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Regeneration of Volatile Compounds in Fuji Apples Following Ultra Low Oxygen Atmosphere Storage and Its Effect on Sensory Acceptability

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The aim of this work was to assess whether extra time spent under AIR conditions after storage in an ultra low oxygen (ULO) atmosphere could allow the regeneration of volatile compound emission without negatively affecting quality parameters and the consumer acceptability of Fuji apples. Fruits were stored for 19 and 30 weeks at 1 °C and 92% RH under ULO atmosphere conditions (1 kPa O₂:1 kPa CO₂) or under ULO conditions followed by different periods (2 and 4 weeks) in cold AIR atmosphere (ULO + 2w or ULO + 4w, respectively). Standard quality and emission of volatile compounds were analyzed after storage plus 1 and 7 days at 20 °C. Sensory attributes and acceptability were also determined after 7 days at 20 °C. The extra period of 30 weeks in an AIR atmosphere after ULO storage resulted in an increase in the concentration of the compounds that most contribute to the flavor of Fuji apples. These fruits were relatively well accepted by consumers despite a slight decline in firmness and acidity.

KEYWORDS: Acceptability; air storage; apple; aroma compounds; regeneration; standard quality parameters; ULO atmosphere

INTRODUCTION

Historically, most guidelines for apple storage conditions have been based on a desire to maintain standard quality specifications such as color, firmness, acidity and sugar content in order to satisfy the goal of maximizing postharvest life and optimizing commercial quality. Even so, over the years, the lack of sensory quality exhibited by a lot of produce has resulted in a failure to satisfy consumer expectations and, consequently, in a significant fall in apple sales. Producers and retailers should both be aware that if consumer satisfaction is achieved, a product will be accepted and repeat purchases will occur (1).

Although current commercial practice often disregards aroma, this aspect is an important attribute influencing the sensory quality of apples. The aroma profile of a fruit is complex and depends on the combination of all the volatile compounds emitted and also on the concentration and odor threshold of each individual compound. In apple, the aroma profile contains more than 300 volatile compounds, mainly including esters, alcohols, aldehydes, ketones, and ethers; of these, the esters are quantitatively and qualitatively the most important volatile compounds contributing to apple aroma, and are associated with the fruity perceptions (2). The esters produced by a ripening apple can be separated into straight-chain and branched-chain types. Whereas straight-chain esters are thought to be derived from fatty acids, branched-chain esters are thought to originate

from the metabolism of branched-chain amino acids (3). The majority of these esters are synthesized during the climacteric phase of ripening (4).

Controlled atmosphere (CA) storage is a well-established technique for maintaining fruit quality and extending the postharvest life of apples. It is well documented that CA with low concentration of O₂ offers great benefits for long-term storage in terms of maintaining texture, soluble solids, and the acidity of apples, but has the drawbacks of reducing the production of some volatiles and consequently producing fruit of poor flavor and aroma compared to that stored in air atmospheres (2, 5–10). The extent and speed of recovery of aroma volatile production after CA varies with the cultivar and storage time (11, 12).

Several different techniques have been studied with the aim of achieving the regeneration of aroma volatile compounds after CA-storage: the treatment of fruit with ethylene during (13) or after storage (14); treatments with precursors of ester compounds such as alcohols, acids, and aldehydes (13, 15–17); exposure to hypoxia atmospheres with 2% O₂ or up to 100% CO₂ (2, 18); and exposure to an extra period under cold air conditions after storage (13). Because of its easy application in cold stores, the last of these possibilities may be interesting for the optimization of storage in order to obtain higher concentrations of aroma volatile compounds and to increase the consumer acceptability of fruits. If this does not result in an excessive reduction of the standard quality parameters of stored fruit, it could be recommended for commercial use.

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Fuji apples stand out for their excellent sensory characteristics (19, 20) and especially for their flavor (21–23) and crunchiness (21, 24), but also for their good storage potential. In previous works (25), we reported that ultra low oxygen (ULO) conditions (1 kPa O₂) had the effect of reducing total volatile production in this cultivar after storage. Moreover, it was concluded that ethyl 2-methylbutanoate, 2-methylbutyl acetate, and hexyl acetate were the compounds that most contributed to Fuji flavor. We also demonstrated the importance of certain aroma volatile compounds for consumer acceptability. In fact, higher acceptability scores were found for fruits with higher emissions of ethanol, *t*-butyl propionate, ethyl butanoate, hexyl acetate, (*E*)-2-hexenol, and butyl hexanoate, although these fruits did not always exhibit the highest total aroma emissions. This suggests that the concentration of some specific volatile compounds is more important than total aroma volatile emission for determining overall fruit acceptability (26).

Because of the lack of recent studies on the regeneration of volatile compounds and their importance in maintaining apple eating quality, we focus this work on ascertaining whether an additional period under cold air conditions after ULO storage could help regenerate some of the volatile compounds in Fuji apples without the loss of firmness, acidity, and solid soluble content, as this would help to improve the sensory acceptance of the fruit.

MATERIALS AND METHODS

Plant Material and Storage Conditions. Apple (*Malus domestica* Borkh. cv. Fuji Kiku-8) fruits were harvested at commercial maturity, 180 days after full bloom (dafb), from 4 year-old trees grown on M-9 EMLA rootstock at the IRTA-Experimental Station, Lleida (NE Spain). Immediately after harvest, 3 lots of 100 kg of apples were selected for uniformity and absence of defects and stored at 1 °C and 92–93% relative humidity (RH) in an ULO atmosphere (1 kPa O₂:1 kPa CO₂) for up to either 19 (S1) or 30 weeks (S2). One lot of ULO-stored fruit remained under these conditions for the whole 19 or 30 week (ULO) period. A second lot was kept for either 17 or 28 weeks under ULO conditions and then stored for 2 weeks in AIR (ULO + 2w). A third batch of fruit was kept for either 15 or 26 weeks in ULO followed by a further 4 weeks in AIR (ULO + 4w). After storage, the fruit was stored at 20 °C, in order to simulate commercial shelf life, during 1 (SL1) and 7 (SL7) days. Instrumental quality measurements and volatile compound emission were measured after 1 and 7 days of fruit storage at 20 °C. Sensory analyses were only carried out after 7 days of storage at 20 °C.

Chemicals. The chemicals obtained were of the highest quality available and were supplied by Sigma-Aldrich (Steinheim, Germany) unless otherwise indicated. Ethyl acetate, *t*-butyl propanoate, propyl acetate, 1-propanol, ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, 2-methyl-1-propanol, 1-butanol, pentyl acetate, 2-methyl-1-butanol, hexyl acetate, 1-hexanol, hexyl 2-methylbutanoate, and 2-ethyl-1-hexanol were obtained from Fluka (Buchs, Switzerland). Ethanol was purchased from Panreac Química, S.A. (Castellar del Vallès, Spain). 2-Methylpropyl acetate and 2-methylciclo pentanone were obtained from Avocado Research Chemicals Ltd. (Madrid, Spain).

Analysis of Volatile Compounds. Eight kilograms of apples (2 kg/replicate × 4 replicates) per treatment (atmosphere × storage period × shelf life period) were selected for analysis of volatile compounds both at harvest and after removal from storage. Intact fruits were placed in an 8 L Pyrex container through which an air stream (900 mL min⁻¹) was passed for 4 h. The resulting effluent was then passed through an ORBO-32 adsorption tube filled with 100 mg of activated charcoal (20/40 mesh), from which volatile compounds were desorbed by agitation for 40 min with 0.5 mL of diethyl ether. The identification and quantification of volatile compounds was performed on a HP 5890 series II gas chromatograph (Hewlett-Packard Co., Barcelona, Spain) equipped with a flame ionization detector (GC-FID), using a cross-linked FFAP capillary column (50 m × 0.2 mm × 0.33 μm) into which

a volume of 1 μL of the extract was injected in all the analyses. The oven program was set at 70 °C (1 min), and the temperature was first raised by 3 °C/min to 142 °C and later by 5 °C/min to 225 °C. It was then kept constant for 10 min at this later temperature. Helium was used as the carrier gas (42 cm³ s⁻¹), with a split ratio of 40:1. The injector and detector were held at 220 and 240 °C, respectively. Compounds were identified by comparing their respective retention indexes with those of standards and by enriching apple extract with authentic samples. Quantification was carried out by adding 25 μL of a 0.2% solution of butylbenzene (assay > 99.5%, Fluka) as an internal standard. A GC-MS system (Hewlett-Packard 5890) was used for compound confirmation, using the same capillary column as in the GC analyses. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as the carrier gas (42 cm³ s⁻¹), following the same temperature gradient program as described previously. Spectrometric data were recorded (Hewlett-Packard 3398 GC Chemstation) and compared with those from the NIST HP59943C original library mass spectra. Results were expressed as μg·kg⁻¹.

Sensory Measurements. For consumer evaluation, the fruit samples removed from each atmosphere and during each storage period were kept in a room at 20 °C for 7 days. Twenty apples per treatment (atmosphere × storage period) were used for sensory analysis. Prior to sensory evaluation, half of each fruit was instrumentally analyzed in relation to its standard quality parameters. Three pieces (one per atmosphere) were placed on white plates and immediately presented to a tasting panel of 40 consumers who conducted a sensory evaluation of fruit for both storage periods. All 40 participants were the same for all treatments assessed. Consumers were volunteers from the staff working at the UdL-IRTA research institute and students from the University of Lleida. All the test participants were habitual (daily) apple consumers. Each piece was identified with a random three-digit code. The order of presentation of the three fruit parts presented on the white plate was randomized for each consumer. Mineral water was used as a palate cleanser between samples. All evaluations were conducted in individual booths under white illumination and at room temperature. Each consumer assessed all three samples and was asked to indicate his/her degree of like/dislike using a 9-point hedonic scale (1-dislike extremely to 9-like extremely). Sweetness, sourness, overall flavor, crispness, firmness, and juiciness were also evaluated by means of a test in which the judges were asked to rank the samples in increasing order (from less to more) for each of the attributes considered. The samples could be retasted as often as required.

Standard Quality Parameter Analyses. As explained in the previous paragraph, parts from 20 fruits per treatment were individually assessed for flesh firmness, soluble solids content (SSC), titratable acidity (TA), and skin color, both at harvest and after removal from cold storage (atmosphere × storage period × shelf life period). Flesh firmness was measured on opposite sides of each fruit with a penetrometer (Effegi, Milan, Italy) equipped with an 11-mm diameter plunger tip; results were expressed in N. SSC and TA were measured in juice pressed from the whole fruit. SSC was determined with a hand refractometer (Atago, Tokyo, Japan), and results were expressed as % sucrose in an equivalent solution. TA was determined by titrating 10 mL of juice with 0.1 N NaOH to pH 8.1 using phenolphthaleine (1%), and results were given as g malic acid/L. Fruit epidermis color was determined with a portable tristimulus colorimeter (Chroma Meter CR-200, Minolta Corp, Osaka, Japan) using CIE illuminant D₆₅ and with an 8 mm measuring aperture diameter. Skin color was measured at two points on the equator of each fruit that were 180° apart: one on the side exposed to sunlight (ES) and the other on the shaded side (SS). Hue angle was measured on both the side exposed to the sun and on the shaded side and the resulting values were respectively used as measurements of superficial and background color.

Statistical Analysis. A multifactorial design was used to statistically analyze the results. The factors considered were storage period, storage atmosphere, shelf life period, and replication. All data were tested by analysis of variance (GLM-ANOVA procedure) applying the SAS program package (27). Means were separated by the LSD test at *P* ≤ 0.05. For multivariate analysis, samples were characterized according to average measurements (instrumental analyses) or by taking average scores for all the consumers (sensory analyses). Two principal

Table 1. Compounds Identified Using GC, Retention Index, Codes Used for PCA and PLS Analyses and Amount at Harvest ($\mu\text{g} \cdot \text{kg}^{-1}$)

compound	RI ^a	code	amount
methyl acetate	834	mA	6.4
ethyl acetate	898	eA	13.7
ethanol	932	eOH	15.2
t-butyl propanoate	964	tbPr	3.1
propyl acetate	984	prA	6.6
methyl butanoate	995	mB	nd
2-methylpropyl acetate	1020	2mprA	4.2
1-propanol	1036	1prOH	7.4
ethyl butanoate	1043	eB	5.8
ethyl 2-methylbutanoate	1059	e2mB	4.6
butyl acetate	1082	bA	27.4
2-methylpropyl propanoate	1086	2mprPr	nd
2-methyl-1-propanol	1091	2m1prOH	2.2
2-methylbutyl acetate	1131	2mbA	246.7
1-butanol	1141	1bOH	6.1
butyl propanoate	1148	bPr	21.0
butyl 2-methylpropanoate	1149	b2mPr	nd
2-methylpropyl butanoate	1165	2mprB	5.3
pentyl acetate	1183	pA	9.0
2-methylbutyl propanoate	1199	2mbPr	14.1
2-methyl-1-butanol	1210	2m1bOH	53.7
D-limonene	1215	D-lim	nd
2-methylciclo pentanone	1218	2mciclona	nd
butyl butanoate	1228	bB	5.4
butyl 2-methylbutanoate	1240	b2mB	12.8
ethyl hexanoate	1241	eHx	nd
pentyl propanoate	1242	pPr	4.4
1-pentanol	1253	1pOH	1.6
hexyl acetate	1283	hxA	57.7
2-methylbutyl 2-methylbutanoate	1288	2mb2mB	nd
2-heptanol	1326	2hOH	nd
hexyl propanoate	1349	hxPr	3.1
6-methyl-5-hepten-2-one	1353	6m5hep2one	nd
1-hexanol	1358	1hxOH	31.8
2-methylpropyl hexanoate	1359	2mprHx	4.0
butyl hexanoate	1423	bHx	34.8
hexyl butanoate	1426	hxB	34.6
hexyl 2-methylbutanoate	1436	hx2mB	97.5
octyl acetate	1484	oA	2.4
2-ethyl-1-hexanol	1494	2ehxOH	28.8
pentyl hexanoate	1520	pHx	7.0
hexyl hexanoate	1621	hxHx	26.2
butyl octanoate	1623	bO	3.9
hexyl octanoate	1854	hxO	nd

^a Kovats retention index in the cross-linked FFAP column (38).

component analysis (PCA) models were performed to provide an easy visualization of the complete data set in a reduced dimension plot. The first PCA model included the 6 samples corresponding to 19 weeks of storage and the second included the 6 samples stored for 30 weeks. Forty-five variables (volatile compounds) were considered in each case. The samples were characterized by their volatile compound emissions. Sample names were coded as described in the Plant Material and Storage Conditions section. The variables analyzed were labeled as specified in **Table 1**. Partial least-squares regression (PLSR) was used as a predictive method to relate consumer acceptability (*Y*) to a set of explanatory variables (*X*) that contains the volatile compounds, instrumental quality measurements, and sensory attributes within a single estimation procedure. Unscrambler, version 6.11a software (28) was used to develop these models. As a pretreatment, data were centered and weighted using the inverse of the standard deviation of each variable in order to avoid the influence of the different scales used for the variables (29). Full cross-validation was run as a validation procedure.

RESULTS AND DISCUSSION

Volatile Compound Emission at Harvest. The volatile compounds identified and quantified at harvest are shown in **Table 1**. A total of 34 compounds were detected, of which 26 were esters (9 acetates, 5 propanoates, 7 butanoates, 4 hexanoates, and 1 octanoate) and 8 alcohols.

The esters 2-methylbutyl acetate, hexyl acetate, hexyl 2-methylbutanoate, butyl hexanoate, and hexyl butanoate, and

alcohols 2-methyl-1-butanol and 1-hexanol were quantitatively the main compounds present. They accounted for 70% of the total volatile fraction. Emissions of 2-methylbutyl and hexyl acetates were related to higher concentrations of their alcohol precursors, as the production of 2-methyl-1-butanol and 1-hexanol paralleled that of their corresponding esters (**Table 1**). This contribution from the alcohol precursors confirmed previous reports for Fuji (30), Gala (31), and Greensleeves apples (32). Moreover, 2-methylbutyl acetate was reported as the predominant volatile compound in Fuji, in agreement with other authors (31, 33).

In previous works, ethyl butanoate, ethyl 2-methylbutanoate, 2-methylbutanoate acetate, ethyl hexanoate, and hexyl acetate were considered to be the compounds that most contribute to Fuji flavor at harvest (30, 33). These compounds also reportedly contribute to fresh-green and fruity odors (34–37).

Standard Quality Parameters at Harvest and after Storage. **Table 2** shows values for standard quality parameters in apples at harvest and after cold storage plus periods of 1 or 7 days at 20 °C (simulating their commercial life and final quality on reaching potential consumers). In all cases, lower values for TA and hue SS (indicating more yellowing on the epidermis) were found for stored fruit than freshly harvested apples (TA, LSD = 0.27; Hue SS, LSD = 7.93). These changes are consistent with the softening, decomposition of organic acids and modification of pigments, which are typically associated with fruit ripening. The values of the quality parameters at harvest have commercial importance as the initial maturity of the fruit is a key factor for successful storage. In this work, fruit values coincided with those recommended for this cultivar (39).

One day after removal from 19-week storage, firmness and superficial red color (Hue ES) remained good in fruit exposed to the ULO + 2w and ULO + 4w conditions in comparison with fruit kept under ULO atmosphere conditions. However, SSC decreased in fruits subjected to the ULO + 2w and ULO + 4w treatments; fruits subjected to the ULO + 4w atmosphere also showed lower values of TA than those stored in a ULO atmosphere, in addition to a higher degree of yellowing, which was indicative of a more advanced stage of ripening. In general, fruits kept under ULO conditions had higher SSC levels than AIR fruits (33). After 7 days of shelf life, no significant changes were observed between the different atmospheres. After 30 weeks of storage (plus 1 day at 20 °C), the atmosphere did not seem to have any significant influence on fruit firmness, superficial color, or background color. After 7 days at 20 °C, a similar retention of firmness was observed, regardless of the storage conditions. In contrast, extending the shelf life period produced an increase in SSC and a reduction in Hue(SS) and Hue(ES) values in fruits subjected to the ULO + 4w treatment. There was also a decline in TA levels in fruits subjected to the ULO + 2w treatment, which indicated more advanced maturity stages in these fruits than in those subjected to ULO.

Volatile Compound Emission after Cold Storage. A total of 45 compounds were detected after cold storage compared with the 34 found at harvest. The compounds that appeared after cold storage but not at harvest were the esters methyl butanoate, ethyl hexanoate, hexyl octanoate, 2-methylpropyl propanoate, butyl 2-methylpropanoate, heptyl 2-methylbutanoate, and 2-methylbutyl 2-methylbutanoate; the alcohol 2-heptanol; and D-limonene, 2-methylciclo pentanone, and 6-methyl-5-hepten-2-one. The total straight-chain ester compounds were divided in 7 acetates, 3 propanoates, 4 butanoates, 4 hexanoates, and 2 octanoates, while the total branched-chain esters comprised 2

Table 2. Standard Quality Parameters of Fuji Kiku 8 Apples at Harvest and after Storage under Different Conditions (ULO, ULO + 2 weeks in Air and ULO + 4 Weeks in Air) Plus 1 and 7 Days at 20°C^a

treatments	storage (weeks)	days at 20 °C	Firmness (N)	SSC (%)	TA (g/L)	Hue (SS)	Hue (ES)
at harvest			74.8	16.0	3.7	99.9	38.2
ULO	19	1	71.0 B	16.5 B	2.7 BC	80.4 B	41.0 C
	19	7	73.1 AB	16.3 BC	3.1 AB	76.2 BC	45.8 BC
	30	1	72.9 AB	15.8 C	3.2 A	77.5 BC	47.6 B
ULO + 2w	30	7	65.3 CD	16.0 BC	2.8 B	78.7 B	49.4 AB
	19	1	68.1 BC	15.4 CD	2.8 B	87.3 AB	45.3 BC
	19	7	74.2 AB	15.8 C	3.2 A	78.0 B	43.1 BC
ULO + 4w	30	1	72.5 AB	15.0 D	2.8 B	79.4 B	48.1 AB
	30	7	63.2 D	15.8 C	2.5 C	80.8 AB	53.1 A
	19	1	67.8 BC	14.9 D	2.1 D	88.7 A	43.7 BC
	19	7	69.1 BC	15.7 C	2.7 BC	86.5 AB	46.6 BC
	30	1	75.8 A	17.4 A	3.1 A	78.2 B	44.2 BC
	30	7	67.0 C	17.6 A	3.0 AB	69.9 C	41.7 C

^a Means followed by different capital letters for each quality parameter are significantly different at $P \leq 0.05$ (LSD test).

acetates, 4 propanoates, 6 butanoates, and 1 hexanoate. A further 12 alcohols were detected after the different storage periods (**Table 1**).

An increase in total emissions of volatile compounds was observed for ULO + 2w and ULO + 4w fruit with respect to samples stored in ULO, regardless of the storage and shelf life periods (ULO, 3079 $\mu\text{g kg}^{-1}$; ULO + 2w, 4621.4 $\mu\text{g kg}^{-1}$; ULO + 4w, 4983.9 $\mu\text{g kg}^{-1}$). Nevertheless, it was necessary to conduct a deeper study of the relationship between volatile compounds and the samples analyzed for different storage and shelf life periods and also for each atmosphere. For this reason, two PCA models were run that considered all 45 volatile compounds emitted by Fuji apples. The first included samples from fruit stored for 19 weeks (**Figure 1A**) and the second included samples subjected to 30 weeks of cold-storage (**Figure 1B**). The variances explained by the first two PCs were 76 and 77% for samples stored for 19 and 30 weeks, respectively. The biplot for short-term storage (**Figure 1A**) revealed that 89% of the volatile compounds were located on the right-hand side of the graph, which indicated a strong correlation with samples stored under ULO + 2w and ULO + 4w conditions after 1 day at 20 °C. A similar pattern was observed for samples subjected to long-term storage (**Figure 1B**). Both biplots showed that samples were separated over the first PC. It can also be observed that the shelf life period was responsible for the main differences between the samples. This was especially detected in the case of samples stored for 30 weeks. We can consequently say that an extra period under AIR conditions after ULO storage increased the amount of the most volatile compounds with respect to ULO after 1 day at 20 °C. This would seem to suggest that the capacity of fruit to synthesize volatile compounds was modified by these treatments. However, this behavior was not observed after 7 days at 20 °C. Previous authors observed the negative influence of shelf life on the production of the acetate compounds that most contribute to Fuji flavor (12).

After 1 day at 20 °C following 19 weeks of cold storage, the concentrations of the following straight-chain esters increased significantly in fruits exposed to the ULO + 2w and ULO + 4w atmospheres: butyl acetate, pentyl acetate, hexyl acetate, butyl butanoate, hexyl butanoate, butyl hexanoate, pentyl hexanoate, and hexyl hexanoate, with respect to those stored in a ULO atmosphere. These compounds contribute a fruity and apple smell (34, 37, 40). Straight-chain organic acid precursors are formed by the oxidation of fatty acids and/or via lipoxigenase activity. Both of these processes require oxygen and are presumably slowed down by ULO storage conditions (41). After 7 days at 20 °C following short-term storage (19 weeks), the concentrations of most straight-chain esters increased in line

with the time that the fruits were stored in AIR. In the case of branched-chain esters, and regardless of the storage period, after 1 day at 20 °C, fruit stored under ULO + 4w and ULO + 2w conditions displayed higher concentrations than those stored under ULO conditions. It is also important to note that emissions of ethyl 2-methylbutanoate were greater in the case of fruit stored under ULO + 2w and ULO + 4w conditions after longer periods of cold storage (30 weeks) plus 7 days at 20 °C than in ULO-stored fruit after 1 day at 20 °C. It has been reported that ethyl 2-methylbutanoate is one of the main contributors to the flavor of Fuji Nagafu 6 (12, 33), Starking Delicious (42), and other Delicious apples (35).

Much effort is made to identify the most important contributors to apple flavor in different apple varieties. **Table 3** shows the evolution of the concentrations of the main contributors to Fuji apple aroma reported by others authors (12, 33). In this way, it is possible to analyze in depth whether concentrations increased with extra time in AIR after storage in a ULO atmosphere. From **Table 3**, it can be generally concluded that after 7 days at 20 °C for both storage periods, the most efficient treatment for increasing the main volatile compounds was ULO + 4w, whereas after 1 day at 20 °C, differences were found for each storage period. After 19 weeks of storage, the ULO + 2w atmosphere helped to increase the amount of hexyl acetate, ethyl 2-methylbutanoate and 2-methylbutyl acetate, while ULO + 4w was the most successful atmosphere for long-term storage. Quantitatively, the most abundant straight-chain ester was hexyl acetate (**Table 3**). From our results, we observed that the atmosphere was only effective for short storage periods, with ULO + 2w and ULO + 4w being the most efficient conditions after 1 and 7 days at 20 °C, respectively. No differences were found for long-term storage. Our results showed an inhibiting effect on the synthesis of hexyl acetate after lengthening the shelf life period to 7 days at 20 °C in the case of short-term storage (**Table 3**). This decrease in hexyl acetate with extended shelf life confirms the findings of previous reports (12) for Fuji Nagafu 6 apples. The inhibiting effect of shelf life could be explained by insufficient substrate availability: substrate is necessary for aroma recovery after cold storage (25). The pool of available precursors may have been consumed earlier in the shelf life period. Straight-chain organic acid precursors are formed by β -oxidation of fatty acids and/or via lipoxigenase activity, both of which require oxygen and are presumably slowed down by ULO storage conditions (30).

Except in the case of 19-week storage, the ULO + 4w atmosphere allowed the regeneration of ethyl butanoate with respect to ULO + 2w and ULO. The same pattern was observed for ethyl hexanoate. Indeed, we should emphasize

Table 3. Compounds ($\mu\text{g} \cdot \text{kg}^{-1}$) That Most Contribute to Fuji Flavor^a

compounds	19 weeks				30 weeks		
	days at 20 °C	ULO	ULO + 2w	ULO + 4w	ULO	ULO + 2w	ULO + 4w
hexyl acetate	1	155.6 B	251.8 A	172.0 B	87.7 CD	119.0 BC	86.0 CD
	7	29.9 D	47.6 CD	93.6 C	24.8 D	43.2 CD	33.5 D
ethyl butanoate	1	3.1 BC	3.1 BC	2.0 BC	nd	nd	1.5 BC
	7	1.6 BC	5.8 AB	6.9 A	0.8 C	3.0 BC	3.6 B
ethyl hexanoate	1	nd	nd	nd	nd	nd	13.9 A
	7	nd	nd	5.4 C	nd	nd	10.9 B
2-methylbutyl acetate	1	503.6 CD	779.0 B	1017.3 A	405.1 D	577.3 C	680.9 BC
	7	290.1 DE	469.2 CD	456.8 CD	194.2 E	299.6 DE	300.0 DE
ethyl 2-methylbutanoate	1	15.5 BC	32.5 A	14.7 BC	10.3 BC	14.7 BC	7.9 C
	7	11.5 BC	13.7 BC	16.8 BC	16.7 BC	16.9 BC	18.8 B

^a Means followed by different capital letters for a given compound are significantly different at $P \leq 0.05$ (LSD test). nd = not detected.

Table 4. Global Acceptability and Sensory Attributes of Fuji Kiku 8 Apples after 19 and 30 Weeks in Cold Storage under Different Conditions (ULO, ULO + 2 Weeks in Air and ULO + 4 Weeks in Air) after 7 Days at 20 °C^a

sensory attributes	ULO		ULO + 2w		ULO + 4w	
	19	30	19	30	19	30
acceptability	6.08 A	6.47 A	6.45 A	6.16 A	6.35 A	6.40 A
flavor	1.71 B	1.90 AB	2.17 A	1.95 AB	2.13 A	2.15 A
sweetness	1.88 B	1.94 B	1.98 B	1.73 B	2.12 AB	2.34 A
sourness	1.95 AB	1.80 B	2.18 A	2.13 AB	1.87 B	2.07 AB
crispness	2.03 AB	2.06 AB	2.23 A	1.81 B	1.75 B	2.14 A
firmness	1.93 B	1.97 AB	2.24 A	1.93 B	1.87 B	2.09 AB
juiciness	1.85 B	2.11 AB	1.99 AB	1.96 AB	2.16 A	1.93 AB

^a Means followed by different capital letters for each sensory attribute are significantly different at $P \leq 0.05$ (LSD test).

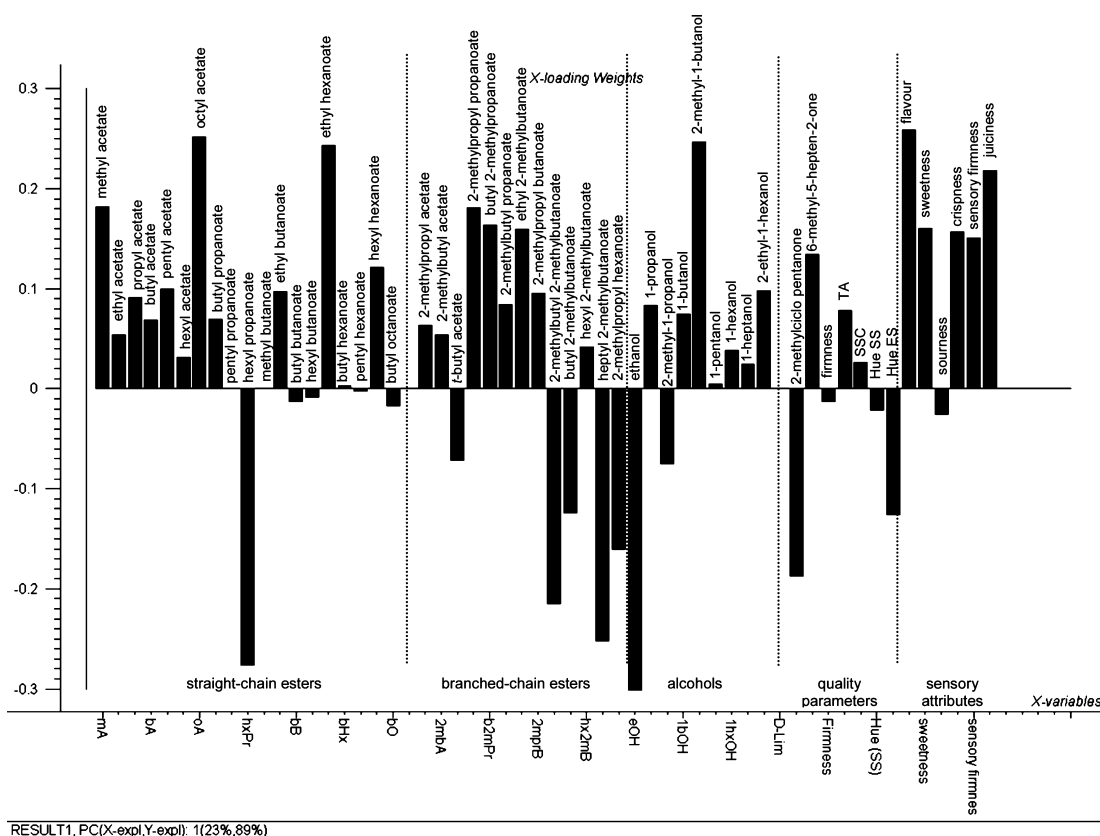


Figure 2. Loading weight plot of PC1 vs PC2 from a PLSR model of volatile compounds; standard quality parameters and sensory attributes (X variables) vs consumer acceptability (Y variable) for Fuji kiku 8 apples after cold storage and 7 days at 20 °C. Aroma volatile compounds are coded as indicated in Table 1. Samples are labeled as described in the Material and Methods section.

storage. Its contribution is also notable in Fuji Nagafu 6 (12) and Starking Delicious (42), and other Delicious apples (35).

It can generally be concluded that the extra period under AIR conditions helped to enhance the aroma profile, adding more intense attributes such as a ripe, fruity smell (34–37).

Acceptability and Sensory Attributes. As far as acceptability was concerned, there were no significant differences associated with the different atmospheres and storage periods (Table 4). With respect to flavor, fruits stored for short periods under ULO conditions registered lower scores than

those subjected to ULO + 2w and ULO + 4w atmospheres. No significant differences were found between ULO + 2w and ULO + 4w. Moreover, we did not detect any influence of storage time. As regards the sweetness perceived by the consumers, all fruit received the same score for short-term storage (19 weeks). However, in the case of long-term storage (30 weeks) the ULO + 4w atmosphere received the highest score, while fruit subjected to the ULO and ULO + 2w conditions scored lowest. For sourness, fruit kept under ULO + 2w conditions scored the highest values for both storage periods. After longer periods (4 weeks) under AIR conditions there was a decrease in the perception of sourness; fruit kept under ULO conditions also received lower scores. Consumers are usually very sensitive to differences in fruit acidity, and recent studies have shown that differences as small as 0.8 g malic acid/L between two apples can be detected by a trained panellist (43). However, no differences in sourness were observed between the two storage periods. With respect to crispness and sensory firmness, both attributes showed the same pattern after 19 weeks of cold storage. The lowest scores were for fruit subjected to ULO + 4w, while the most positive perceptions of crispness and sensory firmness corresponded to fruits subjected to the ULO + 2w treatment. For the ULO + 2w treatment, differences between the two storage periods were found, with lower values for long-term than for short-term storage. For juiciness, fruits stored for shorter periods were perceived as juicier than those subjected to longer-term storage under AIR conditions. In contrast, no significant differences in the perception of juiciness were found for fruit stored for 30 weeks.

PLS Regression Model of Acceptability According to Volatile Compound Emission, Instrumental Quality Measurements, and Sensory Attributes. A PLSR model was developed with the aim of identifying the main variables influencing consumer acceptability. The model considered volatile compounds, instrumental quality measurements, and sensory attributes as *X* variables and acceptability as the *Y* variable. This procedure allowed a rapid assessment of relationships between the dependent variable (*Y*) and a set of potentially explanatory variables (*X*). The validation step indicated that only one PLS factor was relevant in the model. The percentage of explained variance involving this factor was 89% of total variability (**Figure 2**).

Higher acceptability scores were associated with fruit exhibiting higher emissions of the straight-chain esters methyl acetate, octyl acetate, and ethyl hexanoate, and the branched-chain esters 2-methylpropyl propanoate, butyl 2-methylpropanoate, and ethyl 2-methylbutanoate. Within the alcohol group, we concluded that 2-methyl-1-butanol had the greatest influence on acceptability, followed by 1-propanol, 1-butanol, and 2-ethyl-1-hexanol. Of these, ethyl hexanoate and ethyl 2-methylbutanoate stood out for their contribution to the aroma profile of Fuji apples (12, 33). It is important to highlight that ULO + 4w atmosphere caused an increase of the amount of ethyl hexanoate (**Table 3**) as well as of 2-methylpropyl propanoate and butyl 2-methylpropanoate (data not shown). We had also reported the influence of some volatile compounds on consumer acceptability in previous works (44). The combined results suggest that concentrations of certain specific volatile compounds are more important than total aroma volatile emissions in determining overall fruit acceptability. The most important volatile compound that showed the lowest weight on acceptability was ethanol. Ethanol accumulation during storage leads to a disproportionate synthesis of ethanol-derived esters (45). Because of their low organoleptic thresholds (35), disproportionate amounts of these volatiles may be responsible for some of the off-

flavors attributed to fruit stored under hypoxic-controlled atmosphere (CA) conditions (46).

The instrumental quality measurements that positively influenced acceptability were TA and SSC. This result largely confirmed the findings reported by Alavoine et al. (47) and Echeverria et al. (44). There were discrepancies between our study and theirs with respect to fruit firmness and Hue on the shaded side. They reported the influence to be positive in these areas, but according to our results these variables had no positive influence. The weight of the variable Hue(ES) indicated that when red color was lower, fruit acceptability decreased. This result was in accordance with Echeverria et al. (44) and Crasweller and Hollender (48) who reported that red color was the most important instrumental quality parameter influencing apple purchasing patterns.

The sensory attributes that most influenced acceptability were flavor (with the greatest influence) and juiciness. These results are in agreement with Péneau et al. (49) who showed that freshness, together with taste and aroma, was a decisive attribute for selecting apples. They related freshness to crispness, juiciness, aroma, and liking.

Figure 2 shows that some sensory results did not perfectly reflect the corresponding instrumental measurements. Sweetness had a greater influence on acceptability than SSC. This could be due to the fact that perceptions of sugar content can be accentuated by the presence of acids or because some aroma compounds contribute sweet notes to flavor (12, 50, 51).

In summary, the extra period in AIR conditions allowed the regeneration of the characteristic esters for this variety that contribute to fresh-green and fruity odors. Acceptability was mainly determined for some volatile compounds, along with acidity and sugar content as well as the perceived flavor for consumers.

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