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Changes in Volatiles and Glycosides during Fruit Maturation of Two Contrasted Tomato (*Solanum lycopersicum*) Lines

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The relationship between fruit maturation and volatile contents was investigated in two contrasted Cervil (CER) and Levovil (LEV) tomato (*Solanum lycopersicum*) lines. As fruits ripened, their volatile contents mainly increased. Although some compounds displayed contrasting patterns, overall, volatiles were clearly more abundant and conferred stronger aromas to CER than to LEV fruits. This intervarietal difference in volatile contents yielding much lower volatile contents in LEV was further investigated to determine whether it is due to a higher capacity of volatile glycosylation within LEV as compared to CER. Again, glycosides mainly increased during fruit maturation and were more abundant within CER than within LEV. Overall glycoside findings were indicative of a superior capacity to biosynthesize rather than an inferior capacity to glycosylate volatiles of CER. Eugenol and 2-methoxyphenol volatiles were exceptional compounds as they remained at higher levels in maturing LEV than in CER. 2-Methylthioacetaldehyde was for the first time identified as putatively related to differences of aroma between lines, as it was abundant in Cervil but absent in Levovil. Considering the described odor value of these three products, they should contribute differently to the particular olfactive features of LEV and CER fruits.

KEYWORDS: Aroma; ripening; lipoxygenase (LOX); GC-MS; log odor; aglycones

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) fruits are particularly appreciated for their flavor. Their characteristic aromas are developed as fruits ripen. During the fruit maturation phase, genetically regulated processes induce molecular and biochemical changes and lead to phenotypical modifications, including generation of aroma volatiles, color changes, and softening (*1, 2*). Understanding the formation and the evolution of volatile compounds within fruits is essential to ensure and to improve fruit flavor. Although volatiles have been frequently investigated in fully ripened tomatoes (*3*−*6*), their patterns during tomato ripening have rarely been studied (*7*). More than 400 volatile compounds have been identified in tomato fruit (*8*), but only a small number of these produce the characteristic tomato aroma (*9*). These flavor (aroma) volatiles typically identified using GC−olfactometry (GC-O) (*5, 6, 10*) or log odor values (*9*) belong to different chemical families and are formed by an array of biochemical pathways.

A significant proportion of potential contributors to flavor has been reported to occur as nonvolatile compounds, most often as glycosides. They are composed of a glucopyranosyl unit attached through a β-glycosidic linkage to an aglycone (*10*−*12*). This form allows their storage in vacuoles (*12*). On one hand, this conversion removes the aglycone from the pool of volatiles, and therefore reduces their contribution to the fruit flavor. On the other hand, the hydrolysis of glycosides may occur during the ripening process and lead to the release of additional volatile compounds that may reinforce the fruit flavor. Indeed, glycosidase activities increase during fruit ripening (*13, 14*), and maceration of fruit tissues with microbial glycosidase leads to the release of volatiles from glycosides (*15*). However, up to now, no direct evidence was produced for such a reaction *in planta*. Nevertheless, for a large proportion of compounds, free volatiles and their corresponding glycoconjugated forms coexist in fruits. It has previously been shown that glycoside composition and levels differ within ripe fruits of contrasting tomato varieties (*16*). However, to our knowledge, the evolution of the glycoside fraction during the ripening and also the proportions of glycoside-bound and free volatile compounds were not previously considered.

In addition, we were further interested in a series of major volatile compounds, here referred to as lipoxygenase (LOX)-derived volatile products. They are among the principal

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contributors to tomato aroma (*9, 17, 18*). They result from the oxidative degradation of linoleic and linolenic acid that occurs when natural compartmentalizing in the cell is broken down provoked by, for example, mastication or grinding. As a result, the chloroplastic LOX comes in contact with the cytosolic substrate (*19*). LOX volatiles are therefore produced during the sample preparation, and, in addition, they often degrade during long, classic extraction methods (*17*). In this study, we pay attention to experimental conditions, especially through a careful respect of the extraction timing, allowing preservation and better confidence in the measurement of the LOX-derived volatile products.

Here, the aim was to investigate the patterns of volatiles during tomato fruit maturation, regarding the three categories: the free, the LOX-derived, and the glycoside-bound volatiles. The study was performed on two tomato lines previously described as contrasted by their sensory attributes and especially by their flavor (*20*): Levovil (*S. lycopersicum* Mill.), characterized by its large fruits and pharmaceutical sensory attributes, as opposed to Cervil [*S. lycopersicum* var. cerasiforme (Dun.)], which is a cherry tomato line with high intensity of overall aroma. An odor value was calculated for all of the varying volatiles using the log odor method and odor thresholds collected from the literature. The variations between lines were then discussed regarding these values and how they fit to their previously described sensory attributes (*21*).

MATERIALS AND METHODS

Plant Material. Seeds of *S. lycopersicum* var. *cerasiforme* (Cervil, CER) and of *S. lycopersicum* (Levovil, LEV) lines were provided by the seed company Vilmorin. Plants were grown in a heated glasshouse during the spring 2003 and harvested from May 25 to June 15 and belonged to a multiline randomized trial. Plots consisted of a single row of three plants, and each line was represented by two plots. Five fruits were collected at random, over three harvests (separated by 1 week) at four distinguishable maturation stages, ranging from green (G) to breaker (B) to pink (P) and to red (R), that is, 24 samples (2 lines × 3 harvests × 4 stages). Cervil and Levovil weighed on average 7 and 125 g, respectively, on a fresh weight (FW) basis (*22*).

Each sample replicate consisted either of 5 Levovil or 10 Cervil fruits that were ground in a blender. Before grinding, the color of each fruit was estimated through the CIE *L*a*b** system using a Minolta chromameter CM-1000R (Minolta, Ramsey, NJ).

Extraction of Volatiles. Cervil and Levovil volatile and glycoside samples were prepared in triplicates. Fruits were sampled over four maturation stages, thus yielding 12 samples in total per tomato line. Samples were ground in a blender (Waring) for 1 min at 20 °C, 100 g of slurry was then centrifuged at 13000*g* for 30 min at 4 °C, and an aliquot of 50 g of supernatant was sampled and supplemented with an internal standard, 4-nonanol (10 μg). Volatiles were then extracted three times with a mix of pentane/CH_2Cl_2 (2:1, v/v), and the organic phase was concentrated to 1 mL by distillation. The remaining aqueous phase was used for the extraction of glycosides (see the following section).

Extraction of LOX-Derived Volatile Products. Samples were ground in a blender (Waring) for 1 min at 20 °C. Two minutes later, tomato slurry (5 g) was added to 5 g of saturated, cold $CaCl_2$. The mix was vortexed to deactivate the enzyme LOX and filtered through glass wool. The supernatant (5 g) was transferred in a glass tube and supplemented with 10 μg of 4-nonanol. Volatiles were extracted twice with 1 mL of a mixture of pentane/CH_2Cl_2 (2:1, v/v) and centrifuged for 5 min at 1500*g*. The organic phase were pooled and concentrated to 1 mL under a nitrogen stream.

Extraction of Glycosides. The aqueous phase that remained from volatile extraction was passed through a SPE column containing 4 mL of Amberlite XAD-2 resine (Supelco, Bellefonte, PA) according to a modified method reported by Gunata et al. (*23*). After column washing with 50 mL of H_2O that eluted carbohydrates, glycosides were extracted by methanol elution (20 mL). They were then vacuum-dried in a rotary evaporator (Buchi), redissolved in 0.4 mL of citrate−phosphate buffer (0.2 M; pH 5), and washed three times using pentane/CH_2Cl_2 (2:1, v/v). The residue was supplemented with 0.2 mL of an enzymic preparation (AR 2000 Gist-Brocades, 40 mg mL^{-1} citrate−phosphate buffer). The resulting mix was incubated at 37 °C overnight to complete the enzymatic hydrolysis of the glycoside−aglycon bond. The mix was then supplemented with 15 μg of an internal standard (4-nonanol), and the aglycones were extracted three times with 2 mL of CH_2Cl_2. The extracts were pooled and concentrated under a nitrogen stream to 500 μL prior to analysis.

GC-MS Analysis. Volatile and glycoside samples (2 μL) were injected in a port of a GC-MS (CP2010; Shimadzu, Kyoto, Japan) with a CPWAX 52 CB capillary column (30 m, 0.32 mm i.d., 0.5 μm film thickness). The injection port was operated in splitless mode for the first 30 s, then the carrier gas (He) velocity was constant at 35 cm s^{-1}. The initial oven temperature of 40 °C was held for 2 min and then ramped at 4 °C min^{-1} to 230 °C. This final temperature was held for 15 min. The mass spectrometer was operated in the electron impact mode at 70 eV with continuous scans (every 0.5 s) from mass to charge ratio (*m*/*z*) 29 to 250. Data were collected with GC-MS Solution software.

Volatile levels were expressed in micrograms of 4-nonanol equiv kg^{-1} of clarified juice. The concentrations are to be considered as relative data as recoveries after extraction and calibration factors related to the standards were not determined. Note that all data are presented on a fresh weight basis.

Identification of Compounds. Compounds were preliminarily identified by NIST 98 library search, and identities were confirmed for most of them by injection of pure standards. Standards were purchased from Sigma-Aldrich except for (*E*)-2-pentenal, (*E*)-2-octenal, (*E*)-3-hexenoic acid, 3-methyl-2-butenoic acid, and (*Z*)-3-hexenal, which were kindly donated by E. Semon (INRA-UMR FLAVIC, Dijon, France), and 3-oxo-α-ionol, which was donated by J. P. Lepoutre (INRA-UMR SPO, Montpellier, France). 2-Methylthioacetaldehyde was obtained from the aqueous acidic hydrolysis of the commercial 2-methylthioacetaldehyde dimethylacetal.

Analysis of LOX-Derived Volatile Products. Samples (2 μL) were injected in the port of a GC-MS (CP2010; Shimadzu) with a CP SIL 8 CB capillary column (30 m, 0.25 mm i.d., 0.5 μm film thickness). The injection port was operated in split mode (1/15), then the carrier gas (He) velocity was constant at 35 cm s^{-1}. The initial oven temperature of 40 °C was held for 2 min and ramped at 3 °C min^{-1} to 60 °C and then at 10 °C min^{-1} to 230 °C. This final temperature was held for 5 min. The mass spectrometer was operated in the electron impact mode at 70 eV. Mass spectra of LOX-derived volatile products were recorded by single ion monitoring (SIM) mode. Detection quantifications of 1-penten-3-one, hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenal were based on ions 55, 44, 41, and 83, respectively.

Statistical Analyses. For each product, concentrations were analyzed using ANOVA and post hoc comparisons of means. The model for the ANOVA included line and ripening stages as controlled effects. Only significant differences regarding this model are discussed, especially for comments on compounds of **Figure 4**.

Hierarchical clustering according to Euclidean distances was calculated using the multiexperiment viewer (MeV) (*24*), available from the Institute of Genomic Research (http://www.tigr.org/). Using this method, volatiles (**Figure 2**) and glycosides (**Figure 3**) were ordered according to the similarity of their concentration patterns during ripening. For each compound, the average level was estimated across all of the maturation time points in LEV, and then each volatile level at a given time point was divided by the LEV average, converted to a $\log_2$ scale, and is presented in a false color scale (blue = increase, red = decrease) as described by Gibon et al. (*25*).

Log Odor Unit Determination. Log odor units were calculated using the ratio of the concentration of a compound to its odor threshold collected from the literature (*26*). Volatile compounds with positive odor units are assumed to contribute to the flavor (*9*).

RESULTS AND DISCUSSION

Fruit Maturation As Defined by Fruit Color. The color measurements using the tristimulus indicator of color, *L*a*b**,

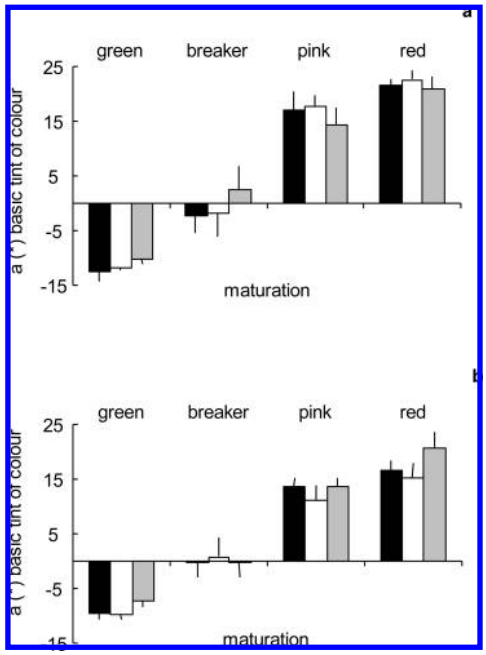

Figure 1. Fruit color changes in CER (**a**) and LEV (**b**) tomatoes during maturation. Black, white, and gray bars show color changes of fruits collected during the first, second, and third harvests, respectively, and ripened until green, breaker, pink, or red colors were attained. Data represent means ± SD of three replicates, consisting of five tomatoes each.

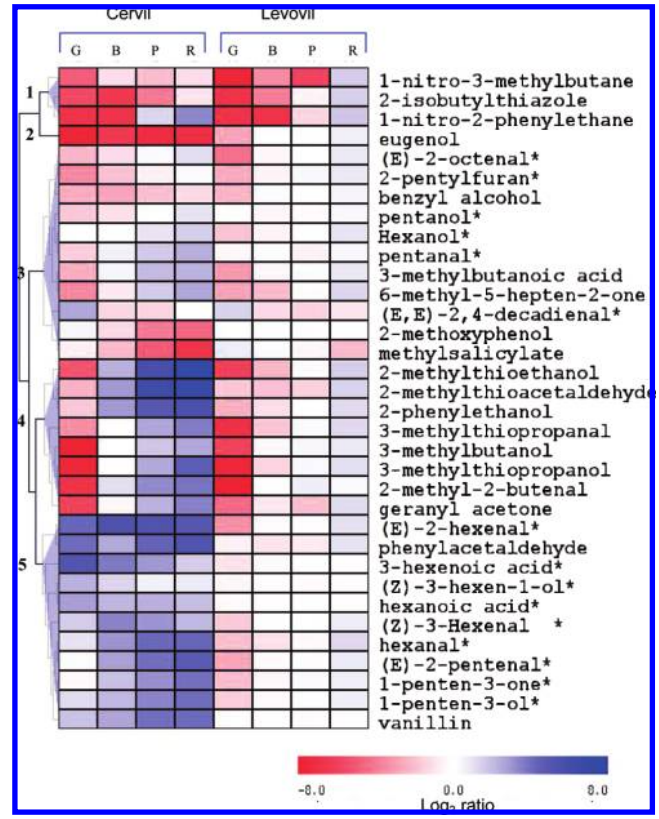

Figure 2. Heat map representing changes in volatile levels in Cervil (CER) and Levovil (LEV) tomatoes during maturation. Log_2 ratios were calculated for each value as described under Materials and Methods. Log_2 ratios give the intensity of the blue or red colors, according to the scale from the legend. Maturation points were defined as G, green; B, breaker; P, pink; and R, red. Volatiles are ranked according to the similarity of their accumulation patterns (hierarchical clustering). The Euclidean distances between volatiles are indicated to the left side of the figure, and identified groups are numbered in reference to the discussion. Stars indicate volatiles putatively issued from the LOX pathway.

were indicative of the accuracy of the sampling. We covered all of the maturation process of both lines regarding their colors, as fruits exhibited an *a** value increasing from negative to positive values, indicative of color variation ranging from green to red (**Figure 1**). Hence, the quantitative change of the color confirmed the relevance of the maturation stages in both LEV and CER tomatoes, here referred to as green (G), breaker (B), pink (P), and red (R) (**Figure 1**). All results here presented came from color analyses of fruits collected over three harvests. Relatively low standard deviations within each maturation stage are indicative of high interharvest homogeneity of samples (**Figure 1**).

CER Tomatoes Are More Abundant in Volatiles than LEV, Especially in LOX-Derived Compounds. More than 50 volatiles were detected in maturing CER and LEV tomato fruits. However, many compounds (~20), notably those with levels of $<30\ \mu g\ kg^{-1}$, could not be quantified with confidence as their signal/noise ratios were insufficient. Therefore, we focused on 34 remaining compounds (**Table 1**), allowing reliable interpretation of their patterns of accumulation during CER and LEV fruit ripening.

Compounds were ranked according to the evolutions of their concentrations to identify groups of volatiles exhibiting similar patterns. According to the Euclidean distances calculated for the clustering, five major groups can be defined (**Figure 2**). A first group of three products (1-nitro-3-methylbutane, 2-isobutylthiazole, 1-nitro-2-phenylethane) shared a drastic increase in Levovil at the red stage. Eugenol was the only compound of the second group as it was absent at all ripening stages in CER but increased with maturation of LEV. Group 3 was composed of the volatiles with similar patterns in LEV and in CER. Most of them increased during ripening, except for (*E*,*E*)-2,4-decadienal, 2-methoxyphenol, and methyl salicylate. The last two were characterized by a marked decrease in Cervil, where they attained trace levels at red ripe maturation stage, whereas their levels remained fairly stable or slightly decreased all during Levovil ripening. 2-Methoxyphenol and eugenol appeared as atypical compounds as they had contrasting patterns between the two maturing lines. Group 4 also reassembled volatiles that increased during ripening, at a much higher extent in Cervil rather than in Levovil. Finally, group 5 corresponded to compounds much more abundant in Cervil than in Levovil at all maturation stages. Interestingly, 9 of 11 compounds of this group putatively issued from the LOX pathway.

Overall, volatile levels mainly increased during fruit maturation in both genotypes. This feature is inherent in fruit (*1*, *2*, *7*, *27*). With regard to eugenol and 2-methoxyphenol, we obtained concentrations at red ripe stages similar to those already described for these lines and notably very low levels in Cervil (*20*). This has been interpreted as the presence of a nonfunctional pathway for these products in CER. However, 2-methoxyphenol was present at the green stage in comparable concentrations in both lines and then decreased in CER to the very low level observed at red ripe stage. Therefore, a nonfunctional pathway is not the cause of its absence.

Finally, the majority of compounds (19 of 34) were more abundant and increased to a greater extent in CER than in LEV fruits (**Figure 2**). For example, the levels in hexanal were 10 times higher at the red ripe stage in CER than in LEV. This is consistent with Saliba-Colombani et al. (*28*), who found higher overall volatile concentrations between red ripe Cervil and Levovil. The CER/LEV differences resulted from a higher

Table 1. Volatile Patterns in CER and LEV Tomatoes during Fruit Maturation[a]

			Cervil[b]				Levovil[b]			
volatile compd	RI[c]	ID	G	B	P	R	G	B	P	R
pentanal	991	A	35 ± 7	152 ± 21	315 ± 55	533 ± 140	76 ± 10	118 ± 38	103 ± 2	173 ± 40
1-penten-3-one	1031	A	18 ± 6	76 ± 1	191 ± 37	328 ± 42	5 ± 1	16 ± 3	27 ± 3	35 ± 13
hexanal	1086	A	138 ± 52	565 ± 192	1211 ± 288	1290 ± 203	17 ± 3	32 ± 5	53 ± 19	159 ± 41
2-methyl-2-butenal	1095	A	tr	17 ± 6	78 ± 35	144 ± 44	tr	7 ± 1	12 ± 3	11 ± 6
(*E*)-2-pentenal	1131	A	4 ± 1	21 ± 2	48 ± 9	83 ± 13	1 ± 0	2 ± 1	3 ± 1	5 ± 2
(*Z*)-3-hexenal	1154	A	11532 ± 3189	43666	31712	17957 ± 8148	1248 ± 461	3385 ± 729	4017 ± 652	6416 ± 1337
1-penten-3-ol	1162	A	26 ± 12	47 ± 2	122 ± 33	180 ± 24	4 ± 0	9 ± 0	13 ± 2	17 ± 5
3-methylbutanol	1205	A	tr	274 ± 41	973 ± 294	1749 ± 644	7 ± 6	222 ± 37	383 ± 42	506 ± 54
(*E*)-2-hexenal	1225	A	470 ± 104	1045 ± 121	954 ± 186	798 ± 16	2 ± 0	20 ± 4	22 ± 3	52 ± 10
2-pentylfuran	1240	A	1 ± 0.1	3.1 ± 0.5	7.6 ± 2	14 ± 1	2 ± 0.3	7.9 ± 1	14 ± 6	21 ± 3
pentanol	1254	A	8 ± 1	13 ± 0	28 ± 5	54 ± 13	25 ± 8	21 ± 10	21 ± 4	37 ± 17
2-methylthioacetaldehyde	1267	A	tr	3 ± 1	27 ± 3	39 ± 3	tr	tr	tr	1 ± 0
1-nitro-3-methylbutane	1339	B	3 ± 3	18 ± 8	8 ± 3	17 ± 5	0 ± 0	3 ± 1	30 ± 30	102 ± 9
6-methyl-5-hepten-2-one	1343	A	1 ± 0	7 ± 1	36 ± 7	73 ± 18	2 ± 0	3 ± 1	11 ± 7	30 ± 10
hexanol	1356	A	10 ± 5	10 ± 3	16 ± 2	25 ± 3	2 ± 0	6 ± 3	8 ± 3	17 ± 5
3-hexen-1-ol	1386	A	267 ± 163	127 ± 25	79 ± 32	87 ± 25	35 ± 3	62 ± 36	42 ± 11	56 ± 5
2-isobutylthiazole	1406	A	tr	tr	tr	3 ± 1	tr	tr	4 ± 2	16 ± 8
(*E*)-2-octenal	1433	A	2 ± 0	4 ± 0	13 ± 5	22 ± 6	1 ± 1	9 ± 7	9 ± 2	19 ± 8
3-methylthiopropanal	1460	A	1 ± 1	3 ± 0	23 ± 8	45 ± 5	tr	1 ± 0	5 ± 2	8 ± 2
2-methylthioethanol	1531	A	tr	11 ± 3	123 ± 42	324 ± 59	tr	tr	2 ± 1	6 ± 1
phenylacetaldehyde	1645	A	53 ± 41	15 ± 3	53 ± 6	99 ± 26	2 ± 1	2 ± 1	2 ± 0	5 ± 1
3-methylbutanoic acid	1675	A	4 ± 2	34 ± 11	98 ± 24	119 ± 59	3 ± 3	18 ± 3	28 ± 1	44 ± 11
3-methylthiopropanol	1717	A	tr	5 ± 2	37 ± 20	138 ± 12	tr	2 ± 0	8 ± 3	12 ± 3
methyl salicylate	1775	A	91 ± 32	32 ± 4	5 ± 2	1 ± 0	237 ± 101	170 ± 42	101 ± 15	40 ± 27
(*E*,*E*)-2,4-decadienal	1809	A	5 ± 2	4.6 ± 0.3	11 ± 2	21 ± 5	2.8 ± 1	6 ± 2	13 ± 2	22 ± 6
hexanoic acid	1848	A	216 ± 75	136 ± 39	153 ± 30	120 ± 21	24 ± 7	27 ± 10	33 ± 8	33 ± 3
geranylacetone	1856	A	tr	1 ± 0	9 ± 2	21 ± 5	tr	1 ± 1	1 ± 1	5 ± 2
2-methoxyphenol	1862	A	212 ± 80	63 ± 12	9 ± 2	6 ± 1	143 ± 14	136 ± 12	152 ± 17	151 ± 14
benzyl alcohol	1877	A	18 ± 8	15 ± 2	26 ± 8	43 ± 5	20 ± 2	124 ± 34	104 ± 54	140 ± 22
2-phenylethanol	1911	A	8 ± 3	183 ± 19	726 ± 113	1145 ± 72	4 ± 0	11 ± 1	24 ± 11	54 ± 3
(*E*)-3-hexenoic acid	1969	A	343 ± 105	135 ± 59	75 ± 14	28 ± 5	5 ± 2	12 ± 3	11 ± 5	10 ± 2
1-nitro-2-phenylethane	2120	B	tr	0 ± 0	8 ± 1	38 ± 22	tr	tr	2 ± 2	12 ± 3
eugenol	2166	A	tr	2 ± 2	1 ± 0	1 ± 1	11 ± 4	77 ± 6	82 ± 21	121 ± 21
vanillin	2561	A	12 ± 8	19 ± 1	49 ± 3	53 ± 5	4 ± 3	3 ± 1	3 ± 0	3 ± 1

[a] Data represent mean ± SD of the three harvests (with the exception of CER 3-hexenal at B and P maturation points, where only two harvests were usable). CER, Cervil; LEV, Levovil. Maturation points are defined as G, green; B, breaker; P, pink; and R, red. The ID identifies the assignment of the components: A, from authentic compounds; B, tentative identification from RI and mass spectrum. tr, traces. [b] In μg/kg equivalents of 4-nonanol. [c] RI, linear retention index based on a series of *n*-hydrocarbons.

biosynthesis at the late stage of development in Cervil for 8 of the 19 compounds (see the fourth group in **Figure 2**). The others were present at higher concentrations in Cervil since the green stage. Interestingly, most of them may issue from the LOX pathway. Cervil, thus, seems to exhibit a more efficient volatile biosynthesis through the LOX pathway compared to Levovil. This advantage seems true even since the early stage of fruit maturation, i.e. the green stage.

These findings encouraged us to further investigate the significance of contrasting volatile patterns and how they may associate with the distinct, variety-specific, tomato aroma. Therefore, contents of different volatiles were further expressed as log odor values that estimate the contribution of each compound to the aroma.

Potential Consequences for Tomato Aroma. To put in perspective the differences in volatiles patterns, we have used the literature values of log odor to identify a potential impact on tomato aroma. Twenty-one compounds were collected from the results in fully ripe (R) CER and LEV fruit as they exhibited positive log odor units, according to the thresholds collected from the literature (*26*) (**Table 2**). Volatiles with the highest log odor values potentially contribute the most to the tomato aroma (*9*). This discussion based on log odor is limited as far as all concentrations reported in the paper are relative to an internal standard. However, relative values are useful to compare differences between lines or stages of ripening. We add to this list β-ionone despite its very low concentration measured in the trial (detected only at red ripe, 0.9 ± 0.2 μg kg^{-1} in Cervil and 0.4 ± 0.05 μg kg^{-1} in Levovil). Its very low odor threshold indicated that even at that concentration, it may contribute to the fruit aroma. The scale that we obtained matched satisfactorily with the result obtained by the more accurate GC-O method (*6*, *12*, *29*). Only three compounds, 1-nitro-2-phenylethane, (*E*)-2-octenal,

Table 2. Volatile Compounds Contributing to the Tomato Aroma As Defined by Their Positive Log Odor Values in Cervil and Levovil Mature (R) Fruits[a]

	odor threshold	log odor		
volatile compd	(pg L^{-1})	Cervil	Levovil	aroma
(*Z*)-3-hexenal*	0.25	4.8	4.4	leaf-like
pent-1-en-3-one*	1	2.7	2.2	pungent, fish-like
hexanal*	4.5	2.5	2.4	tallowy, leaf-like
(*E*,*E*)-2,4-decadienal*	0.07	2.5	2.5	deep-fried
3-methylthiopropanal	0.2	2.4	1.6	potato-like
β-ionone	*0.007*	*2.1*	*1.7*	*floral*
pentanal*	12	1.6	1.1	pungent, almond-like
(*E*)-2-hexenal*	17	1.4	0.9	green, apple-like
phenylacetaldehyde	4	1.4	0.1	honey-like, flowery
1-nitro-2-phenylethane	2	1.3	0.8	flower, spicy
(*E*)-2-octenal*	3	0.9	0.8	fatty, nutty
3-methylbutanol	250	0.8	0.3	malty
2-pentylfuran*	6	0.4	0.5	buttery, green bean-like
2-methylthioacetaldehyde	16	0.4	*	almond, aldehyde
2-methylthioethanol	120	0.4	*	unknown
vanillin	20	0.4	*	vanilla
2-methoxyphenol	3	0.3	1.7	smoky, sweet
6-methyl-5-hepten-2-one	50	0.2	*	fruit-like
3-hexen-1-ol*	70	0.1	*	leaf-like, grass
2-phenylethanol	1000	0.1	*	honey-like, spicy
2-isobutylthiazole	3.5	*	0.7	green, tomato leaf
eugenol	6	*	1.3	spicy, clove

[a] Only log odor values >0 are reported as compounds putatively involved in the overall aroma. Log values were calculated from the data of **Table 1** and odor threshold in water collected from the literature (*26*).

Table 3. Glycoside Patterns in CER and LEV Fruit during Maturation[a]

			Cervil[b]				Levovil[b]			
glycoside compd	RI[c]	ID	G	B	P	R	G	B	P	R
3-methylbutanol	1210	A	9 ± 1	35 ± 7	160 ± 58	902 ± 145	5 ± 1	29 ± 3	147	178 ± 17
4-methylpentanol	1319	A	1 ± 0	1 ± 0	3 ± 1	10 ± 1	1 ± 0	1 ± 0	3	4 ± 0
3-methylpentanol	1332	A	1 ± 0	2 ± 1	6 ± 2	36 ± 7	1 ± 0	3 ± 1	16	34 ± 5
(*E*)-3-hexen-1-ol	1386	A	19 ± 4	10 ± 1	9 ± 4	13 ± 1	3 ± 0	2 ± 0	2	2 ± 0
6-methyl-5-hepten-2-ol	1469	A	tr	tr	1 ± 0	10 ± 5	1 ± 0	1 ± 0	1	5 ± 1
benzaldehyde	1527	A	20 ± 5	25 ± 4	43 ± 38	29 ± 8	16 ± 5	13 ± 2	12	15 ± 2
methyl benzoate	1624	A	8 ± 9	26 ± 16	21 ± 9	79 ± 12	tr	1 ± 1	2	3 ± 3
3-methylbutanoic acid	1675	A	3 ± 1	10 ± 4	51 ± 9	193 ± 101	4 ± 0	16 ± 5	81	73 ± 16
methyl salicylate	1775	A	4 ± 1	5 ± 2	5 ± 2	9 ± 2	2 ± 1	2 ± 1	2	2 ± 1
3-methyl-2-butenoic acid	1801	A	1 ± 0	3 ± 1	14 ± 1	43 ± 21	tr	tr	tr	0 ± 0
2-methyl-2-butenoic acid	1851	A	2 ± 0	3 ± 1	13 ± 5	29 ± 10	tr	tr	tr	0 ± 0
2-methoxyphenol	1862	A	21 ± 1	14 ± 5	9 ± 4	12 ± 1	7 ± 2	5 ± 1	9	7 ± 2
benzyl alcohol	1877	A	100 ± 14	73 ± 10	82 ± 34	123 ± 17	129 ± 14	283 ± 95	536	511 ± 110
2 -phenylethanol	1911	A	23 ± 6	113 ± 23	408 ± 101	1068 ± 122	24 ± 3	32 ± 7	56	79 ± 9
eugenol	2166	A	1 ± 0	1 ± 1	1 ± 0	1 ± 0	4 ± 1	19 ± 4	46	58 ± 4
benzoic acid	2438	A	45 ± 5	37 ± 14	111 ± 45	134 ± 46	14 ± 3	17 ± 8	30	27 ± 6
phenylacetic acid	2560	A	51 ± 21	36 ± 11	53 ± 34	82 ± 38	8 ± 1	5 ± 2	8	7 ± 1
3-oxo-α-ionol	*2627*	B	35 ± 10	50 ± 20	41 ± 20	50 ± 16	27 ± 3	36 ± 6	39 ± 5	43 ± 12
3-oxo-retro-α-ionol (isomer 1)[d]	2725	B	21 ± 8	29 ± 11	32 ± 18	47 ± 11	15 ± 2	22 ± 4	19	25 ± 8
2-(2-hydroxyphenyl) ethanol	2839	A	53 ± 41	15 ± 3	53 ± 6	99 ± 26	0 ± 0	1 ± 1	2	3 ± 3
3-oxo-retro-α-ionol (isomer II) [d]	2880	B	31 ± 13	48 ± 20	54 ± 27	83 ± 19	20 ± 1	32 ± 8	27	38 ± 13

[a] Data represent mean ± SD of the three harvests (with the exception of compounds measured in LEV at P maturation point, for which only two replicates were usable). Maturation points are as defined in **Table 1**. The ID identifies the assignment of the components: A, from authentic compounds; B, tentative identification from RI and mass spectrum. tr, traces. [b] In μg/kg equivalents of 4-nonanol. [c] RI, linear retention index based on a series of *n*-hydrocarbons. [d] As described in ref *33*.

and 2-pentylfuran, exhibited a positive log odor, whereas they were not detected by GC-O. This could be due to higher concentrations in our extracts or to an underevaluated odor threshold reported for these compounds. To the contrary, some compounds identified by GC-O are absent from our list. The reason is either that the compounds were not detected in our extracts (for example, 1-octen-3-one) or that the corresponding threshold was not available. The Furaneol detected as olfactive by Krumbein et al. (*30*) had a negative log odor in our study. Finally, β-ionone also illustrated that minor compounds in terms of concentration may be major contributors to the aroma perception.

All but five compounds exhibited a higher concentration in Cervil than in Levovil. The majority of them (9 of 17) were LOX-derived products, especially (*Z*)-3-hexenal, pent-1-en-3-one, and hexanal, the three products exhibiting the highest log odor values in Cervil. In addition, Cervil also synthesized a set of products, such as phenylacetaldehyde, 2-methylthioacetaldehyde, and 2-methylthioethanol, exhibiting positive log odor values, whereas, in contrast, the log was close to zero or negative in Levovil. Finally, only three compounds were detected as major contributors of the aroma in LEV but not in CER: 2-methoxyphenol, eugenol, and 2-isobutylthiazole.

These results are in agreement with sensory analysis performed on these lines and revealing CER as significantly superior to LEV for the overall aroma intensity (ARO), the lemon aroma (LEM), and the candy aroma (CAN) (*22*). On the one hand, the preeminent log odor values of the LOX-derived compounds may explain the best ARO score of Cervil. Indeed, a combination of these compounds, including (*Z*)-3-hexenal, hexanal, (*E*)-2-hexenal, and 1-penten-3-one, was already reported as conferring the aroma of fresh ripe tomato (*31*). On the other hand, one can assume that the compounds associated with floral or fruity aroma and preeminent in CER may explain the best scores of this line for LEM and CAN. With regard to eugenol and 2-methoxyphenol, our results were in agreement with the correlation already reported between the score obtained for a "pharmaceutical" descriptor and the concentration of these two compounds in the fruits (*20*). Accordingly, in our trial, their log odor was high in Levovil but low or negative in Cervil.

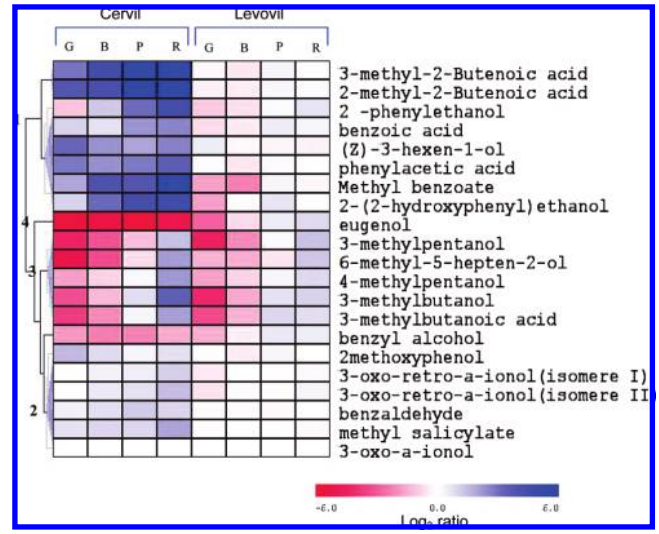

Figure 3. Patterns of Cervil (CER) and Levovil (LEV) glycosides during fruit maturation. Presented data were obtained using the same method as in **Figure 2**.

Glycoside-Bound Volatiles Plays an Active Role in the Contrast between Lines. Glycosides were analyzed throughout the trial to check whether the glycoside and volatile patterns paralleled (i.e., glycosides are strictly a buffer for volatiles storage) or if they differed (i.e., glycosides play an active role in the differences between LEV and CER by differentially storing the volatiles). Twenty-one compounds were detected with confidence all during maturation for the two lines (**Table 3**). As for the volatiles, we cluster the compounds according to their patterns to reassemble compounds exhibiting similar evolutions (**Figure 3**).

Two main groups, at each side of the clustering tree, reassemble compounds more abundant in Cervil. The first group of eight compounds exhibited a much higher concentration in Levovil, especially at the red ripe stage, whereas the second group (five compounds) exhibited a weaker difference between

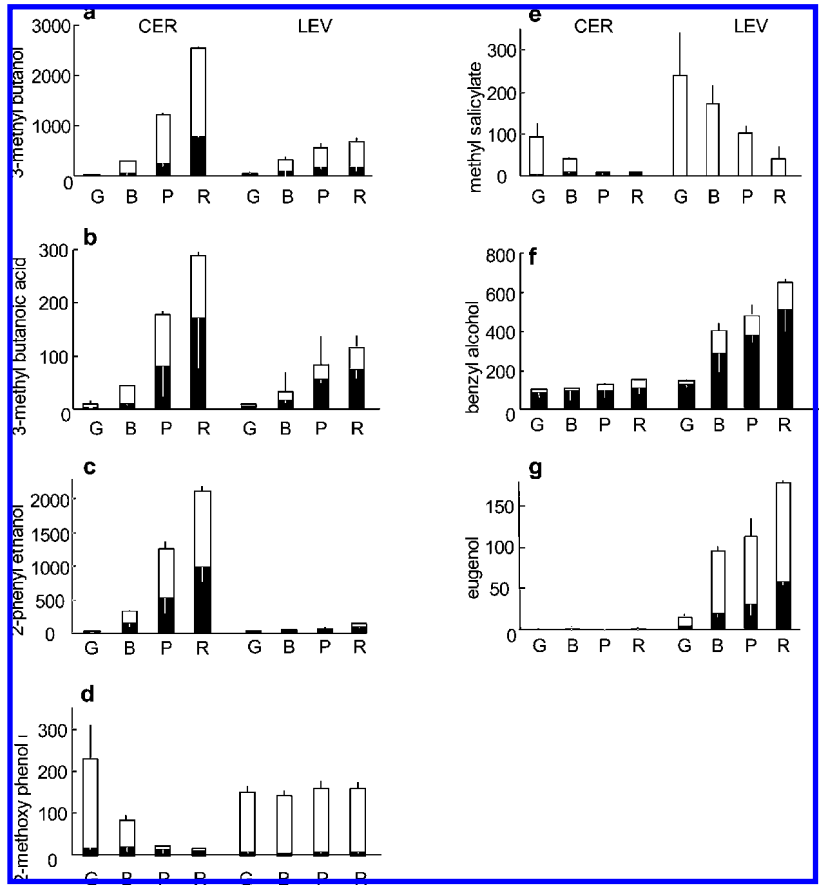

Figure 4. Changes of volatiles (white bars) and glycosides (black bars) in maturing Cervil (CER) and Levovil (LEV) fruits. Maturation stages were as defined in **Figure 1**. Data represent means ± SD of the three harvests. Concentrations are in micrograms per kilogram.

lines and little difference during the ripening. Anyway, 14 of the 21 products reported in **Table 3** were more abundant in Cervil than in Levovil. The two groups were separated by another one composed of compounds differing only slightly between lines, but exhibiting large variations from green to red ripe stages. Again, as with volatiles, eugenol was isolated in a specific group as it was not detected in Cervil glycosides.

Nine of the 21 reported products were detected only as glycosides, but for the others, as they were detected either as volatiles or as glycosides, it was possible to consider both glycosides and their corresponding volatiles. **Figure 4** shows the three cases observed. Glycosides of 3-methylbutanol, 3-methylbutanoic acid, and 2-phenylethanol followed the same increasing pattern as their corresponding volatiles. They showed higher levels in CER than in LEV by 2-, nearly 3-, and >10-fold, respectively, indicating a higher capacity of biosynthesis of these compounds within CER than within LEV (**Figure 4a**−**c**). Note that for 3-methylbutanoic acid, the proportion volatile/glycoside varied between LEV and CER: mature LEV fruits (P and R stages) exhibit relatively more glycoside form than Cervil. The second case was illustrated by the glycosides of 2-methoxyphenol and methyl salicylate, which remained at low levels during maturation of both CER and LEV fruits, whereas the corresponding volatiles decreased to trace levels between immature (G) and ripe (R) CER (**Figure 4d**,**e**). Methyl salicylate levels decreased between immature (G) and mature (R) LEV fruits, but remained more abundant in ripening LEV than in CER fruits (**Figure 4e**). Conversely, 2-methoxyphenol remained abundant in LEV all during the maturation (**Figure 4d**). For these two compounds, the glycosides did not follow the volatile variations. The last case was illustrated by eugenol and benzyl alcohol; both shared a large difference between lines for their glycoside patterns. For benzyl alcohol the glycosides remained unchanged in CER all during the ripening, whereas it quadrupled in LEV. For the eugenol, it remained at trace levels for volatiles as for glycosides in CER, whereas both glycoside and volatiles were multiplied by 10 in LEV (**Figure 4f**,**g**).

Glycoside patterns permitted further understanding of the volatile levels within CER and LEV fruits. Overall, the conversion of volatiles to glycosides and the reverse reaction are not common features for all volatiles as demonstrated by the different cases highlighted in **Figure 4**. There is a clear difference between lines for the glycoside biosynthesis as was also the case for the free volatiles. Moreover, glycosides did not compensate the difference between the volatile levels within the two varieties as the sum (volatile + glycoside) did not yield the total concentration between CER and LEV. CER exhibited a better biosynthesis capacity for the glycosides as it did for the volatiles, as most measured compounds (14 of 21) were more abundant in Cervil. In addition to exhibiting a superior aroma as fresh fruit, Cervil should also benefit from its higher glycoside content when processed. Indeed, the glycoside of 3-methylbutanoic acid, at least twice more abundant in CER than in LEV, may be hydrolyzed into its volatile during tomato processing, and the corresponding volatile has been reported to confer a strong aroma in tomato paste (*31*).

Finally, the glycoside/volatile comparisons of eugenol and 2-methoxyphenol, biosynthetically close, illustrated a clear difference for the mechanism involved in the contrast observed between the lines. CER was unable to accumulate eugenol, and both glycoside and free volatiles are present at only trace levels. The 2-methoxyphenol glycosides were low in both lines, although LEV did synthesize the volatiles. This behavior denies

the hypothesis of a simple and straightforward biochemical relationship for the genetic control of this variation as already mentioned (*20*).

To our knowledge, this is the first time that volatile and volatile−glycoside patterns were simultaneously investigated in two tomato lines during fruit maturation. We have confirmed that tomato lines with distinct physical and biochemical features may also be distinguished by their volatile fingerprints (*32*) and that this difference is maintained throughout fruit maturation. Although volatiles generally accumulate during fruit maturation, we have outlined compounds that may have contrasting patterns between the two studied lines. Whereas volatiles were overall more abundant within CER, certain compounds, including eugenol, remained at higher concentrations in LEV. Overall, higher volatile levels within CER were due to its superior capacity of biosynthesis rather than to an inferior capacity for glycoside biosynthesis. The volatile variations that we observed, according to log odor of the components, are in accordance with the sensory attribute already compiled for these two lines.

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LITERATURE CITED

(1) Obando-Ulloa, J. M.; Moreno, E.; Garcia-Mas, J.; Nicolai, B.; Lammertyn, J.; Monforte, A. J.; Fernandez-Trujillo, J. P. Climacteric or non-climacteric behavior in melon fruit−1. Aroma volatiles. *Postharvest Biol. Technol.* **2008**, *49*, 27–37.

(2) Supriyadi; Shimizu, K.; Suzuki, M.; Yoshida, K.; Muto, T.; Fujita, A.; Tomita, N.; Watanabe, N. Maturity discrimination of snake fruit (*Salacca edulis* Reinw.) cv. Pondoh based on volatiles analysis using an electronic nose device equipped with a sensor array and fingerprint mass spectrometry. *Flavour Fragrance J.* **2004**, *19*, 44–50.

(3) Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C., Fresh tomato volatiles. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; ACS Symposium Series 288; American Chemical Society: Washington, DC, 1989; pp 213−222.

(4) Buttery, R. G.; Teranishi, R.; Ling, L. C. Fresh tomato aroma volatiles: a quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540–544.

(5) Krumbein, A.; Auerswald, H. Characterization of aroma volatiles in tomatoes by sensory analyses. *Nahrung* **1998**, *42*, 395–399.

(6) Langlois, D.; Etievant, P. X.; Pierron, P.; Jorrot, A. Sensory and instrumental characterisation of commercial tomato varieties. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 534–540.

(7) Baldwin, E. A.; Nisperos-Carriedo, M. O.; Moshonas, M. G. Quantitative analysis of flavor and other volatiles and for certain constituents of two tomato cultivars during ripening. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 265–269.

(8) Petro-Turza, M. Flavor of tomato and tomato product. *Food Rev. Int.* **1987**, *2*, 309–351.

(9) Baldwin, E. A.; Scott, J. W.; Shewmaker, C. K.; Schuch, W. Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. *HortScience* **2000**, *35*, 1013–1022.

(10) Marlatt, C.; Ho, C. T.; Chien, M. J. Studies of aroma constituents bound as glycosides in tomato. *J. Agric. Food Chem.* **1992**, *40*, 249–252.

(11) Winterhalter, P.; Skouroumounis, G. K. Glycoconjugated aroma compounds: occurence, role and biotehnological transformation. In *Advances in Biochemical Engineering/Biotechnology*; Scheper, Ed.; Spring-Verlag: Heildelberg, Germany, 1997; Vol. 55, pp 74−105.

(12) Stahl-Biskup, E.; Intert, F.; Holthuijzen, J.; Stengele, M.; Schulz, G. Glycosidically bound volatiles—a review 1986−1991. *Flavour Fragrance J.* **1993**, *8*, 61–80.

(13) Fils-Lycaon, B.; Buret, M. Changes in glycosidase activities during development and ripening of melon. *Postharvest Biol. Technol.* **1991**, *1*, 143–151.

(14) Odoux, E.; Escoute, J.; Verdiel, J. L.; Brillouet, J. M. Localization of β-D-glucosidase activity and glucovanillin in vanilla bean (*Vanilla planifolia* Andrews). *Ann. Bot.* **2003**, *92*, 437–444.

(15) Sarry, J. E.; Gunata, Z. Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. *Food Chem.* **2004**, *87*, 509–521.

(16) Ortiz-Serrano, P.; Gil, J. V. Vicente quantitation of free and glycosidically bound volatiles in and effect of glycosidase addition on three tomato varieties (*Solanum lycopersicum* L.). *J. Agric. Food Chem.* **2007**, *55*, 9170–9176.

(17) Buttery, R. G.; Ling, L. C., Volatile components of tomato fruit and plant parts: relationship and biogenesis. In *Bioactive Volatile Compounds from Plants*; Teranishi, R., Buttery, R., Sugisaiva, H., Eds.; American Chemical Society: Washington, DC,1993; pp 23−34.

(18) Yilmaz, E.; Tandon, K. S.; Scott, J. W.; Baldwin, E. A.; Shewfelt, R. L. Absence of a clear relationship between lipid pathway enzymes and volatile compounds in fresh tomatoes. *J. Plant Physiol.* **2001**, *158*, 1111–1116.

(19) Chen, G.; Hackett, R.; Walker, D.; Taylor, A.; Lin, Z.; Grierson, D. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiol.* **2004**, *136*, 2641–2651.

(20) Causse, M.; Saliba-Colombani, V.; Lecomte, L.; Duffe, P.; Rousselle, P.; Buret, M. QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. *J. Exp. Bot.* **2002**, *53*, 2089–2098.

(21) Saliba-Colombani, V.; Causse, M.; Langlois, D.; Philouze, J.; Buret, M. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. *Theor. Appl. Genet.* **2001**, *102*, 259–272.

(22) Causse, M.; Saliba-Colombani, V.; Lesschaeve, I.; Buret, M. Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. *Theor. Appl. Genet.* **2001**, *102*, 273–283.

(23) Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83–90.

(24) Saeed, A. I.; Sharov, V.; White, J.; Li, J.; Liang, W.; Bhagabati, N.; Braisted, J.; Klapa, M.; Currier, T.; Thiagarajan, M.; Sturn, A.; Snuffin, M.; Rezantsev, A.; Popov, D.; Ryltsov, A.; Kostukovich, E.; Borisovsky, I.; Liu, Z.; Vinsavich, A.; Trush, V.; Quackenbush, J. TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **2003**, *34*, 374+.

(25) Gibon, Y.; Usadel, B.; Blaesing, O. E.; Kamlage, B.; Hoehne, M.; Trethewey, R.; Stitt, M. Integration of metabolite with transcript and enzyme activity profiling during diurnal cycles in *Arabidopsis* rosettes. *Genome Biol.* **2006**, *7*, R76.

(26) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München: Garching, Germany, 1998; p 63.

(27) Mastelic, J.; Jerkovic, I. Gas chromatography−mass spectrometry analysis of free and glycoconjugated aroma compounds of seasonally collected *Satureja montana* L. *Food Chem.* **2003**, *80*, 135–140.

(28) Saliba-Colombani, V.; Causse, M.; Langlois, D.; Philouze, J.; Buret, M. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. *Theor. Appl. Genet*. **2001**, *102*, 259–272.

(29) Mayer, F.; Takeoka, G. R.; Buttery, R. G.; Whitehand, L. C.; Naim, M.; Rabinowitch, H. D. Studies on the aroma of five fresh tomato cultivars and the precursors of cis- and trans-4,5-epoxy-(*E*)-2-decenals and methional. *J. Agric. Food Chem*. **2008**, *56*, 3749–3757.

(30) Krumbein, A.; Peters, P.; Bruckner, B. Flavour compounds and a quantitative descriptive analysis of tomatoes (*Lycopersicon esculentum* Mill.) of different cultivars in short-term storage. *Postharvest Biol. Technol*. **2004**, *32*, 15–28.

(31) Buttery, R. G. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In *Flavor Science—Sensible Principles and Techniques*; Acree, T. E., Teranishi, R., Eds.; American Chemical Society: Washington, DC, 1993; pp 258–286.

(32) Tikunov, Y.; Lommen, A.; de Vos, C. H. R.; Verhoeven, H. A.; Bino, R. J.; Hall, R. D.; Bovy, A. G. A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiol*. **2005**, *139*, 1125–1137.

(33) Aubert, C.; Ambid, C.; Baumes, R.; Gunata, Z. Investigation of bound aroma constituents of yellow-fleshed nectarines (*Prunus persica* L. Cv. Springbright). Changes in bound aroma profile during maturation. *J. Agric. Food Chem*. **2003**, *51*, 6280–6286.

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