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Quantitation of Free and Glycosidically Bound Volatiles in and Effect of Glycosidase Addition on Three Tomato Varieties (*Solanum lycopersicum* L.)

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The volatile fractions of three tomato cultivars (p73, Jorge, and Durinta) were studied in both free and glycosidically bound forms. The possibility of increasing the concentration of free volatile compounds by adding selected glycosidases was also tested. The free volatile fraction (FVF) of tomato juice was directly determined by headspace solid-phase microextraction (SPME). To analyze the glycosidically bound fraction (GBF), tomato juice samples were extracted with C18 cartridges and the resulting glycoside extracts were enzymatically hydrolyzed. The released aglycons were determined by headspace SPME. Of these compounds, six were not previously reported to belong to the tomato GBF. The concentration of 21 of 24 compounds detected in the FVF was significantly different between cultivars, the majority of them being greater in p73 than in Durinta and Jorge cultivars. In the GBF, 19 of 26 compounds that were detected were significantly different between cultivars but only the amount of *trans*-linalool oxide was significantly the greatest in the p73 cultivar. The addition of *Candida molischiana* β-glucosidase (BGLN) and *Saccharomyces cerevisiae* exoglucanase (EXG1) to tomato juice samples led to increases in the concentration of 10 compounds, with variations depending on the cultivar or enzyme. These results provide scientific support for using glycosidases as a tool to improve tomato aroma.

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

INTRODUCTION

Consumers are greatly displeased by the aroma of fresh tomatoes, which is mainly due to the use of cultivars improved only for their agronomical features and effects derived from pre- and postharvest treatments. Tomato flavor is formed by a complex mixture of sugars, acids, amino acids, minerals, and volatile compounds (*1*, *2*). The relative contributions of taste and aroma to tomato flavor have not been clearly defined; however, a number of authors consider that the composition of the volatile fraction plays an important role in the final flavor of fresh and processed tomato (for a review, see ref *3*). Volatiles in tomato are formed in the intact fruit during ripening, as well as upon tissue disruption (*4*). More than 400 volatile compounds have been identified in tomato fruits (*5*, *6*). Log odor units are the ratio of the concentration of a component in a food to its odor threshold. Compounds with positive odor units are assumed to contribute to the flavor of a food (*7*). Only 16 tomato compounds were found with a log odor unit of >0, and among these, Buttery (*4*) 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, and other compounds with negative odor units may also contribute to the overall flavor of tomato as background notes. Recently, Tandon et al. (*8*) 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 concentration of volatile compounds of fruits and vegetables can be increased enzymatically with nonvolatile precursors that occur naturally in these products. A growing number of reports have demonstrated that volatile precursors make up a reserve of aroma to be exploited in tomato (*3*, *4*, *9*, *10*) and in a range of fresh and processed fruits, as well as in wines (*11–15*) and related beverages and in some other consumed plant and leaf products (*16–23*). Most of the precursor compounds identified in plants and fruits are glycosidic deriva-

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tives, mainly *O*-β-D-glucosides or *O*-diglycosides. The common structural element is a glucopyranosyl unit attached through a β-glycosidic linkage to an aglycone, which, in turn, can be a monoterpene (linalool, geraniol, or nerol), a C_{11}/C_{13}-norisoprenoid (β-damascenone, β-ionone, etc.), a benzene derivative (benzyl alcohol, 2-phenylethanol, etc.), or a linear alcohol. In the case of diglycosides, the glucose moiety was further substituted with α-L-arabinofuranose, α-L-arabinopyranose, α-L-rhamnopyranose, β-D-glucopyranose, β-D-apiofuranose, or β-D-xilopyranose (*11, 12*).

Acid or enzymatic hydrolysis of glycosides leads to the release of volatiles. The enzymatic release of volatiles from glycosides is catalyzed by *O*-glycoside hydrolases. They are distributed among plants and microorganisms, mainly yeasts and filamentous fungi. Among the glycoside hydrolases, β-glucosidases have been the subject of much work because of their importance. They catalyze the hydrolysis of the glycosidic bond between the glucopyranosyl unit and the aglycone moiety. With regard to enzymatic hydrolysis of diglycosidic precursors, it can take place in one step, where diglycosidase catalyzes the cleavage of the aglyconic linkage (*24*), or in two steps. In sequential mode, first one exoglycosidase makes the cleavage of the intersugar linkage, releasing corresponding sugars and β-D-glucosides. In the second step, β-glucosidase catalyzes the hydrolysis of β-D-glucoside and releases the corresponding aglycone and glucose (*25*). The activity of exoglycosidases is not affected by the aglycone moiety of the diglycosides. However, the aglycone structure of β-D-glucosides greatly affects β-glucosidase activity (*12*).

Enzyme source can influence the pattern of freed aglycons and, therefore, the sensory properties of the products. Due to the fact that most fungal β-glucosidases are inhibited by glucose, few studies have focused on using exogenous enzymes for aroma enhancement in fruit juices (*12*). On the other hand, there is evidence that β-glucosidase from *Candida molischiana* significantly increases the levels of linalool, benzyl alcohol, and 2-phenylethanol in wine and in the juice of several fruits (peach, white grape, mango, cherry, strawberry, passion fruit, orange, apple, and papaya) (*26*).

The effect of glycosides on tomato flavor, however, is still not completely understood (*3, 10–12*). In fact, although several studies have focused on the aromatic composition of tomato fruits, we still lack a quantifiable definition for tomato flavor (*3*).

The aim of this work is to study the volatile and glycosidic fraction of three tomato cultivars, Jorge, Durinta, and p73, and the putative role of glycosidic activities in the enhancement of the free volatile fraction.

MATERIALS AND METHODS

Plant Material and Treatments. Tomatoes of the cultivars Jorge, Durinta, and p73 were grown in hydroponics in a greenhouse, under standardized conditions in Mediterranean climate, in the Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Murcia (Spain). Ripened fruits in the plants at breaker stage of maturity according to size, color, and firmness (partially vine ripened to a light pink color and firm textured) were hand-harvested at random from different plants (three pieces by plant) in July and transported to the laboratory within 24 h in isothermal boxes. Ten to 12 visually defect free tomatoes per cultivar, uniform in color and size, were selected and manually homogenized. After centrifugation, the supernatant was aliquoted and kept at −20 °C as a clear juice for further analyses. Jorge and Durinta are commercial cultivars of economic importance, and p73 is a useful cultivar for further genetic transformation.

Enzyme Production. Yeast strains T_{73}/YCEXG1a and T_{73}/YCB3$_5$ were used to produce exoglucanase EXG1 and β-glucosidase BGLN, respectively (*27, 28*). Yeasts were grown for 48 h in 4.75 L of YPD-rich medium containing 1 μg/mL cycloheximide for 48 h using a Bioflo III fermentor (New Brunswick Scientific, Edison, NJ). Cultures were stored overnight at 4 °C to permit yeast sedimentation, and the supernatant was concentrated to 500 mL in a Pellicon Ultrafiltration System (Millipore Corp., Bedford, MA) using a 10000 Da molecular mass cutoff polysulfone filter. Proteins were precipitated at 0 °C with ammonium sulfate (80% saturation) and separated by sedimentation at 4 °C overnight.

Glycosidase Activity Measurement. β-Glucosidase activity was assayed using *p*-nitrophenyl β-D-glucopyranoside (pNPG) (Sigma-Aldrich, St. Louis, MO) as the substrate. Briefly, reactions were conducted in 75 mM citrate-phosphate buffer (pH 5.0) containing 5 mM pNPG and appropriate amounts of the sample in a final volume of 250 μL. Incubation was carried out at 30 °C for 20 min, and the reaction was terminated by adding 0.5 mL of 0.2 M Na_2CO_3 (pH 10.2). The absorbance was measured at 405 nm. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per min at 30 °C in citrate-phosphate buffer (pH 5). All enzymatic measurements were taken in triplicate.

Standards. Hexanal, 3-methyl-1-butanol, *trans*-2-hexenal, 3-octanone, octanal, 3-methyl-1-pentanol, 4-methyl-1-pentanol, 1-hexanol, *cis*-3-hexenol, nonanal, *trans*- and *cis*-linalool oxides, 1-heptanol, (*R*/*S*)-2-ethyl-1-hexanol, decanal, benzaldehyde, (*R*/*S*)-linalool, 1-octanol, (*R*)-α-terpineol, (*S*)-β-citronellol, nerol, geraniol, benzyl alcohol, 2-phenylethanol, β-ionone, and eugenol were purchased from Fluka (Buchs, Switzerland); 6-methyl-5-hepten-2-one, 2-isobutylthiazole, 6-methyl-5-hepten-2-ol, *cis*-4-decenal, methyl salicylate, 3,5-dimethylbenzaldehyde, guaiacol, and 2-methoxy-4-vinylphenol were purchased from Sigma-Aldrich.

Experimental Procedure. Aliquots of clear tomato juice of the three cultivars were randomized and separated into three groups. The first one was used to analyze the content of free volatiles. From the second group were obtained the glycoside extracts needed for the characterization of the bound fraction after exhaustive hydrolysis with AR2000 and analysis of the released volatile aglycons. Finally, the third group of samples was used for the treatments with BGLN and EXG1 and without enzyme to determine the effect of these glycosidases on the free volatile fraction. All treatments were carried out in triplicate.

Analysis of Volatile Compounds. Sample extraction was performed in headspace mode, for 30 min with magnetic stirring, using a solid-phase microextraction (SPME) device (Supelco, Bellefonte, PA) with a 10 mm fiber coated with 100 μm polydimethylsiloxane. After extraction, the SPME device was introduced into a gas chromatograph (GC) splitless injector and maintained at 240 °C for 4 min.

A HP5890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a HP-Innowax capillary column [30 m × 0.25 mm (inside diameter) × 0.25 μm] was used. The operating conditions were as follows: detector (FID), 300 °C; injector, 240 °C; and an oven temperature increased from 40 (5 min) to 150 °C at a rate of 5 °C/min, increased from 150 to 250 °C at a rate of 20 °C/min, and held at 250 °C for 10 min.

Identification of all compounds was performed using reference compounds for comparing the retention times and the mass spectra obtained by the use of an Agilent 5973N MS detector coupled to an Agilent 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany).

For calibration and quantitative analysis, FID response factors were determined by GC analysis of solutions containing defined concentrations of the respective reference compounds and 2-octanol (0.3 μg) as the internal standard.

Extraction and Hydrolysis of Tomato Glycosides. For the isolation of glycosides, 40 mL of clear juice was passed through a 500 mg C18 Sep-Pack cartridge (Waters Corp., Milford, MA), previously activated with ethanol (10 mL) and water (20 mL), and washed with water (10 mL). The fraction containing free volatile 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

Table 1. Free and Glycosidically Bound Volatile Compound Concentrations (micrograms per liter) in the Three Tomato Cultivars, p73, Jorge, and Durinta[a]

	free			bound		
compound	p73	Jorge	Durinta	p73	Jorge	Durinta
hexanal	599.6 a	177.7 c	455.8 b	48.24	75.30	101.8
3-methyl-1-butanol	370.8 a	227.4 b	437.4 a	324.3 b	335.5 b	483.2 a
trans-2-hexenal	133.9 a	59.16 b	75.70 b	154.8	145.5	168.8
3-octanone	1.561 a	0.735 b	0.847 b	nd[b]	nd[b]	nd[b]
octanal	5.435 a	1.245 b	2.155 b	nd[b]	nd[b]	nd[b]
6-methyl-5-hepten-2-one	20.65 a	8.189 b	6.456 b	nd[b]	nd[b]	nd[b]
4-methyl-1-pentanol	nd[b]	nd[b]	nd[b]	0.169	0.257	0.219
3-methyl-1-pentanol	nd[b]	nd[b]	nd[b]	0.451 b	0.786 a	0.502 b
1-hexanol	966.4 a	789.7 ab	605.8 b	15.22	26.07	22.14
cis-3-hexenol	3056 a	1931 b	1603 b	nd[b]	nd[b]	nd[b]
nonanal	0.877 a	0.609 ab	0.413 b	nd[b]	nd[b]	nd[b]
2-isobutylthiazole	0.795 a	0.572 b	0.433 c	tr[c]	tr[c]	tr[c]
cis-linalool oxide	nd[b]	nd[b]	nd[b]	1.578 a	1.264 ab	0.828 b
1-heptanol	6.934 a	3.562 b	tr[c]	0.337 b	0.787 a	0.863 a
6-methyl-5-hepten-2-ol	nd[b]	nd[b]	nd[b]	tr[c]	0.265 a	0.119 b
trans-linalool oxide	nd[b]	nd[b]	nd[b]	5.165 a	nd[b]	0.573 b
2-ethyl-1-hexanol	4.824 a	2.307 b	2.817 ab	4.084	5.764	6.800
decanal	0.425	0.365	0.278	nd[b]	nd[b]	nd[b]
cis-4-decenal	0.986 a	0.128 b	0.362 c	tr[c]	tr[c]	tr[c]
benzaldehyde	nd[b]	nd[b]	nd[b]	33.54 b	47.69 a	43.62 a
linalool	0.488 a	0.206 b	0.238 ab	6.968 a	5.869 ab	3.712 b
1-octanol	11.41 a	4.427 b	1.584 c	0.299 b	0.600 a	0.473 ab
α-terpineol	nd[b]	nd[b]	nd[b]	1.943 a	1.992 a	1.061 b
β-citronellol	nd[b]	nd[b]	nd[b]	0.172 c	0.619 a	0.340 b
methyl salicylate	4.226 b	3.449 b	5.636 a	3.088 b	6.677 b	15.73 a
nerol	2.836 a	1.229 b	0.857 c	1.773 ab	2.045 a	1.499 b
geraniol	nd[b]	nd[b]	nd[b]	2.216 a	2.016 a	1.223 b
3,5-dimethylbenzaldehyde	8.011 a	3.723 b	1.813 c	nd[b]	nd[b]	nd[b]
guaiacol	944.9	521.9	503.9	70.55 b	102.8 a	113.5 a
benzyl alcohol	537.9	153.4	128.8	533.8 b	810.1 a	930.1 a
2-phenylethanol	502.8 a	332.4 b	263.8 b	582.7 b	714.5 a	694.3 ab
β-ionone	1.346 a	0.544 b	0.739 b	nd[b]	nd[b]	nd[b]
eugenol	99.75 a	89.21 a	39.09 b	58.31 b	188.6 a	81.02 c
2-methoxy-4-vinylphenol	nd[b]	nd[b]	nd[b]	1.023 a	0.465 b	1.317 a

[a] Different letters for the entries in the free and bound columns indicate significant differences ($p < 0.05$) among the three cultivars as determined by Tukey's HSD procedure at the 95% confidence level. [b] Not detected. [c] Less than 0.1 μg/L.

°C, and the dry extract was dissolved in 3 mL of 75 mM citrate-phosphate buffer (pH 5) and defined as the glycoside extract. Subsequently, exhaustive hydrolysis by Pectinase AR2000 (Gist-Brocades, Seclin, France) was performed following a previously reported method with some modifications (*29*); 100 μL of an AR2000 solution (2.5%, w/v) was added to 1 mL aliquots of the glycoside extract, and the mixture was incubated at 40 °C for 48 h. After hydrolysis, free volatile compounds that were released were analyzed by SPME as previously reported.

Incubation of Tomato Juice with BGLN and EXG1. Tomato juice samples (3 mL) were added with sodium benzoate (0.2%, w/v) as a preservative and incubated (6 days, 15 °C) without enzyme addition (blank) or with addition of 50 mU of BGLN or EXG1. After the hydrolysis period, the volatile compounds that were released were analyzed by SPME as previously reported.

Statistical Analysis. Statistical significance was determined by analysis of the variance (ANOVA). For mean comparisons, Tukey's HSD procedure was performed.

RESULTS AND DISCUSSION

Characterization of the Free and Bound Volatile Fraction of p73, Jorge, and Durinta Tomato Cultivars. The free volatile fraction (FVF) of p73, Jorge, and Durinta cultivars was analyzed using solid-phase microextraction and gas chromatography. All compounds that have been identified have previously been reported in literature as components of free aroma fraction in tomato (*4–6*, *31*). **Table 1** shows the concentrations of the 34 volatile compounds detected on the free and bound fractions and the statistically significant differences among the three cultivars.

In the FVF, linalool, nonanal, 2-isobutylthiazole, decanal, *cis*-4-decenal, nerol, 1-heptanol, and β-ionone were found, at least in one of the tomato cultivars, at a concentration lower than 1 μg/L. It has been reported that a volatile compound does not necessarily have to be abundant to have an important impact on tomato flavor. For example, β-ionone, a volatile present at one of the lowest concentrations in tomato fruits, is included in the mixture of 10 compounds that reproduces the aroma of fresh tomato (*4*).

Only the concentrations of decanal, guaiacol, and benzyl alcohol were not significantly different among the three cultivars in the FVF. For the rest of them, there were significant differences at least between two cultivars, and only the concentrations of hexanal, 2-isobutylthiazole, 1-heptanol, *cis*-4-decenal, 1-octanol, nerol, and 3,5-dimethylbenzaldehyde were significantly different among the three cultivars that were analyzed. The amount of six of the 10 compounds whose combination of appropriate concentrations was suggested to produce the aroma of a fresh, ripe tomato (*4*) (hexanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, 2-isobutylthiazole, and β-ionone) was greater in p73 than in Durinta and Jorge cultivars. Sensory studies would need to be conducted to determine if these analytical differences lead to a better organoleptic appreciation of the p73 cultivar. Carbonell-Barrachina et al. (*30*) comparing three tomato cultivars reported that the one with the highest values of odor and aroma in the sensory evaluation was the cultivar with largest amount of hexanal and *trans*-2-hexenal, which is the same as the case for

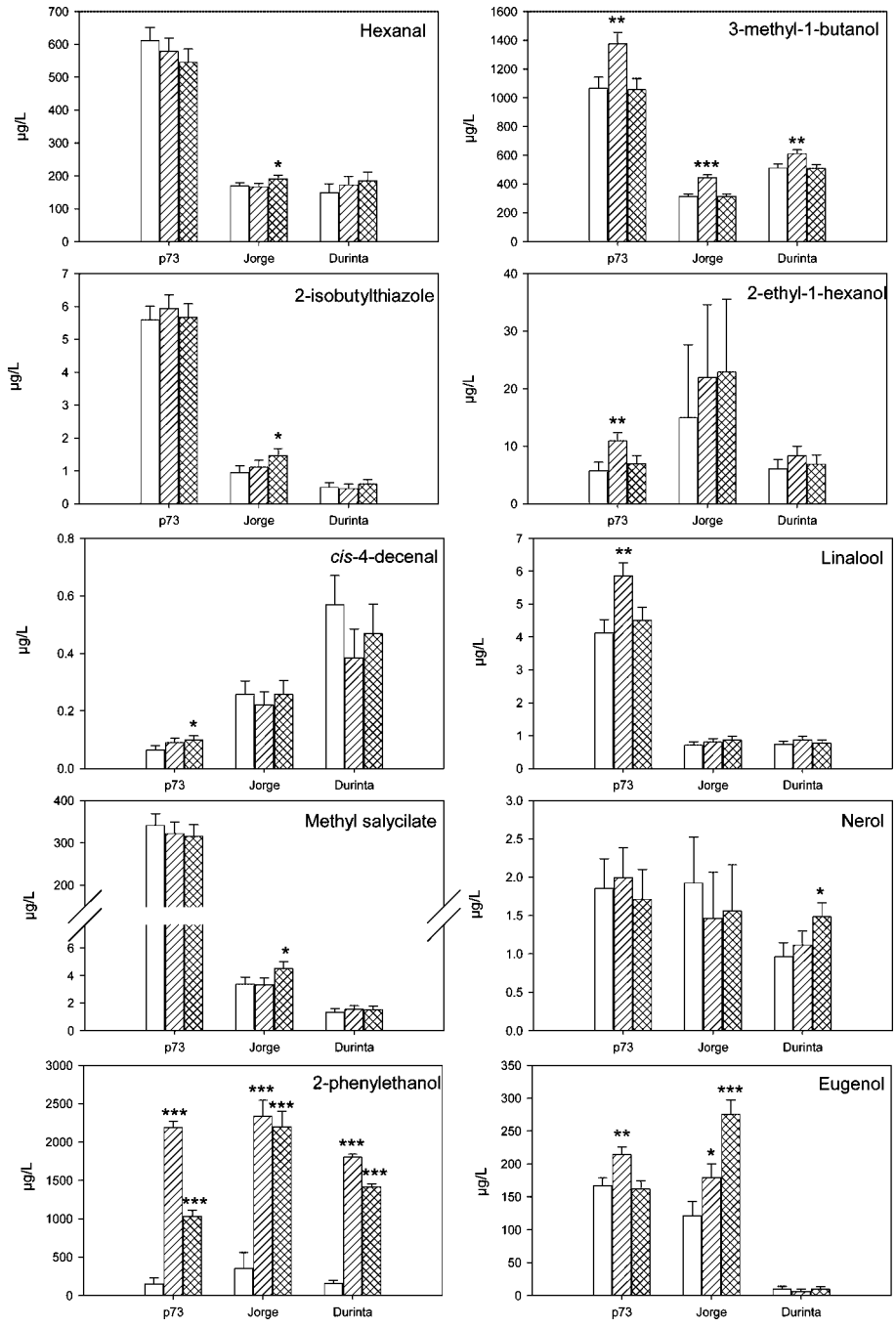

Figure 1. Concentrations of free volatile compounds after incubation for 6 days expressed as mean values of three replicates and Tukey's honestly significant difference intervals at the 95% confidence level: $p < 0.05$ (one asterisk), $p < 0.01$ (two asterisks), and $p < 0.001$ (three asterisks) compared with the untreated sample of each cultivar. White, striped, and cross-hatched bars indicate data from enzymatically untreated, BGLN-treated, and EXG1-treated samples, respectively.

p73. To support the results related to cultivar comparisons, it would be necessary to conduct more experiments taking into account other important factors such as growing location, environmental conditions, and cultural practices. The strong influence of the cultivar on the concentration of volatile free components has also been reported in previous studies, supporting the possibility of using the volatile profile as a tool for cultivar identification, for example, in tomato breeding programs (*1*, *2*, *8*, *30*, *31*, *33*–*37*).

The log odor value has been employed as a useful tool for measuring the hypothetical individual contribution of each compound to the final flavor of food (*3*–*5*, *37*–*42*), although this value does not consider the strong influence that the food matrix can exert on the final aroma (*37*, *39*–*41*). Log odor values have been calculated for all the compounds that have been detected, except for *cis*-4-decenal and 3,5-dimethylbenzaldehyde (data not shown), using the odor thresholds in water reported in the literature (*4*, *5*, *7*, *42*, *43*). In the FVF, hexanal, *trans*-2-hexenal, octanal, 1-hexanol, *cis*-3-hexenol, decanal, guaiacol, and eugenol exhibited a positive log odor value for the three cultivars. The p73 cultivar achieved the greatest log odor value of these compounds. 3-Methyl-1-butanol had a positive log odor value for only p73 and Durinta cultivars.

It is important to bear in mind that the homogenization of tomato fruits may enhance the formation of enzymatic oxidation products (*2*). Although the samples were processed quickly, the contents of some volatile compounds could have increased or decreased due to this procedure. It has been reported that *cis*-

3-hexenal is a key odorant in the tomato flavor but also an unstable compound (*44*, *45*). It was apparently largely isomerized to *trans*-2-hexenal during isolation and analysis (*4*, *46*) and rapidly degrades if frozen fruits are used (*44*). *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.

Glycoside extracts were obtained from juice samples of the three cultivars and incubated with the commercial enzyme preparation AR2000 for the exhaustive release of the corresponding aglycons. Subsequently, the concentration of the released free volatiles was determined, representing the glycosidically bound fraction (GBF) (**Table 1**). The concentrations of the aglycons hexanal, *trans*-2-hexenal, 4-methyl-1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol did not differ significantly among any of the three cultivars. The rest of the bound compounds detected were significantly different at least between two of the cultivars analyzed, and only the concentrations of β-citronellol and eugenol glycosides were significantly different among the three cultivars. Ten of the 24 aglycons detected in the GBF were not found in free form, with special mention of the terpenols geraniol, β-citronellol, α-terpineol, and *trans*- and *cis*-linalool oxides, which are traditionally related to floral and fruity flavor notes. The potential release of aglycons can be especially relevant in the case of linalool since its concentration in the GBF can be from 14- to 28-fold greater than that in the FVF, depending on the cultivar. The concentration of benzyl alcohol was 1–7-fold greater in the GBF, and the concentrations of *trans*-2-hexenal, methyl salicylate, nerol, 2-phenylethanol, and eugenol were 0.6–2.8-fold higher in the GBF, too. These compounds, more abundant as bound forms in tomato, could represent an especially relevant source for enhancing the volatile profile. The use of glycosidases for this objective has been widely studied and successfully implemented in the improvement of wine aroma (*15*, *47–50*).

It is interesting to note that in the FVF, the levels of 14 of 21 compounds that exhibited statistically significant differences between cultivars were significantly greater in the p73 cultivar than in Durinta and Jorge cultivars. In contrast, in the GBF, of 19 compounds that showed statistically significant differences between cultivars, only the concentration of *trans*-linalool oxide was significantly greater in p73 than in Durinta and Jorge cultivars. Our results indicate that the cultivars with lower free volatile concentrations (Jorge and Durinta) exhibited higher concentrations of bound compounds. Moreover, the variability found in the concentrations of glycosidic compounds among cultivars was lower than for the free volatiles. More studies that include more cultivars and agronomic variables are necessary to confirm these preliminary results about comparisons of tomato cultivars.

Addition of BGLN and EXG1 Glycosidases to Tomato Juice. *C. molischiana* β-glucosidase BGLN and *Saccharomyces cerevisiae* exoglucanase EXG1 were selected for their contrasted ability to release volatile compounds from their respective glycosidic precursors (*14*, *27*, *28*). Extracts enriched in each activity were obtained from grown medium cultures of the respective recombinant yeast strain (see Materials and Methods for details). BGLN or EXG1 (50 mU) was added to 3 mL of tomato juice and the mixture incubated for 6 days at 15 °C. The concentration of 10 of the 24 compounds detected was significantly increased by at least one of the enzymes added to the tomato juice (**Figure 1**). The amount of three compounds increased significantly only after incubation with BGLN (3-methyl-1-butanol in the three cultivars and 2-ethylhexanol and linalool in only p73). On the other hand, the concentration of five compounds increased significantly only after the incubation with EXG1 (hexanal, 2-isobutylthiazole, and methyl salicylate in Jorge cultivar, *cis*-4-decenal in p73, and nerol in Durinta). The concentration of 2-phenylethanol and eugenol increased significantly by both enzymes, although there were differences in the quantities released by each enzyme depending on the cultivar. In p73 and Durinta, BGLN released a significantly greater quantity of 2-phenylethanol than EXG1. With regard to eugenol, the incubation of p73 juice with BGLN produced a greater concentration than EXG1, while in Jorge juice, the opposite happened.

Gueguen et al. (*26*) had already observed an increase in the content of linalool and 2-phenylethanol when the fruit juices were treated with *C. molischiana* β-glucosidase BGLN, and Marlatt et al. (*10*) showed that 2-phenylethanol was one of the major components found in the bound fraction of tomato. Our data confirm these results.

The only compound with an increased concentration in the three cultivars, with both enzymes, was 2-phehylethanol. This increase was 6–14-fold (in the Jorge–EXG1 and p73–BGLN combinations, respectively) greater than in the control (tomato juice incubated for 6 days at 15 °C without enzyme addition). As a result, the negative log odor units of the 2-phehylethanol in tomato extracts became positive in the extracts incubated with enzymes.

Although there have been a large number of studies carried out that aimed to identify the volatile components of fresh tomatoes, very few have focused on components bound as glycosides and their possible impact on the final flavor (*9*, *10*). In this work, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, methyl salicylate, guaiacol, and 2-methoxy-4-vinylphenol have been reported for the first time as part of the glycosidic fraction of tomato. This preliminary information about the putative role of microbial glycosidases in the enhancement of the free volatile profile in tomato fruits could be very interesting for further construction of transformed plants overexpressing target enzymes. Our work has highlighted the relevance of the tomato GBF as a potential source of volatile compounds and the possible use of selected enzymatic activities as a tool to increase the quantity of free volatiles.

Supporting Information Available: A table of response factors for FID and linear regression coefficients and a table of log odor units and odor thresholds for the volatile compounds detected in the free fraction of p73, Jorge, and Durinta cultivars. This material is available free of charge via the Internet at http://pubs.acs.org.

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