Quantitative Comparison of Free and Bound Volatiles of Two Commercial Tomato Cultivars (*Solanum lycopersicum* L.) during Ripening

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The changes in the profile of both free and glycosidically bound volatiles were studied in Money-maker and Raf tomato cultivars during fruit ripening. The concentrations of 20 of 24 and of 27 of 30 compounds detected in the free volatile fraction (FVF) and glycosidically bound fraction (GBF), respectively, differed significantly between cultivars during ripening. Most free and bound volatiles increased during ripening in both cultivars. The contribution of each free volatile compound to the overall aroma was estimated by calculating its log *U* value, which indicated that only 11 compounds seem to exert a strong influence. Nine volatiles were detected exclusively in the GBF, among them geraniol, β-citronellol, α-terpineol, and *trans*- and *cis*-linalool oxides. Nine other compounds were found to be more abundant in the GBF than in the FVF, their absolute levels varying between cultivars and stages of ripening. According to the log *U* values reached, of these nine compounds, linalool, 3-methyl-1-butanol, *trans*-2-hexenal, eugenol, and 2-phenylethanol may have an impact on tomato aroma upon release from their glycosidic conjugates. Sugars resulting from the enzymatic hydrolysis of the GBF were detected by high-performance liquid chromatography. Rhamnose was the most abundant followed by arabinose, glucose, and xylose.

KEYWORDS: Tomato (*Solanum lycopersicum* L.); volatiles; glycosidically bound; SPME; ripening

INTRODUCTION

The cultivated tomato is both popular and frequently consumed worldwide. Its consumer acceptability is mainly derived from its unique flavor, which comprises sugars, acids, minerals, and aroma volatiles (*1*, *2*). The importance of taste and aroma in tomato flavor has never been firmly established, but tomato aroma volatiles play an important role in consumer acceptability (for a review, see ref *3*). In recent decades, the lack of fresh "tomato-like" aroma and flavor of tomatoes purchased in supermarkets is a common consumer complaint. The reasons for this dissatisfaction range from poor genetic material (*1*, *3*) to pre- and postharvest procedures, like tomato-fruit storage at low temperatures (*4*, *5*) or harvesting immature or mature-green fruits (*6*−*8*).

In tomato, volatiles are formed in the intact fruit during ripening, as well as upon tissue disruption. It has been reported that *trans*-2-pentenal and geranial appear for the first time after tissue disruption, some volatiles such as hexanal and 2-iso-

butylthiazole increase after tissue disruption, and others such as 3-methyl-1-butanol, hexanol, *cis*-3-hexenol, 6-methyl-5-hepten-2-one, or 2-phenylethanol do not show significant changes due to tissue disruption (*3*). Fruit ripening is accompanied by changes in flavor, texture, color, and aroma, which altogether produce fruit with desirable quality attributes (*9*). In general, the concentration of individual volatiles in tomato fruits increases during ripening, peaking at the mature-breaker or mature-red stages (*2*, *10*, *11*). Only certain tomato volatiles decrease as the fruit ripens, for example, methyl salicylate (*12*). Tomato is a climacteric fruit, and certain changes in aroma compounds during ripening are considered to be ethylene-mediated; however, no direct relationship has been reported. McGlasson et al. (*13*) reported that 15 compounds with medium or very strong odor intensities, such as hexanal, 3-methyl-1-butanol, 1-hexanol, or 2-isobutylthiazole, were deficient or absent in fruits of the nonripening mutants *rin* and *nor*. Gao et al. (*12*) showed that ethylene regulates the production of lipid-derived alcohol volatiles, 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol in tomato fruits, but does not influence the production of *cis*-3-hexenal and *trans*-2-hexenal. The biochemical and genetic regulation of fruit aroma has only recently become a focus of attention (*14*, *15*).

Although more than 400 volatile compounds have been identified in tomato fruits (*16*, *17*), only a limited number are

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considered essential to tomato flavor. The log odor unit (log U) is the ratio of the volatile concentration in a food to its odor threshold and has a practical use in the selection of the most important aroma contributors in a mixture (*3, 7, 16, 18−23*). Compounds with positive log odor units are assumed to contribute to the flavor of a food, and compounds with negative odor units may still contribute to the overall flavor of tomato but as background notes (*24*). Only 16 tomato volatiles were found with a log odor unit of > 0, and among these, Buttery (*10*) suggested that a combination of appropriate concentrations of *cis*-3-hexenal, *cis*-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β-ionone produces the aroma of a fresh, ripe tomato. Recently, Tandon et al. (*25*) reported that tomatoes described as full-flavored were characterized by a low level of titratable acidity, a high content of total sugars and soluble solids, and an intermediate content of hexanal, *cis*-3-hexenal, 2- and 3-methyl-1-butanol, *trans*-2-hexenal, *cis*-3-hexenol, geranyl acetone, β-ionone, and 1-penten-3-one.

The presence of flavor precursors in tomato has been demonstrated, representing a reserve of aroma to be exploited in this fruit (*3, 10, 26−28*). Most of the precursor compounds identified in plants and fruits are glycosidic derivatives, mainly *O*-β-D-glucosides or *O*-diglycosides (*29, 30*). Acid or enzymatic hydrolysis of glycosides leads to the release of volatiles known as aglycons. The enzymatic release of aglycons from glycosides is catalyzed by *O*-glycoside hydrolases. Among glycoside hydrolases, β-glucosidases have been the subject of many studies because of their importance, as they catalyze the hydrolysis of the glycosidic bond between the glucopyranosyl unit and the aglycon moiety. Enzymatic hydrolysis of diglycosidic flavor precursors can occur in one step when a diglycosidase catalyzes the cleavage of the aglyconic linkage (*31*), or in two steps. In sequential mode, first, one exoglycosidase makes the cleavage of the intersugar linkage releasing the corresponding sugar (arabinose, rhamnose, apiose, glucose, and xylose) and β-D-glucoside. In the second step, a β-glucosidase catalyzes the hydrolysis of the β-D-glucoside and releases the corresponding aglycon and glucose (*30, 32, 33*).

Although tomato volatiles have been extensively investigated, we have limited knowledge of the glycosidically bound volatiles present in this fruit (*3, 11, 27−30*). Recently, Birtic et al. (*11*) described changes in free and glycosidically bound flavor volatiles in tomato during ripening but without quantitative data. The purpose of this work is to study the volatile and glycosidic fractions of two commercial tomato cultivars, Moneymaker and Raf, providing a quantitative comparison during on-plant ripening and to estimate the possible contribution of each compound to the overall aroma by means of log U values.

MATERIALS AND METHODS

Plant Material and Treatments. Tomato cultivars were grown in a greenhouse, in a Mediterranean climate, at the Instituto de Biología Molecular y Celular de Plantas (IBMCP, Valencia, Spain). There were three rows of each cultivar with 20 plants by row. Five fruits free of visual defects were chosen at random from each plant. The fruits were hand-picked at three different ripeness stages according to size, color, and firmness: green (fruit at full size, on average 5 or 6 cm in diameter, bright green, and hard texture), breaker (partially vine ripened to a light pink color and firm texture), and red (fully vine ripened, red, and soft texture). Selected fruits of each cultivar were blended and centrifuged. The supernatant was preserved at −20 °C as a clear juice. Moneymaker is a well-known commercial cultivar of smooth, medium-size, and intense-red fruits without special sensory characteristics, and Raf is a cultivar of economic importance greatly appreciated for its organoleptic features.

Standards. The following were purchased from Sigma-Aldrich (St. Louis, MO): hexanal, 3-methyl-1-butanol, *trans*-2-hexenal, 1-hexanol, *cis*-3-hexenol, *trans*- and *cis*-linalool oxides, 1-heptanol, benzaldehyde, linalool, 1-octanol, α-terpineol, β-citronellol, nerol, benzyl alcohol, 2-phenylethanol, β-ionone, nonanal, octanal, 3-octanone, decanal, 3-methyl-1-pentanol, 4-methyl-1-pentanol, eugenol, 6-methyl-5-hepten-2-one, *cis*-4-decenal, 3,5-dimethylbenzaldehyde, 6-methyl-5-hepten-2-ol, 2-isobutylthiazole, methyl salicylate, geraniol, guaiacol, D-(+)-glucose, D-(+)-arabinose, D-(+)-rhamnose, and D-(+)-xylose.

Analysis of Volatile Compounds. Samples (3 mL of clear tomato juice) were extracted by headspace mode, for 30 min at 30 °C with magnetic stirring, using a SPME device (Supelco, Bellefonte, PA) with a 10 mm fiber coated with 100 μm polydimethylsiloxane. After extraction, the SPME device was inserted into a gas chromatograph (GC) splitless injector and maintained at 240 °C for 4 min. A HP5890 GC (Hewlett-Packard, Waldbronn, Germany) equipped with an HP-INNOWax capillary column [30 m × 0.25 mm (inside diameter) × 0.25 μm (Hewlett-Packard)] was used. The operating conditions were as follows: detector temperature (FID), 300 °C; injector temperature, 240 °C; oven temperature from 40 (5 min) to 150 °C at a rate of 5 °C/min and then from 150 to 250 at 20 °C/min and held at 250 °C for 10 min.

Compounds were identified by comparing retention times with those of standard compounds and by using an Agilent 5973N MS detector coupled to an Agilent 6890 GC (Agilent Technologies, Waldbronn, Germany).

Volatile compounds were quantified using linear regression analysis from solutions with known concentrations of the commercial standards. Three repeated measurements were made for each sample.

Extraction and Hydrolysis of Tomato Glycosides. Glycoside isolation was performed according following previous works with some modifications (*27, 28, 31*). Clear juice (40 mL) was passed through a 500 mg C18 Sep-Pack cartridge (Waters Corp., Milford, MA), previously activated with methanol (10 mL) and water (20 mL), and washed with water (10 mL). The fraction containing free aroma compounds was then eluted with pentane (10 mL). Both the water and pentane eluents were discarded. The tomato glycoside fraction was then eluted with 10 mL of methanol. The methanol was removed under reduced pressure at 45 °C, and the dry extract was dissolved in 3 mL of 75 mM citrate-phosphate buffer (pH 5) and defined as glycoside extract.

Exhaustive hydrolysis of the tomato glycosidically bound fraction was performed following previously reported methods with some modifications (*28, 34*). An AR2000 solution (100 μL, 2.5%, w/v) (Gist-Brocades, Seclin, France) was added to 1 mL aliquots of the glycoside extract, and the mixture was incubated at 40 °C for 48 h. After hydrolysis, free aroma compounds released were analyzed as previously described. All treatments were performed in triplicate.

Analysis of Sugar Moieties. After enzymatic hydrolysis of the tomato glycosidically bound fraction by Pectinase AR2000, liquid chromatography was used to identify released sugars; 100 μL of hydrolyzed extract samples, diluted accordingly for chromatographic analysis, was injected into a Dionex System (Sunnyvale, CA) consisting of a P680A pump and a 200s ELSD detector (SofTA Corp.). An Aminex HPX-87P column (300 mm × 7.8 mm) was use for separation. The column oven temperature was set at 60 °C. Sugar elution was performed with Milli-Q H_2O at a rate of 0.6 mL/min under isocratic conditions for 25 min. Peaks were identified using commercial standards (Sigma). All treatments were performed in triplicate.

Statistical Analysis. Statistical significance was determined by analysis of variance (ANOVA). For mean comparisons, Tukey's HSD procedure was performed. Data were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of the Free Volatile Fraction. The free volatile fraction (FVF) of Moneymaker and Raf cultivars was analyzed using solid-phase microextraction and gas chromatography. The concentrations of the 23 volatile compounds detected in the FVF of Moneymaker and Raf cultivars and the statistically significant differences among the three ripening stages are listed in **Table 1**. All identified compounds have previously been reported in the

Table 1. Free Volatile Compound Concentrations (micrograms per liter) of Moneymaker and Raf Tomato Cultivars in Three Ripening Stages, Green, Breaker, and Red[a]

free compound	Moneymaker			Raf			CC[b]		
	green	breaker	red	green	breaker	red			
hexanal	194.6 a	826.6 b	1827 c	255.9 b	117.8 a	2679 c	G	B	R
3-methyl-1-butanol	nd[c]	1536 b	623.6 a	nd[c]	401.4 a	596.8 a		B	
trans-2-hexenal	252.4 b	261.8 b	122.4 a	253.6 c	49.39 a	152.0 b		B	
3-octanone	0.208 a	0.398 a	0.841 b	0.351 a	0.542 a	1.140 b			R
octanal	3.365 b	4.009 c	2.733 a	3.632 a	4.575 a	2.732 a			
6-methyl-5-hepten-2-one	1.570 a	18.62 b	121.2 c	1.648 a	9.439 b	160.7 c		B	R
1-hexanol	35.79 a	245.1 b	3735 c	55.80 a	184.3 b	2359 c			R
cis-3-hexenol	88.86 a	695.6 b	3121 c	192.7 a	983.5 b	2817 c	G		R
nonanal	0.295 a	0.258 a	0.532 a	0.198 a	0.229 a	0.686 b			
2-isobutylthiazole	0.186 a	2.837 b	11.99 c	0.202 a	0.208 a	2.114 b		B	R
1-heptanol	7.571 ab	4.698 a	9.254 b	24.20 b	8.655 a	26.12 b	G	B	R
decanal	tr[d]	0.122 a	0.272 a	0.161 a	0.128 a	0.278 a			
cis-4-decenal	tr[d]	0.135 a	0.353 b	0.117 a	0.093 a	0.228 b			
linalool	1.088 a	1.418 ab	1.595 b	1.200 a	2.055 ab	2.603 b			R
1-cctanol	2.411 a	1.771 a	5.840 b	1.362 a	2.094 a	6.911 b			R
methyl salicylate	1.756 a	1.662 a	1.252 a	181.9 b	1.883 a	nd[c]	G		
nerol	1.753 a	1.650 a	2.025 a	1.616 a	1.586 a	1.803 a			
3,5-dimethyl benzaldehyde	1.422 a	1.782 b	3.418 c	3.878 a	2.845 a	2.536 a		B	R
guaiacol	125.2 a	550.9 b	3240 c	250.7 a	150.5 a	4712 b	G	B	R
benzyl alcohol	263.2 a	416.6 b	291.9 a	323.6 ab	296.8 a	505.1 b		B	R
2-phenylethanol	71.22 a	77.76 a	67.00 a	116.0 a	136.0 a	280.0 b			R
β-ionone	tr[d]	tr[d]	2.003	tr[d]	tr[d]	2.190			
eugenol	12.35 a	175.5 b	305.4 c	6.811 a	10.29 a	25.50 b		B	R

[a] Different letters for the entries in the Moneymaker and Raf columns indicate significant differences ($p < 0.05$) among the three ripening stages. [b] For the cultivar comparison (CC), G, B, and R indicate significant differences ($p < 0.05$) between both cultivars at green (G), breaker (B), and red (R) ripening stages. [c] Undetected. [d] Less than 0.1 μg/L.

literature as components of the tomato free aroma fraction (*3*, *7*, *26*, *27*, *35*, *36*), except for 3-octanone, *cis*-4-decenal, and 3,5-dimethyl benzaldehyde.

In the FVF, nonanal, decanal, and *cis*-4-decenal were always found at concentrations of <1 μg/L in both cultivars, as were 3-octanone, 2-isobutylthiazole, and β-ionone at different ripening stages. In addition, 3-methyl-1-butanol was not detected at the mature-green stage of both cultivars.

Changes in individual flavor volatiles during ripening were analyzed (**Table 1**). The only concentrations not to change significantly during ripening were nerol and decanal in both cultivars, nonanal, methyl salicylate, and 2-phenylethanol in Moneymaker, and 1-octanal and 3,5-dimethylbenzaldehyde in Raf. In general terms, the concentration of individual volatiles increased during ripening and peaked at the mature-breaker or mature-red stages. The highest concentration increases were recorded for hexanal, 6-methyl-5-hepten-2-one, 1-hexanol, *cis*-3-hexenol, 2-isobutylthiazole, guaiacol, and eugenol as ripening progressed in both cultivars, ranging from 4- to 104-fold higher at the red stage than at the green stage. The only exceptions to this ripening-associated increase were *trans*-2-hexenal in both cultivars and methyl salicylate in Raf, in which concentrations decreased significantly during ripening, peaking at the mature-green stage.

The ripening process has been extensively studied in economically important fruit crops like the tomato, and the fact that the concentration of most individual volatiles increases during ripening is well-known (*2*, *10*, *15*). Previous works conducted with different tomato cultivars have reported different patterns in time course changes of the same volatile compounds during ripening (*2*, *11*, *12*, *15*, *37*), pointing to cultivar as a variability factor.

The number of compounds for which concentrations differed significantly between Moneymaker and Raf increased as the fruits ripened. Only the concentrations of 1-octanal, nonanal, decanal, and nerol did not differ significantly between the

two cultivars. For the others, it was particularly relevant that 2-isobutylthiazole and eugenol were significantly more abundant at the mature-breaker and mature-red stages in Moneymaker, as were linalool and 2-phenylethanol at the mature-red stage of Raf. These four compounds are supposed to have a positive organoleptic influence on tomato aroma, according to the literature (*10*, *38*, *39*).

These results, along with previous studies (*1*, *22*, *28*, *40*−*43*), suggest that the cultivar constitutes a source of variability to tomato FVF and to time course changes during ripening. This fact supports the possibility of using the volatile profile as a tool for tomato cultivar identification (*2*, *25*, *35*, *44*). To confirm this, further experiments would be necessary, taking into account other important factors such as growing location, environmental conditions, and cultural practices.

The extent to which each monitored compound contributed to the overall aroma of the tomato was estimated by converting concentrations into odor units (*3*, *10*, *19*, *21*). The odor units and their logarithms (log *U*) were calculated, except for those of *cis*-4-decenal and 3.5-dimethylbenzaldehyde, using the odor thresholds in water reported in the literature (*10*, *16*, *23*, *45*, *46*) (**Figures 1** and **2**). In the FVF, hexanal, 3-methyl-1-butanol, *trans*-2-hexenal, octanal, *cis*-3-hexenol, guaiacol, and eugenol gave a positive log *U* value in Moneymaker and Raf cultivars during ripening, as well as decanal in Raf. Among them, the log *U* values of hexanal, *cis*-3-hexenol, guaiacol, and eugenol increased as fruits ripened. According to their positive log *U* values, these seven compounds should contribute to tomato aroma at any stage of ripening, especially hexanal and guaiacol, which reached the highest log *U* values in both cultivars with concentrations ~1000-fold greater than their odor thresholds, as well as eugenol, whose concentration was ~100-fold greater than its odor threshold in Moneymaker.

Compounds with large negative log *U* values probably do not contribute to the overall flavor of tomato, such as 3-octanone, 1-heptanol, 1-octanol, nerol, and benzyl alcohol in both cultivars
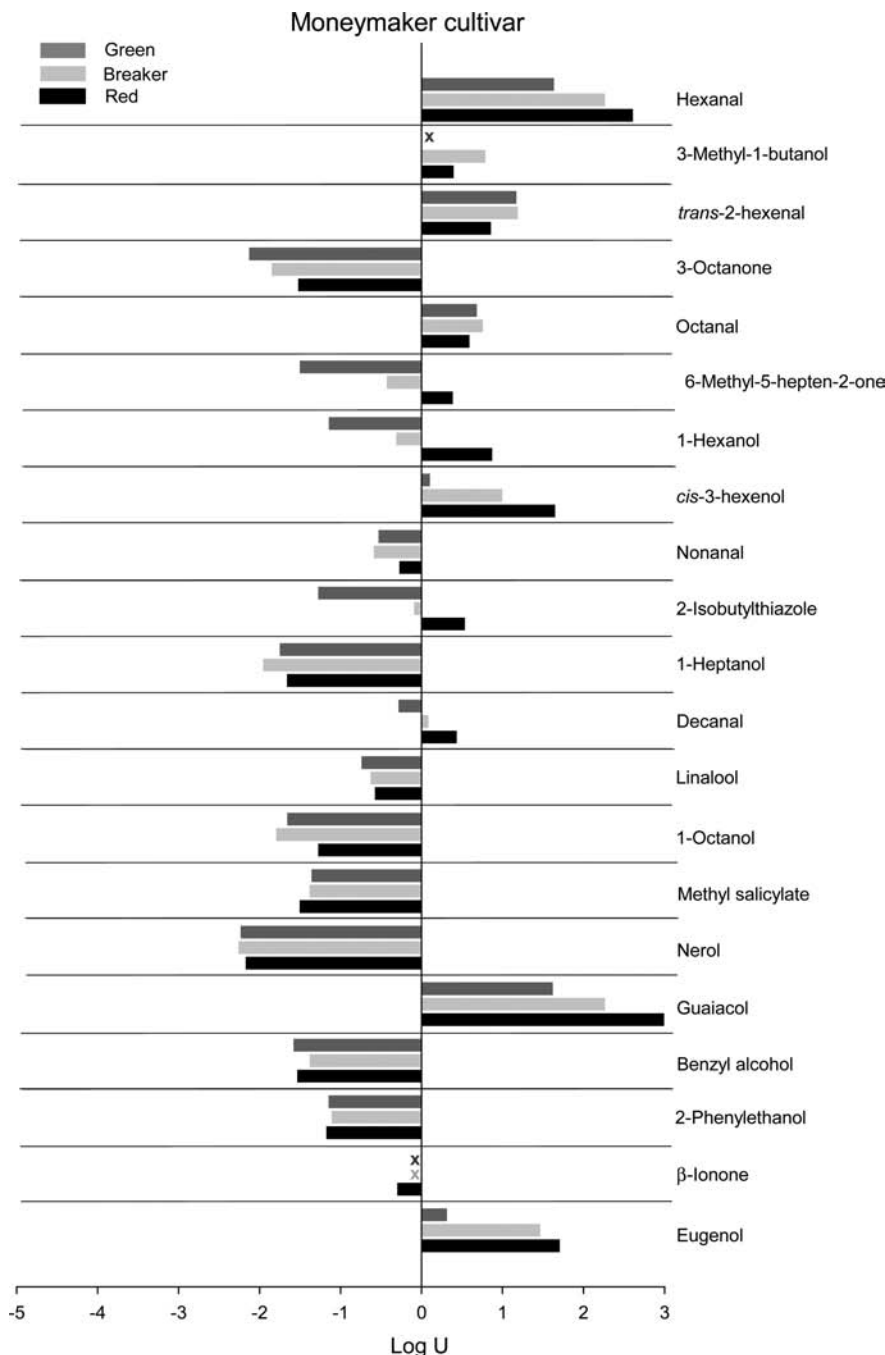
Figure 1. Log U values of the volatile compounds detected in the FVF of the Moneymaker cultivar in three ripening stages (green, breaker, and red). An x indicates the compound was undetected or its concentration was <1 μg/L.

and methyl salicylate and 2-phenylethanol in Moneymaker (**Figures 1** and **2**), concentrations of which ranged from 10- to 100-fold below their odor threshold.

It is difficult to establish the extent to which compounds with log U values between −1 and 1 affect overall tomato flavor, for instance, nonanal and linalool in both cultivars and 2-iso-butylthiazole and 2-phenylethanol in Raf (**Figures 1** and **2**). It has been reported that the subthreshold odor constituents play an important role in the characteristic flavor of jasmine tea because of the synergistic effects between them (*39*).

It is of particular interest that the concentrations of 6-methyl-5-hepten-2-one and 1-hexanol increased in both cultivars, while the concentrations 2-isobutylthiazole and decanal increased in Moneymaker, leading to negative to positive log U values during ripening (**Figures 1** and **2**). At the mature-red stage, the concen-

tration of these four compounds ranged from 2- to 8-fold higher than their odor thresholds; therefore, they may play a role in the aroma of ripe tomatoes. On the other hand, in the Raf cultivar the concentration of methyl salicylate was 7-fold greater than its odor threshold at the mature-green stage and 20-fold lower at the mature-breaker stage (**Figure 2**); hence, methyl salicylate could be involved in the changes in the sensory perception experienced from mature-green to ripened tomatoes.

Changes in the sign of log U values during ripening could contribute to explaining the great aromatic differences perceived between mature-green and mature-breaker or mature-red toma-toes. These results also suggest that only a limited number of volatiles may be essential to tomato aroma and flavor, while the others could be considered to have little or no odor impact (*3*). One must take into account the fact that the log U values do
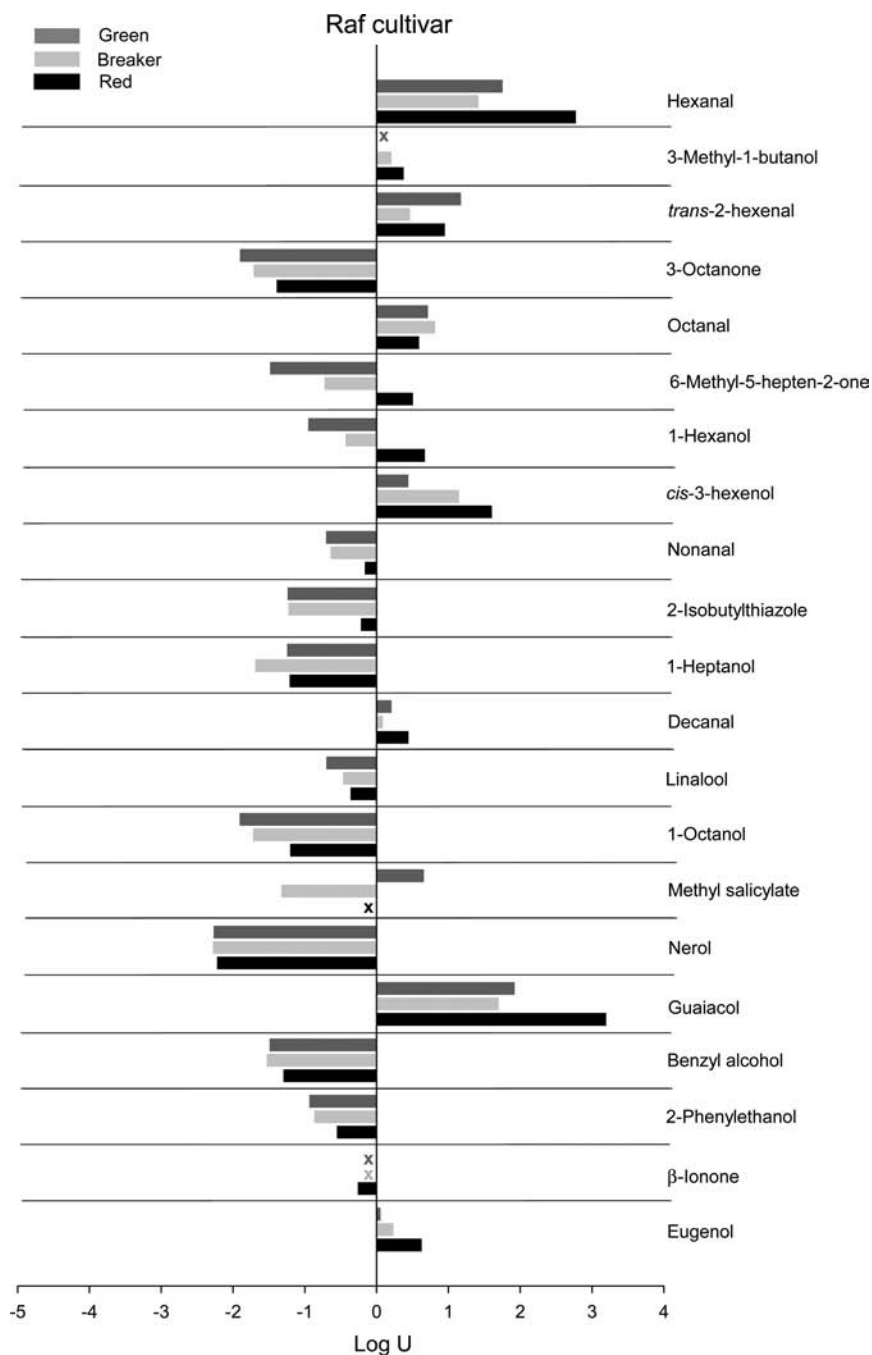
Figure 2. Log *U* values of the volatile compounds detected in the FVF of the Raf cultivar in three ripening stages (green, breaker, and red). An x indicates the compound was undetected or its concentration was <1 μg/L.

not consider the possible influence exerted by the interactions between the different components of a food and by the food matrix itself on the final aroma (*19–22*).

Finally, it is important to bear in mind that the homogenization of tomato fruits may enhance the formation of enzymatic oxidation products, something that seems to change with the progression of fruits ripening (*2*). Although care was taken to process samples quickly, the concentrations of some volatile compounds could have increased or decreased during sample preparation. It has been reported that *cis*-3-hexenal is a key odorant in the tomato flavor but also an unstable compound (*7, 46*). It was apparently largely isomerized to *trans*-2-hexenal during isolation and analysis (*10, 47*) and rapidly degrades if frozen fruits are used (*24*). *cis*-3-Hexenal was probably not detected due to *cis–trans* isomerization and because of the use

of frozen tomato juice samples, and consequently, the level of *trans*-2-hexenal could be higher under our experimental conditions.

Characterization of the Glycosidic Fraction. Extracts enriched in glycosidically bound compounds, and lacking free volatile compounds, were obtained from juice samples of Moneymaker and Raf cultivars at three ripening stages (green, breaker, and red) and incubated with the commercial enzyme preparation AR2000 for exhaustive release of the corresponding aglycons and sugars. After hydrolysis, the concentrations of the released free volatiles were determined in the same way as for the FVF. The concentrations of 29 volatile compounds were quantified from the glycosidically bound fractions (GBF) of Moneymaker and Raf at three ripening stages. The concentrations and the statistically significant differences among them are listed in **Table 2**.

Table 2. Glycosidically Bound Volatile Compound Concentrations (micrograms per liter) of Moneymaker and Raf Tomato Cultivars at Three Ripening Stages, Green, Breaker, and Red[a]

aglycon	Moneymaker			Raf			CC[b]		
	green	breaker	red	green	breaker	red			
hexanal	22.76 a	95.04 b	402.9 c	19.11 a	70.11 a	311.3 b	G		R
3-methyl-1-butanol	28.41 a	261.3 b	7240 c	18.36 a	390.6 b	1762 c		B	R
trans-2-hexenal	371.6 b	1071 c	192.8 a	389.2 a	741.5 a	356.9 a			R
3-octanone	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]			
octanal	0.472	tr[c]	tr[c]	0.467	tr[c]	tr[c]			
4-methyl-1-pentanol	nd[d]	0.269 a	1.196 b	tr[c]	0.746 a	0.763 a		B	R
3-methyl-1-pentanol	nd[d]	1.939 a	13.64 b	nd[d]	1.241 a	4.422 b		B	R
1-hexanol	8.484 a	7.270 a	109.6 b	11.06 a	54.83 a	25.73 a			R
cis-3-hexenol	16.47 b	7.772 a	23.43 c	16.06 b	13.58 ab	9.463 a		B	R
nonanal	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]			
2-isobutylthiazole	nd[d]	tr[c]	tr[c]	nd[d]	nd[d]	tr[c]			
cis-linalool oxide	28.15 b	16.36 a	10.55 a	22.34 c	18.36 b	6.959 a	G		
1-heptanol	0.484 b	0.298 a	2.815 c	1.070 a	0.424 a	0.951 a			R
6-methyl-5-hepten-2-ol	0.127 a	0.132 a	7.946 b	tr[c]	0.163 a	3.557 b		B	R
trans-linalool oxide	35.46 b	17.82 a	nd[d]	22.48 a	20.42 a	nd[d]	G		
decanal	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]			
benzaldehyde	33.06 c	14.20 a	24.84 b	29.74 a	10.71 a	15.87 a			R
linalool	63.86 b	39.48 a	28.79 a	77.32 c	48.58 b	35.59 a	G	B	R
1-octanol	0.334 b	0.232 a	0.436 c	0.330 a	0.368 a	0.809 b			R
α-terpineol	3.088 a	3.064 a	5.299 b	4.811 a	5.383 ab	6.136 b	G	B	
β-citronellol	0.260 a	0.154 a	4.888 b	0.140 a	0.171 a	2.577 b	G		R
methyl salicylate	3.903 b	4.363 b	2.464 a	9.820 a	22.52 b	33.00 c	G	B	R
nerol	1.844 a	1.560 a	9.345 b	1.198 a	1.244 a	4.999 b	G	B	R
geraniol	4.443 b	3.331 a	9.771 c	3.087 a	3.944 a	7.254 b	G	B	R
guaiacol	36.60 a	25.31 a	18.57 a	61.10 b	47.16 a	43.60 a	G	B	
benzyl alcohol	252.1 a	683.1 b	2030 c	436.5 a	1087 b	2168 c	G	B	
2-phenylethanol	291.4 b	260.9 a	539.8 c	311.2 a	403.7 b	894.2 c	G	B	R
β-ionone	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]	tr[c]			
eugenol	6.024 a	75.49 b	212.1 c	6.835 a	189.6 b	492.8 c		B	R

[a] Different letters for the entries in the Moneymaker and Raf columns indicate significant differences ($p < 0.05$) among the three ripening stages. [b] For the cultivar comparison (CC), G, B, and R indicate significant differences ($p < 0.05$) between both cultivars at green (G), breaker (B), and red (R) ripening stages. [c] Less than 0.1 μg/L. [d] Undetected.

The aglycons 3-octanone, nonanal, decanal, 2-isobutylthiazole, and β-ionone were always detected at a concentration of < 0.1 μg/L in the GBF of both cultivars. Only the concentrations of 2-isobutylthiazole, decanal, and β-ionone were not significantly different between Raf and Moneymaker during ripening, demonstrating high variability in the concentration of GBF components between the two cultivars.

The compounds that did not show significant changes in their concentrations during ripening were guaiacol in Moneymaker and *trans*-2-hexenal, 3-octanone, 1-hexanol, 1-heptanol, *trans*-linalool oxide, benzaldehyde, and β-ionone in Raf. For the rest, the concentrations of most of the individual aglycons increased with ripening, peaking at the mature-breaker or mature-red stages, as in the FVF. In both cultivars, hexanal, 3-methyl-1-butanol, 6-methyl-5-hepten-2-ol, β-citronellol, benzyl alcohol, and eugenol concentrations increased most during ripening, ranging from 5- to 259-fold higher in the mature-red stage than in the mature-green stage. Similar ripening-associated changes have been described for benzaldehyde, 3-methyl-1-butanol, 6-methyl-5-hepten-2-ol, benzyl alcohol, and eugenol in other tomato cultivars (*11*).

Conversely, exceptions to this ripening-associated increase were octanal, *cis*- and *trans*-linalool oxides, and linalool in both cultivars, *cis*-3-hexenol and guaiacol in Raf, and methyl salicylate and benzaldehyde in Moneymaker, for which concentrations decreased during ripening, peaking at the mature-green stage. Among them, the potential release of *cis*- and *trans*-linalool oxides, benzaldehyde, and linalool may be particularly relevant. These four compounds are traditionally related to floral and fruity flavor notes that lead to an appreciation sweetness in

tomatoes (*38*), and sweeter tomatoes are more acceptable to consumers (*48*).

These data show great variability in the concentration of volatile components of the GBF in tomato with the progression of fruit ripening. This has also been reported in previous works with mango (*49*) and nectarine (*31*), suggesting the tomato volatile profile could potentially be enhanced if the hydrolysis of glycosidic linkage and consequent aglycons release is achieved.

The potential effect of the released aglycons on aroma could be greater for compounds that are more abundant in the bound form than in the free form. Eight of the 29 aglycons of the GBF (**Table 2**) were not found in the FVF (**Table 1**), in particular the terpenols geraniol, β-citronellol, α-terpineol, and *trans*- and *cis*-linalool oxides, which are traditionally related to floral and fruity flavor notes, even though they were always present at low concentrations (< 36 μg/L), according to the results previously reported for the tomato cultivars p73, Jorge, and Durinta (*28*). The volatiles 3-methyl-1-butanol, *trans*-2-hexenal, linalool, methyl salicylate, nerol, benzyl alcohol, 2-phenyl-1-ethanol, and eugenol were more abundant in the GBF than in the FVF of both cultivars at least in one of the ripening stages. **Figure 3** shows a comparison of their concentrations in free and bound forms. The concentration of bound linalool was from 14- to 64-fold greater than that of its free form, depending on the cultivar and the stage of ripening. The hypothetical release of linalool from glycoside could be especially relevant since its concentration could increase from 3 to > 70 μg/L and its odor threshold in water is 6 μg/L.

The concentrations of the bound forms of 3-methyl-1-butanol, *trans*-2-hexenal, and eugenol reached 12-, 15-, and 19-fold greater
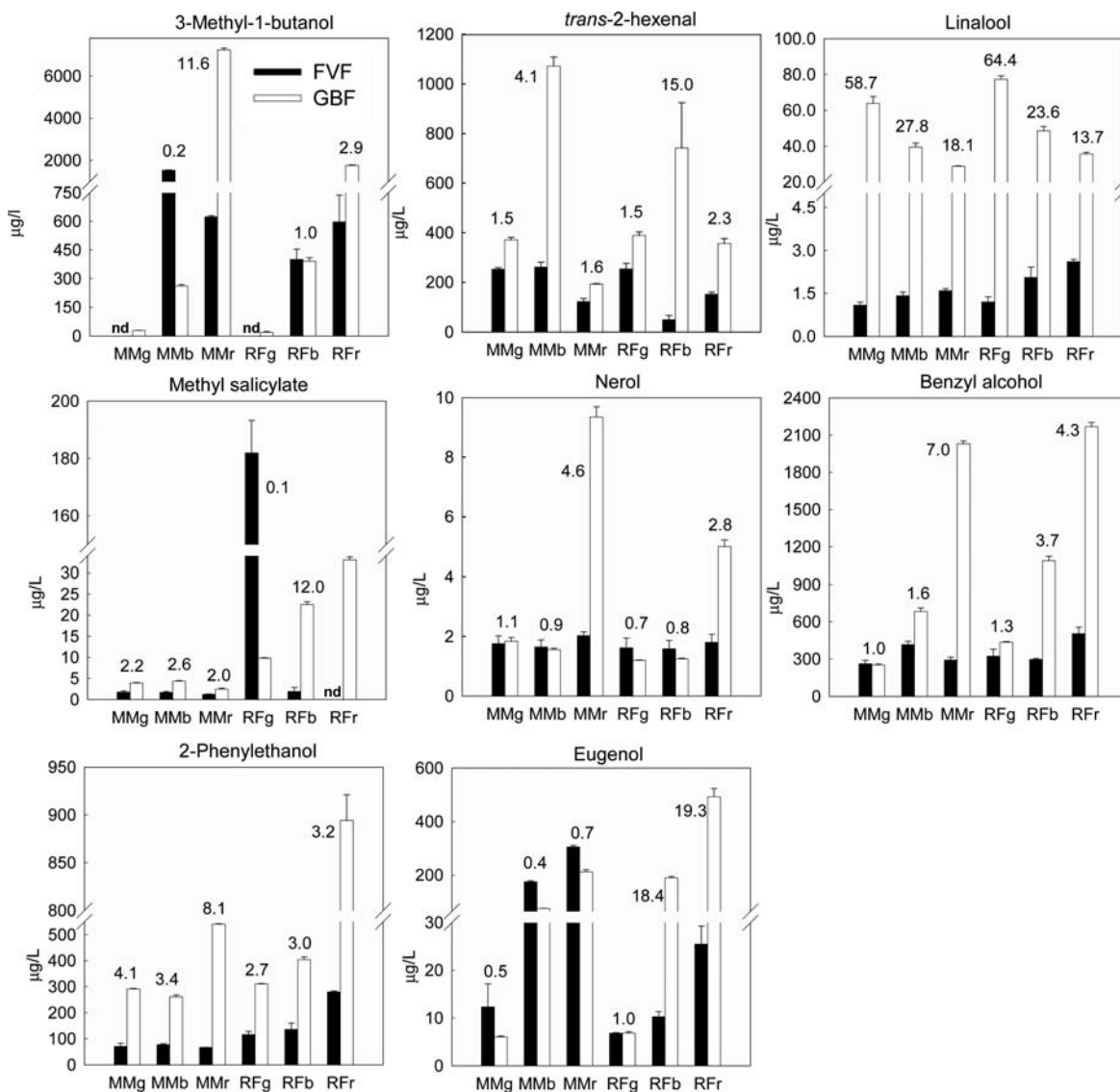
Figure 3. Concentrations of the eight volatile compounds that are more abundant in the glycosidically bound form (white bars) than in the free form (black bars) expressed as mean values of three replicates with a standard error bar. Numeric values above the bars are the ratios between the concentrations of free and glycosidically bound forms. MMg, MMb, and MMr indicate the green, breaker, and red stages of the Moneymaker cultivar, respectively. RFg, RFb, and RFr indicate the green, breaker, and red stages of the Raf cultivar, respectively. nd indicates not detected.

than those of their free forms, respectively. These compounds had positive log U values in the FVF; therefore, their hydrolysis could lead to considerably higher positive log U values, thereby enhancing their effect on overall aroma. The volatiles benzyl alcohol, nerol, and methyl salicylate gave negative log U values in the FVF, and even if their hypothetical release from the glycosidically bound forms is achieved, they will still remain subthreshold odor compounds. Nevertheless, the concentration of methyl salicylate at the mature-red stage of Raf could increase from undetected (< 0.01 μg/L) to more than 30 μg/L, and its odor threshold in water is 40 μg/L. On the other hand, 2-phenylethanol also had negative log U values in the FVF but the hydrolysis of its glycosidic precursor at the mature-red stage of Raf could lead to a concentration slightly higher than its odor threshold in water (1000 μg/L).

There are many studies of the use of glycosidases to enhance the volatile profile, which have led to the successful improvement of wine aroma (50−54). Currently, similar studies focus on the tomato (28), showing that the stage of tomato fruit ripening is another important parameter to consider.
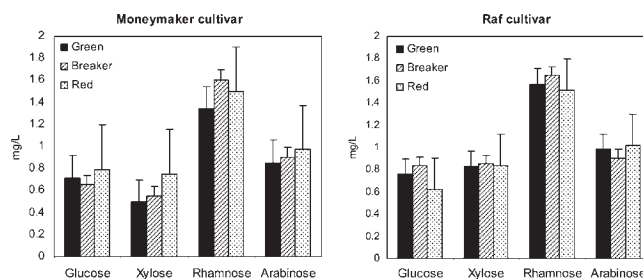

Figure 4. Concentrations of the sugars detected in the enzymatically hydrolyzed GBF of Moneymaker and Raf cultivars at three ripening stages (green, breaker, and red) expressed as mean values of three replicates with a standard error bar.

In this study, the sugars glucose, xylose, rhamnose, and arabinose were detected after the enzymatic hydrolysis of the GBF of Moneymaker and Raf cultivars during ripening by HPLC (**Figure 4**). All sugars peaked at the mature-breaker or mature-red stages. Rhamnose was the most abundant sugar at all

three ripening stages of both tomato cultivars, followed by arabinose, glucose, and xylose. Particularly noticeable was the presence of xylose which has been described only as a component of diglycosides of volatile compounds in tea (*30*).

In summary, this study has quantified the free and bound volatiles of Raf and Moneymaker cultivars during fruit ripening and discussed their hypothetical impact on aroma according to their log *U* values. The concentrations of most free volatile compounds detected in both cultivars increased during ripening; however, only 11 compounds reached a concentration higher than their odor thresholds, and thus, they could exert a major influence on the overall aroma. Changes in glycosidically bound volatiles have been analyzed in tomato during ripening, and for the first time, the sugars glucose, xylose, rhamnose, and arabinose have been reported as a part of the glycosidic conjugates. The relevance of tomato GBF as a potential source of interesting aroma compounds, such as alcohols and terpenols, has been highlighted. Further sensory studies would be needed to relate the analytical differences between the free volatiles of both cultivars to consumer preferences, as well as to evaluate the potential impact of the released aglycons on the final flavor of the tomato fruits.

ABBREVIATIONS USED

FVF, free volatile fraction; GBF, glycosidically bound fraction; SPME, solid-phase microextraction; log *U*, logarithm of the odor units.

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