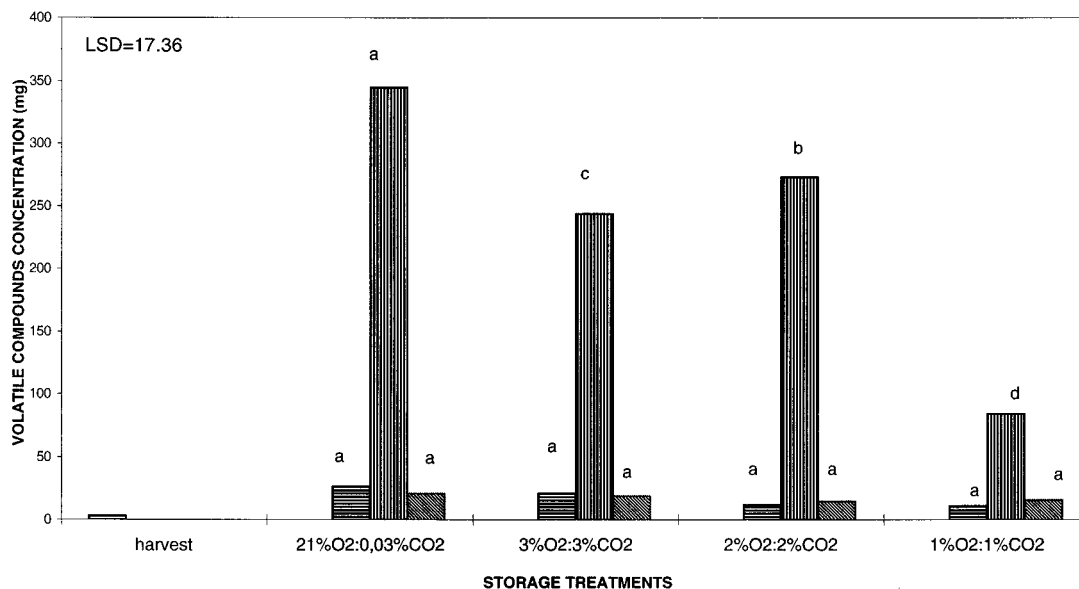
<sup>a</sup> Kovats retention index (RI) in column cross-linked FFAP (Poole and Poole, 1993). <sup>b</sup> Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg) and are the result of 24-h collection. The compounds not detected are indicated as ND.

These results confirm that extending storage from 5 to 7 months decreases the total volatile fraction as obtained by Streif and Bangerth (1988), but no decrease was found during the period from 3 to 5 months as obtained by Brackmann (1989) in Golden Delicious with CA combinations of 3% O<sub>2</sub>/CO<sub>2</sub>, 1% O<sub>2</sub>/3% CO<sub>2</sub>, and normal cold storage. On the other hand, in this work the conditions of 2% O<sub>2</sub>/CO<sub>2</sub> gave higher production of volatiles compared with 3% O<sub>2</sub>/CO<sub>2</sub> (CA) and 1% O<sub>2</sub>/CO<sub>2</sub> (ULO). In other studies on Golden Delicious it was shown that 1% O<sub>2</sub> and 3% CO<sub>2</sub> were less favorable to aromatic production. Our results seem to show that the conditions used compensate for the negative effects of oxygen reduction up to 1% and the increase of carbon dioxide up to 3%.

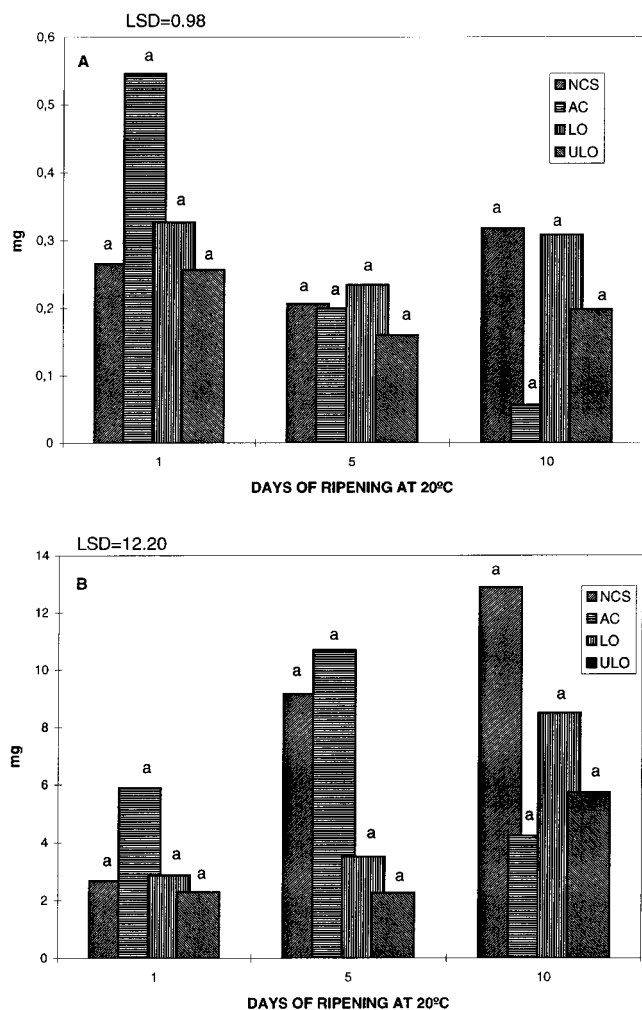
The ester production was always higher than that of alcohols in all periods studied after transfer of the fruit to room temperature (1, 5, and 10 days) and for samples kept during 3, 5, and 7 months (Figures 2–4). This higher ester production was also found by Mattheis et al. (1995) in Bisbee Delicious apples obtained at three different harvest dates and after 6 months of storage with different oxygen concentrations and a fixed 2% CO<sub>2</sub>. Analyses were done in fruit kept for 10 days at room temperature after storage.

Statistical analysis shows that the storage period, the combination of oxygen and carbon dioxide, and days of ripening in air significantly affect the total ester and alcohol productions. The storage period and ripening in air at 20 °C have the highest effect.

In short storage periods (3 months) the different conditions do not induce significant differences in total alcohols and esters (Figure 2). These results are similar to those obtained with Law Rome apples (Fellman et al., 1993) and McIntosh and Cortland apples in 3% O<sub>2</sub>/CO<sub>2</sub> and normal cold storage (Yahia et al., 1990).



**Figure 1.** Total aromatic volatile emission after 10 days of ripening poststorage at 20 °C of Starking Delicious apples. Bars with the same letter within each cold-storage period are not significantly different ( $p > 0.05$ ) according to Tukey's LSD test.



**Figure 2.** Total alcohol emission (A) and total ester emission (B) after 3 months of storage of Starking Delicious apples. Temperature was 1 °C in all storage treatments: 21% O<sub>2</sub>/0.03% CO<sub>2</sub> (NCS); 3% O<sub>2</sub>/3% CO<sub>2</sub> (AC); 2% O<sub>2</sub>/2% CO<sub>2</sub> (LO); 1% O<sub>2</sub>/1% CO<sub>2</sub> (ULO). Values are means of four replicate extract samples each obtained from four or five apples (~1 kg). Bars with the same letter within each day of ripening at 20 °C are not significantly different ( $p > 0.05$ ) according to Tukey's LSD test.

The highest ester production was obtained after 5 months in NCS but only after 5 and 10 days of ripening at 20 °C (Figure 3B). However, the combination 2% O<sub>2</sub>/CO<sub>2</sub> (LO) produced the highest ester production after only 1 day of ripening at 20 °C. Mattheis et al. (1995) obtained the highest ester production in NCS after 6 months of storage and 10 days at room temperature.

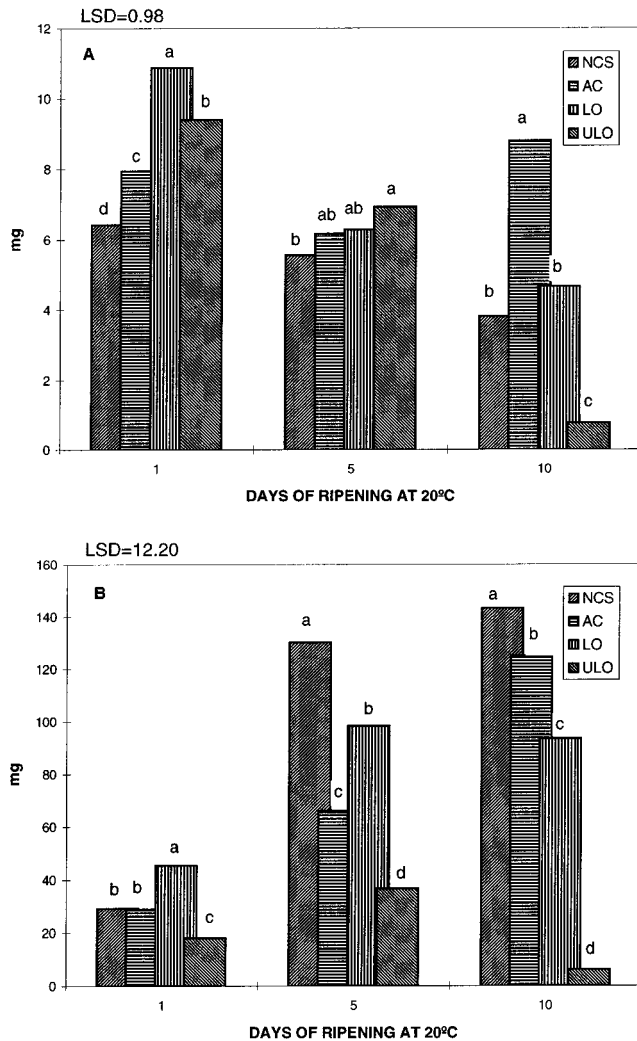
After 7 months, the four storage conditions do not show a significant influence on the total ester production (Figure 4B) and no decrease was observed due to LO concentration as was described in other works (Streif and Bangerth, 1988; Brackmann et al., 1993; Fellman et al., 1993).

The individual esters and alcohols were influenced by storage conditions, period of storage, and days at 20 °C. (Tables 4–6). The highest effect is due to the storage period. After 3 months of storage and 1 day of ripening at 20 °C, pentyl acetate and hexyl propionate were detected for the first time.

The highest acetate concentrations were obtained after 5 months of storage. The cold storage with 3% and 2% O<sub>2</sub>/CO<sub>2</sub> gave the highest production of propyl, butyl, pentyl, and hexyl acetates (Table 5). The suppressive effect of 3% O<sub>2</sub> and 1% CO<sub>2</sub> found by Brackmann et al. (1993) for straight-chain esters in Golden Delicious was not shown when the oxygen and carbon dioxide levels were equal. Ripening in air at 20 °C induced a significant increase in propyl acetate after CA storage at 3% and 2% O<sub>2</sub>/CO<sub>2</sub>, while hexyl acetate decreased with time. Other straight-chain acetate esters show a variable behavior.

Shorter or longer storage periods (3 or 7 months) do not induce higher amounts of acetates in fruit kept at NCS, except for hexyl and butyl acetates (Tables 4 and 6).

The branched-chain esters, 2-methylpropyl and 2-methylbutyl acetates, ethyl 2-methylbutyrate, and hexyl 3-methylbutyrate, show the highest amounts after 5 months of storage at 3% and 2% O<sub>2</sub>/CO<sub>2</sub>. These results are similar to those shown by Tressl et al. (1970) and Mattheis et al. (1995), confirming the favorable effect of CA. In addition, 2-methylpropyl acetate increased during storage at ambient temperature.

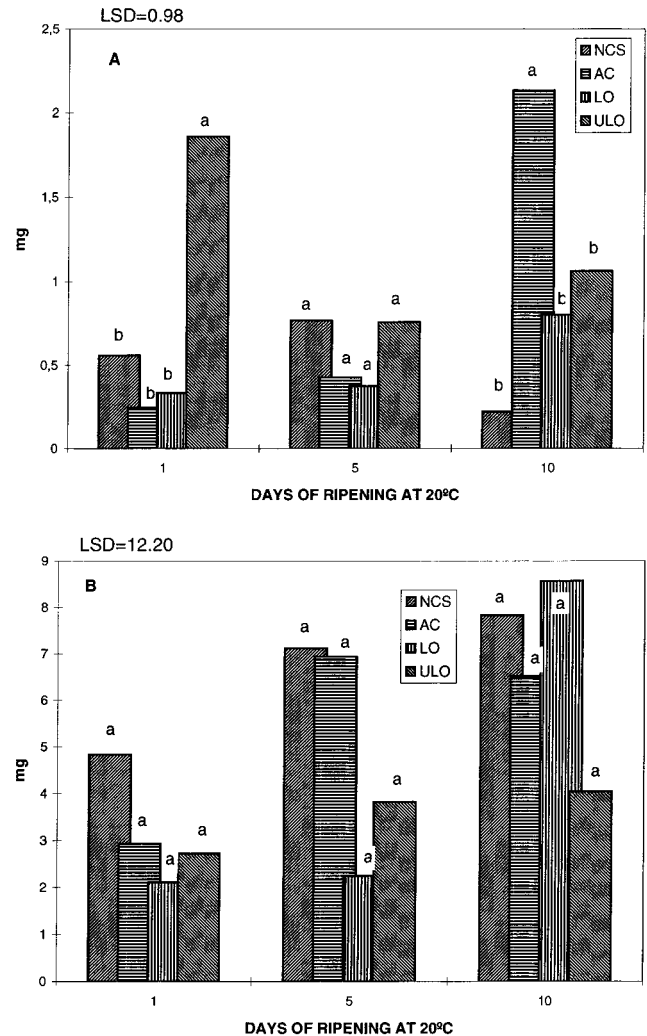


**Figure 3.** Total alcohol emission (A) and total ester emission (B) after 5 months of storage of Starking Delicious apples. Temperature was 1 °C in all storage treatments: 21% O<sub>2</sub>/0.03% CO<sub>2</sub> (NCS); 3% O<sub>2</sub>/3% CO<sub>2</sub> (AC); 2% O<sub>2</sub>/2% CO<sub>2</sub> (LO); 1% O<sub>2</sub>/1% CO<sub>2</sub> (ULO). Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg). Bars with the same letter within each day of ripening at 20 °C are not significantly different ( $p > 0.05$ ) according to Tukey's LSD test.

The highest amounts of ethyl 2-methylbutyrate were obtained after 5 months of storage at 3% and 2% O<sub>2</sub>/CO<sub>2</sub>, and 10 days of ripening at 20 °C. There was also a spectacular increase during the first 5 days at 20 °C (Table 5). This compound has a direct impact on apple flavor, since it has been identified as the volatile compound that contributes most to the flavor of the group Delicious (Flath et al., 1967); moreover, it is already the major component at commercial harvest in Starking Delicious (Table 3) and after cold storage.

The relative contribution of volatile compounds to the overall aroma is determined by calculating the number of odor units (Takeoka et al., 1992). Guadagni et al. (1966) defined the odor unit as the compound concentration divided by its odor threshold. Ethyl 2-methylbutyrate reaches 639 440 odor units at the highest concentration obtained.

The delaying effect of CA storage on the synthesis of ethyl 2-methylbutyrate reported by Mattheis et al. (1995) was observed in this work only after 7 months and 1 day of ripening at 20 °C (Table 6). In these



**Figure 4.** Total alcohol emission (A) and total ester emission (B) after 7 months of storage of Starking Delicious apples. Temperature was 1 °C in all storage treatments: 21% O<sub>2</sub>/0.03% CO<sub>2</sub> (NCS); 3% O<sub>2</sub>/3% CO<sub>2</sub> (AC); 2% O<sub>2</sub>/2% CO<sub>2</sub> (LO); 1% O<sub>2</sub>/1% CO<sub>2</sub> (ULO). Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg). Bars with the same letter within each day of ripening at 20 °C are not significantly different ( $p > 0.05$ ) according to Tukey's LSD test.

conditions its amount in all storage systems was lower compared with the day of harvest. Longer storage at 20 °C (10 days), however, leads to higher levels than those at the harvest date. All fruit produce more volatiles than at harvest. Although it is known that different varieties show different behaviors under cold storage, our results show a positive effect of a long (10 days) stage at 20 °C after long storage periods at different CA on the synthesis of ethyl 2-methylbutyrate.

Substrate availability is an important factor in the flavor recovery of fruits stored in CA for long periods (Fellman et al., 1993). Since the amino acid content is relatively constant during storage (Ackermann et al., 1992) and they are the precursors of branched-chain esters, this could explain the increase in ethyl 2-methylbutyrate after 10 days of ripening in air.

After 5 months, the hexyl esters such as hexyl acetate, propionate, butyrate, and 3-methylbutyrate show a decrease in their amount at ambient temperature in all storage conditions. This effect was also observed by Yahia (1991). However, there was an increase in ethyl hexanoate, giving a maximum of 42 298 odor units in

**Table 4. Effect of O<sub>2</sub> and CO<sub>2</sub> Treatments on Ester and Alcohol Amounts (Micrograms) from Starking Delicious Apples after 3 Months of Storage plus 1, 5, and 10 Days of Ripening at 20 °C<sup>a</sup>**

compound	days at <i>T</i> <sub>amb</sub> <sup>b</sup>	21% O <sub>2</sub> /0.03% CO <sub>2</sub>	3% O <sub>2</sub> /3% CO <sub>2</sub>	2% O <sub>2</sub> /2% CO <sub>2</sub>	1% O <sub>2</sub> /1% CO <sub>2</sub>
Esters					
ethyl acetate	1	40.0 ± 19.4 a B	202.4 ± 113.3 a A	68.3 ± 15.6 a A	88.6 ± 20.6 a A
LSD = 698.5	5	1900.7 ± 446.2 a A	526.5 ± 109.7 b A	213.0 ± 54.6 b A	223.7 ± 135.6 b A
	10	1284.1 ± 430.0 a A	290.9 ± 137.5 b A	184.8 ± 116.6 b A	403.1 ± 70.0 b A
propyl acetate	1	187.0 ± 45.1 a A	160.0 ± 0.0 a A	84.3 ± 41.6 a A	291.0 ± 66.0 a A
LSD = 390.0	5	133.1 ± 10.3 a A	229.7 ± 66.4 a A	148.4 ± 26.1 a A	173.6 ± 47.0 a A
	10	396.2 ± 159.9 a A	203.6 ± 119.1 a A	351.2 ± 253.7 a A	224.4 ± 61.5 a A
butyl acetate	1	212.6 ± 30.6 c B	940.9 ± 142.0 b A	1116.0 ± 383.9 a A	340.9 ± 63.4 c A
LSD = 165.4	5	290.0 ± 92.7 b B	490.0 ± 47.1 a B	484.8 ± 31.5 a B	141.2 ± 35.8 b B
	10	487.4 ± 167.3 a A	188.1 ± 14.3 b C	382.6 ± 171.0 a B	338.5 ± 33.5 ab A
pentyl acetate	1	16.0 ± 5.2 b A	32.5 ± 8.0 a A	36.9 ± 9.6 a A	15.8 ± 3.0 b A
LSD = 11.8	5	12.9 ± 2.61 a A	17.1 ± 2.5 a B	19.9 ± 2.6 a B	8.1 ± 2.4 a A
	10	13.5 ± 2.4 a A	0 a C	18.7 ± 7.4 a B	15.4 ± 0.9 a A
hexyl acetate	1	521.1 ± 53.9 b A	1458.9 ± 270.7 a A	1513.0 ± 469 a A	550.9 ± 173.4 b A
LSD = 280.9	5	318.4 ± 10.4 a A	632.2 ± 102.5 a B	369.6 ± 118.1 a B	294.8 ± 55.5 a A
	10	498.3 ± 7.0 a A	123.5 ± 54.8 b C	177.2 ± 9.1 b B	235.8 ± 31.4 b A
2-methylpropyl acetate	1	11.9 ± 5.4 a A	18.3 ± 5.6 a A	24.9 ± 9.6 a A	28.6 ± 14.8 a A
LSD = 161.9	5	11.9 ± 5.6 a A	20.2 ± 3.3 a A	27.2 ± 2.7 a A	10.2 ± 2.0 a A
	10	19.3 ± 9.6 a A	18.0 ± 6.2 a A	27.1 ± 12.6 a A	33.0 ± 3.2 a A
2-methylbutyl acetate	1	178.1 ± 50.3 c A	578.0 ± 47.4 b A	777.2 ± 160.4 a A	332.1 ± 62.4 c A
acetate	5	111.8 ± 28.4 d A	396.0 ± 3.4 b A	527.3 ± 54.0 a A	229.4 ± 32.3 c A
LSD = 1039.0	10	364.7 ± 27.9 b A	188.5 ± 19.3 c A	321.8 ± 41.3 b A	737.0 ± 28.1 a A
ethyl propionate	1	64.8 ± 19.5 a A	168.7 ± 42.1 a A	50.2 ± 21.4 a A	60.0 ± 11.4 a A
LSD = 275.4	5	76.4 ± 16.7 a A	80.7 ± 4.2 a A	80.0 ± 7.2 a A	70.9 ± 32.4 a A
	10	90.8 ± 32.2 a A	61.3 ± 21.4 a A	84.4 ± 25.0 a A	55.5 ± 21.0 a A
<i>tert</i> -butyl propionate	1	0 a A	69.1 ± 24.9 a A	21.3 ± 6.3 a A	30.0 ± 10.6 a A
LSD = 1700.7	5	340.5 ± 81.6 a A	275.7 ± 68.8 a A	70.2 ± 15.4 a A	37.4 ± 16.2 a A
	10	649.6 ± 238.3 a A	224.1 ± 44.9 a A	88.9 ± 56.6 a A	26.2 ± 9.6 a A
hexyl propionate	1	9.2 ± 4.3 c AB	41.5 ± 6.0 b A	66.1 ± 8.5 a A	0 c A
LSD = 14.5	5	0 b B	18.8 ± 2.0 a B	18.3 ± 5.2 a B	12.2 ± 2.6 ab A
	10	18.3 ± 4.8 b A	12.8 ± 2.3 b B	31.5 ± 5.3 a B	13.0 ± 1.3 b A
ethyl butyrate	1	130.5 ± 78.4 a A	365.1 ± 68.0 a A	228.4 ± 68.3 a A	155.2 ± 42.6 a A
LSD = 7121.8	5	3729.8 ± 1083.2 a A	3477.1 ± 437 a A	747.7 ± 112.9 a A	610.5 ± 25.1 a A
	10	4347.3 ± 1681.4 a A	1598.1 ± 608 a A	1543.9 ± 1174.1a A	1275.2 ± 135 a A
ethyl 2-methylbutyrate	1	100.1 ± 42.6 a A	410.1 ± 60.9 a A	71.8 ± 28.6 a A	138.2 ± 50.3 a A
LSD = 3645.0	5	1144.4 ± 437.6 a A	3477.6 ± 475.2 a A	718.3 ± 119.2 a A	425.8 ± 107.0 a A
	10	3086.1 ± 957.2 a A	1534.2 ± 210.3 a A	1001.5 ± 567.8.a A	2155.9 ± 179.1 a A
butyl butyrate	1	76.7 ± 23.0 a A	76.4 ± 14.2 a A	53.9 ± 29.1 a A	18.2 ± 4.6 a A
LSD = 133.1	5	19.2 ± 0.6 a A	26.9 ± 1.7 a A	33.5 ± 7.6 a A	0 a A
	10	49.2 ± 0.2 a A	0 a A	49.1 ± 23.9 a A	27.8 ± 4.6 a A
hexyl 3-methylbutyrate	1	15.9 ± 4.9 b A	100.8 ± 25.8 a A	52.7 ± 23.0 b A	56.5 ± 4.3 ab A
LSD = 47.3	5	24.7 ± 2.6 a A	60.3 ± 2.9 a AB	16.3 ± 1.4 a A	29.5 ± 3.2 a A
	10	57.1 ± 16.3 a A	18.3 ± 1.9 a B	36.9 ± 9.4 a A	48.9 ± 2.3 a A
hexyl butyrate	1	19.8 ± 7.2 c A	137.8 ± 13.9 a A	97.6 ± 53.0 ab A	79.7 ± 13.5 b A
LSD = 41.9	5	23.9 ± 2.2 a A	46.8 ± 2.3 a B	30.3 ± 12.4 a B	36.1 ± 5.3 a B
	10	40.7 ± 5.2 ab A	10.7 ± 1.8 b B	58.0 ± 28.4 a AB	42.2 ± 4.8 ab AB
ethyl hexanoate	1	117.8 ± 33.9 a A	163.4 ± 19.8 a A	40.6 ± 10.8 a A	46.0 ± 6.3 a A
LSD = 2599.4	5	1073.3 ± 362.0 a A	854.6 ± 105.6 a A	98.0 ± 23.8 a A	96.9 ± 16.4 a A
	10	914.1 ± 94.9 a A	205.9 ± 71.7 a A	44.4 ± 8.8 a A	121.4 ± 26.2 a A
Alcohols					
ethanol	1	0 a A	27.4 ± 6.0 a A	16.5 ± 5.0 a A	13.2 ± 3.6 a A
LSD = 153.2	5	27.7 ± 2.4 a A	19.2 ± 4.3 a A	16.8 ± 5.3 a A	7.3 ± 1.9 a A
	10	26.0 ± 4.8 a A	23.4 ± 11.3 a A	13.5 ± 2.9 a A	11.2 ± 2.2 a A
1-propanol	1	47.3 ± 3.3 a A	62.4 ± 22.3 a A	42.6 ± 5.9 a A	49.8 ± 10.0 a A
LSD = 363.0	5	26.0 ± 1.7 a A	40.2 ± 4.6 a A	44.8 ± 3.0 a A	28.2 ± 9.7 a A
	10	30.9 ± 4.8 a A	29.6 ± 3.2 a A	33.3 ± 15.0 a A	32.2 ± 9.8 a A
2-methyl-1-propanol	1	39.0 ± 8.2 a A	51.1 ± 16.8 a A	0 a A	0 a A
LSD = 75.8	5	21.5 ± 5.0 a A	33.0 ± 4.6 a A	41.1 ± 1.2 a A	38.5 ± 12.8 a A
	10	41.0 ± 8.3 a A	25.4 ± 4.2 a A	45.7 ± 9.2 a A	0 a A
1-butanol	1	37.6 ± 20.8 c A	128.9 ± 15.6 a A	90.0 ± 30.5 ab A	65.2 ± 4.9 bc A
LSD = 49.5	5	49.8 ± 7.3 a A	0 b B	50.6 ± 4.1 a A	9.4 ± 0.9 b B
	10	41.6 ± 4.5 a A	0 a B	40.9 ± 10.1 a A	29.4 ± 8.1 a AB
2-methyl-1-butanol	1	22.6 ± 3.2 a A	74.2 ± 9.8 a A	70.6 ± 39.5 a A	51.6 ± 5.0 a A
LSD = 159.3	5	27.9 ± 4.5 a A	44.7 ± 3.0 a A	51.2 ± 7.0 a A	35.0 ± 3.8 a A
	10	59.5 ± 3.8 a A	0 a A	25.9 ± 0.1 a A	74.7 ± 4.5 a A
1-hexanol	1	54.6 ± 5.4 a A	96.7 ± 24.2 a A	36.2 ± 16.2 a A	49.9 ± 6.2 a A
LSD = 108.6	5	36.6 ± 8.7 a A	48.9 ± 3.3 a A	22.1 ± 8.0 a A	22.7 ± 9.7 a A
	10	37.2 ± 5.7 a A	0 a A	33.5 ± 7.6 a A	15.4 ± 1.7 a A

<sup>a</sup> Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg) and are the result of 24-h collection. Means within the same day of ripening followed by the same small letters are not significantly different at *p* = 0.05 (LSD Tukey's test). Means within the same cold-storage condition followed by the same capital letters are not significantly different at *p* = 0.05 (LSD Tukey's test). <sup>b</sup> Days of ripening at 20 °C.

**Table 5. Effect of O<sub>2</sub> and CO<sub>2</sub> Treatments on Ester and Alcohol Amounts (Micrograms) from Starking Delicious Apples after 5 Months of Storage plus 1, 5, and 10 Days of Ripening at 20 °C<sup>a</sup>**

compound	days at <i>T</i> <sub>amb</sub> <sup>b</sup>	21% O <sub>2</sub> /0.03% CO <sub>2</sub>	3% O <sub>2</sub> /3% CO <sub>2</sub>	2% O <sub>2</sub> /2% CO <sub>2</sub>	1% O <sub>2</sub> /1% CO <sub>2</sub>
Esters					
ethyl acetate	1	2772.2 ± 958.4 a C	977.3 ± 267.6 b C	2149.0 ± 382.7 a C	856.7 ± 125.0 b B
LSD = 698.5	5	8047.6 ± 827.8 a A	4399.0 ± 852.9 c B	5928.8 ± 770.4 b A	3598.6 ± 314.9 d A
	10	4176.4 ± 906.4 b B	5576.5 ± 1788.8 a A	3144.5 ± 468.1 c B	478.4 ± 31.73 d B
propyl acetate	1	1706.6 ± 326.2 c B	2777.1 ± 398.1 ab C	2654.1 ± 806.1 b C	3119.2 ± 303.6 a A
LSD = 390.0	5	2419.3 ± 168.3 b A	3575.4 ± 397.4 a B	3390.9 ± 111.1 a B	2692.5 ± 998.3 b B
	10	1734.2 ± 357.9 b B	4263.8 ± 670.2 a A	4452.0 ± 224.3 a A	417.8 ± 132.1 c C
butyl acetate	1	1307.4 ± 279.8 c A	2085.1 ± 243.5 a A	2060.7 ± 144.3 a A	549.2 ± 15.8 b A
LSD = 165.4	5	651.9 ± 19.2 c B	1481.7 ± 101.4 a B	901.1 ± 63.5 b B	400.7 ± 34.9 d A
	10	357.6 ± 76.6 b C	935.7 ± 131.3 a C	1023.9 ± 74.7 a B	322.6 ± 49.4 b A
pentyl acetate	1	61.94 ± 16.49 c AB	124.4 ± 22.5 b A	158.3 ± 16.9 a B	62.3 ± 5.0 c A
LSD = 11.8	5	63.1 ± 6.2 c A	119.8 ± 13.3 a A	93.4 ± 6.2 b C	60.9 ± 5.1 c A
	10	50.5 ± 8.3 b B	117.5 ± 17.8 a A	114.5 ± 15.4 a B	15.9 ± 2.1 c B
hexyl acetate	1	3473.8 ± 411.0 b A	5860.1 ± 683.2 a A	5791.7 ± 236.9 a A	1520.9 ± 186.2 c A
LSD = 280.9	5	2342.3 ± 269.8 b B	3066.8 ± 307.4 a B	2832.2 ± 114.7 a B	844.6 ± 103.5 c B
	10	1215.8 ± 235.8 b C	1957.6 ± 71.1 a C	1786.0 ± 283.1 a C	205.6 ± 17.5 c C
2-methylpropyl acetate	1	222.0 ± 107.1 c C	773.3 ± 113.4 b C	970.8 ± 103.9 a B	639.6 ± 102.3 b B
LSD = 161.9	5	486.5 ± 70.4 c B	1257.6 ± 331.1 a B	1058.0 ± 219.3 b B	959.1 ± 192.0 b A
	10	959.5 ± 206.7 b A	1998.9 ± 24.9 a A	1854.8 ± 332.4 a A	81.9 ± 12.0 c C
2-methylbutyl acetate	1	2001.9 ± 836.7 c A	7440.0 ± 625.5 a AB	8412.8 ± 1241.7 a A	4475.0 ± 335.9 b B
LSD = 1039.0	5	1450.3 ± 397.5 b A	6908.6 ± 1312.0 a B	6556.0 ± 506.6 a B	5966.0 ± 404.4 a A
	10	1241.1 ± 251.8 b A	7994.1 ± 2001.5 a A	7608.9 ± 663.4 a A	1452.2 ± 168.1 b C
ethyl propionate	1	1081.0 ± 130.0 d B	1504.1 ± 284.1 c A	2284.8 ± 535.6 a A	1833.2 ± 96.2 b A
LSD = 275.4	5	1387.0 ± 177.8 a A	1264.7 ± 141.5 a A	1322.9 ± 134.7 a B	1490.2 ± 595.1 a B
	10	817.7 ± 296.3 b B	1289.6 ± 580.9 a A	1062.7 ± 84.7 ab B	263.0 ± 136.2 c C
tert-butyl propionate	1	1322.2 ± 687.8 a C	756.1 ± 220.2 a C	1509.7 ± 228.8 a C	726.0 ± 103.6 a B
LSD = 1700.7	5	11014.5 ± 1461.2 a B	4133.9 ± 1785.2 b B	10506.4 ± 1873.5 a B	3876.5 ± 1753.0 b A
	10	13721.2 ± 3142.0 b A	17298.9 ± 345.0 a A	16856.1 ± 5810.2 a A	240.2 ± 59.0 c B
hexyl propionate	1	61.0 ± 7.9 d A	268.1 ± 34.9 a A	248.5 ± 29.4 b A	88.2 ± 9.7 c A
LSD = 14.5	5	61.9 ± 9.7 c A	143.2 ± 18.8 a B	119.0 ± 11.6 b B	48.2 ± 6.7 c B
	10	46.2 ± 1.5 b B	117.1 ± 11.2 a C	126.0 ± 10.8 a B	21.1 ± 1.9 c C
ethyl butyrate	1	4840.8 ± 869.5 a B	2395.7 ± 824.6 a C	6082.2 ± 323.6 a B	1832.8 ± 174.1 a A
LSD = 7121.8	5	54348.9 ± 1732.3 a A	14792.2 ± 6728.2 c B	25208.2 ± 2675.9 b A	5761.9 ± 1553.6 d A
	10	49256.1 ± 6962.2 a A	29976.7 ± 544.1 b A	22812.1 ± 6994.5 c A	792.8 ± 144.2 d A
ethyl 2-methylbutyrate	1	3124.5 ± 772.7 b C	2957.7 ± 783.4 b C	7317.2 ± 1820.2 a B	3246.2 ± 1389.0 b B
LSD = 3645.0	5	18687.7 ± 2415.2 b B	14118.0 ± 6184.0 c B	31285.1 ± 1511.0 a A	13574.6 ± 5006.8 c A
	10	32010.9 ± 2849.4 b A	42203.1 ± 2062.5 a A	33186.4 ± 6077.9 b A	1243.3 ± 197.1 c B
butyl butyrate	1	1136.0 ± 159.6 b A	1584.3 ± 40.3 a A	721.0 ± 444.7 c B	356.5 ± 44.4 d A
LSD = 133.1	5	409.3 ± 50.9 c B	918.7 ± 108.1 a B	631.3 ± 66.6 b B	443.4 ± 288.0 c A
	10	270.2 ± 67.3 c C	707.7 ± 204.3 b C	1020.2 ± 90.6 a A	27.8 ± 5.2 d B
hexyl 3-methylbutyrate	1	282.7 ± 72.5 c A	347.2 ± 84.4 b B	643.8 ± 66.7 a A	285.5 ± 29.0 c A
LSD = 47.3	5	206.8 ± 17.9 c B	520.5 ± 130.2 a A	366.8 ± 45.6 b B	160.3 ± 21.0 c B
	10	109.7 ± 6.0 c C	222.3 ± 33.8 b C	347.1 ± 37.7 a B	28.8 ± 1.9 d C
hexyl butyrate	1	423.7 ± 83.6 b A	312.0 ± 37.4 c A	507.9 ± 97.0 a A	150.7 ± 13.4 d B
LSD = 41.9	5	194.4 ± 11.7 c B	266.9 ± 46.6 b B	220.9 ± 21.1 c B	359.9 ± 58.0 a A
	10	92.9 ± 5.5 b C	121.9 ± 9.4 b C	245.9 ± 19.9 a B	17.5 ± 4.5 c C
ethyl hexanoate	1	5321.1 ± 215.5 a B	1828.5 ± 585.0 b C	4841.0 ± 684.2 a A	0 b A
LSD = 2599.4	5	42297.8 ± 1595.6 a A	4414.6 ± 144.6 bc A	6091.7 ± 1968.4 b A	2275.6 ± 390.6 c A
	10	40197.8 ± 9018.0 a A	11516.6 ± 1364.1 b B	5686.0 ± 1676.8 c A	180.8 ± 43.5 d A
Alcohols					
ethanol	1	30.3 ± 3.5 a A	41.1 ± 5.3 a A	49.9 ± 10.5 a A	48.7 ± 6.6 a A
LSD = 153.2	5	43.2 ± 3.5 a A	33.1 ± 1.8 a A	36.7 ± 3.1 a A	58.4 ± 11.7 a A
	10	35.9 ± 12.3 a A	37.3 ± 13.1 a A	39.7 ± 12.8 a A	12.5 ± 8.7 a A
1-propanol	1	1947.7 ± 366.1 c A	2960.5 ± 384.0 b A	3228.9 ± 704.5 ab A	3430.6 ± 311.8 a A
LSD = 363.0	5	2098.3 ± 285.7 b A	2329.1 ± 160.3 b B	2237.8 ± 149.4 b B	3377.7 ± 637.8 a A
	10	1375.4 ± 443.0 c B	2281.1 ± 699.6 a B	1772.0 ± 215.9 b C	82.9 ± 12.6 d B
2-methyl-1-propanol	1	507.0 ± 32.8 c A	677.2 ± 82.0 b A	732.3 ± 165.0 b A	826.8 ± 134.7 a A
LSD = 75.8	5	454.6 ± 28.0 c A	597.9 ± 48.2 b B	480.0 ± 5.2 c B	702.2 ± 152.8 a B
	10	366.3 ± 61.9 b B	563.0 ± 156.6 a B	429.8 ± 37.0 b B	34.2 ± 7.6 c C
1-butanol	1	731.1 ± 160.7 a A	231.4 ± 85.8 c A	352.1 ± 45.6 b A	157.1 ± 18.7 d A
LSD = 49.5	5	490.1 ± 34.9 a B	278.9 ± 67.7 b A	116.4 ± 31.7 c B	107.5 ± 26.7 c B
	10	744.8 ± 50.2 a A	96.7 ± 5.0 b B	94.7 ± 5.7 b B	67.8 ± 11.8 b B
2-methyl-1-butanol	1	344.5 ± 110.9 c A	955.8 ± 161.9 b A	1718.6 ± 324.5 a A	1867.5 ± 205.5 a B
LSD = 159.3	5	287.2 ± 33.5 c A	1078.7 ± 120.1 b A	1175.8 ± 274.4 b B	2075.3 ± 219.0 a A
	10	296.4 ± 43.1 c A	708.0 ± 187.6 b B	1035.7 ± 162.7 a B	126.9 ± 24.3 d C
1-hexanol	1	701.4 ± 235.1 d A	1071.5 ± 200.4 c A	2143.4 ± 83.7 a A	1195.8 ± 132.9 b A
LSD = 108.6	5	717.8 ± 163.5 b A	675.1 ± 26.6 b B	897.5 ± 64.2 a B	391.8 ± 92.5 c B
	10	451.5 ± 62.2 b B	368.2 ± 45.0 b C	588.6 ± 60.3 a C	30.0 ± 2.7 c C

<sup>a</sup> Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg) and are the result of 24-h collection. Means within the same day of ripening followed by the same small letters are not significantly different at *p* = 0.05 (LSD Tukey's test). Means within the same cold-storage condition followed by the same capital letters are not significantly different at *p* = 0.05 (LSD Tukey's test). <sup>b</sup> Days of ripening at 20 °C.

**Table 6. Effect of O<sub>2</sub> and CO<sub>2</sub> Treatments on Ester and Alcohol Amounts (Micrograms) from Starking Delicious Apples after 7 Months of Storage plus 1, 5, and 10 Days of Ripening at 20 °C<sup>a</sup>**

compound	days at T <sub>amb</sub> <sup>b</sup>	21% O <sub>2</sub> /0.03% CO <sub>2</sub>	3% O <sub>2</sub> /3% CO <sub>2</sub>	2% O <sub>2</sub> /2% CO <sub>2</sub>	1% O <sub>2</sub> /1% CO <sub>2</sub>
Esters					
ethyl acetate	1	169.4 ± 13.8 b A	44.0 ± 8.4 b B	54.0 ± 5.7 b A	1074.9 ± 458.9 a A
LSD = 698.5	5	208.6 ± 178.0 a A	198.4 ± 66.9 a B	95.8 ± 19.3 a A	784.5 ± 146.1 a AB
	10	753.5 ± 306.3 b A	1594.3 ± 136.4 a A	503.5 ± 128.5 b A	319.0 ± 81.3 b B
propyl acetate	1	136.3 ± 44.9 a A	90.1 ± 23.2 a A	82.4 ± 15.3 a A	96.7 ± 12.8 a B
LSD = 390.0	5	75.2 ± 18.9 a A	192.6 ± 45.7 a A	79.5 ± 12.8 a A	251.5 ± 34.5 a AB
	10	89.8 ± 43.2 b A	133.4 ± 9.5 b A	265.5 ± 98.8 ab A	548.5 ± 124.3 a A
butyl acetate	1	808.1 ± 120.0 a A	464.9 ± 118.8 b A	319.3 ± 104.5 b A	88.0 ± 8.7 c A
LSD = 165.4	5	263.8 ± 57.1 a B	276.2 ± 74.0 a B	91.2 ± 35.1 b B	108.1 ± 7.6 ab A
	10	183.9 ± 21.6 a B	147.1 ± 5.7 a B	191.7 ± 37.1 a AB	182.3 ± 23.2 a A
pentyl acetate	1	22.7 ± 2.3 a A	18.7 ± 3.8 a B	13.3 ± 4.9 a B	14.8 ± 1.6 a AB
LSD = 11.8	5	17.0 ± 4.1 bc A	61.0 ± 4.9 a A	11.5 ± 2.4 c B	23.8 ± 3.4 b A
	10	0 b B	9.1 ± 1.4 b B	27.6 ± 9.2 a A	8.2 ± 1.5 b B
hexyl acetate	1	807.8 ± 18.4 a A	488.0 ± 129.7 b A	218.9 ± 111.4 bc A	113.1 ± 21.8 c A
LSD = 280.9	5	329.8 ± 71.6 ab B	479.4 ± 124.0 a A	175.7 ± 27.3 b A	142.2 ± 15.0 b A
	10	150.5 ± 12.6 a B	193.2 ± 8.9 a B	228.3 ± 15.4 a A	148.4 ± 18.8 a A
2-methylpropyl acetate	1	41.4 ± 5.0 a A	34.1 ± 9.7 a A	39.9 ± 8.9 a A	65.1 ± 26.5 a A
LSD = 161.9	5	28.4 ± 8.3 a A	71.3 ± 15.1 a A	25.1 ± 8.7 a A	97.7 ± 12.1 a A
	10	47.7 ± 4.2 a A	135.9 ± 13.6 a A	127.9 ± 21.1a A	31.9 ± 8.9 a A
2-methylbutyl acetate	1	612.8 ± 116.5 a A	966.4 ± 264.6 a A	897.5 ± 166.8 a A	596.3 ± 103.5 a A
LSD = 1039.0	5	231.9 ± 37.5 a A	958.3 ± 284.1 a A	346.9 ± 138.2 a A	583.2 ± 15.8 a A
	10	183.3 ± 19.1 a A	465.7 ± 70.9 a A	742.7 ± 94.5 a A	532.3 ± 115.5 a A
ethyl propionate	1	130.2 ± 46.3 a A	36.5 ± 11.2 a A	49.8 ± 10.8 a A	14.6 ± 1.9 a B
LSD = 275.4	5	64.7 ± 22.9 a A	180.0 ± 58.0 a A	54.3 ± 7.2 a A	42.4 ± 5.8 a B
	10	38.1 ± 5.6 b A	0 b A	186.7 ± 8.6 b A	561.1 ± 172.1 a A
<i>tert</i> -butyl propionate	1	48.9 ± 7.4 a A	0 a A	35.9 ± 9.9 a A	21.0 ± 0.8 a A
LSD = 1700.7	5	252.5 ± 77.0 a A	217.6 ± 59.0 a A	36.8 ± 17.9 a A	260.7 ± 2.9 a A
	10	705.0 ± 96.9 a A	8.2 ± 0.4 a A	563.6 ± 94.8 a A	189.7 ± 36.6 a A
hexyl propionate	1	49.9 ± 4.6 a A	61.5 ± 13.5 a A	50.0 ± 8.2 a A	20.1 ± 4.3 b A
LSD = 14.5	5	0 a B	56.7 ± 12.9 a A	30.6 ± 7.0 b B	19.5 ± 0.7 b A
	10	0 b B	20.7 ± 4.3 a B	28.2 ± 4.9 a B	14.3 ± 1.3 ab A
ethyl butyrate	1	964.4 ± 230.7 a A	55.1 ± 13.2 a A	77.6 ± 17.7 a A	55.5 ± 4.6 a A
LSD = 7121.8	5	3006.6 ± 669.1 a A	1876.1 ± 523.0 a A	323.8 ± 53.7 a A	481.0 ± 79.8 a A
	10	3377.9 ± 336.8 a A	1321.1 ± 187.5 a A	1815.4 ± 406.1 a A	640.6 ± 125.9 a A
ethyl 2- methylbutyrate	1	222.2 ± 65.2 a A	48.1 ± 7.9 a A	74.0 ± 6.3 a A	127.2 ± 26.1 a A
LSD = 3645.0	5	770.8 ± 391.1 a A	1562.0 ± 16.4 a A	489.4 ± 73.9 a A	854.3 ± 85.3 a A
	10	1456.0 ± 334.7 a A	1638.9 ± 62.7 a A	2858.8 ± 261.2 a A	1225.0 ± 317.8 a A
butyl butyrate	1	159.7 ± 45.5 ab A	244.2 ± 44.7 a A	31.0 ± 14.6 bc A	0 c A
LSD = 133.1	5	31.1 ± 7.7 a AB	54.5 ± 17.8 a B	18.4 ± 6.5 a A	0 a A
	10	0 a B	10.4 ± 2.2 a B	35.9 ± 12.3 a A	4.7 ± 1.3 a A
hexyl 3-methylbutyrate	1	57.7 ± 6.0 a A	41.9 ± 6.3 a B	42.4 ± 18.7 a A	21.5 ± 6.0 a A
LSD = 47.3	5	17.0 ± 4.1 b AB	127.1 ± 27.7 a A	83.9 ± 2.4a A	37.6 ± 8.5 b A
	10	0 b B	17.6 ± 3.1 ab B	60.7 ± 14.5 a A	35.5 ± 2.5 ab A
hexyl butyrate	1	169.9 ± 20.6 a A	54.9 ± 3.7 a B	38.5 ± 9.4 a A	26.7 ± 6.7 a A
LSD = 41.9	5	53.3 ± 3.3 b B	99.8 ± 16.3 a A	60.0 ± 3.3 ab A	32.2 ± 9.4 b A
	10	0 b C	17.5 ± 4.5 ab B	45.7 ± 6.8 a A	32.9 ± 1.5 ab A
ethyl hexanoate	1	297.2 ± 75.3 a A	73.0 ± 22.1 a A	31.3 ± 8.0 a A	37.1 ± 5.3 a A
LSD = 2599.4	5	1408.8 ± 447.2 a A	446.8 ± 200.0 a A	148.0 ± 31.0 a A	171.9 ± 7.2 a A
	10	956.3 ± 173.3 a A	464.2 ± 39.9 a A	502.5 ± 58.6 a A	219.8 ± 39.0 a A
Alcohols					
ethanol	1	0 b A	0 b B	0 b A	1168.9 ± 571.3 a A
LSD = 153.2	5	0 a A	0 a B	0 a A	482.9 ± 50.8 a B
	10	16.0 ± 2.2 b A	1075.6 ± 135.4 a A	14.7 ± 5.2 b A	0 b C
1-propanol	1	54.0 ± 21.0 a A	29.5 ± 9.5 a A	30.2 ± 4.1 a A	27.9 ± 2.3 a A
LSD = 363.0	5	30.5 ± 9.0 a A	60.0 ± 16.1 a A	26.3 ± 2.6 a A	47.8 ± 4.1 a A
	10	28.5 ± 16.8 a A	34.3 ± 28.3 a A	63.0 ± 21.3 a A	179.4 ± 15.1 a A
2-methyl-1-propanol	1	27.1 ± 6.4 a A	0 a A	0 a A	13.9 ± 1.4 a A
LSD = 75.8	5	0 a A	0 a A	0 a A	14.6 ± 4.2 a A
	10	0 a A	10.9 ± 2.6 a A	30.6 ± 1.4 a A	59.2 ± 4.9 a A
1-butanol	1	153.6 ± 13.9 a A	28.0 ± 10.0 b A	34.6 ± 4.9 b A	25.9 ± 1.5 b A
LSD = 49.5	5	38.5 ± 11.5 a B	19.1 ± 12.4 a A	12.9 ± 2.8 a A	31.3 ± 4.4 a A
	10	44.0 ± 8.7 a B	18.5 ± 2.3 a A	23.1 ± 2.6 a A	26.9 ± 5.1 a A
2-methyl-1-butanol	1	150.8 ± 21.5 a AB	126.7 ± 16.2 a A	100.7 ± 11.6 a A	1.9 ± 23.6 a A
LSD = 159.3	5	213.1 ± 28.0 ab A	56.4 ± 14.6 b A	259.4 ± 70.1 a A	66.7 ± 12.4 b A
	10	3.5 ± 2.7 b B	28.8 ± 6.0 b A	278.0 ± 118.4 a A	83.9 ± 20.2 b A
1-hexanol	1	147.3 ± 24.8 b A	35.1 ± 8.4 b A	51.3 ± 21.4 ab A	52.1 ± 5.4 ab A
LSD = 108.6	5	57.8 ± 6.5 a AB	95.6 ± 8.8 a A	62.0 ± 11.8 a A	29.3 ± 2.7 a A
	10	30.6 ± 3.2 a B	21.9 ± 2.1 a A	28.2 ± 4.9 a A	14.3 ± 1.3 a A

<sup>a</sup> Values are means of four replicate extract samples each obtained from four or five apples (≈1 kg) and are the result of 24-h collection. Means within the same day of ripening followed by the same small letters are not significantly different at  $p = 0.05$  (LSD Tukey's test). Means within the same cold-storage condition followed by the same capital letters are not significantly different at  $p = 0.05$  (LSD Tukey's test). <sup>b</sup> Days of ripening at 20 °C.



NCS and after 5 days at 20 °C. Since this compound has a lower odor threshold than the other hexyl esters, it may favorably compensate for their decrease (Table 5). Furthermore, the ambient temperature (20 °C) may favor the synthesis of ethyl hexanoate compared to the other hexyl esters.

The favorable effect of higher temperature after 5 months of storage is especially significant in *tert*-butyl propionate and ethyl butyrate, showing a large increase after 5 days in all storage conditions except in ULO. Three or seven months of storage and a stage at ambient temperature do not induce significant differences in the amounts of 2-methylpropyl and 2-methylbutyl acetates, *tert*-butyl propionate, or ethyl butyrate or hexanoate.

Alcohol production is favored by our CA compared to NCS (Figure 3A). The highest productions were observed in 2% O<sub>2</sub>/CO<sub>2</sub> (LO) after 1 day of ripening at 20 °C and in 3% O<sub>2</sub>/CO<sub>2</sub> (CA) after 10 days of ripening at 20 °C. After 7 months, all storage conditions influence the total alcohol concentration (Figure 4A) after 1 and 10 days of ripening at 20 °C. ULO gives the higher concentration after 1 day of ripening at 20 °C and 3% O<sub>2</sub>/CO<sub>2</sub> (CA) after 10 days. The storage time has a higher effect on the alcohol concentrations (Tables 4–6a). As with the esters (Tables 4–6), 5 months of storage gave the highest alcohol concentrations.

Differences in ethanol content after 3 and 5 months of storage (Tables 4 and 5) are not due to storage conditions or time at 20 °C. After 7 months (Table 6), ethanol is detected after 10 days of ripening at 20 °C except with ULO, for which the highest concentration is obtained the first day and it decreases afterward. After 5 months of storage (Table 5), there is a decrease of 1-propanol at ambient temperature at the same time that an increase of propyl acetate in fruit coming from CA and LO is found. This may confirm the biochemical origin of propyl acetate from 1-propanol and the favorable effect of poststorage permanence at ambient temperature as observed by Hansen et al. (1992) with Jonagold apples.

Production of 2-methyl-1-propanol and 1-hexanol (Tables 4–6a) is influenced by both storage conditions and stay at 20 °C. Five months of storage gives the highest concentration after 1 day of ripening at 20 °C. In the same conditions there is a decrease in hexyl acetate. According to Harb et al. (1994), 1-hexanol favors production of hexyl acetate, and although results confirm  $\beta$ -oxidation of 1-propanol to propyl acetate, it is not the case with 1-hexanol. Propyl esters are synthesized differently from other straight-chain esters (Hansen et al., 1992), and different aliphatic alcohols such as 1-hexanol may be found as conjugated glucosides in apples (Schwab and Schreier, 1990). NCS gives the higher 1-butanol concentration. After 3 and 7 months, 1-propanol and 2-methyl-1-propanol concentrations are not influenced by storage conditions. However, after 7 months there are differences in production of 1-butanol and 1-hexanol (Table 6).

The conditions that produce anaerobic metabolism, with high contents of ethyl acetate and ethanol in CA storage with very low oxygen (ULO), are observed after 7 months of storage and not before (Table 6).

The esters were the main compounds at harvest date and during ripening at 20 °C after cold storage in all conditions. Ethyl 2-methylbutyrate, ethyl butyrate, and ethyl hexanoate were the ones that contributed most to the Starking Delicious overall aroma in this work.

Volatile emission after 5 months of storage was the highest. We can conclude that this storage period was optimum for maximum volatile production. The suppressive effect of CA on total volatile emission compared with normal cold storage was significant only after 5 months of storage.

LO (2% O<sub>2</sub>/CO<sub>2</sub>) was the best combination within CA conditions, and ULO (1% O<sub>2</sub>/CO<sub>2</sub>) had a depressing effect on total volatile emission. CA (3% O<sub>2</sub>/CO<sub>2</sub>) and LO (2% O<sub>2</sub>/CO<sub>2</sub>) conditions did not suppress the synthesis of branched-chain esters or some straight chain esters.

Ripening in air at 20 °C favored the synthesis of the majority of volatile compounds after cold storage, especially after 5 months of storage, but the ability of fruits to produce volatile compounds during shelf life after cold storage declined as storage time increased beyond 5 months.

#### ABBREVIATIONS USED

CA, controlled atmosphere; FID, flame ionization detector; I<sub>2</sub>-IK, iodine and potassium iodure solution; LO, low oxygen; NCS, normal cold storage; RH, relative humidity; SSC, soluble solids content; TA, titratable acidity; ULO, ultralow oxygen.

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