

Relationships between Volatile Production, Fruit Quality, and Sensory Evaluation in Granny Smith Apples Stored in Different Controlled-Atmosphere Treatments by Means of Multivariate Analysis

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Aroma compounds, quality parameters, and sensory evaluation of Granny Smith apples were analyzed after 3, 5, and 7 months of cold storage in three controlled-atmosphere (CA) treatments, in which oxygen and carbon dioxide were held at 1, 2, and 3%. During poststorage ripening, the apples were kept at 20 °C for 1, 5, and 10 days before analytical measurements were made. The highest volatile emission was obtained after 5 months of storage in all CA treatments, reaching its highest value when a low-oxygen CA (LO) was used. Ultralow-oxygen CA (ULO) showed the highest ability to maintain apple firmness. The correlation among analytical and sensory parameters suggests that ethyl 2-methylbutyrate, 1-butanol, pentyl acetate, and *tert*-butyl propionate are the aroma compounds with the highest influence in the sensorial score. Concerning CA treatments, LO and ULO appear to be very valuable technologies for maintaining the sensorial quality even after 7 months of storage.

Keywords: *Granny Smith; poststorage quality; aroma compounds; CA treatments; principal components analysis; partial least-squares regression*

INTRODUCTION

Most studies on the Granny Smith variety of apple are concerned with scald development during cold storage (Ingle and D'Souza, 1989; Truter et al., 1994; Bauchot et al., 1995), but there are a few studies on its aroma composition (Mosandl et al., 1991) or changes during cold storage.

Controlled-atmosphere (CA) storage significantly decreases the flavor of apples because the production of the volatile compounds responsible for apple aroma is reduced (Hansen et al., 1992; Brackmann et al., 1994; Song and Bangerth, 1994), although only a few of the compounds emanating from apples have a decisive impact on the sensory quality (Cunningham et al., 1986). The suppressive effect depends on the composition of the atmosphere and on the length of storage, particularly in an ultralow-oxygen and high-CO₂ atmosphere (Brackmann et al., 1993). Furthermore, the CA influences the poststorage ripening of fruit at ambient temperatures (Streif and Bangerth, 1988; Yahia, 1991).

Aroma volatile compounds are responsible for aroma, but the organoleptic quality of a food also depends on the taste. In apples the taste is determined by the acid/sugar ratio and texture. Under CA treatments, minimal loss of firmness, titratable acidity, and soluble solids concentration occurs in comparison with normal cold-storage conditions (Chen et al., 1985; Kader, 1986).

Research correlating analytical measurements with sensory measurements is limited. Bourne (1979) out-

lined the difficulties associated with correlating analytical and sensory measurements due to high fruit-to-fruit variation. In building up sensory models based on measurements of aroma components by gas chromatography (GC), a conventional multiple linear regression approach is not advisable because many problems of multicollinearity have been found. Researchers have thus turned to bilinear multivariate procedures, of which the most common are principal component regression and partial least-squares regression (Hoerl and Kennard, 1970; Martens and Naes, 1989; Brockhoff et al., 1993). Among the advantages of such techniques we have found that collinearity effects are avoided.

The aim of this study was to analyze the relationships between sensory evaluation and aroma volatile compounds of Granny Smith apples to determine which volatile compounds mainly influence aroma, the relationships between quality parameters and volatile compounds, and the influence on such relationships of the CA treatment in order to conserve the quality of fruit for a long time.

MATERIALS AND METHODS

Plant Material and Fruit Quality Analysis. Apples from 10-year-old Granny Smith (*Malus communis* Borkh.) trees on MM-106 rootstock grown in Lleida (northeastern Spain) were harvested at commercial maturity (183 days after full bloom). Firmness, soluble solids concentration (SSC), titratable acidity (TA), starch hydrolysis, and fruit size were analyzed at harvest and after removal from cold storage. Firmness was measured with an 11-mm-tip penetrometer (Effegi, Milan, Italy) on two opposite sides of the apples. SSC and TA were measured in juice pressed from the whole fruit. The SSC was determined with a hand refractometer (Atago, Tokyo, Japan) and the TA by titrating 10 mL of juice with 0.1 N NaOH to pH 8 using

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phenolphthaleine and calculating the result as malic acid. Starch hydrolysis was rated visually using a 1–6 scale (1, full starch; 6, no starch) after an equatorial section had been stained with a 0.5% I₂–KI solution.

Determination of Ethylene Production and Respiration. To measure respiration (as carbon dioxide production) and ethylene, six apples divided into two replicates were weighed, placed in 5-L jars, and continuously aerated with humidified air at 20 °C. Ethylene production was measured by taking gas samples from the effluent air with a 1 mL syringe from respiration jars where the fruit was continuously aerated with humidified air at a rate of ~2 L/h at 20 °C. Gas samples were injected into a gas chromatograph (Hewlett-Packard 5890 Series II, Barcelona, Spain) equipped with an flame ionization detector (FID) and an alumina column (1.5 m × 3 mm). Gas analyses were conducted isothermally at 100 °C. N₂ carrier, air, and H₂ flows were 45, 400, and 45 mL/min, respectively. The injector and detector were held at 120 and 180 °C, respectively. Carbon dioxide production was measured directly by connecting the effluent tube to an infrared analyzer unit.

Storage Conditions and Experimental Assay. Immediately after harvest, three lots of 42 kg of apples were selected for uniformity and stored at 1 °C in semicommercial cold-storage rooms with different CA treatments. Relative humidity (RH) was between 92 and 95% throughout the experiment in all cold-storage rooms. Storage conditions were as follows: standard CA (SCA), 2.8–3% O₂/2.8–3% CO₂; low-oxygen CA (LO), 1.8–2% O₂/1.8–2% CO₂; and ultralow-oxygen CA (ULO), 0.8–1% O₂/0.8–1% CO₂.

Samples of fruit were taken from each CA storage room after 3, 5, and 7 months of storage. Four replicates of ~1 kg of apples were used to analyze the volatile compounds after 1, 5, and 10 days of shelf life at room temperature (20 °C). Ten apples were used to measure the fruit quality parameters and another six were used to determine CO₂ and ethylene production as described above. Sensory evaluation was performed with 12 apples.

Analysis of Aroma Volatile Compounds. For the extraction of aroma volatile compounds from intact apples, the dynamic headspace method was used, and the volatile compounds were quantified by reference to an internal standard (butylbenzene) described previously by López et al. (1998a). The identification and quantification of the volatile compounds was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a capillary column [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 (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: hold at 70 °C for 1 min, raised from 70 to 142 °C at 3 °C/min, hold at 142 °C for 2 min, raised from 142 to 230 °C at 25 °C/min, and hold at 230 °C for 5 min. A volume of 1 µL was injected in all analyses.

For the identification and confirmation of the different compounds a Hewlett-Packard 5890 GC coupled to a mass spectrometer (GC/MS) equipped with the same capillary column as in the GC analyses was used. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as the 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, raised from 40 to 210 °C at 5 °C/min, and 210 °C for 15 min was used in the GC. The spectrometric data were compared with those from the NIST HP59943C original library mass-spectra (Hewlett-Packard).

Sensory Analysis. For sensory evaluation, fruit from the three CA treatments was removed to an air-conditioned room at 20 °C. Apple samples were peeled and quartered, and one part of each one (corresponding to each CA treatment) was put on a white plate and immediately presented to a taste panel of 30 untrained judges (18 female, 12 male). Each part was identified by a random three-digit code. The order of presentation of the three parts on the white plates was randomized for each of the judges, and they assessed all samples at the same time (each one was from a different CA

Table 1. Meaning of the X, Y, and Z Values of the Sample Generic Labels

	1	2	3
X	3 months	5 months	7 months
Y ^a	ULO	LO	SCA
Z	1 day	5 days	10 days

^a ULO (1% O₂/1% CO₂), LO (2% O₂/2% CO₂), and SCA (3% O₂/3% CO₂).

Table 2. Aroma Volatile Compound Production by Granny Smith Apples at Harvest Date

compound	code ^a	amount, ^b µg/kg	RI ^c
methyl acetate	AMETIL	ND	841
ethyl acetate	ACETIL	15.1 ± 1.5	895
propyl acetate	APRIL	53.8 ± 12.7	990
butyl acetate	ABUTIL	30.2 ± 0.8	1086
pentyl acetate	ACPENT	13.0 ± 1.1	1194
hexyl acetate	ACHEXI	9.7 ± 0.4	1283
2-methylpropyl acetate	A2MEPR	24.8 ± 8.2	1023
2-methylbutyl acetate	A2MBUT	5.1 ± 0.2	1134
ethyl propionate	PRETIL	64.9 ± 10.1	925
tert-butyl propionate	PRTBUT	10.9 ± 2.6	967
ethyl butyrate	BUETIL	ND	1047
butyl butyrate	BUTBUT	ND	1229
hexyl butyrate	BUHEXIL	ND	1433
ethyl 2-methylbutyrate	M2BUET	10.7 ± 2.7	1061
ethyl 3-methylbutyrate	M3BUET	ND	1069
hexyl 3-methylbutyrate	M3BUHEX	8.4 ± 0.9	1442
ethyl hexanoate	HEXETIL	ND	1245
ethanol	ETANOL	4.3 ± 1.1	936
1-propanol	PROPOL	9.2 ± 2.4	1042
1-butanol	BUTOL	ND	1148
2-methyl-1-propanol	MEPROL	18.9 ± 0.1	1095
2-methyl-1-butanol	MEBUTOL	ND	1213
1-hexanol	HEXAOL	ND	1363
nerol	NEROL	13.4 ± 3.2	1819
α-farnesene	FARNESE	ND	1923

^a Code of samples used in analyses of PCA and PLS. ^b Values are means ± SD of four replicate extract samples from four to five apples (~1 kg) 24 h after collection. Values at less than minimum detection capacity are indicated as ND (López et al., 1998). ^c Kovats retention index (Poole and Poole, 1993) in column cross-linked FFAP.

treatment) and were asked to rate overall fruit preference on a 9-point verbal scale (1, dislike very much; 9, like very much). All evaluations were conducted in individual booths under white illumination and at room temperature.

Statistical and Multivariate Analysis. A factorial design with storage time, CA treatments, poststorage shelf life, and replication as factors was used for volatile compound analyses. For quality parameters and sensory evaluation the same design was used, without the poststorage shelf-life factor. All data were tested by analysis of variance (GLM-ANOVA procedure) with the SAS program package (SAS Institute, 1988). Means were separated by Tukey's least significant difference test at $p < 0.05$ (LSD).

To provide a global overview of the samples, a principal component analysis (PCA) was developed with Unscrambler vers. 6.11a (CAMO ASA, 1997). Samples were characterized by the volatile emissions and measures of the quality parameters as described above. Sample names were coded XYZ, where X, Y, and Z refer to storage period, CA treatment, and days of shelf life, respectively, taking the values 1, 2, and 3 with the meaning indicated in Table 1. Quality parameters were coded as FIRM (firmness), TA (titratable acidity), SSC (solid soluble concentration), C2H4 (ethylene production), CO₂ (carbon dioxide production), and SENSE (sensory score). The codes for volatile aroma compounds are indicated in Table 2. Partial least-squares regression (PLS) was used to correlate quality parameters, sensory score, and volatile compounds. At first, volatile compounds as *x* variables were correlated with quality parameters as *y* variables by PLS2 regression and then both volatile compounds and quality parameters were used

Table 3. Quality Parameters of Granny Smith Apples Determined at Harvest Date and 1 Day after Removal from CA Storage^a

storage (months)	CA treatment ^b	firmness (N) LSD = 0.45	acidity (g L ⁻¹ of malic acid)		SSC (°Brix) LSD = 0.95
			LSD = 0.71	LSD = 0.71	
0	harvest	76.12	9.02	10.92	
3	SCA	73.40 a	7.09 b	11.88 a	
	LO	74.45 a	8.36 a	11.76 a	
	ULO	77.40 a	8.78 a	12.32 a	
5	SCA	61.90 b	6.95 b	13.31 a	
	LO	68.60 a	7.53 b	13.14 a	
	ULO	69.55 a	8.35 a	12.16 b	
7	SCA	61.90 c	6.27 b	12.56 a	
	LO	73.10 b	7.40 a	11.52 b	
	ULO	85.93 a	7.43 a	10.41 c	

^a Values are means from 10 apples that had remained at room temperature (20 °C for 1 day). Means followed by the same letter are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

as x variables to explain sensory score as y variable by PLS1 regression. Centering and weighting of the data by the inverse of the standard deviation (SD) of each variable was used as data pretreatment to avoid dependence on the measurement units (Martens and Naes, 1989).

RESULTS AND DISCUSSION

Fruits were harvested at commercial maturity when ethylene production and respiration were $3.6 \pm 0.3 \mu\text{L}/\text{kg}\cdot\text{h}$ and $8.6 \pm 0.9 \text{ mL}/\text{kg}\cdot\text{h}$, respectively, the starch index was 1.9 ± 0.2 , and fruit size was $84.6 \pm 1.5 \text{ mm}$. Firmness, TA, and SSC are shown in Table 3.

The volatile fraction emitted by the Granny Smith variety was collected and analyzed before the fruit was

placed in cold-storage conditions. The aroma compounds were identified and quantified at harvest date (Table 2), and the amounts of these were lower than obtained after different cold-storage periods (Tables 4–6). A total of 15 volatile compounds were detected, 11 of which were esters, which constitute >84% of the total aroma compounds emitted. Ethyl propionate, propyl acetate, butyl acetate, and 2-methylpropyl acetate represent 59.4% of the total aroma fraction.

Aroma Composition of Apples. A fast classification of the samples and a global overview of the relationships between each volatile compound and the CA treatments was obtained with a PCA of the samples described by the volatile measurements. Figure 1 shows the scores of the samples in the subspace defined by the first two principal components (PC1 and PC2), which explain up to 50% of the total variance. Scores of samples corresponding to $X = 1$ (3 months of storage) are clustered on the right of the plot. When storage time was increased to 5 months ($X = 2$), the scores moved to the left of the plot, becoming widely spread. Samples taken after 7 months of storage ($X = 3$) appear clustered just between the groups corresponding to $X = 1$ and $X = 2$. Within each storage period, the shelf-life period increases the score with respect to PC2 [samples with $Z = 3$ (10 days of shelf life) have the highest PC2 score]. This indicates that the storage time and the shelf life had the highest influence on the differences between samples. Conversely, the CA treatment did not present a clear pattern with respect to PC1 or PC2. Thus, CA treatment is not well correlated with the main PCs, which indicates a lower influence of this factor on the differentiation of these samples.

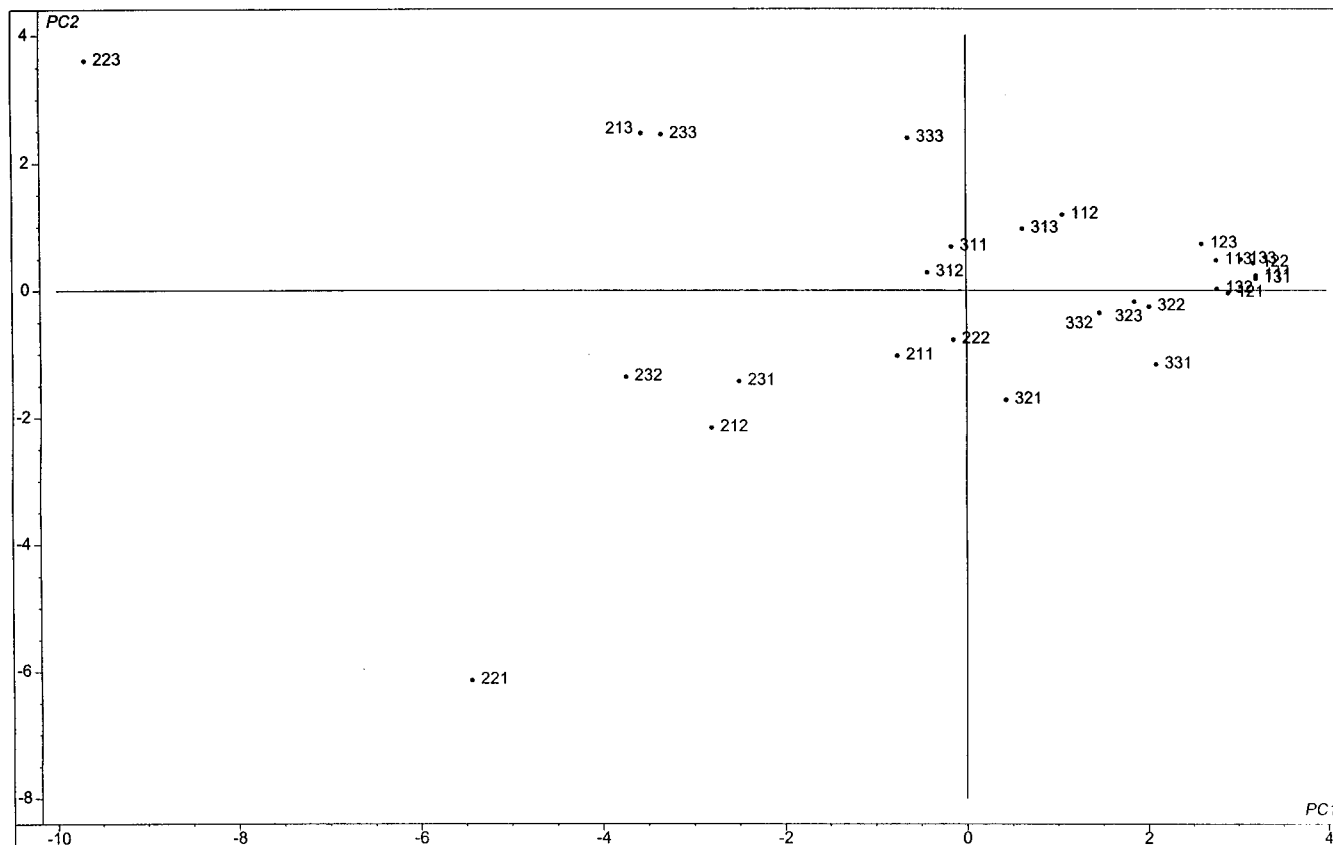


Figure 1. Scores plot corresponding to the PCA of Granny Smith apples characterized by the volatile compounds. Sample names were coded $X, Y,$ and Z , where $X, Y,$ and Z mean storage period, CA treatment, and days of shelf life, respectively, and those take the values 1, 2, and 3 with the meaning indicated in Table 1.

Table 4. Ester and Alcohol Production (Micrograms per Kilogram) by Granny Smith Apples during 1, 5, and 10 Days of Shelf Life (20 °C) after 3 Months of Cold Storage under Different CA Treatments^a

compound	shelf life ^b	SCA ^c	LO	ULO
Ester Production				
ethyl acetate LSD = 506.2	1	103.0 ± 49.7 a A	178.5 ± 78.5 a A	67.3 ± 23.4 a A
	5	157.3 ± 52.5 a A	210.0 ± 59.5 a A	384.0 ± 147.1 a A
	10	177.8 ± 54.7 a A	213.8 ± 34.9 a A	309.7 ± 106.0 a A
propyl acetate LSD = 81.7	1	70.5 ± 27.6 a B	0 a B	0 a A
	5	168.0 ± 102.5 a A	75.3 ± 30.6 b AB	0 b A
	10	75.0 ± 11.0 ab B	108.3 ± 12.0 a A	73.8 ± 23.7 b A
butyl acetate LSD = 253.5	1	0 a A	47.5 ± 0.7 a A	0 a A
	5	66.7 ± 25.3 a A	0 a A	59.0 ± 22.6 a A
	10	34.3 ± 8.1 a A	46.3 ± 9.7 a A	81.0 ± 19.8 a A
hexyl acetate LSD = 686.6	1	142.8 ± 29.6 a A	155.0 ± 1.4 a A	182.3 ± 6.4 a A
	5	121.0 ± 33.9 a A	70.5 ± 18.9 a A	378.0 ± 39.6 a A
	10	107.3 ± 20.8 a A	119.5 ± 31.3 a A	100.3 ± 23.6 a A
2-methylpropyl acetate LSD = 66.3	1	0 a A	0 a A	0 a A
	5	0 a A	0 a A	0 a A
	10	0 a A	24.5 ± 3.5 a A	24.0 ± 7.3 a A
2-methylbutyl acetate LSD = 218.1	1	64.3 ± 18.7 a A	81.0 ± 2.8 a A	49.5 ± 14.8 a B
	5	73.0 ± 7.2 ab A	0 b A	290.0 ± 23.2 a A
	10	0 b A	0 b A	269.0 ± 36.8 a A
ethyl propionate LSD = 50.6	1	31.8 ± 8.5 b A	120.0 ± 69.3 a A	36.5 ± 13.4 b A
	5	66.0 ± 25.9 b A	23.0 ± 7.1 bc B	0 c
	10	75.6 ± 4.7 a A	65.3 ± 24.3 a B	27.3 ± 18.1 a A
<i>tert</i> -butyl propionate LSD = 434.0	1	0 a A	0 a A	0 a B
	5	59.0 ± 25.9 b A	71.3 ± 21.0 b A	1967.0 ± 622.3 a A
	10	157.7 ± 28.0 a A	256.0 ± 53.0 a A	321.8 ± 47.4 a B
ethyl butyrate LSD = 1382.6	1	65.0 ± 15.7 a A	147.5 ± 48.8 a A	0 a A
	5	359.5 ± 27.7 a A	229.0 ± 103.4 a A	1371.0 ± 391.7 a A
	10	770.8 ± 206.5 a A	1006.8 ± 300.9 a A	945.0 ± 144.4 a A
hexyl butyrate LSD = 156.5	1	0 a A	25.5 ± 0.7 a A	0 a A
	5	19.0 ± 4.0 a A	0 a A	0 a A
	10	0 a A	0 a A	0 a A
ethyl 2-methylbutyrate LSD = 2245.0	1	100.3 ± 19.7 a A	167.0 ± 19.8 a A	78.3 ± 16.3 a B
	5	527.7 ± 150.2 b A	695.0 ± 78.9 b A	5788.0 ± 513.4 a A
	10	1231.8 ± 383.3 a A	1909.3 ± 590.3 a A	1307.5 ± 336.7 a B
hexyl 3-methylbutyrate LSD = 82.8	1	0 a A	35.0 ± 5.7 a A	38.3 ± 10.3 a AB
	5	33.3 ± 13.2 ab A	0 b A	90.5 ± 19.1 a A
	10	0 a A	20.0 ± 1.0 a A	0 a B
ethyl hexanoate LSD = 616.0	1	146.0 ± 62.1 a A	169.0 ± 67.9 a A	0 a A
	5	113.7 ± 40.1 a A	50.3 ± 13.5 a A	254.0 ± 33.9 a A
	10	109.8 ± 31.6 a A	152.0 ± 21.7 a A	42.7 ± 14.2 a A
Alcohol Production				
ethanol LSD = 204.2	1	37.5 ± 0.7 a A	0 a A	0 a A
	5	0 a A	97.7 ± 21.6 a A	103.5 ± 37.5 a A
	10	0 a A	0 a A	95.0 ± 36.3 a A
1-propanol LSD = 202.0	1	76.3 ± 16.5 a A	56.5 ± 7.8 a A	45.3 ± 14.3 a A
	5	85.0 ± 16.1 a A	76.3 ± 19.5 a A	0 a A
	10	42.8 ± 16.2 a A	49.3 ± 9.6 a A	38.3 ± 14.2 a A
1-butanol LSD = 105.5	1	0 a A	0 a A	0 a B
	5	0 b A	0 b A	137.0 ± 72.1 a A
	10	0 a A	67.5 ± 12.0 a A	33.0 ± 4.2 a AB
2-methyl-1-butanol LSD = 211.9	1	0 a A	0 a A	94.5 ± 45.7 a A
	5	29.0 ± 1.4 a A	58.0 ± 14.2 a A	188.0 ± 14.1 a A
	10	56.3 ± 5.7 a A	121.5 ± 41.3 a A	62.5 ± 20.3 a A
1-hexanol LSD = 181.6	1	0 a A	0 a A	58.7 ± 15.0 a AB
	5	47.7 ± 15.0 ab A	33.5 ± 6.4 b A	221.5 ± 16.3 a A
	10	51.7 ± 14.6 a A	74.5 ± 17.1 a A	0 a B
nerol LSD = 247.3	1	241.5 ± 10.6 a A	41.3 ± 16.8 a A	32.8 ± 6.1 a A
	5	71.0 ± 12.5 a A	46.7 ± 12.7 a A	0 a A
	10	62.7 ± 20.1 a A	50.5 ± 12.4 a A	68.0 ± 5.6 a A

^a Values are means ± SD of four replicate extract samples from four to five apples (≈1 kg) 24 h after collection. Means within the same day of shelf life followed by the same lower case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). Means within the same CA treatments followed by the upper case capital letters are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b Days of ripening at 20 °C. ^c SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

Influence of Different Storage Periods on the Aroma Composition of Apples. For a more detailed analysis of the relationships between volatile compounds and the quality parameters and to assess the influence of different CA treatments, a PLS2 regression was developed. Figures 2 and 3 show the corresponding scores and loadings plots for the first two PCs. PC1 appears to be mainly determined by volatile emission

and SSC, whereas PC2 is mainly explained by respiration (CO₂ production).

Figure 2 shows that after 3 months of storage ($X = 1$) samples did not show great differences in PC1 or PC2, as before. After 5 months ($X = 2$), the effect of the CA treatment appeared. Major differences were explained by the score in PC2 (CO₂), this being lower in ULO samples ($Y = 1$), as expected, due to the negative score

Table 5. Ester and Alcohol Production (Micrograms per Kilogram) by Granny Smith Apples during 1, 5, and 10 Days of Shelf Life (20 °C) after 5 Months of Cold Storage under Different CA Treatments^a

compound	shelf life ^b	SCA ^c	LO	ULO
Ester Production				
methyl acetate	1	0 b B	0 b A	251.3 ± 66.6 a B
LSD = 86.2	5	0 a B	0 a A	0 a C
	10	414.5 ± 177.4 a A	0 b A	366.0 ± 48.0 a A
ethyl acetate	1	529.8 ± 200.8 b B	993.7 ± 331.7 ab AB	1142.8 ± 195.4 a B
LSD = 506.2	5	848.5 ± 289.5 a B	614.7 ± 64.7 ab B	300.5 ± 7.8 b C
	10	2768.0 ± 1122.6 a A	1590.5 ± 247.6 b A	1838.0 ± 187.9 b A
propyl acetate	1	475.3 ± 79.0 b A	253.0 ± 96.2 c B	661.0 ± 38.2 a B
LSD = 81.7	5	389.0 ± 63.6 b B	122.0 ± 29.7 c C	824.0 ± 107.5 a A
	10	356.0 ± 179.6 b B	582.3 ± 110.5 a A	251.5 ± 96.9 c C
butyl acetate	1	415.0 ± 57.6 b B	1509.0 ± 650.5 a A	206.0 ± 72.9 b A
LSD = 253.5	5	873.3 ± 280.0 a A	245.0 ± 56.7 b B	182.7 ± 35.3 b A
	10	341.8 ± 129.5 a B	454.0 ± 75.8 a B	228.0 ± 34.0 a A
pentyl acetate	1	0 a B	0 a B	0 b B
LSD = 30.3	5	81.5 ± 10.6 a A	0 b B	0 b B
	10	65.5 ± 12.0 c A	183.0 ± 41.4 a A	119.0 ± 9.9 b A
hexyl acetate	1	709.3 ± 408.0 b AB	2827.0 ± 246.3 a A	443.0 ± 116.6 b A
LSD = 686.6	5	1370.3 ± 675.5 a A	776.0 ± 77.6 a B	924.5 ± 294.9 a A
	10	588.0 ± 121.7 a B	1014.3 ± 211.6 a B	584.3 ± 344.9 a A
2-methylpropyl acetate	1	282.5 ± 197.3 a A	0 c C	113.5 ± 14.8 b C
LSD = 66.3	5	259.0 ± 42.4 a A	122.5 ± 12.0 b B	181.0 ± 36.4 b B
	10	133.0 ± 2.8 c B	336.0 ± 108.9 a A	268.5 ± 64.3 b A
2-methylbutyl acetate	1	1281.5 ± 403.7 b A	1811.5 ± 402.3 a A	302.0 ± 29.2 c B
LSD = 218.1	5	627.3 ± 252.4 a B	837.7 ± 95.8 a B	292.0 ± 89.4 b B
	10	495.0 ± 77.8 b B	783.0 ± 172.6 a B	606.3 ± 58.7 ab A
ethyl propionate	1	0 b B	107.7 ± 37.6 a A	0 b C
LSD = 50.6	5	68.5 ± 30.4 c A	150.5 ± 60.1 b A	666.0 ± 135.8 a A
	10	113.0 ± 14.0 ab A	157.3 ± 40.8 a A	66.0 ± 27.9 b B
<i>tert</i> -butyl propionate	1	1354.7 ± 238.3 a B	782.0 ± 284.3 b B	142.0 ± 9.9 c B
LSD = 434.0	5	1198.8 ± 663.0 a B	770.3 ± 64.3 a B	251.5 ± 24.7 b B
	10	3042.3 ± 776.8 b A	8200.7 ± 224.4 a A	1930.5 ± 95.5 c A
ethyl butyrate	1	2407.5 ± 890.8 a B	2328.7 ± 172.6 a B	264.3 ± 28.8 b C
LSD = 1382.6	5	2977.5 ± 1414.7 a B	1778.0 ± 72.7 a B	1790.0 ± 878.6 a B
	10	6714.7 ± 2005.1 b A	12313.8 ± 1215.2 a A	6969.5 ± 1673.7 b A
hexyl butyrate	1	0 b B	328.3 ± 220.6 a A	0 b B
LSD = 156.5	5	197.5 ± 111.3 a A	0 b B	161.0 ± 54.0 a A
	10	0 b B	276.0 ± 148.5 a A	95.3 ± 41.1 b AB
ethyl 2-methylbutyrate	1	3294.0 ± 227.9 ab C	4564.7 ± 1803.7 a B	862.3 ± 128.5 b B
LSD = 2245.0	5	5566.8 ± 3477.7 a B	3681.0 ± 2080.8 ab B	1839.7 ± 508.1 b B
	10	10782.7 ± 2135.4 b A	17674.5 ± 1955.9 a A	7046.0 ± 224.8 c A
ethyl 3-methylbutyrate	1	0 a A	0 a A	222.5 ± 9.2 a A
LSD = 283.8	5	0 a A	0 a A	87.3 ± 35.2 a A
	10	0 a A	0 a A	228.0 ± 32.5 a A
hexyl 3-methylbutyrate	1	145.8 ± 21.4 b A	287.3 ± 91.9 a A	183.0 ± 105.7 b B
LSD = 82.8	5	146.3 ± 62.9 b A	165.0 ± 23.1 b B	421.0 ± 186.4 a A
	10	74.0 ± 21.2 c A	358.8 ± 73.9 a A	191.5 ± 30.4 b B
ethyl hexanoate	1	1523.0 ± 93.1 a A	1235.0 ± 648.0 a B	378.0 ± 73.6 b B
LSD = 616.0	5	664.0 ± 174.3 a B	709.7 ± 231.2 a B	1074.7 ± 583.1 a A
	10	944.0 ± 446.4 b AB	1914.0 ± 148.0 a A	1545.7 ± 850.2 ab A
Alcohol Production				
ethanol	1	715.5 ± 197.4 a AB	436.0 ± 110.3 b B	654.3 ± 255.7 a B
LSD = 204.2	5	599.8 ± 155.0 b B	388.0 ± 59.4 c B	872.0 ± 155.9 a A
	10	865.0 ± 141.1 a A	745.5 ± 67.0 ab A	557.3 ± 131.1 b B
1-propanol	1	632.0 ± 117.0 b B	1715.0 ± 553.7 a A	198.7 ± 54.5 c B
LSD = 202.0	5	966.0 ± 240.8 a A	97.5 ± 26.2 c C	580.5 ± 58.7 b A
	10	414.0 ± 135.2 a C	364.7 ± 66.0 a B	127.3 ± 17.0 b B
1-butanol	1	123.0 ± 29.7 a AB	0 b B	0 b A
LSD = 105.5	5	207.0 ± 91.9 a A	0 b B	0 b A
	10	95.0 ± 45.5 b B	401.0 ± 70.9 a A	0 b A
2-methyl-1-butanol	1	208.5 ± 36.1 b B	540.5 ± 117.7 ab B	235.7 ± 29.5 b B
LSD = 211.9	5	307.5 ± 168.8 ab AB	119.0 ± 28.3 b C	335.7 ± 179.5 a B
	10	464.5 ± 183.4 b A	808.8 ± 188.2 a A	674.0 ± 33.9 ab A
1-hexanol	1	195.7 ± 56.2 b B	672.3 ± 301.2 a B	250.0 ± 77.8 b B
LSD = 181.6	5	448.0 ± 221.1 a A	255.7 ± 92.1 b C	410.3 ± 86.5 ab AB
	10	421.0 ± 107.1 b A	991.0 ± 207.9 a A	554.0 ± 116.0 b A
nerol	1	1770.0 ± 825.0 a A	311.3 ± 112.4 c B	855.8 ± 190.8 b A
LSD = 247.3	5	776.3 ± 366.2 a B	51.3 ± 4.6 b C	1003.7 ± 320.9 a A
	10	369.8 ± 201.9 ab C	590.3 ± 157.9 a A	141.7 ± 50.3 b B

^a Values are means ± SD of four replicate extract samples from four to five apples (≈1 kg) 24 h after collection. Means within the same day of shelf life followed by the same lower case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). Means within the same CA treatments followed by the same upper case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b Days of ripening at 20 °C. ^c SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

of PC2. Differences in the score of PC1 can also be seen. LO samples ($Y = 2$) produced the greatest emission of

aroma compounds and SSC [which correlates well with PC1 (Figure 3)], whereas ULO samples produced the

Table 6. Ester and Alcohol Production (Micrograms per Kilogram) by Granny Smith Apples during 1, 5, and 10 Days of Shelf Life (20 °C) after 7 Months of Cold Storage under Different CA Treatments^a

compound	shelf life ^b	SCA ^c	LO	ULO
Ester Production				
methyl acetate LSD = 86.2	1	0 b B	383.7 ± 180.7 a A	0 b A
	5	0 b B	0 b B	0 b A
	10	392.0 ± 158.4 a A	0 b B	0 b A
ethyl acetate LSD = 506.2	1	567.5 ± 88.4 b A	1083.3 ± 81.1 a A	587.5 ± 78.6 ab AB
	5	836.7 ± 266.3 a A	321.7 ± 19.6 b B	943.3 ± 126.6 a A
	10	770.0 ± 193.1 a A	695.0 ± 7.1 ab AB	214.5 ± 64.3 b B
propyl acetate LSD = 81.7	1	0 b B	0 b B	360.0 ± 0.7 a A
	5	223.3 ± 28.9 a A	0 b B	0 b B
	10	0 b B	380.0 ± 14.1 a A	0 b B
butyl acetate LSD = 253.5	1	200.0 ± 70.7 a A	322.5 ± 59.1 a A	177.5 ± 53.0 a A
	5	206.7 ± 90.7 a A	285.0 ± 63.6 a A	215.0 ± 21.2 a A
	10	180.0 ± 26.5 a A	0 a B	140.0 ± 28.3 a A
hexyl acetate LSD = 686.6	1	595.3 ± 242.8 a A	983.3 ± 235.4 a A	435.0 ± 152.9 a A
	5	427.5 ± 146.8 a A	350.0 ± 70.7 a A	757.5 ± 189.3 a A
	10	543.3 ± 136.5 a A	760.0 ± 56.6 a A	185.0 ± 21.2 a A
2-methylpropyl acetate LSD = 66.3	1	0 b A	0 b A	210.0 ± 65.6 a B
	5	0 b A	0 b A	276.7 ± 61.1 a C
	10	0 a A	0 a A	58.5 ± 12.0 a A
2-methylbutyl acetate LSD = 218.1	1	745.0 ± 134.4 a A	652.5 ± 115.6 a A	235.0 ± 48.2 b A
	5	210.0 ± 101.5 ab B	0 b B	250.0 ± 70.7 a A
	10	180.0 ± 56.6 b B	0 b B	400.0 ± 28.3 a A
ethyl propionate LSD = 50.6	1	0 b B	0 b A	70.0 ± 42.4 a A
	5	82.0 ± 33.6 a A	0 b A	0 b B
	10	46.7 ± 11.5 a AB	0 a A	9.5 ± 3.5 a B
<i>tert</i> -butyl propionate LSD = 434.0	1	0 a B	0 a A	206.7 ± 30.6 a A
	5	326.7 ± 102.6 a B	0 a A	380.0 ± 160.9 a A
	10	2133.3 ± 418.8 a A	0 b A	269.0 ± 41.0 b A
ethyl butyrate LSD = 1382.6	1	0 a B	275.3 ± 86.9 a A	298.3 ± 94.1 a B
	5	357.5 ± 109.0 b B	204.0 ± 5.7 b A	2633.3 ± 510.7 a A
	10	7398.3 ± 998.9 a A	210.0 ± 28.3 b A	796.0 ± 59.4 b B
butyl butyrate LSD = 48.6	1	265.5 ± 77.1 a A	306.7 ± 70.9 a A	0 b B
	5	0 b C	0 b C	235.0 ± 35.4 a A
	10	210.0 ± 70.7 a B	220.0 ± 28.3 a B	0 b B
hexyl butyrate LSD = 156.5	1	0 a A	0 a A	75.0 ± 7.1 a AB
	5	0 a A	0 a A	0 a B
	10	0 b A	0 b A	176.7 ± 11.5 a A
ethyl 2-methylbutyrate LSD = 2245.0	1	395.5 ± 246.8 a C	389.0 ± 146.9 a A	1253.8 ± 592.9 a A
	5	2692.5 ± 1545.2 a B	1258.0 ± 158.4 a A	1026.3 ± 177.5 a A
	10	7660.0 ± 228.7 a A	460.0 ± 56.6 b A	1775.0 ± 417.2 b A
ethyl 3-methylbutyrate LSD = 283.8	1	0 b B	588.0 ± 257.4 a A	470.0 ± 135.3 a A
	5	0 b B	0 b B	718.5 ± 87.0 a A
	10	519.3 ± 135.2 a A	0 b B	106.5 ± 9.2 b B
hexyl 3-methylbutyrate LSD = 82.8	1	0 b A	0 b A	101.7 ± 29.3 a B
	5	53.3 ± 5.8 b A	0 b A	216.7 ± 47.3 a A
	10	0 b A	0 b A	120.0 ± 28.3 a B
ethyl hexanoate LSD = 616.0	1	361.0 ± 152.1 a B	670.0 ± 234.3 a A	245.0 ± 88.6 a AB
	5	0 b B	240.0 ± 56.6 ab A	817.5 ± 217.5 a A
	10	1140.0 ± 175.8 a A	510.0 ± 42.4 b A	181.0 ± 15.6 b B
Alcohol Production				
ethanol LSD = 204.2	1	250.0 ± 0.7 b B	780.0 ± 165.2 a A	385.0 ± 85.3 b B
	5	927.5 ± 165.2 a A	660.0 ± 169.7 b A	790.0 ± 42.4 ab A
	10	323.3 ± 160.4 a B	350.0 ± 70.7 a B	97.5 ± 3.5 c C
1-propanol LSD = 202.0	1	435.5 ± 190.2 b A	1567.5 ± 227.7 a A	263.8 ± 75.2 b A
	5	347.3 ± 178.1 b A	556.3 ± 150.6 a B	230.0 ± 42.4 b A
	10	246.7 ± 75.7 a A	310.0 ± 28.3 a C	240.0 ± 42.4 a A
2-methyl-1-propanol LSD = 39.7	1	0 b A	0 b A	203.3 ± 20.8 a A
	5	0 a A	0 a A	0 a B
	10	0 b A	0 b A	165.0 ± 35.4 a A
1-butanol LSD = 105.5	1	0 b B	0 b A	135.0 ± 21.2 a B
	5	0 a B	0 a A	0 a C
	10	220.0 ± 81.9 a A	0 b A	250.0 ± 70.7 a A
2-methyl-1-butanol LSD = 211.9	1	0 c B	279.7 ± 101.0 b B	812.5 ± 225.0 a B
	5	200.0 ± 60.0 c AB	649.0 ± 29.7 b A	970.0 ± 103.9 a AB
	10	346.7 ± 215.0 b A	265.0 ± 21.2 b B	1075.0 ± 134.4 a A
1-hexanol LSD = 181.6	1	0 b B	0 b B	502.5 ± 108.5 a A
	5	0 a B	0 a B	121.5 ± 12.8 a B
	10	333.3 ± 92.4 a A	185.0 ± 49.5 ab A	95.0 ± 35.4 b B

^a Values are means ± SD of four replicate extract samples from four to five apples (≈1 kg) 24 h after collection. Means within the same day of shelf life followed by the same lower case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). Means within the same CA treatments followed by the same upper case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b Days of ripening at 20 °C. ^c SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

lowest emission of volatiles. After 7 months of storage ($X = 3$), differences in the score of the second PLS factor reached their maximum. Samples coming from the ULO

treatment ($Y = 1$) showed the highest CO₂ production, this indicating the effectiveness of the ULO treatment in delaying respiration.

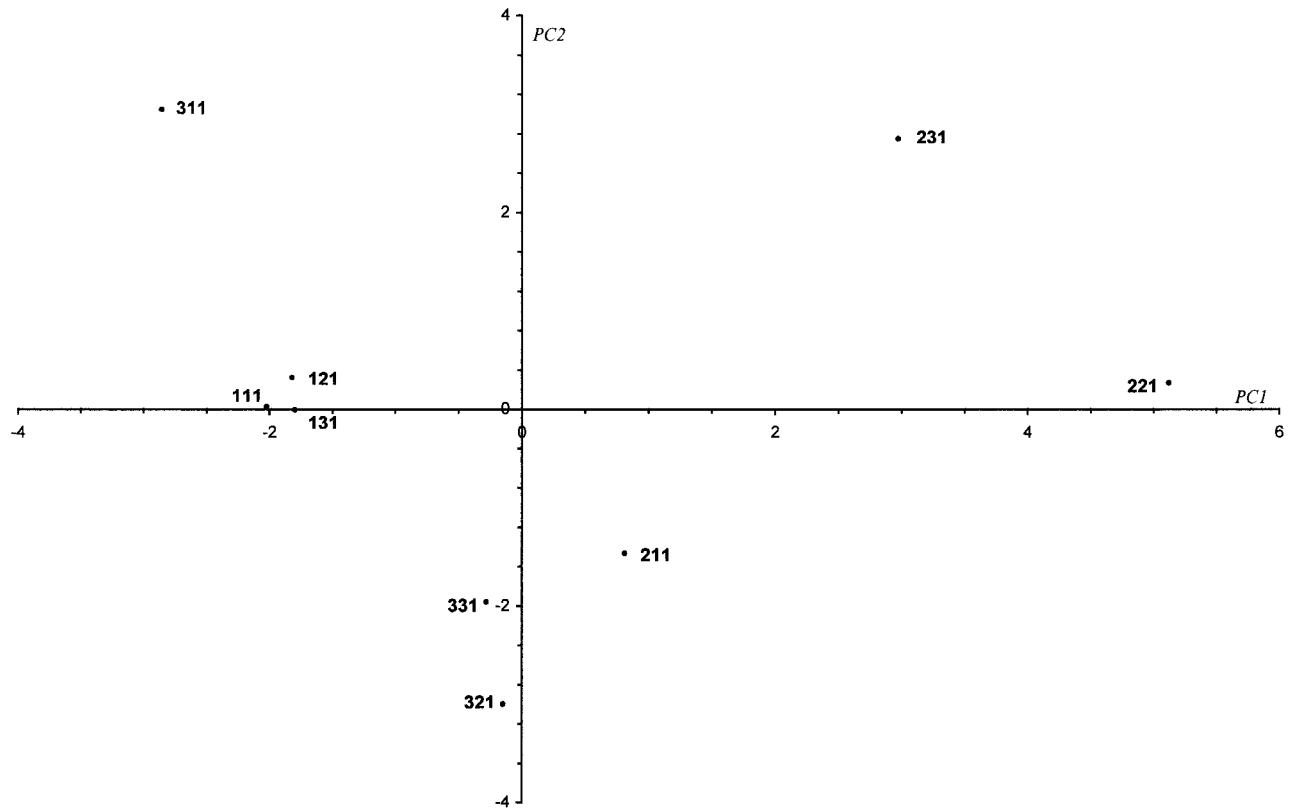


Figure 2. Scores plot of a PLS2 analysis among quality parameters (*y* variables) and volatile compounds (*x* variables). Sample names were coded as indicated in Figure 1. Both sets of parameters were measured after 1 day of shelf life ($Z = 1$).

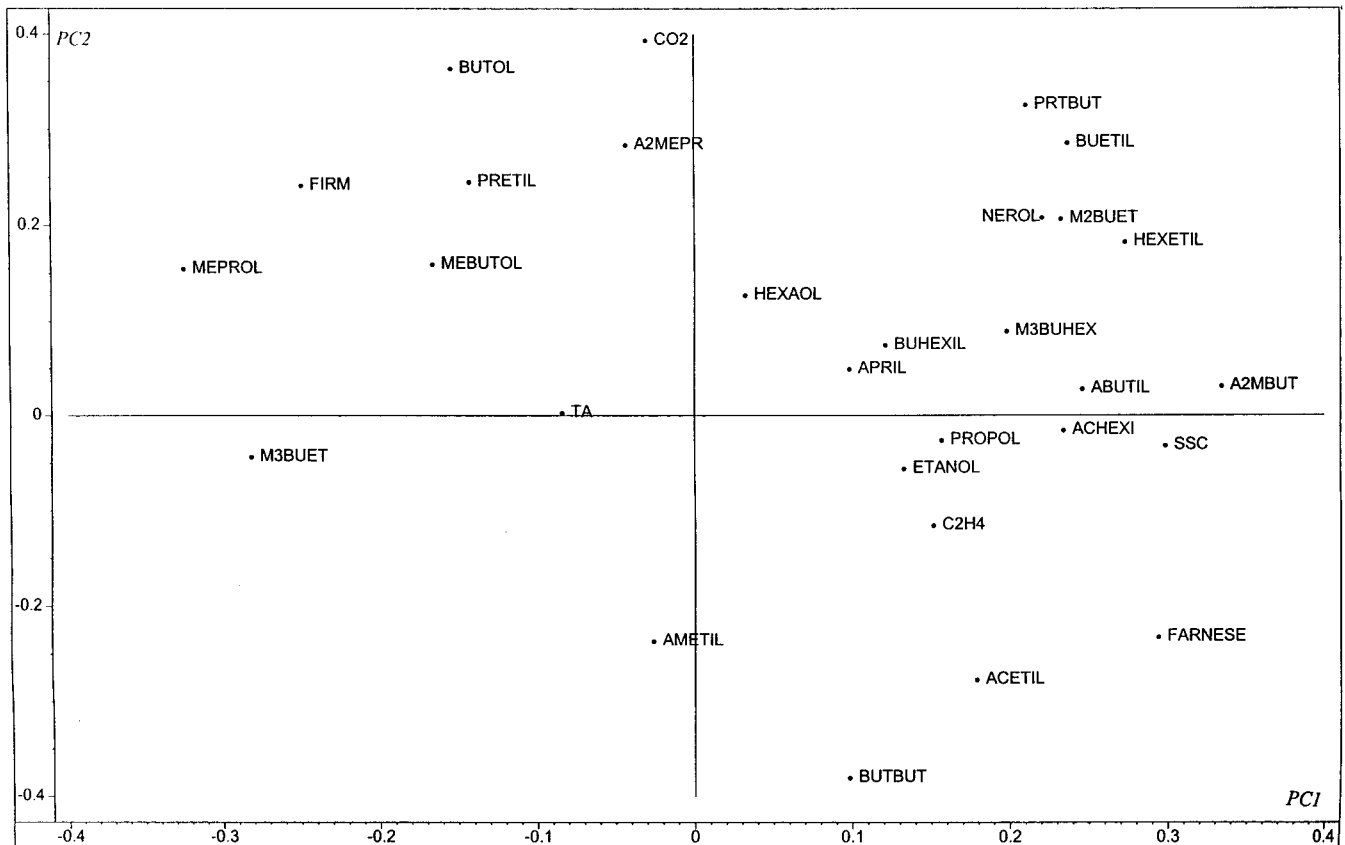


Figure 3. Plot of the *x*-loading and *y*-loading weights of a PLS2 analysis among quality parameters (*y* variables) and volatile compounds (*x* variables). Quality parameters were labeled as FIRM (firmness), TA (titratable acidity), SSC (solid soluble concentration), C2H4 (ethylene production), and CO2 (carbon dioxide production). Labels of volatile compounds are indicated in Table 2.

Table 7. α -Farnesene Production (Micrograms per Kilogram) by Granny Smith Apples during 1, 5, and 10 Days of Shelf Life (20 °C) after 3, 5, and 7 Months of Cold Storage under Different CA Treatments^a

storage (months)	shelf life ^b	SCA ^c	LO	ULO
3	1	535.0 ± 97.1 b B	3777.5 ± 14.8 a A	1790.3 ± 572.0 ab B
	LSD = 2613.9	5	642.5 ± 41.7 c B	6159.0 ± 762.3 a A
		10	433.0 ± 261.3 a B	1033.8 ± 748.7 a B
5	1	5805.7 ± 187.1 b B	19908.3 ± 3996.9 a A	18873.3 ± 3289.4 a A
	5	10271.7 ± 928.4 a A	10037.0 ± 6423.3 a C	4580.3 ± 1696.5 b B
	10	1710.3 ± 163.3 b C	14891.3 ± 2977.3 a B	1345.3 ± 648.8 b C
7	1	5844.7 ± 1127.3 a A	5502.5 ± 1964.5 a A	3731.7 ± 1002.8 a A
	5	2096.7 ± 843.1 ab B	981.7 ± 303.3 b B	3927.5 ± 221.5 a A
	10	3253.3 ± 1498.6 a AB	814.3 ± 212.1 a B	2865.0 ± 183.8 a A

^a Values are means ± SD of four replicate extract samples from four to five apples (\approx 1 kg) 24 h after collection. Means within the same day of shelf life followed by the same lower case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). Means within the same CA treatments followed by the same upper case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b Days of ripening at 20 °C. ^c SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

The CA treatments greatly influenced volatile production. After 3 months of storage (Table 4), ethyl butyrate, hexyl butyrate, ethyl hexanoate, 1-butanol, 1-hexanol, and nerol were detected for the first time. During this period the CA treatments influenced the production of propyl acetate, 2-methylbutyl acetate, ethyl propionate, *tert*-butyl propionate, ethyl 2-methylbutyrate, hexyl 3-methylbutyrate, 1-butanol, and 1-hexanol (Table 4). Shelf life of 5 days at room temperature (20 °C) increased aroma production. Thus, the different CA treatments influenced some aroma compounds of Granny Smith apples after short storage periods, as also observed for other varieties (Streif and Bangerth, 1988; Brackmann et al., 1993).

After 5 months of storage, methyl acetate was detected for the first time. All aroma components, except ethyl 3-methylbutyrate, were influenced by the CA treatment, leading to the widely spread scores plot (Figure 2). With the exception of methyl, ethyl, and propyl acetate, the ester production was significantly higher ($p < 0.05$) in apples stored in the SCA and LO treatments, both exhibiting a higher score on PC1 (Table 5). Moreover, the LO treatment produced an increase in all alcohols except 1-propanol during the shelf-life period (Table 5). Thus, the suppressive effect of CA with low oxygen on aroma production was found here with ULO (Streif and Bangerth, 1988; Yahia et al., 1990) but not with our LO treatment after 5 months of storage. The higher production of aroma compounds in the LO and SCA treatments was previously observed with Starking Delicious apples (López et al., 1998).

Hexyl esters, which are responsible for the fruity aroma of apples (De Pooter and Schamp, 1989), reached their highest value in the SCA and LO treatments. The position of hexyl esters in the scores plot (Figure 2) was equivalent to the position of the SCA and LO variables in the loadings plot (Figure 3). Conversely, the ULO treatment shows a higher correlation with the emission of ethyl acetate, 1-propanol, α -farnesene, and ethanol (Figure 2).

There was a clear correlation between hexyl esters (mainly hexyl butyrate and hexyl 3-methylbutyrate) and 1-hexanol (Figure 3). This relationship supports the suggestion of some authors (Paillard, 1979; De Pooter et al., 1987), who indicate that once the alcohol has been produced, it can be used to obtain the corresponding esters. There is no such correlation for 1-butanol and its esters, which supports the evidence that the butyl radical is obtained from a different source (Yahia et al., 1990; Hansen et al., 1992).

In terms of the linear-chain acetates (Table 5) after 1 day of shelf life, ULO gave the highest production of

short-chain acetates (methyl, ethyl, and propyl acetate) and LO gave the highest production of butyl and hexyl acetate. There was no suppressive effect on Granny Smith apples by low-oxygen CA, as found with other varieties (Hansen et al., 1992; Brackmann et al., 1993; Fellman et al., 1993).

The CA treatments tested had a different influence on the production of branched-chain esters. The ULO treatment increased the production of these esters during the shelf-life period, whereas in LO 2-methylpropyl acetate increased and 2-methylbutyl acetate decreased (Table 5).

After 7 months of storage, the fruit had a lower ester content according to the PC1 score. Samples from SCA and LO were closely correlated with the butyl butyrate content, which along with methyl acetate was detected only after this period (Table 6; Figure 3). Of all the esters only butyl and hexyl acetate did not show significant differences due to the CA treatment.

Apples coming from ULO show significantly higher concentrations of 2-methylpropyl acetate, 2-methyl-1-propanol, and 1-butanol, as observed in the scores plot of PLS2 (Figure 2). In the same CA treatment, higher amounts of both linear- and branched-chain alcohols, except ethanol and 1-propanol, were obtained (Table 6). Consequently, after long CA storage in ULO the capacity of ester synthesis is reduced (Streif and Bangerth, 1988; Brackmann et al., 1993), not because of alcohol content (Table 6), as suggested by other authors (Knee and Hatfield, 1989), but perhaps because these CA treatments do not provide appropriate conditions for the activity of certain enzymes, such as the acylCoA alcohol transferase (Fellman et al., 1993).

The LO treatment gave the highest α -farnesene emission (Table 7), indicating that this treatment could prevent oxidation of α -farnesene to triene conjugated compounds, which are known to be responsible for the development of scald (Chen et al., 1993; Lau, 1997). Furthermore, α -farnesene is highly correlated with ethyl acetate (Figure 2), which indicates that in this variety α -farnesene may be synthesized from ethyl acetate (Figure 3), the precursor of isoprene (Belitz and Grosch, 1988).

In general, it was observed that during the shelf-life period the apples that had been kept under SCA increased their aroma production. Those stored in LO showed less aroma production, and those stored in ULO showed irregular behavior, except for the ethyl esters, which had a tendency to increase (Table 6), as observed by Mattheis et al. (1991). LO and ULO did not favor the production of branched esters during shelf life.

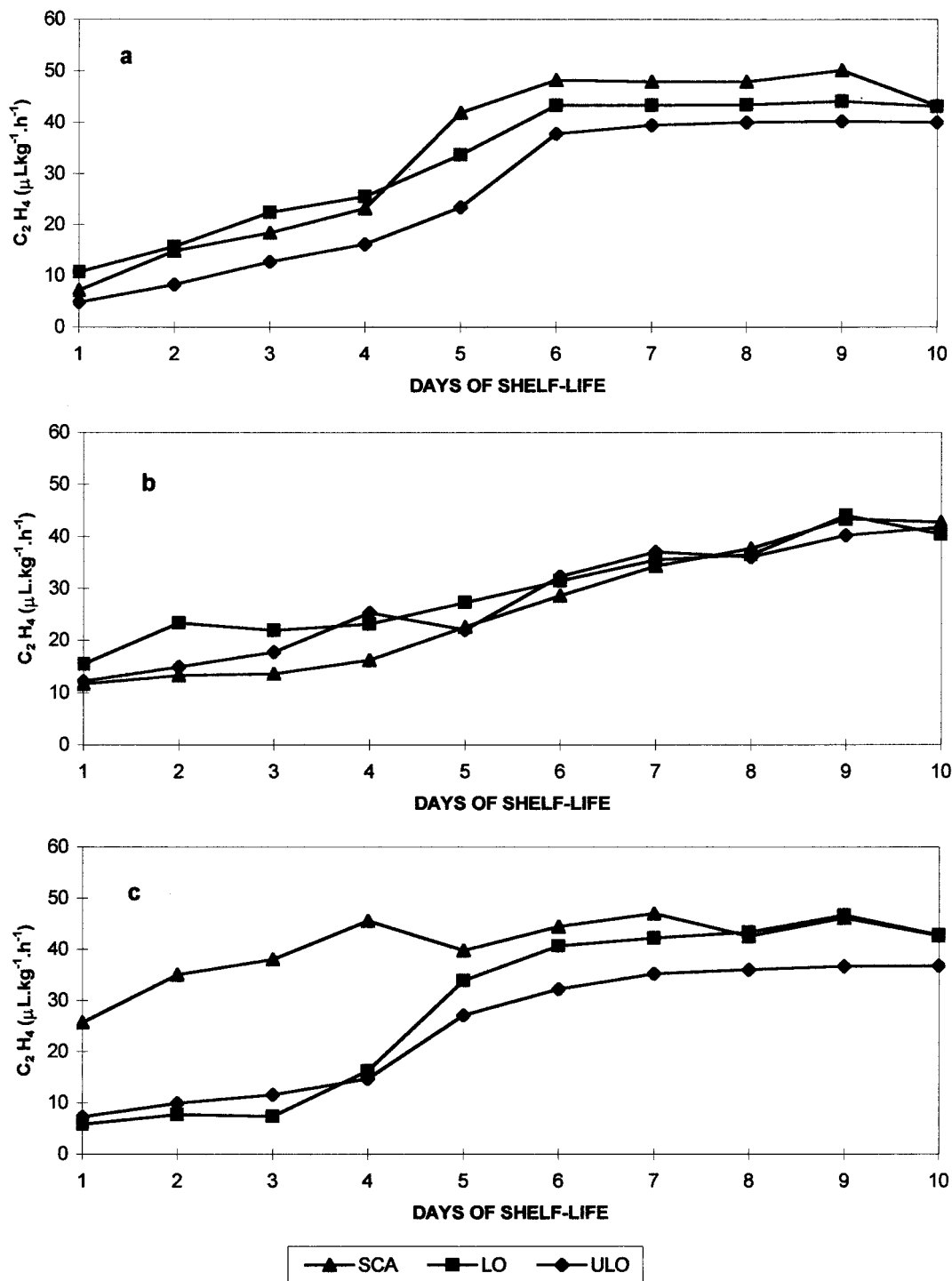


Figure 4. Ethylene production by Granny Smith apples during 10 days of shelf life at 20 °C, after 3 months (a), 5 months (b), and 7 months (c) of cold storage in different CA treatments: SCA (3% O_2 /3% CO_2), LO (2% O_2 /2% CO_2), and ULO (1% O_2 /1% CO_2).

These results differ from those of other authors (Brackmann et al., 1993).

Influence of Different CA Treatments on Organoleptic Quality, Respiration, and Ethylene Production of Apples. Different CA treatments and storage period significantly affected ($p < 0.01$) all maturity parameters studied. The main effect was due to the storage period.

Figure 3 shows that SSC and firmness covary negatively, both showing weak correlation with acidity. During storage, firmness decreased while SSC increased up to 5 months (Table 3). Up to 5 months, the synthesis of sugars was predominant, probably from starch, and

the subsequent decrease could be due to its use in the respiratory metabolism (Tucker and Grierson, 1987).

After 5 months, LO and ULO gave apples that were firmer than those stored in SCA (Table 3). Firmness appeared as the main characteristic of ULO samples after 7 months of storage, as shown in the corresponding positions in both Figures 2 and 3; the firmness of ULO apples differed clearly from that of LO and SCA apples. Acidity decreased from harvest up to 3 months of storage, showing a slight decrease during the rest of the storage period. ANOVA did not show significant differences ($p > 0.05$) between acidity in LO and ULO apples at 3 or 7 months, although both treatments led to

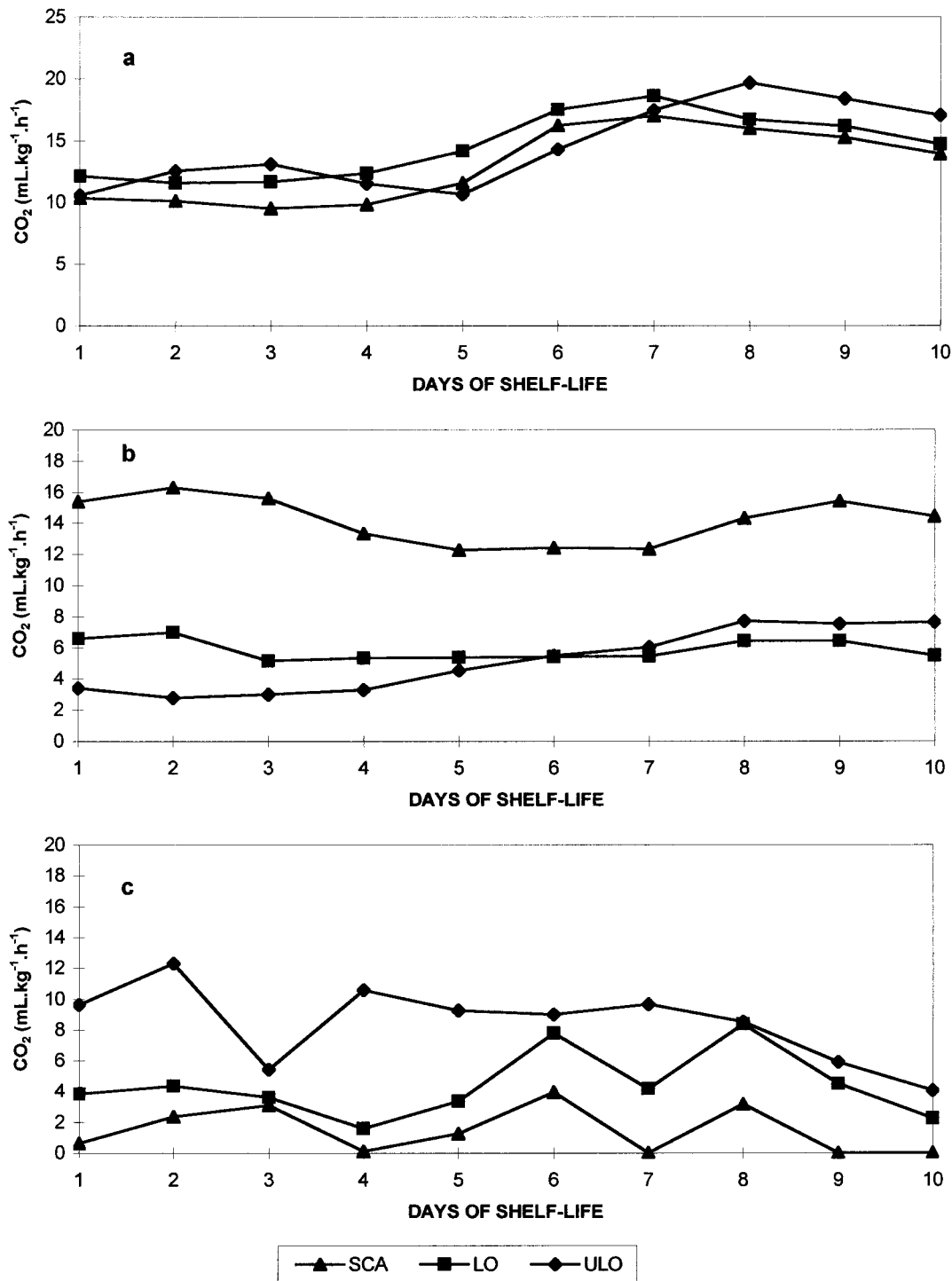


Figure 5. Respiration (CO₂ production) by Granny Smith apples during 10 days of shelf life at 20 °C, after 3 months (a), 5 months (b), and 7 months (c) of cold storage in different CA treatments: SCA (3% O₂/3% CO₂), LO (2% O₂/2% CO₂), and ULO (1% O₂/1% CO₂).

significantly more sour apples than in SCA samples. After 5 months, the best treatment for acidity maintenance was ULO (Table 3).

Firmness is related to 1-butanol, ethyl propionate, 2-methyl-1-butanol, and 2-methyl-1-propanol (Figure 3). The LO and SCA treatments were mainly correlated with methyl acetate and butyl butyrate; the first component could come from pectin disintegration, and the second could be obtained from lipid β -oxidation of membrane (Bartley, 1986; Harb et al., 1994), thus explaining the loss of firmness in these treatments.

The values for firmness and acidity were similar to

those reported elsewhere and with other apple varieties (Brackmann et al., 1994; Drake et al., 1991; Truter et al., 1994).

It is interesting that after 5 months of storage in LO there was a correlation between butyl acetate, hexyl acetate, 2-methylbutyl acetate, and SSC in agreement with the fruity sensation given by these aroma compounds in sensory analysis (Rizzolo et al., 1989; Young et al., 1996). Also, there is a good correlation between ethylene and SSC, indicating that ethylene is responsible for the changes in carbohydrate metabolism.

After 3 months of storage, ethylene production in-

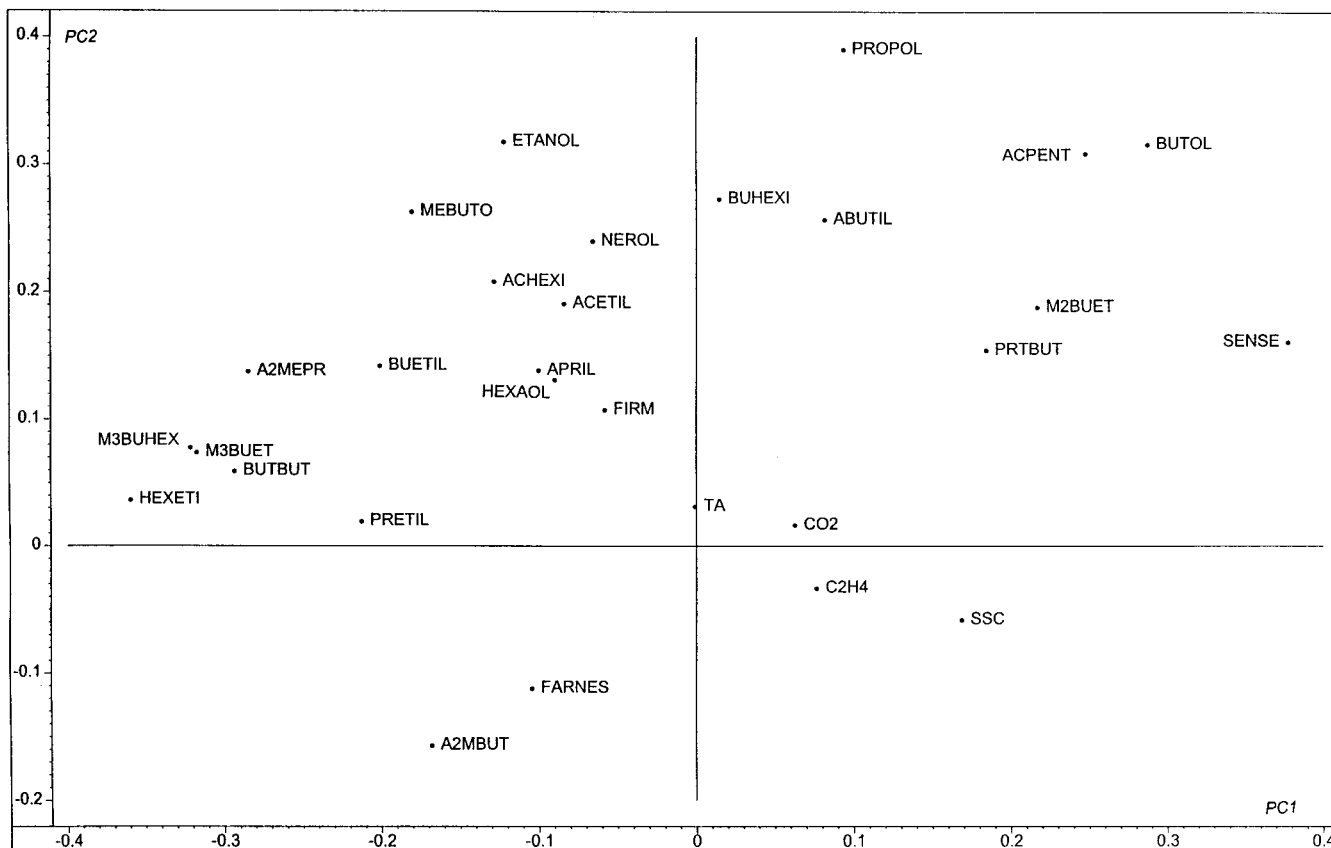


Figure 6. Loadings plot of a PLS1 analysis among quality parameters and volatile compounds as x variables and average sensory score as the y variable (SENSE). Labels of volatile compounds and quality parameters are indicated in Table 2 and Figure 3, respectively.

creased at 20 °C up to day 6. The increase was more noticeable in apples from the SCA treatment. These results are similar to those shown by Bauchot et al. (1995). Apples stored in LO and ULO showed a similar behavior (Figure 4a). After 5 months, there was an increase in ethylene production almost until the last day studied in all treatments (Figure 4b). Apples from 7 months of storage also showed an increase in ethylene production. In SCA, almost all of this increase occurred after the first day, as seen in Figure 4c.

Ethylene production and respiration (CO_2 production) were weakly correlated, which indicates that in this variety ethylene production is not followed by an increase in respiration. After 3 months, all apples had a similar pattern of respiration (Figure 5a). In all cases a maximum was obtained after 7 or 8 days. After 5 months, apples from SCA showed higher CO_2 production than those from LO and ULO (Figure 5b). Respiration after 7 months was lower and more irregular during the shelf life in all CA treatments, but it was more similar in those samples from SCA and LO (Figure 5c).

A delay of ripening of apples in CA treatments, especially in LO and ULO, has already been reported (Lidster et al., 1987; Lange, 1988). Our results show that the fruit retained the capacity to produce ethylene but that respiration decreased during storage with a significant decrease in carbon dioxide production after 7 months (Figure 5c).

Influence of Different CA Treatments on Sensory Analysis of Apples. From the evaluation of the results of the sensory analysis, it can be concluded that neither the different CA treatments nor storage periods have a significant effect on sensory score (Table 8). The

Table 8. Mean Sensory Scores for Granny Smith Apples Stored in Different CA Conditions for 3, 5, and 7 Months^a

CA treatment ^b		3 months	5 months	7 months
LSD =	SCA	5.12 ± 2.96 a	5.68 ± 3.21 a	5.64 ± 3.02 a
1.13	LO	5.56 ± 2.41 a	4.64 ± 2.85 a	5.56 ± 3.09 a
	ULO	5.84 ± 2.45 a	4.84 ± 2.65 a	4.56 ± 2.91 a

^a Means within the same storage period followed by the same lower case letters are not significantly different at $p < 0.05$ (LSD Tukey's test). ^b SCA (3% O_2 /3% CO_2), LO (2% O_2 /2% CO_2), and ULO (1% O_2 /1% CO_2).

cause was the high variability among judges in rating overall preference. This leads to the high value of the residual mean square error of the analysis of variance (ANOVA). Whole fruit sensory evaluation is complicated by many sources of variation including within-tree and within-apple (Dever et al., 1995). Moreover, the time required for sensory evaluation makes it difficult to evaluate enough fruits to be representative. Nevertheless, it is important to identify the factors that influence sensory perception and then to describe the sampled apples in terms of these factors.

In an attempt to assess the role of the different quality parameters and volatile compounds in the flavor of Granny Smith apples, a PLS1 model was conducted to regress the sensory scores with volatile and quality parameters. The loadings for the first two PLS factors, which explain up to 73% of the variance of the sensory score, are plotted in Figure 6. As can be seen, the sensory score is negatively correlated with 2-methylbutyl acetate, ethyl hexanoate, butyl butyrate, hexyl 3-methylbutyrate, and ethyl 3-methylbutyrate, among others. It is positively correlated with ethyl 2-methyl-

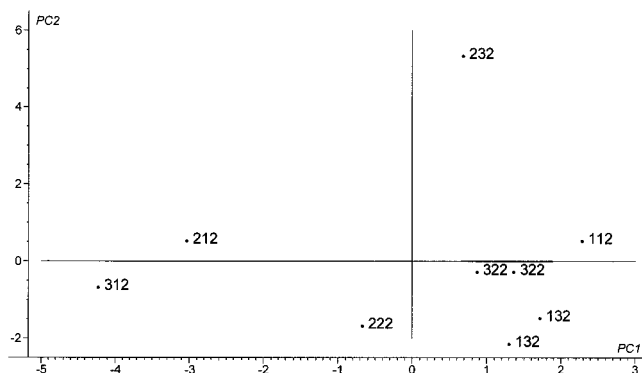


Figure 7. Scores plot of a PLS1 analysis among quality and volatile measurements as x variables and average sensory score as y variable. Samples are labeled as described in Figure 1.

butyrate, *tert*-butyl propionate, pentyl acetate, and 1-butanol. Ethyl 2-methylbutyrate has a direct impact on apple flavor, because it has been identified as the volatile compound that contributes most to the flavor of Golden Delicious apples (Flath et al., 1967; Kakiuchi et al., 1986; Song and Bagerth, 1996).

Among the quality parameters the most correlated with sensory score was SSC.

Apples after 3 months of storage received a good sensorial score (compare Figures 6 and 7), the differences between the CA treatments being of little importance according to the results obtained in the preceding section. Apples after 5 months of storage showed the highest differences due to the CA treatment, ULO samples scoring the best. After 7 months, samples from SCA received the lowest sensorial score, whereas apples from the LO and ULO treatments received a positive sensorial score similar to those of the $X = 1$ samples (3 months of storage).

Our results show that storage in LO and ULO did not produce such a high effect on decreasing aroma compound production of Granny Smith apples. A shelf-life period after 5 months of storage permits high aroma compound production, mainly of hexyl esters. Also, the CA treatments keep quality parameters at acceptable levels and decrease respiration, and apples can ripen properly during the shelf-life period.

The regression analysis used has revealed the correlations between aroma compounds, quality parameters, and sensory evaluation of Granny Smith apples and its importance for characterizing the apples during CA storage.

ABBREVIATIONS USED

CA, controlled atmosphere; FID, flame ionization detector; GC, gas chromatograph; I_2 -IK, iodine and potassium iodure solution; LO, low-oxygen controlled atmosphere; PCA, principal component analysis; PC1, first principal component; PC2, second principal component; PLS, partial least-squares regression; SCA, standard controlled atmosphere; SSC, soluble solids concentration; TA, titratable acidity; ULO, ultralow-oxygen controlled atmosphere.

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