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Effect of the Commercial Ripening Stage and Postharvest Storage on Microbial and Aroma Changes of 'Ambrunés' Sweet Cherries

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The purpose of this work was to investigate the effect of the commercial ripening stage and postharvest storage on microbial and aroma changes of 'Ambrunés' sweet cherries. The microbial counts and volatile profile of sweet cherry batches automatically sanitized and classified in three commercial ripening stages were studied for five postharvest storages. The batches were also evaluated sensorially, and the correlations between volatile compounds and aroma quality were determined. The microbial counts provided evidence that 21 days of cold storage is near the maximum extension of 'Ambrunés' sweet cherry storage in maintaining the minimal microbial quality during their shelf-life period. Relevant changes associated with longer cold storages were found in different aroma constituents with a negative impact on flavor. These changes were more evident in less ripened sweet cherries, including a decrease of (*E*)-2-hexenal and 1-hexanol and an increase of 2-methyl-propanal and 2-methyl-butanal. These compounds could constitute a good tool to predict flavor quality in 'Ambrunés' sweet cherries during the cold-storage process.

KEYWORDS: Sweet cherry; microbial quality; volatile compounds; ripening stage; postharvest storage

INTRODUCTION

Sweet cherry "Cereza del Jerte" is a high-quality fruit of *Prunus avium* L. varieties from the "Jerte Valley" region, in the central west of Spain. Autochthonous varieties, mainly 'Ambrunés', are hand-picked without stems (type "Picota") and marketed under the Registry of the Protected Designation of Origin "Cereza del Jerte" (Valdastilla, Cáceres, Spain). The environmental conditions give the "Cereza del Jerte" a more highly valued flavor than other commercial sweet cherry varieties.

There has been growing interest in the study of fruit flavor components and their influencing factors (*1*−*3*). In the sweet cherry, there are several studies of the aroma compounds (*4*−*6*). Among the volatile compounds causing the characteristic aroma of sweet cherries, aldehydes, esters, and alcohols have been identified. C_6 aldehydes [hexanal and (*E*)-2-hexenal], (*E*)-2-hexenol, and benzaldehyde have been described as the most important aromas of sweet cherries (*4*, *5*, *7*). These volatile compounds have also been suggested as a useful tool to segregate commercial and new cherry selections into various subgroups (*5*). However, most of these studies concern fresh fruit (*4*−*7*) or study changes in aroma components during fruit development (*6*). Usually, after mechanical classification of the fruit into commercial ripening stages, they are kept in cold storage in sweet-cherry-producing areas, to preserve fruit quality and decrease the incidence of physiological disorders. Good sensory and microbial qualities are critical factors in maintaining the commercial marketability of sweet cherries after cold storage (*8*).

The objective of our research was to ascertain the effect of the commercial ripening stage and postharvest storage on microbial and aroma changes of 'Ambrunés' sweet cherries.

MATERIALS AND METHODS

Sample Collection. The samples of sweet cherry (*Prunus avium* L.) used in this study were obtained from 12-year-old sweet cherry trees of the 'Ambrunés' cultivar on *Prunus avium* L. rootstock from an experimental orchard at an altitude of 400 m above sea level in Cabrero (latitude, 40° 06′ 40″ N; longitude, 5° 53′ 20″ W), the Jerte Valley (Cáceres, Spain). Fruits were harvested totally at random from multiple trees and transported to the distribution center in less than 1 h. They were then hydrocooled at a water temperature of 1 °C in a 1000 L immersion hydro-cooler equipped with a water recirculation system. Sodium hypochlorite was added to the water to achieve a chlorine concentration of 100 μL L^{-1}. Then, the cherries were grouped into three commercial ripening stages based on the size and color of the fruit by an I20 automatic color sorter (Multiscan Technologies, Alicante, Spain; **Table 1**). Fruits (approximately 500 g) were packaged in polypropylene trays and sealed with macro-perforated film. Five batches of five fruit trays for each ripening stage were randomly selected and stored in a chamber at 1 °C with a relative humidity

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Table 1. Sweet Cherry Batches Studied

	ripening stage[a]			
batches	1	2	3	total
storage[b]				
A	5[c]	5	5	15
B	5	5	5	15
C	5	5	5	15
D	5	5	5	15
E	5	5	5	15
total	20	20	20	60

[a] Color parameters: stage 1, 34−49 (I20 automatic color sorter, Multiscan Technologies, Alicante, Spain) corresponding to mean values of L^* = 35.68, a^* = 34.03, b^* = 14.16, C^* = 36.89, and hue = 22.33 (Minolta CR-400 chroma meter, Osaka, Japan); stage 2, 25.5−34 (L^* = 31.15, a^* = 22.47, b^* = 7.79, C^* = 23.78, and hue = 18.94); stage 3, 0−25.5 (L^* = 28.51, a^* = 14.62, b^* = 3.28, C^* = 14.98, and hue = 12.67). [b] Storage A, 0 days at 1 °C + 2 days at 5 °C (transport simulation) + 2 days at 20 °C (shelf life); storage B, 7 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage C, 14 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage D, 21 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage E, 28 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C. [c] Number of fruit trays per batch.

(RH) of 95% in darkness for 0, 7, 14, 21, and 28 cold-storage days (**Table 1**). After cold storage, the batches were subjected to a period of 2 days at 5 °C and then a shelf-life period of 2 days at 20 °C to simulate commercial practices. Furthermore, additional samples of each ripening stage were collected immediately after hydro-cooler treatment for microbial analysis.

Physicochemical Analysis. For each batch, 25 fruits of each tray were homogenized and assayed for total soluble solids (TSS), titratable acidity (TA), pH, and maturation index (TSS/TA) for each sampling date. TSS was determined with an automatic temperature-compensated DR101 digital refractometer (Optic Ivymen System, Barcelona, Spain), and the results were expressed as °Brix. TA and pH were determined in a 5 g aliquot diluted to 50 mL with deionized water obtained with a Milli-Q water purification system (Millipore, Bedford, MA), using a 716 DMS automatic titrator (Metrohm, Herisau, Switzerland). Values determined are the means of two measurements. Samples were titrated with 0.1 mol L^{-1} NaOH until the pH reached 8.1. Results are expressed as a percentage of malic acid. The maturation index was calculated as the ratio between TSS (°Brix) and TA (percentage of malic acid).

Microbiological Analysis. For the counts, three independent replicates of 10 g of sweet cherry sample per batch were homogenized in 90 mL of sterile 0.1% (p/v) peptone water in a Stomacher (lab blender, model 4001, Seward Medical, London, U.K.) for 30 s. Appropriate dilutions were made with 0.1% (p/v) peptone water, and 1 mL aliquots were plated onto the culture media under the following conditions: the mesophilic and psychrotrophic aerobic bacteria counts on plate count agar (PCA, Oxoid, Unipath, Basingstoke, U.K.) for 48 h at 30 ± 1 °C and 7 days at 5 ± 1 °C, respectively, yeasts and molds on malt extract agar (MEA, Oxoid) for 4 days at 25 ± 1 °C, *Pseudomonas* spp. on *Pseudomonas* agar base (PsAB, Oxoid) for 48 h at 30 ± 1 °C, *Enterobacteriaceae* spp. on violet red bile glucose agar (VRBGA, Oxoid) for 24 h at 30 ± 1 °C, and coliforms on violet red bile lactose agar (VRBLA, Oxoid) for 24 h at 37 ± 1 °C. The colonies were selected according to their morphology from plates that had counts between 30 and 300 colonies (2−5 colonies for each plate) and were then subcultured on the same medium on which they had been isolated. Each isolate was examined for colony and cell morphology under a microscope and, in the case of bacteria, was tested for its Gram reaction. Complementarily, catalase, oxidase, citrate, methyl red, and urease activities and glucose and lactose fermentation were tested to characterize the colonies at the microbial group level.

Volatile Compound Extraction. Three independent replicates of 30 fruits selected at random per batch were minced after removing the pit. A total of 1 g was weighed into a 10 mL headspace vial (Hewlett-Packard, Palo Alto, CA) and sealed with a polytetrafluoroethylene (PTFE) butyl septum (Perkin-Elmer, Foster City, CA) in an aluminum cap. Volatile compounds were extracted by solid-phase microextraction (SPME) (*9*) with a 10 mm long, 100 μm thick fiber coated with carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA). Prior to collection of volatiles, the fiber was preconditioned at 220 °C for 50 min at the GC injection port. It was then inserted into the headspace vial for 30 min at 40 °C in a water bath. Blank runs were performed to discard possible volatile contamination during the volatile compound analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analyses. GC/MS analyses were performed as described by Ruiz et al. (*9*), using an Agilent 6890 GC−5973 MS system (Agilent Technologies, Little Falls, DE). A 5% phenyl−95% polydimethylsiloxane column (30 m × 0.32 mm inner diameter, 1.05 μm film thickness, Hewlett-Packard) was used for the separation of volatile compounds. To calculate the Kovats index of the compounds, *n*-alkanes (R-8769, Sigma Chemical Co., St. Louis, MO) were run under the same conditions. The NIST/EPA/NIH mass spectrum library (comparison quality >90%) and Kovats indices were used to identify the volatile compounds (*10*). Additionally, the identity of certain analytes was confirmed by a comparison of the retention time and mass spectra using a laboratory-built MS spectral database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. Quantitative data were obtained from the total ion current chromatograms by integration of the GC peak areas.

Sensorial Analysis. A total of 15 panelists previously trained with commercial samples of sweet cherries were asked to perform a sensory characterization of the batches studied. For that, 10 fruits selected at random from each batch were presented to each panelist. Descriptive and hedonic analyses were performed according to international standard methods (*11*). The sensory descriptors used were external appearance, skin color, sweet cherry taste, firmness, and overall score. During each session, three samples were presented in randomized order to the panelists, who judged the descriptors using a numbered scale (from 1 to 10 points).

Statistical Methods. Statistical analysis of the data was carried out using SPSS for Windows, version 15.0 (SPSS, Inc., Chicago, IL). Mean values of the microbial counts, physicochemical parameters, and the area of volatile compounds were studied by two-way analysis of variance (ANOVA) and separated by Tukey's honestly significant difference test ($p \leq 0.05$). The relationships between sensorial analysis and volatile compound values were evaluated by Pearson correlation coefficients and verified by linear regression analysis.

RESULTS AND DISCUSSION

Physicochemical Analysis. In addition to color, relevant inter-maturation stage differences were found in other physicochemical parameters studied, such as TSS (**Table 2**). TSS was greater in the samples of ripening stage 3. Serrano et al. (*12*) observed a significant increase of TSS among three different maturity stages of several sweet cherry cultivars (from the least to most ripening stage). Similar values of TSS, TA, pH, and maturation index have been reported for different sweet cherry varieties, including the "Picota" type (*7*, *13*, *14*). **Table 2** also lists the physicochemical characteristics of the sweet cherries after the five different storages studied. The pH and maturation index were greater in the samples of storage D, whereas the TA was lower in samples of storage C, D, and E. The increase of maturation index values in sweet cherry batches with a long period of cold storage is coherent with the results reported in other work (*12*). Finally, the influence of the ripening stage on the evolution of the physicochemical parameters during cold storage was low because the *p* interaction values were not significant ($p_{int} > 0.05$).

Counts of the Different Microbial Groups. No relevant differences were found in microbial counts of sweet cherry samples harvested at different ripening stages during the storage periods (data not shown). After the hydro-cooling treatment, total viable counts in these batches showed mean values of 2.48 and 0.85 log CFU g^{-1} for total mesophilic aerobic bacteria and yeasts, respectively (**Table 3**). For the remaining culture media, the counts were lower than 1 log CFU g^{-1}. Similar results have been reported in fresh sweet cherries for other cultivars, such as 'Burlat' and 'Sweetheart' (*15*). With respect to the storages studied, the microbial counts are also presented in **Table 3**. Significant increases in the counts of most culture media occurred after

Table 2. Physicochemical Parameters of Sweet Cherry Fruits at Different Ripening Stages

	ripening stages				storage (days)						
physicochemical parameters	1[a]	2	3	p[b]	A[c]	B	C	D	E	p	p_{int}[b]
TSS	14.31 c	16.30 b	19.33 a	0.000	16.84	16.66	16.62	17.05	16.01	0.951	0.526
TA (% malic acid)	0.35	0.34	0.35	0.954	0.43 a	0.41 ab	0.37 b	0.37 b	0.36 b	0.000	0.189
pH	4.41	4.46	4.44	0.188	4.35 c	4.45 ab	4.42 b	4.49 a	4.46 ab	0.000	0.092
maturation index (arbitrary units)	44.18	51.76	60.51	0.171	39.51 b	40.63 ab	44.98 a	45.75 a	44.57 a	0.000	0.095

[a] Color parameters: stage 1, 34−49 (I20 automatic color sorter, Multiscan Technologies, Alicante, Spain) corresponding to mean values of L^* = 35.68, a^* = 34.03, b^* = 14.16, C^* = 36.89, and hue = 22.33 (Minolta CR-400 chroma meter, Osaka, Japan); stage 2, 25.5−34 (L^* = 31.15, a^* = 22.47, b^* = 7.79, C^* = 23.78, and hue = 18.94); stage 3, 0−25.5 (L^* = 28.51, a^* = 14.62, b^* = 3.28, C^* = 14.98, and hue = 12.67). [b] p values; p_{int}, p interaction values. [c] Storage A, 0 days at 1 °C + 2 days at 5 °C (transport simulation) + 2 days at 20 °C (shelf life); storage B, 7 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage C, 14 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage D, 21 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage E, 28 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C.

Table 3. Microbial Counts in the Sweet Cherry Storage Batches Studied

										microbial count in storage (log CFU g^{-1})					
	biochemical characterization[a]								HT[b]	A[c]	B	C	D	E	
culture medium	G	C	O	Ci	M	U	GL	L	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	p[d]
PCA									2.48 ± 1.44 b[e]	3.84 ± 0.87 b	4.10 ± 0.57 b	5.30 ± 0.48 ab	6.68 ± 0.28 a	6.38 ± 1.48 ab	0.017
PCA (psi)									0.00 ± 0.00 c	2.97 ± 2.57 bc	4.55 ± 0.75 b	5.33 ± 0.59 ab	6.65 ± 0.68 a	5.92 ± 0.74 ab	0.000
EM (yeast)									0.85 ± 1.33 c	4.24 ± 0.26 b	4.42 ± 0.24 b	4.99 ± 0.59 b	6.44 ± 0.83 a	6.79 ± 0.73 a	0.000
EM (molds)									0.00 ± 0.00 c	0.00 ± 0.00 c	1.13 ± 0.98 bc	2.57 ± 0.81 ab	4.25 ± 1.48 a	5.04 ± 2.53 ab	0.004
Pseudomonas	−	+	+	−	−	−	−	−	0.00 ± 0.00 b	0.00 ± 0.00 b	3.35 ± 2.91 ab	4.56 ± 1.33 ab	5.67 ± 1.72 a	5.65 ± 1.01 a	0.000
VRBG	−	+	−	−	−	−	+	−	0.67 ± 1.03 b	2.07 ± 2.01 b	2.96 ± 1.22 ab	4.41 ± 0.72 ab	5.99 ± 0.24 a	4.61 ± 1.15 a	0.003
VRBA	−	+	−	−	−	−	+	+	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.57 ± 0.98	2.85 ± 3.01	1.69 ± 2.73	0.188

[a] G, Gram reaction; C, catalase; O, oxidase; Ci, citrate; M, methyl red; U, urease; GL, glucose fermentation; L, lactose fermentation. [b] Microbial counts after the hydro-cooling treatment. [c] Storage A, 0 days at 1 °C + 2 days at 5 °C (transport simulation) + 2 days at 20 °C (shelf life); storage B, 7 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage C, 14 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage D, 21 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage E, 28 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C. [d] p values. [e] For a given row, values with different letters are significantly different.

21 days of cold storage plus 2 days at 5 °C plus 2 days at 20 °C (storage D). Yeasts, total mesophilic aerobic bacteria, and total psychrotrophic bacteria presented counts greater than 6 log CFU g^{-1}, whereas in the culture media for *Pseudomonas* spp., *Enterobacteriaceae* spp., and molds, the counts were at levels of 4.25−5.99 log CFU g^{-1}. Spoilage in both fresh fruit and fresh-cut fruit and vegetables is usually detected by consumers when yeast counts reach levels above 5 log CFU g^{-1} (*16*, *17*). Thus, our results are evidence that storage D is near the maximum extension of 'Ambrunés' storage in maintaining the minimal microbial quality during their shelf-life period. Indeed, similar counts were found in fruits with 28 days in cold storage, which showed clear signs of alteration by proliferation of molds (**Table 3**). This batch was discarded for the volatile compounds and sensory analysis.

Volatile Compounds. Over 65 volatile compounds were identified in the samples, including 17 hydrocarbons, 14 carbonyls, 21 alcohols, 10 carboxylic acids, and 3 esters (**Table 4**). Most compounds identified have been reported in fresh sweet cherry fruits (*4*, *6*, *7*).

Several of the aforementioned volatile compounds were detected at very low levels. Hydrocarbons were less than 4% of the total area of volatile compounds in both the maturation and storage batches. Hexane and 2-methyl-*trans*-1,3-pentadiene were the most abundant hydrocarbons, showing maximum levels after storages D and A, respectively (**Table 4**). Low amounts of alkanes and alkenes in sweet cherry fruits have also been reported in other studies (*6*, *7*), and the origin of these compounds can be attributed to lipid oxidation (*18*). Regardless of their origin, aliphatic and branched hydrocarbons have been considered as noncontributors to food flavor (*19*) and are not among the most odor-active compounds described for fruits.

Table 4 also lists the carbonyl compounds: aliphatic aldehydes (31.99−37.29% of total area), aromatic aldehydes (7.40−17.40%), branched aldehydes (0.16−1.16%), and ketones (0.21−0.85%). The carbonyl compounds with high relative contents included hexanal, (*E*)-2-hexenal, and benzaldehyde. Several workers report that these compounds are among the most important aroma components of sweet cherry fruit (*5*−*7*, *20*). Aliphatic C_6 aldehydes come from fatty acid oxidation promoted by lipoxygenase (*21*), whereas benzaldehyde originates from the enzymatic hydrolysis of the amygdalin in the fruit. However, other minority compounds, such as branched aldehydes, may influence the sweet cherry flavor because these substances have a very low odor threshold. Branched aldehydes are formed from precursor amino acids, such as L-leucine, L-isoleucine, and L-valine (*22*, *23*). At the ripening stages studied, there were few statistically significant differences among batches for any of the carbonyl compounds. Only the (*E*)-2-hexenal concentration increased between ripening stages 1 and 3 (**Table 4**). However, with respect to the storages studied, most relevant aliphatic aldehydes had the highest concentration in the shortest storage (A), whereas benzaldehyde, 3-methyl-butanal, 2-methyl-butanal, and 2-propanone showed the highest concentrations after 21 days of storage (storage D; **Table 4**). Storage time has been described as a determining factor for the variation of volatile aldehydes in sweet cherries (*20*). Coherent with our results, Mattheis et al. (*4*) found a decrease of hexanal and an increase of benzaldehyde in sweet cherries after 7 days postharvest. In addition, the influence of the ripening stage on the evolution of some aldehydes during cold storage were significant according to the p interaction values. Among these compounds, aromatic and branched aldehydes showed p_{int} values lower than 0.05 (**Table 4**). Benzaldehyde increased significantly during storage for the samples of stages 1 and 2, whereas the amount of this compound showed no relevant difference for fruits classified in stage 3 (**Figure 1**). A similar evolution was observed for branched aldehydes, with the highest concentration shown in samples of stage 1 stored for 28 days.

Alcohols detected included aliphatic (34.66−50.09% of total area), aromatic (2.56−10.93%), and branched compounds (0.57−1.41%;

Table 4. Volatile Compounds from 'Ambrunés' Sweet Cherries at Different Ripening Stages and Storage Times

	maturation stages[a]							storage [days at 1 °C + days at 5 °C + shelf life (SL)][b]										
	1		2		3			A (0 + 2 + SL)		B (7 + 2 + SL)		C (14 + 2 + SL)		D (21 + 2 + SL)				
volatile compounds	mean[c]	%	mean	%	mean	%	p^d	mean	%	mean	%	mean	%	mean	%	p	p_{int}	ID[e]
alkanes	9.34	0.47	40.42	1.35	41.44	1.38		32.86 ab	0.72	6.93 b	0.42	5.21 b	0.23	76.61 a	3.63	**		
hexane	7.40	0.38	37.63	1.26	39.07	1.31		23.42 ab	0.51	6.93 b	0.42	5.16 b	0.23	76.61 a	3.63	**		A
undecane	0.82	0.04	0.73	0.02	1.53	0.05		4.07 a	0.09	0.00 b	0.00	0.05 b	0.00	0.00 b	0.00	**		A
tetradecane	0.53	0.03	0.49	0.02	0.16	0.01		1.57	0.03	0.00	0.00	0.00	0.00	0.00	0.00	*		A
hexadecane	0.26	0.01	1.33	0.04	0.47	0.02		1.39	0.03	0.00	0.00	0.00	0.00	0.00	0.00			A
heneicosane	0.33	0.02	0.24	0.01	0.00	0.00		0.76	0.02	0.00	0.00	0.00	0.00	0.00	0.00	+		A
pentacosane	0.00	0.00	0.00	0.00	0.22	0.01		0.30	0.01	0.00	0.00	0.00	0.00	0.00	0.00			A
alkenes	12.10	0.61	38.65	1.29	34.02	1.14		109.46	2.40	0.63	0.04	1.03	0.05	1.89	0.09	**		
(*Z*)-1,3-pentadiene	1.29	0.07	3.07	0.10	0.82	0.03		5.54 a	0.12	0.00 b	0.00	0.00 b	0.00	1.37 ab	0.07	*		B
2-methyl-*trans*-1,3-pentadiene	8.37	0.43	30.13	1.01	29.10	0.97		90.14 a	1.98	0.00 b	0.00	0.00 b	0.00	0.00 b	0.00	**		C
1,4-hexadiene	0.10	0.01	0.79	0.03	0.22	0.01		1.34	0.03	0.00	0.00	0.00	0.00	0.13	0.01			B
cyclohexene	0.06	0.00	0.06	0.00	0.00	0.00		0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	+		B
2,3-dimethyl-1,3-butadiene	0.00	0.00	1.02	0.03	1.62	0.05		3.52	0.08	0.00	0.00	0.00	0.00	0.00	0.00	+		C
2-methyl-1,3-pentadiene	0.04	0.00	1.65	0.06	0.26	0.01		2.20	0.05	0.00	0.00	0.34	0.02	0.06	0.00			C
(*Z*)-3-methyl-1,3-pentadiene	0.05	0.00	1.59	0.05	1.74	0.06		4.23	0.09	0.06	0.00	0.22	0.01	0.00	0.00	+		C
2,4-hexadiene	1.55	0.08	0.07	0.00	0.12	0.00		2.32	0.05	0.00	0.00	0.00	0.00	0.00	0.00			B
3-methyl-cyclohexene	0.63	0.03	0.26	0.01	0.14	0.00		0.00	0.00	0.57	0.03	0.47	0.02	0.33	0.02			C
alkynes	0.01	0.00	0.00	0.00	0.15	0.00		0.00	0.00	0.01	0.00	0.20	0.01	0.00	0.00			
1-heptyne	0.01	0.00	0.00	0.00	0.15	0.00		0.00	0.00	0.01	0.00	0.20	0.01	0.00	0.00			B
aldehydes	629.83	31.99	971.38	32.52	1039.50	34.73		1521.74	33.36	576.64	34.61	843.67	37.29	578.89	27.46	**		
acetaldehyde	27.11	1.38	21.37	0.72	25.51	0.85		17.89	0.39	20.62	1.24	30.21	1.34	29.95	1.42		+	A
butanal	1.20	0.06	0.85	0.03	0.60	0.02		0.00	0.00	1.19	0.07	0.00	0.00	2.33	0.11			A
pentanal	4.28	0.22	4.81	0.16	2.10	0.07		13.72 a	0.30	1.03 b	0.06	0.00 b	0.00	0.18 b	0.01	**		A
(*E*)-2-pentenal	0.00	0.00	1.16	0.04	0.82	0.03		2.64 a	0.06	0.00 b	0.00	0.00 b	0.00	0.00 b	0.00	**	+	B
hexanal	382.27	19.41	580.46	19.44	551.51	18.43		949.58 a	20.82	276.40 b	16.59	531.47 b	23.49	261.54 b	12.41	**		A
(*E*)-2-hexenal	212.92 b	10.81	358.70 a,b	12.01	451.87 a	15.10	*	524.57 a	11.50	274.37 b	16.47	281.07 ab	12.42	284.64 ab	13.50	*		A
(*E*,*E*)-2,4-hexadienal	2.05	0.10	4.04	0.14	7.09	0.24		13.35 b	0.29	3.03 a	0.18	0.93 a	0.04	0.26 a	0.01	**		B
aromatic aldehydes	222.87	11.32	264.77	8.87	221.37	7.40		217.36	4.77	145.19	8.71	216.13	9.55	366.67	17.40	*		
benzaldehyde	222.87	11.32	264.77	8.87	221.37	7.40		217.36 ab	4.77	145.19 b	8.71	216.13 ab	9.55	366.67 a	17.40	*	*	A
branched aldehydes	19.82	1.01	10.50	0.35	4.72	0.16		7.79	0.17	0.78	0.05	13.72	0.61	24.44	1.16	**	*	
2-methyl-propanal	6.05	0.31	2.16	0.07	1.60	0.05		2.28	0.05	0.00	0.00	6.10	0.27	4.70	0.22		+	A
3-methyl-butanal	4.33	0.22	3.59	0.12	1.93	0.06		2.98 ab	0.07	0.78 b	0.05	3.13 ab	0.14	6.24 a	0.30	**	**	A
2-methyl-butanal	9.44	0.48	4.75	0.16	1.20	0.04		2.52 b	0.06	0.00 b	0.00	4.49 b	0.20	13.50 a	0.64	**	*	A
ketones	9.15	0.46	10.70	0.36	13.87	0.46		9.76	0.21	5.52	0.33	11.75	0.52	17.94	0.85	*		
2-propanone	7.88	0.40	7.77	0.26	13.41	0.45		7.24 b	0.16	3.84 b	0.23	11.04 ab	0.49	16.63 a	0.79	**		A
3-hydroxy-2-butanone	1.26	0.06	2.92	0.10	0.46	0.02		2.52	0.06	1.65	0.10	0.72	0.03	1.31	0.06			A
3-methyl-2-cyclopenten-1-one	0.02	0.00	0.01	0.00	0.00	0.00		0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00			B
alcohols	853.56	43.35	1299.15	43.50	1442.20	48.19		2284.81	50.09	773.01	46.40	1004.84	44.42	730.58	34.66	**		
ethanol	14.64	0.74	17.82	0.60	38.22	1.28	+	8.42 b	0.18	20.60 ab	1.24	21.56 ab	0.95	43.66 a	2.07	+		A
2-butanol	0.78	0.04	0.44	0.01	0.00	0.00		1.03	0.02	0.00	0.00	0.58	0.03	0.00	0.00			A
1-butanol	0.25	0.01	0.00	0.00	0.34	0.01		0.13	0.00	0.00	0.00	0.06	0.00	0.60	0.03			A
1-penten-3-ol	1.03	0.05	6.38	0.21	7.15	0.24	+	10.80 a	0.24	0.86 b	0.05	3.33 ab	0.15	4.43 ab	0.21	*	+	A
(*Z*)-2-penten-1-ol	0.00	0.00	2.25	0.08	2.29	0.08		6.05 a	0.13	0.00 b	0.00	0.00 b	0.00	0.00 b	0.00	*		B
(*E*)-2-hexen-1-ol	764.76	38.84	1067.92	35.76	1121.06	37.46		1873.27 a	41.07	637.39 b	38.26	870.21 b	38.47	557.44 b	26.45	**		B
1-hexanol	72.10	3.66	203.96	6.83	272.83	9.12	+	385.10 a	8.44	113.87 b	6.83	108.96 b	4.82	123.92 b	5.88	**		A
1-tetradecanol	0.00	0.00	0.00	0.00	0.26	0.01		0.00	0.00	0.28	0.02	0.07	0.00	0.00	0.00			B
unknown alcohol	0.00	0.00	0.39	0.01	0.05	0.00		0.00	0.00	0.00	0.00	0.07	0.00	0.52	0.02			D
aromatic alcohols	92.39	4.69	205.44	6.88	93.90	3.14		116.97	2.56	107.63	6.46	67.27	2.97	230.45	10.93			
cyclobutanol	0.31	0.02	1.71	0.06	0.00	0.00		0.08	0.00	2.06	0.12	0.39	0.02	0.16	0.01			C
cyclopentanol	0.00	0.00	0.08	0.00	0.17	0.01		0.00	0.00	0.22	0.01	0.11	0.00	0.00	0.00			C
cyclohexanol	38.68	1.96	127.83	4.28	34.26	1.14		46.87	1.03	59.39	3.56	9.31	0.41	152.13	7.22			C
benzenemethanol	47.09	2.39	69.49	2.33	50.96	1.70		46.92	1.03	44.33	2.66	54.10	2.39	78.04	3.70		*	A
2,6-dimethoxy-phenol	1.45	0.07	0.39	0.01	0.00	0.00		0.00	0.00	0.00	0.00	2.45	0.11	0.00	0.00	+		C
2,2′-[(1-methyl-1,2-ethanediyl]b-phenol	0.80	0.04	0.52	0.02	0.35	0.01		0.00 b	0.00	1.17 a	0.07	0.93 ab	0.04	0.13 ab	0.01	*		C
4,4′-(1-methylethylidene)bis-phenol	4.07	0.21	5.43	0.18	8.17	0.27		23.10 a	0.51	0.46 b	0.03	0.00 b	0.00	0.00 b	0.00	**	+	C
branched alcohols	16.60	0.84	25.35	0.85	26.60	0.89		35.20	0.77	9.49	0.57	17.08	0.75	29.64	1.41	**		
2-methyl-1-propanol	0.29	0.01	0.00	0.00	1.18	0.04		0.00	0.00	0.00	0.00	0.19	0.01	1.77	0.08	+		A
3-methyl-3-buten-1-ol	8.94	0.45	13.98	0.47	12.45	0.42		20.13 a	0.44	4.24 b	0.25	9.72 ab	0.43	13.06 ab	0.62	**		B
3-methyl-1-butanol	1.24	0.06	0.89	0.03	3.24	0.11		1.48	0.03	0.00	0.00	0.00	0.00	5.67	0.27			B
3-methyl-2-buten-1-ol	4.44	0.23	6.12	0.21	7.71	0.26		6.93	0.15	4.19	0.25	4.44	0.20	8.81	0.42			A
2-methyl-2-buten-1-ol	1.70	0.09	4.36	0.15	2.03	0.07		6.66	0.15	1.06	0.06	2.73	0.12	0.34	0.02			A
acids	80.27	4.08	108.64	3.64	46.47	1.55		193.24	4.24	32.78	1.97	72.10	3.19	15.72	0.75	+		
acetic acid	10.61	0.54	9.69	0.32	4.94	0.17		1.73 b	0.04	16.99 a	1.02	11.60 ab	0.51	3.34 b	0.16	**		A
hexanoic acid	4.86	0.25	47.12	1.58	8.68	0.29		14.43	0.32	3.92	0.24	57.98	2.56	4.55	0.22			A
octanoic acid	0.00	0.00	0.48	0.02	1.01	0.03		0.76	0.02	0.00	0.00	0.00	0.00	1.23	0.06			A
nonanoic acid	0.00	0.00	1.92	0.06	5.03	0.17		4.40	0.10	0.64	0.04	0.00	0.00	4.24	0.20			A
tetradecanoic acid	4.73	0.24	4.10	0.14	0.98	0.03		12.09	0.26	0.00	0.00	0.27	0.01	0.72	0.03	*		A
pentadecanoic acid	3.02	0.15	0.22	0.01	1.82	0.06		6.07	0.13	0.68	0.04	0.00	0.00	0.00	0.00			B

Table 4. Continued

	maturation stages[a]							storage [days at 1 °C + days at 5 °C + shelf life (SL)][b]										
	1		2		3			A (0 + 2 + SL)		B (7 + 2 + SL)		C (14 + 2 + SL)		D (21 + 2 + SL)				
volatile compounds	mean[c]	%	mean	%	mean	%	p^d	mean	%	mean	%	mean	%	mean	%	p	p_{int}	ID[e]
9-hexadecenoic acid	15.06	0.76	11.76	0.39	4.90	0.16		41.95 a	0.92	0.35 b	0.02	0.00 b	0.00	0.00 b	0.00	*		B
hexadecanoic acid	29.50	1.50	29.38	0.98	17.52	0.59		87.76 a	1.92	10.21 ab	0.61	2.25 b	0.10	1.65 b	0.08	*		A
(*Z*)-9-octadecenoic acid	9.01	0.46	0.82	0.03	1.60	0.05		15.24	0.33	0.00	0.00	0.00	0.00	0.00	0.00			B
octadecanoic acid	3.47	0.18	3.14	0.11	0.00	0.00		8.82	0.19	0.00	0.00	0.00	0.00	0.00	0.00	+		A
esters	0.00	0.00	3.37	0.11	3.21	0.11		4.42	0.10	3.90	0.23	0.44	0.02	0.00	0.00			
hexyl acetate	0.00	0.00	0.80	0.03	0.25	0.01		0.95	0.02	0.00	0.00	0.44	0.02	0.00	0.00			B
(*E*)-2-hexenyl acetate	0.00	0.00	2.14	0.07	0.46	0.02		3.47	0.08	0.00	0.00	0.00	0.00	0.00	0.00			C
(*Z*)-methyl-9-octadecenoate	0.00	0.00	0.43	0.01	2.50	0.08		0.00	0.00	3.90	0.23	0.00	0.00	0.00	0.00			C

[a] Color parameters: stage 1, 34−49 (I20 automatic color sorter, Multiscan Technologies, Alicante, Spain) corresponding to mean values of L^* = 35.68, a^* = 34.03, b^* = 14.16, C^* = 36.89, and hue = 22.33 (Minolta CR-400 chroma meter, Osaka, Japan); stage 2, 25.5−34 (L^* = 31.15, a^* = 22.47, b^* = 7.79, C^* = 23.78, and hue = 18.94); stage 3, 0-25.5 (L^* = 28.51, a^* = 14.62, b^* = 3.28, C^* = 14.98, and hue = 12.67). [b] Storage A, 0 days at 1 °C + 2 days at 5 °C (transport simulation) + 2 days at 20 °C (shelf life); storage B, 7 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage C, 14 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C; storage D, 21 days at 1 °C + 2 days at 5 °C + 2 days at 20 °C. [c] Mean values in area arbitrary units. [d] p values: +, $p < 0.1$; ∗, $p < 0.05$; ∗∗, $p < 0.001$. [e] Reliability of identification: A, identified by a comparison to standard compounds; B, tentatively identified by the NIST/EPA/NIH mass spectrum library (comparison quality > 90%) and Kovats index; C, tentatively identified by the NIST/EPA/NIH mass spectrum library (comparison quality > 90%); D, tentatively identified by the NIST/EPA/NIH mass spectrum library (comparison quality < 90%).

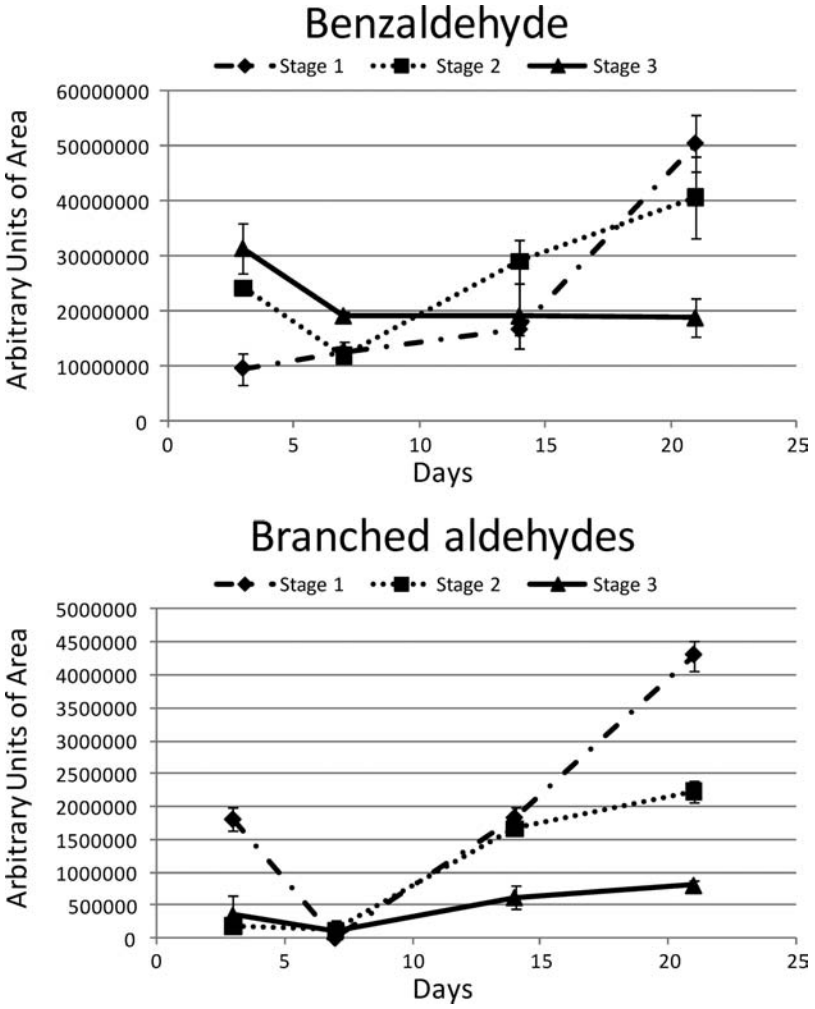

Figure 1. Effect of the ripening stage on the evolution of benzaldehyde and branched aldehydes.

Table 4). No significant differences were found among the maturation stage batches studied for these volatile compounds. With respect to the storage time, the sweet cherries with the longest storage (D) showed the highest values of ethanol. The increase in the ethanol concentration indicates a fermentative metabolism, and it has been considered a symptom of degradation in fruits, including sweet cherries (*6*). (*E*)-2-Hexenol (26.45−41.07% of total area) was the most abundant volatile compound found, and this alcohol and hexenal, (*E*)-2-hexenal, hexanal, and benzaldehyde are considered to be predominant flavor volatiles in sweet cherries (*5*). As was the case with their respective precursor aldehydes, the aliphatic C_6 and C_5 alcohols detected had the highest concentration in the shortest storage (A; **Table 4**). Additionally, some minority aromatic and branched alcohols showed differences between

storages A and B. Low amounts of branched alcohols have also been reported in sweet cherries, although these volatile compounds have not previously been related with storage or ripening time (*4*, *20*). Among aromatic alcohols, the amount of benzenemethanol showed a relevant interaction between the maturation stage and storage factors (**Table 4**). The influence of the ripening stage on the evolution of this compound during cold storage was similar to its precursor benzaldehyde (data not shown).

The acids represented less than 5% of the total area of volatile compounds. Acetic acid (0.04−1.02% of total area) can make an important contribution to the characteristic flavor because of its relatively low odor threshold. The amount of this acid was highest in storage B. Mattheis et al. (*20*) described acetic acid as a relevant volatile constituent of 'Bing' sweet cherries, whereas in other studies, this compound is a minor component or simply not mentioned for different sweet cherry cultivars (*5*−*7*). The concentrations of hexanoic, octanoic, and nonanoic acids were not significantly different among either the ripening stages or the storages, whereas several fatty acids detected (from C_{14} to C_{18}) presented the highest values in the shortest storage (A). The detection of fatty acids in the volatile fraction extracted with the headspace/solid-phase microextraction method has been described in different foods, including fruits (*22*). Progressive fatty acid enzymatic degradation can explain, at least partly, the low amount of these compounds found in the longest storages.

With respect to esters, hexyl acetate, (*E*)-2-hexenyl acetate, and (*Z*)-methyl-9-octadecenoate were found at very low levels (0.00−0.23% of total area). Hexyl acetate and (*E*)-2-hexenyl acetate have also been described in several other sweet cherry cultivars (*5*). The presence of these esters was in accordance with their alcohol precursors, which were the most predominant alcohols found in this survey.

Relationship between the Volatile Compounds and the Sensorial Analysis. The values of all of the sensory descriptive attributes studied showed a strong correlation with the overall scores in the hedonic test, including the "sweet cherry flavor" descriptor (**Table 5**). A total of 10 volatile compounds (5 aldehydes, 4 alcohols, and 1 hydrocarbon) showed significant correlations with sweet cherry flavor and the overall score. In particular, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, hexanal, 1-hexanol, and 1-penten-3-ol were positively correlated with sweet cherry flavor and the overall valuation of the fruits, confirming that these lipid-related volatile compounds are the most important aromas of 'Ambrunés' sweet cherries (**Table 5**). Some C_6 aldehydes are associated with green/grassy notes, while (*E*)-2-hexenal has a fruity/sweet/fresh odor (*24*). On the contrary, 3-methyl-cyclohexene and branched aldehydes, mainly 2-methyl-propanal and 2-methyl-butanal, were negatively correlated with sweet cherry flavor and the overall valuation of the fruits. These amino-acid-derived volatiles have not previously

Table 5. Sensory Descriptors and Volatile Compounds Positively Correlated with Sweet Cherry Flavor and Sensory Global Valuation in "Ambrunés" Sweet Cherries

	correlations	
sensory descriptors/volatile compounds	sweet cherry flavor	overall valuation
sensory evaluation		
color		0.965[a]
texture		0.913[a]
external appearance		0.793[a]
sweet cherry flavor		0.965[a]
volatile compounds		
hydrocarbons		
3-methyl-cyclohexene	−0.401[b]	−0.423[c]
carbonyls		
hexanal	0.358[b]	0.390[b]
(*E*)-2-hexenal	0.578[a]	0.553[a]
2-methyl-propanal	−0.492[c]	−0.435[c]
3-methyl-butanal		−0.349[b]
2-methyl-butanal	−0.496[c]	−0.481[c]
alcohols		
1-penten-3-ol	0.434[c]	0.439[c]
cyclopentanol	0.360[b]	0.360[b]
(*E*)-2-hexen-1-ol		0.408[c]
1-hexanol	0.469[c]	0.472[c]

[a] Correlation is significant at the 0.01 level. [b] Correlation is significant at the 0.1 level. [c] Correlation is significant at the 0.05 level.

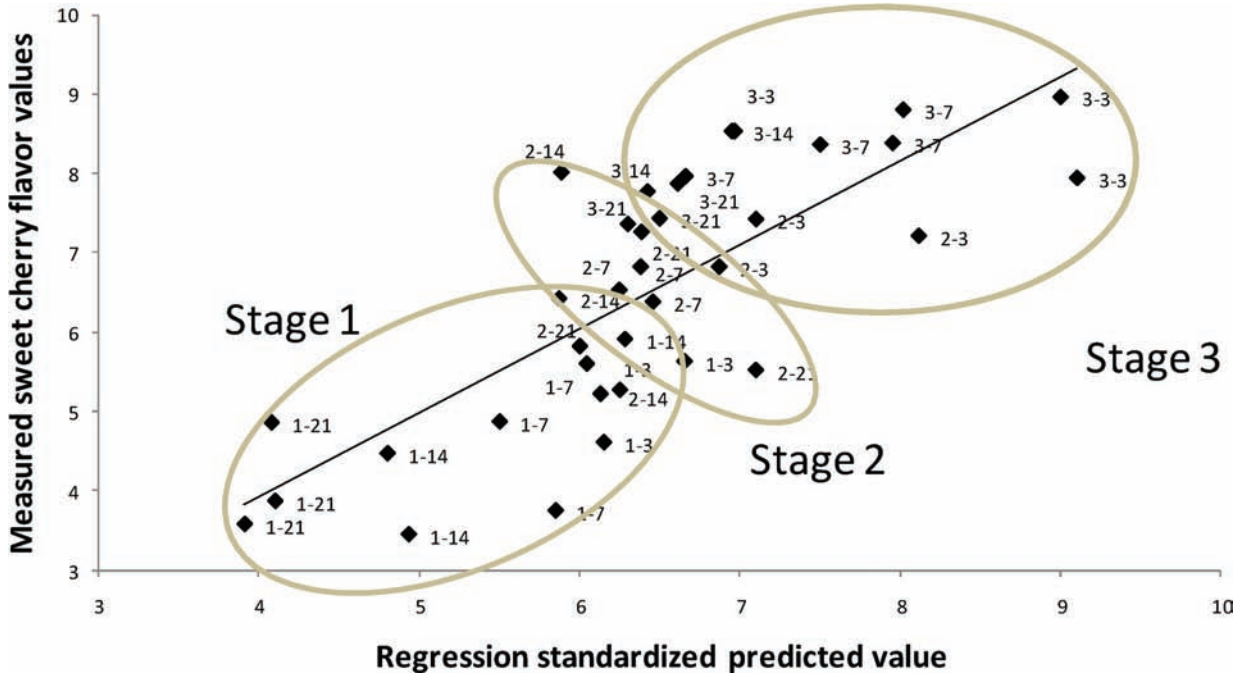

Figure 2. Scatter plot of measured versus predicted sweet cherry flavor values derived from PLSR analysis of (*E*)-2-hexenal, 1-hexanol, 2-methyl-propanal, and 2-methyl-butanal.

been described as aroma components in sweet cherries, although in other fruits, they play a relevant role in flavor (*1*, *23*). Alcohol dehydrogenase and alcohol acetyltransferase convert the branched aldehydes to their respective alcohols and esters. An increase in these last compounds contributing to sweet/fresh notes or a decrease in branched aldehydes, which contribute to stale/rotten notes, as a result of these enzymatic activities would likely be beneficial to the sweet cherry flavor.

With the aim of confirming (*E*)-2-hexenal, 1-hexanol, 2-methyl-propanal, and 2-methyl-butanal as the volatile compounds mainly influencing the sweet cherry flavor differences between samples, a partial least-squares regression (PLSR) method was used. The model ($r = 0.703$) indicated that up to 49% of variation in the descriptive "sweet cherry flavor" could be predicted from the amounts of the selected volatile compounds. The biplot of the predicted versus measured sweet cherry flavor values shows three groups of samples according to their maturation stage (**Figure 2**). These results confirmed that the most ripened commercial stage (stage 3) is the adequate option for cold storage, including for long periods of time up to 21 days. In addition, the plot also shows that the aroma of sweet cherries was negatively impacted by the longest storage studied, mainly in the samples of the least ripened stage (stage 1). This may be explained by a partial loss of enzymatic activity or tissue capacity for biosynthesis of some relevant lipid-derived volatiles.

We can conclude that the hydro-cooled 'Ambrunés' sweet cherries showed an acceptable microbial quality at least for 21 days in cold storage, independent of their commercial ripening level. However, relevant changes associated with the longer cold storages were found in different aroma constituents, with a negative impact on the sweet cherry flavor. These changes were less intense in fruit in the commercial ripening stage 3. Monitoring aroma compounds, such as (*E*)-2-hexenal, 1-hexanol, 2-methyl-propanal, and 2-methyl-butanal, could constitute a good tool to predict flavor quality in 'Ambrunés' sweet cherries during the cold-storage process.

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