Characterization of the free and glycosidically bound aroma potential of two important tomato cultivars grown in Turkey

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Abstract Free and glycosidically bound volatiles from two major tomato cultivars (*Lycopersicon esculantum* L. cv. Alida and Merve) of Turkey were determined. Free volatile compounds were extracted using liquid–liquid microextraction, while bound volatiles were extracted using solid phase extraction. The compounds were analyzed using GC–FID and GC–MS. Alida showed presence of, 39 free and 32 bound aroma compounds again 38 free and 31 bound aroma compounds is Merve. The odor activity values of the volatile compounds suggested that hexanal, (Z)-3-hexenal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal and 2-phenylethanol were most significant odorants in both cultivars. Guaiacol and eugenol were flavor contributors for Merve. The norisoprenoids 5,6-epoxy- β -ionone and 3-hydroxy- β -ionone were observed in free form in tomato. Norisoprenoids, terpenoids, volatile phenols and higher alcohols were present in the glycosidic extract. Among the glycosidically bound compounds, 2-phenylethanol, guaiacol and eugenol were found to be potential contributors to overall tomato flavor upon hydrolysis.

Keywords Tomatoes · Chemical composition · Aromas · GC–MS

Introduction

Tomato (*Lycopersicon esculantum*) is a fruit-bearing vegetable that belongs to the Solanaceae family. Tomato is one of the most widely consumed vegetables and is consumed either fresh or industrially processed. According to FAO, Turkey, with an annual production of 12,600,000 tons in 2016, ranks fourth in the World and is the top country for tomato production in Europe (FAO 2018).

Turkey has unique microclimate conditions for tomato production, particularly in Mersin province, which is located in the upper part of the Mediterranean region. Although many tomato cultivars are produced in this area, the cultivation of the Merve and Alida cultivars has been increasingly expanded in recent years, particularly for tomato export during July and August. Alida is a very suitable variety in terms of growing in the field and in the greenhouse. The fruit of this cultivar has a dark red color, a slightly flattened round shape and a long shelf-life. Merve is grown in the field in the summer, and its fruit is hard with a slightly flattened round shape. Interestingly, both cultivars have a long shelf-life storage capability (Syn-genta-Turkey 2016).

When quality is evaluated in terms of consumer acceptance, the first factors that come to mind are sensorial characteristics, such as color, texture, taste and aroma (Özkaya et al. 2013). The aroma attributes of tomatoes are of the utmost importance for their appreciation (Mayer et al. 2004). More than 400 volatile compounds have been identified in tomato fruits (Baldwin et al. 2000). Odor-active compounds in tomato have been identified through

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gas chromatography–olfactometry (GC–O) analysis in several studies (Selli et al. 2014). Fresh tomato flavor could be reproduced for the most part with 10 compounds: (Z)-3-hexenal, (Z)-3-hexanol, hexanal, 1-penten-3-one, 3-methylbutanal, (E)-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole and β -ionone. In particular, hexanal, (E)-2-hexenal, (Z)-3-hexenal, 1-hexanol, (Z)-3-hexenol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, geranylacetone, 1-penten-3-one, 3-methylbutanal, methyl salicylate, and β -ionone were considered to be potent aroma contributors because of their high odor activity values (OAVs) (Cebolla-Cornejo et al. 2011). The OAV is the ratio of the volatile concentration in the material to the odor threshold (Baldwin et al. 2000). Volatile compounds with high OAVs are often found to be important for flavor (Grosch 2001).

In plants and fruits, many volatile compounds are accumulated as non-volatile and flavorless glycoconjugates, most often as O-glycosides (Winterhalter and Skouroumounis 1997). Many aglycones have long been recognized as important contributors to fruit flavor (Winterhalter and Skouroumounis 1997). The release of volatile compounds from these glycosidic precursors is possible through acid or enzymatic hydrolysis (Winterhalter and Skouroumounis 1997; Gunata et al. 1988). Despite the large number of investigations of free volatile compounds from tomato cultivars, studies that aim to evaluate both the free and bound aroma potential of tomato are scarce (Baldwin et al. 2000; Ortiz-Serrano and Gil 2007, 2010). More than 20 glycosidically bound aroma compounds were identified in tomato cultivars (Marlatt et al. 1992; Ortiz-Serrano and Gil 2007, 2010). The aglycone moiety consisted of the compounds from monoterpenes, norisoprenoids and shikimate derivatives that were able to impact tomato flavor when released.

The aim of this work was to study the flavor potential of two important tomato cultivars from Turkey (Alida and Merve) by analyzing both the free and glycosidically bound volatile composition. The contribution of volatile compounds to tomato flavor is discussed in terms of their OAVs.

Materials and methods

Solvent and chemicals

Analytical grade dichloromethane (Pestanal, $\geq 99.8\%$), chloroform (Chromasolv[®] Plus, $\geq 99.9\%$), *n*-pentane (Chromasolv[®] Plus, $\geq 99\%$), methanol (Chromasolv[®] Plus, $\geq 99.9\%$), anhydrous sodium sulfate, ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$ (pure, p.a., $\geq 99\%$), absolute ethanol (Spectralanal, $\geq 99.8\%$), and *n*-alkane standards (C8–C40)

were from Sigma-Aldrich. The Supelclean[™] LC-18 cartridge was from Supelco (France), and the enzymatic preparation AR2000 was from DSM (France). Reference compounds were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) (2-Octanol, 4-nonanol, hexanal, (E)-2-pentenal, (Z)-3-hexenal, (E)-2-hexenal, 2-octanone, (E)-2-heptenal, (E)-2-octenal, benzaldehyde, acetophenone, geranial, linalool, nerol, acetovanillone, 2-methyl butanol, pentanol, hexanol, (Z)-3-hexenol, 6-methyl-5-hepten-2-one, benzyl alcohol, 2-phenyl ethanol, guaiacol, eugenol, hexanoic acid, methyl hexanoate, nonanoic acid, methyl pentanoate, octanoic acid, sucrose, glucose, fructose, citric acid, malic acid, ascorbic acid, lycopene, and β -carotene).

Samples

The Alida and Merve cultivars grown in the Mersin province were harvested at the pink mature stage, which is considered to be the commercial maturity stage. Two kilograms of fruit were sampled from different areas in the same orchard. The fruit was cut into small cubes ($\sim 1 \text{ cm}^3$), immediately frozen in liquid nitrogen and reduced to a powder using a mortar grinder (Pulverisette 2, Fritsch) under liquid nitrogen.

Soluble solids content, titratable acidity, sugars and organic acids

Total soluble solids content ($^\circ\text{Brix}$) and titratable acidity (TA) in tomato samples were performed according to Majidi et al. (2014). The individual sugars (glucose, fructose, and sucrose) and organic acids (malic and citric) were simultaneously analyzed by HPLC from 1 mL of supernatant diluted 20-fold with deionized water (Aubert and Chanforan 2007). Quantifications of sugars and organic acids were carried out using five-point calibration curves prepared for sucrose (0.05–1 g/L), glucose (1–20 g/L), fructose (1–20 g/L), malic acid (0.1–2 g/L), and citric acid (1–10 g/L) using the corresponding standards. For sugars and organic acids, the LOD and LOQ were estimated to 0.01 g/L and 0.05 g/L, respectively.

Ascorbic acid (AA)

Ascorbic acid was analyzed according to a previously reported protocol, with some modifications (Nour et al. 2015). 1 g of frozen powder was used in the analysis. For HPLC analysis of AA, Varian ProStar 230 HPLC equipment coupled with a photodiode area detector (Varian PDA detector 330) was used. The PDA was set to 245 nm. Ten microliters of each sample were injected onto a 250 mm $\times$ 4.6 mm i.d. (5 μm) Supelcosil C18 column

(Sigma-Aldrich) using a Varian 410 autosampler. The column oven temperature was set to 25 °C. The mobile phase (0.2 M KH_2PO_4 ; pH adjusted to 3.0 with H_3PO_4) flow was 1 mL/min. Compounds were identified by comparing retention times with those of standards and by spiking the samples with reference compounds. Quantifications of ascorbic acid were carried out using five-point calibration curves prepared for ascorbic acid (10–100 mg/L) using the corresponding standard. The LOD and LOQ were estimated to 0.1 mg/L and 0.5 mg/L, respectively.

Carotenoids

Carotenoids were extracted according to a previously reported protocol, with some modifications (Uçan et al. 2016). Approximately 500 mg of frozen powder was used in analysis. The HPLC equipment and column were the same as those described above. The PDA was set to 450 nm. The volume of injection was 10 µL. The column oven temperature was set to 25 °C. A binary solvent system was used at a 1 mL/min flow rate with solvent A (acetone) and solvent B (water). The elution gradient was as follows: 0–2 min, isocratic 15% B; 2–7 min, linear 15–5% B; and 7–20 min, isocratic 5% B. Compounds were identified by comparing retention times with those of standards and by spiking the samples with reference compounds. Quantifications of carotenoids were carried out using five-point calibration curves prepared for lycopene (10–100 mg/L) and β -carotene (1–10 mg/L) using the corresponding standards. For carotenoids, the LOD and LOQ were estimated to 0.1 mg/L and 0.5 mg/L, respectively.

Extraction of free aroma compounds via LLME

Free aroma compounds were extracted according to a previously reported protocol, with some modifications (Aubert and Chanforan 2007). Briefly, 50 grams of frozen powder, 50 mL of a saturated $(\text{NH}_4)_2\text{SO}_4$ solution, and 50 µL of 2-octanol (401.3 µg/mL) (internal standard) were homogenized in a Waring blender for 90 s. The mixture was centrifuged (14,000g, 5 min, 4 °C) and the supernatant was filtered through glass wool. Forty milliliters of supernatant were then introduced into a 50 mL screw-capped conical centrifuge tube (34–98 mm glass borosilicate), followed by the addition of 150 µL of chloroform. The mixture was subjected to magnetic stirring (stir bar of 15 × 6 mm) at room temperature for 60 min. After removal of the magnetic stir bar, the tube was sonicated for 1 min in a Branson Ultrasonic Cleaner 5510 and then centrifuged (10,000g, 5 min, 4 °C). The chloroform layer was then recovered with a 50 µL syringe, transferred to a 100 µL vial, and immediately analyzed via GC–MS and

GC–FID. The expressed volatile concentrations are presented as the internal standard equivalent of three replicates.

Isolation of glycosidic extracts

A previously reported protocol was used (Aubert et al. 2003). Briefly, 100 g of frozen tomatoes, 50 mL of distilled water, and 66.7 g of $(\text{NH}_4)_2\text{SO}_4$ were homogenized in a Waring blender for 3 min. The mixture was then centrifuged (10,000g; 15 min; 4 °C). The supernatant was filtered through Whatman Glass Microfibre Filters. The clear juice was loaded onto a RP18 reverse-phase column. The column was rinsed with 25 mL of H_2O and 50 mL of a pentane/dichloromethane mixture (2:1, v/v). The glycosidic fraction was eluted with 50 mL of MeOH. The methanolic extract was concentrated to 1 mL under reduced pressure (rotavapor). The extract was then transferred into a small vial and concentrated to dryness at 40 °C under a nitrogen stream.

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out according to a previously reported protocol (Meret et al. 2011). One hundred microliters of citrate phosphate buffer (0.2 M, pH 5.0) were added to the glycosidic extract. The mixture was washed five times using 1 mL aliquots of pentane/dichloromethane (2:1, v/v). After the addition of 200 µL of Pectinase AR 2000 (70 mg/mL; DSM, Denmark), the mixture was incubated for 12 h at 40 °C. The sample was then mixed with a 4-nonanol solution as an internal standard. The liberated aglycones were extracted five times with 1 mL of a pentane/dichloromethane mixture (2:1, v/v). The organic layer was recovered, dried over anhydrous Na_2SO_4 and concentrated at 40 °C to a final volume of 400 µL using a Dufton column. The organic extract was stored at – 20 °C until the time of analysis. All analyses were performed in triplicate.

GC and MS analysis of free aroma compounds

GC conditions were adjusted according to a previously reported protocol, with some modifications (Aubert and Chanforan 2007). A Varian 3800 gas chromatograph mounted with a SolGel-Wax (SGE) capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used. The flow of the hydrogen carrier gas was 1 mL/min. The oven temperature was kept at 35 °C for 5 min, then programmed to increase to 150 °C at 3 °C/min and to 250 °C at 5 °C/min, and finally kept at 250 °C for 10 min. The injections (2 µL) were performed at 220 °C in splitless mode (3 min) using a CombiPAL autosampler (CTC

Analytics). The FID detector was kept at 250 °C. Detection sensitivity of FID was 30 ng/ μ L of C₁₄, C₁₅, and C₁₆ in iso-octane. The levels of the volatile compounds were expressed as 2-octanol equivalents.

To identify the volatiles, a Varian 3800 gas chromatograph equipped with a saturn ion-trap mass spectrometer was used. The capillary column was the same as described above. Injections (1 μ L) were performed at 220 °C in splitless mode (3 min) using a CombiPAL autosampler (CTC Analytics). The flow of the carrier gas (helium) was 1 mL/min. The oven temperature program was as described above. Mass spectra were recorded in electron impact (EI) ionization mode. The ion trap, the manifold, and the transfer line temperatures were set to 150, 45, and 250 °C, respectively. Mass spectra were scanned in the m/z range of 30–350 amu at 1 s intervals. The average signal noise ratio (SNR) for 400 fg of tetrachlorodibenzo-p-dioxin (TCDD) at m/z 257 + 259 was 49, corresponding to an instrumental minimum detection level of approximately 25 fg at 3/1 SNR. Compounds were identified using the NIST mass spectral database, linear retention indices and the injection of reference compounds when available (see Tables 2 and 3).

GC–MS conditions for bound aroma compounds

GC–MS conditions were adjusted according to a previously reported protocol, with some modifications (Yilmaztekin et al. 2011). All analyses were carried out on a CP-3800 gas chromatograph coupled with a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA, USA). Chromatographic separation was achieved with a DB-Wax (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m length, 0.32 mm I.D., 0.5 μ m film thickness). The oven temperature was set at 60 °C for 3 min, increased from 60 to 220 °C at 2 °C/min, increased from 220 to 245 °C at 3 °C/min, and maintained at 245 °C for 20 min. Helium was used as the carrier gas in constant flow mode (1 mL/min). The ion trap temperature was 150 °C; the manifold and transfer line temperatures were 45 and 250 °C, respectively. The mass range scanned was m/z 60–120. Ionization was performed in electronic impact (EI) mode. Enzymatically released bound aroma compounds were quantified as 4-nonanol equivalents.

Statistical analysis

Analyses of variance were performed using the Statbox 6.3 program at various probability levels.

Results and discussion

Chemical composition

The chemical composition of the Alida and Merve tomato cultivars are given in Table 1. Total titratable acidity value was 3.85 meq/100 g for Alida and 5.69 meq/100 g for Merve. The main organic acids were malic and citric acids. The total soluble solid material was higher in Alida (4.80%) than in Merve (3.80%). Ascorbic acid content was 5.98 and 7.71 mg/100 g for Merve and Alida, respectively, which was in the range of values detected in tomato cultivars (4.28–31.51 mg/100 g) (Kavita et al. 2015; Figàs et al. 2015). Fructose and glucose were the main sugars. Total sugar content was higher for Merve (32.07 g/kg) than Alida (24.52 g/kg). The values were within the range of those observed for tomato cultivars (Özkaya et al. 2013). Alida was significantly richer in carotenoids. Merve Lycopene was the major carotenoid, followed by β -carotene, which was consistent with the reported composition of carotenoids of tomato cultivars (Kavita et al. 2015; Figàs et al. 2015).

Composition of free aroma compounds

Thirty nine volatile compounds, consisting of 16 aldehydes and ketones, 9 higher alcohols, 4 esters, 3 volatile acids, 3 volatile phenols, 2 norisoprenoids, 1 lactone and 1 furan, were detected in Alida (Table 2). The same compounds occurred in Merve except acetosyringone. The levels of most of the compounds differed significantly between tomato cultivars ($p \leq 0.05$). The detected volatiles compounds were also reported for different tomato cultivars in previous studies (Carbonell-Barrachina et al. 2005; Heredia et al. 2012; Selli et al. 2014). Total amounts aroma compounds content was 666.85 μ g/kg and 600.67 μ g/kg for Merve and Alida tomato cultivars, respectively.

In both cultivars, aldehydes were amongst the most dominant compounds. The levels of 6 out of 12 aldehydes were greater than their odor thresholds. Hexanal and (Z)-3-hexenal levels were quite high in both cultivars. While the hexanal concentrations were 32.85 and 46.30 μ g/kg for Alida and Merve, respectively. The concentrations of (Z)-3-hexenal was 3.20 and 2.62 μ g/kg for Alida and Merve, respectively. These compounds could impart fresh and green notes to the flavor of the Alida and Merve as reported for other tomato cultivars (Heredia et al. 2012; Selli et al. 2014). Indeed, the concentrations of those compounds were higher than their odor thresholds, which were reported to be 4.5 μ g/kg for hexanal and 0.25 μ g/kg for (Z)-3-hexenal (Mayer et al. 2008). For the odor thresholds of the volatiles, the values obtained in water were taken from the literature

Table 1 Chemical composition of the tomato cvs. Alida and Merve

	ALIDA	MERVE	<i>p</i> ^a
Ascorbic acid (mg/100 g)	7.71 ± 0.02	5.98 ± 0.20	***
Titrate acidity (meq/100 g)	3.85 ± 0.02	5.69 ± 0.04	***
Total soluble solid content (%Brix)	3.80 ± 0.00	4.80 ± 0.00	***
Sugars and organic acids g/kg			
Fructose	12.87 ± 0.24	16.84 ± 0.10	***
Glucose	11.65 ± 0.21	15.23 ± 0.09	***
Sucrose	0.09 ± 0.00	0.14 ± 0.01	**
Malic acid	0.33 ± 0.07	0.17 ± 0.02	*
Citric acid	2.84 ± 0.03	4.23 ± 0.08	***
Carotenoids (mg/100 g)			
Lycopene	4.17 ± 0.15	3.38 ± 0.04	**
β-carotene	0.48 ± 0.01	0.27 ± 0.01	***

^a**p* < 0.05; ***p* < 0.01; ****p* < 0.001

(Table 2). The OAVs of both aldehydes were superior to 1, between 7.3 and 12.8 according to the cultivar. The (E,Z)-2,4-decadienal and (E,E)-2,4-decadienal concentrations ranged from 1.16 to 3.43 µg/kg in the Alida and Merve cultivars. The odor threshold value of both aldehydes was found to be 0.11 µg/kg (Bezman et al. 2003). With OAVs between 10.6 and 31.2, these compounds are possible contributors to fatty and frying attributes in both cultivars. These compounds are considered to be potent flavor compounds of tomatoes (Bezman et al. 2003; Mayer et al. 2008; Selli et al. 2014). (E)-2-heptenal and (E)-2-octenal presented moderate OAVs between 1.2 and 1.4.

Among 5 ketones detected, only 6-methyl-5-hepten-2-one, which has green and citrus-like attributes, had an OAV greater than 1 in the tomato cultivars. The total amounts of higher alcohols were 282.55 µg/kg and 341.38 µg/kg in the cv. Alida and the cv. Merve, respectively. Only 2-phenylethanol, which imparts a floral, roselike odor, was detected at a concentration higher than its odor threshold value (68 µg/kg) (Bezman et al. 2003). In the Moneymaker and Raf cultivars, this compound was found at concentrations below its odor threshold (Ortiz-Serrano and Gil 2010). Other higher alcohols, including 1-penten-3-ol, 2-methyl butanol, pentanol, hexanol, (Z)-3-hexenol, 6-methyl-5-hepten-2-ol and benzyl alcohol, exhibited OAVs inferior to 1. The contribution of these compounds to the flavors of the Alida and Merve cultivars may be negligible.

Alida was distinguished from Merve by significantly higher levels of the volatile phenols. The total amounts of volatile phenol was 49.36 µg/kg for Alida and 3.31 µg/kg for Merve. Guaiacol and eugenol was 17.55 µg/kg and 22.95 µg/kg, respectively, for Alida and 0.98 µg/kg and 2.33 µg/kg, respectively, for Merve. Guaiacol imparts a phenolic, smoky and burning odor with an odor threshold

of 3 µg/kg (Buttery et al. 1989). Eugenol is characterized by a flowery, cloves fragrance and an odor threshold of 6 µg/kg (Buttery et al. 1990). In contrast to Merve, both compounds may contribute to the flavor to Alida because their OAVs were superior to 1. In three tomato cultivars, the eugenol and guaiacol concentrations were also found at levels that significantly exceeded their thresholds (Ortiz-Serrano and Gil 2007). The guaiacol concentration was decreased and the eugenol concentration increased during tomato maturity. The OAV of acetosyringone for Alida could not be determined because its odor threshold was not available.

Regarding volatile acids, hexanoic, octanoic, and nonanoic acids were detected in both cultivars, with total amounts of 49.77 µg/kg in Alida and 61.94 µg/kg in Merve. Due to their high odor thresholds, these compounds may not contribute directly to the flavor of tomatoes (Zhu et al. 2016).

Among the esters, methyl pentanoate, methyl hexanoate, methyl salicylate and methyl dihydro jasmonate were identified. The total concentration of esters was 14.4 µg/kg in Alida and 28.94 µg/kg in Merve. Ester compounds are responsible for the fruity flavors and impact odorants of several fruits (Komes et al. 2005). However, their odor activity values were below 1 in both tomato cultivars, suggesting that these compounds may not contribute to their flavors, consistent with earlier studies (Selli 2007).

5,6-epoxy-β-ionone and 3-hydroxy-β-ionone were observed for the first time in the free aroma fraction of tomato. The OAVs of those compounds were not determined because no data was available regarding their odor thresholds.

Table 2 Free volatile compound concentrations ($\mu\text{g/kg}$) in two tomato cultivars, Alida and Merve

Aroma compounds	RI ^a	ID ^b	Alida	OAV ^e of Alida	Merve	OAV of Merve	Odor threshold ($\mu\text{g/kg}$ of water)	<i>p</i> ^f
<i>Aldehydes and ketons</i>								
Hexanal	1079	A	32.85 $\pm$ 3.56	7.3	46.30 $\pm$ 2.26	10.3	4.5	**
(E)-2-pentenal	1137	A	4.57 $\pm$ 0.77	< 1	3.56 $\pm$ 0.64	< 1	1500	
(Z)-3-hexenal	1146	A	3.20 $\pm$ 0.13	12.8	2.62 $\pm$ 0.09	10.5	0.25	**
3-methyl-2-butenal	1206	B	3.39 $\pm$ 0.43	n.c. ^c	1.01 $\pm$ 0.16	n.c.	Unknown	**
(E)-2-hexenal	1213	A	2.34 $\pm$ 0.24	< 1	4.21 $\pm$ 0.61	< 1	17	**
(E)-2-heptenal	1229	A	11.83 $\pm$ 0.76	< 1	15.47 $\pm$ 1.76	1.2	13	*
2-octanone	1285	A	4.68 $\pm$ 0.75	< 1	4.74 $\pm$ 0.03	< 1	50	
6-methyl-5-hepten-2-one	1333	B	86.17 $\pm$ 2.69	1.7	112.67 $\pm$ 3.27	2.3	50	**
(E)-2-octenal	1345	A	3.96 $\pm$ 0.36	1.3	4.30 $\pm$ 0.77	1.4	3	
Benzaldehyde	1518	A	6.12 $\pm$ 4.59	< 1	7.50 $\pm$ 2.02	< 1	350	
(E,Z)-2,4-decadienal	1555	B	1.38 $\pm$ 0.36	12.6	3.43 $\pm$ 0.21	31.2	0.11	**
Acetophenone	1615	A	2.72 $\pm$ 0.99	< 1	7.50 $\pm$ 1.09	< 1	65	**
2-hydroxybenzaldehyde	1675	B	9.15 $\pm$ 1.77	n.c.	3.80 $\pm$ 0.91	n.c.	Unknown	*
Geranial	1715	A	2.23 $\pm$ 0.81	< 1	2.10 $\pm$ 0.39	< 1	32	
(E,E)-2,4-decadienal	1840	B	2.28 $\pm$ 1.32	20.7	1.16 $\pm$ 0.32	10.6	0.11	
Acetovanillone	2604	A	18.96 $\pm$ 3.45	n.c.	0.92 $\pm$ 0.33	n.c.	Unknown	**
Total			195.83		221.29			
<i>Higher alcohols</i>								
Pentanol	1107	A	28.70 $\pm$ 1.89	n.c.	31.49 $\pm$ 5.11	n.c.	Unknown	
2-methyl butanol	1109	A	42.32 $\pm$ 2.08	n.c.	40.71 $\pm$ 5.87	n.c.	Unknown	
1-penten-3-ol	1170	B	10.76 $\pm$ 0.61	< 1	5.05 $\pm$ 7.89	< 1	400	
6-methyl-5-hepten-2-ol	1317	B	6.90 $\pm$ 0.31	n.c.	6.01 $\pm$ 0.12	n.c.	Unknown	*
Hexanol	1340	A	3.66 $\pm$ 0.45	< 1	5.49 $\pm$ 0.45	< 1	2500	**
(Z)-3-hexenol	1366	A	0.65 $\pm$ 0.01	< 1	1.45 $\pm$ 0.31	< 1	70	*
Benzyl alcohol	1851	A	45.84 $\pm$ 3.03	< 1	22.74 $\pm$ 3.89	< 1	10,000	**
2-phenylethanol	1883	A	139.95 $\pm$ 5.59	2.1	222.60 $\pm$ 26.04	3.3	68	**
Guaiacylpropanol	2850	B	3.77 $\pm$ 0.74	n.c.	5.84 $\pm$ 2.25	n.c.	Unknown	
Total			282.55		341.38			
<i>Volatile phenols</i>								
Guaiacol	1859	A	17.55 $\pm$ 0.72	5.9	0.98 $\pm$ 0.31	< 1	3	***
Eugenol	2143	A	22.95 $\pm$ 4.46	3.8	2.33 $\pm$ 0.76	< 1	6	**
Acetosyringone	2953	B	8.86 $\pm$ 2.24	n.c.	n.d. ^d	n.c.	Unknown	
Total			49.36		3.31			
<i>Volatile acids</i>								
Hexanoic acid	1874	A	18.79 $\pm$ 1.61	< 1	23.14 $\pm$ 2.89	< 1	3000	
Octanoic acid	2066	A	22.77 $\pm$ 1.78	< 1	27.47 $\pm$ 1.89	< 1	3000	*
Nonanoic acid	2202	A	8.21 $\pm$ 4.23	< 1	11.33 $\pm$ 3.44	< 1	3000	
Total			49.77		61.94			
<i>Esters</i>								
Methyl pentanoate	1087	A	1.90 $\pm$ 0.23	< 1	13.25 $\pm$ 1.73	< 1	20	***
Methyl hexanoate	1575	A	2.71 $\pm$ 0.58	< 1	5.41 $\pm$ 0.82	< 1	70	*
Methyldihydrojasmonate	1644	B	6.25 $\pm$ 2.84	< 1	10.16 $\pm$ 3.23	< 1	15	
Methylsalicylate	1745	B	3.62 $\pm$ 1.56	n.c.	0.12 $\pm$ 0.11	n.c.	Unknown	*
Total			14.48		28.94			
<i>C13 Norisoprenoids</i>								
5,6-epoxy-beta-ionone	1979	B	0.85 $\pm$ 0.45	n.c.	2.67 $\pm$ 1.00	n.c.	Unknown	*

Table 2 continued

Aroma compounds	RI ^a	ID ^b	Alida	OAV ^c of Alida	Merve	OAV of Merve	Odor threshold (µg/kg of water)	<i>p</i> ^f
3-hydroxy-beta-ionone	2693	B	0.57 ± 0.17	n.c.	1.16 ± 0.24	n.c.	Unknown	*
Total			1.42		3.83			
<i>Lactones</i>								
Dihydroactinidiolide	2307	B	5.90 ± 1.18	n.c.	5.96 ± 0.66	n.c.	Unknown	
Total			5.90		5.96			
<i>Furans</i>								
2-acetylfurane	1497	B	1.36 ± 0.41	n.c.	0.08 ± 0.02	n.c.	Unknown	**
Total			1.36		0.08			
General total			600.67		666.85			

^aRI: Linear retention index based on a series of n-hydrocarbons for DB-WAX

^bID: A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; B, identity tentatively assigned by comparing mass spectra, and LRI

^cn.c., not calculated; no odor threshold available

^dn.d., not detected

^eOdor activity value = average concentration of samples/odor threshold in water

^f**p* < 0.05, ***p* < 0.01, ****p* < 0.001

Composition of bound aroma compounds in the Alida and Merve cultivars

A total of 31 glycosidically bound aroma compounds were observed in both Alida and Merve, including 13 C₁₃-norisoprenoids, 6 terpenoids, 7 higher alcohols, 3 volatile phenols, 1 aldehyde and 1 ester (Table 3). The same compounds were detected in both cultivars, but the levels were significantly different for the majority of the compounds. The total concentration of bound volatiles was higher for Merve (977.73 µg/kg) than Alida (816.24 µg/kg).

The total norisoprenoid content of Merve (648.10 µg/kg) was nearly three-fold higher than that of Alida (210.91 µg/kg). A total of 32% of the total norisoprenoid content of Alida was made up of 3-oxo- α -ionol. This value was followed by vomifoliol (24.8%), 3-oxo-7,8-dihydro- α -ionol (16.2%) and 3-oxo-retro- α -ionol (4.7%). In the cv. Merve, 39.1% of the total norisoprenoid content consisted of 3-oxo- α -ionol, followed by 3-oxo-7,8-dihydro- α -ionol (23.6%), vomifoliol (17.3%), and 3-hydroxy-7,8-dihydro- β -ionone (3.6%). All of the detected norisoprenoids have been previously reported in the glycosidic fractions of tomato cultivars (Marlatt et al. 1992; Ortiz-Serrano and Gil 2007, 2010).

The total concentrations of bound terpenoids were 39.32 µg/kg and 88.13 µg/kg for Alida and Merve, respectively. Linalool and linalool derivatives were the predominant bound terpenoids. The bound linalool concentration was three-fold higher than its odor threshold

(6 µg/kg) (Fabre et al. 2004). Linalool was distinguished by a floral-woody, lilac, lavender odor (Selli et al. 2011). (*E*)-linalool oxide, (*Z*)-linalool oxide, linalool, α -terpineol, β -citronellol, nerol and geraniol were also reported in the glycosidic fraction of tomato cultivars (Marlatt et al. 1992; Ortiz-Serrano and Gil 2007).

The total concentrations of bound higher alcohols were 454.56 µg/kg for Alida and 186.21 µg/kg for Merve. The level of 2-methyl-1-butanol was significantly higher for Alida (140.89 µg/kg) than Merve (98.28 µg/kg). The opposite was observed for the 2-phenylethanol level. 2-methyl-1-butanol, benzyl alcohol and 2-phenyl ethanol were the major bound higher alcohols in both cultivars, as reported for other tomato cultivars (Marlatt et al. 1992). The level of 2-phenylethanol exceeded its threshold value (68 µg/kg) (Bezman et al. 2003) in both cultivars, with values of 140.89 µg/kg in the cv. Alida and 98.28 µg/kg in the cv. Merve. Pentanol, hexanol, (*Z*)-3-hexenol, and 6-methyl-5-hepten-2-ol were among the other bound alcohols detected.

With respect to free volatile phenols, Alida was richer in bound volatile phenols than Merve. The bound volatile phenol content was 77.37 µg/kg for Alida and 35.71 µg/kg for Merve. Guaiacol, vinylguaiacol and eugenol were the detected compounds, with eugenol latter being the major compound. In both cultivars, the concentration of bound volatile phenols was above their odor threshold. High levels of bound guaiacol and eugenol were also observed previously in three tomato cultivars (Ortiz-Serrano and Gil 2007, 2010).

Table 3 Glycosidically bound volatile compound concentrations ($\mu\text{g/kg}$) in two tomato cultivars, Alida and Merve

Aroma compounds	RI ^a	ID ^b	Alida	OAV ^d for Alida	Merve	OAV for Merve	Odor threshold ($\mu\text{g/kg}$ of water)	<i>p</i> ^e
<i>C₁₃ Norisoprenoids</i>								
7,8-Dihydro- β -ionol	1995	B	2.29 $\pm$ 0.23	n.c. ^c	5.04 $\pm$ 0.08	n.c.	Unknown	
4-oxo- β -ionone	2459	B	3.06 $\pm$ 0.24	n.c.	9.63 $\pm$ 0.51	n.c.	Unknown	*
4-oxo-7,8-dihydro- β -ionone	2492	B	6.68 $\pm$ 0.09	n.c.	21.24 $\pm$ 0.55	n.c.	Unknown	*
3-hydroxy- β -damascone	2544	B	0.55 $\pm$ 0.02	n.c.	1.17 $\pm$ 0.06	n.c.	Unknown	*
3-hydroxy-7,8-dihydro- β -ionone	2557	B	6.09 $\pm$ 0.22	n.c.	23.59 $\pm$ 1.30	n.c.	Unknown	*
3-oxo- α -ionol	2590	A	67.18 $\pm$ 3.54	n.c.	253.53 $\pm$ 16.65	n.c.	Unknown	**
3-hydroxy-7,8-dihydro- β -ionone	2608	B	6.14 $\pm$ 0.60	n.c.	10.15 $\pm$ 0.83	n.c.	Unknown	*
4-oxo- β -ionol	2620	A	5.23 $\pm$ 0.27	n.c.	9.33 $\pm$ 0.63	n.c.	Unknown	*
3-hydroxy- β -ionone	2633	B	6.26 $\pm$ 0.54	n.c.	20.96 $\pm$ 0.65	n.c.	Unknown	**
3-oxo-retro- α -ionol	2677	B	9.92 $\pm$ 0.37	n.c.	16.12 $\pm$ 0.74	n.c.	Unknown	*
3-oxo-7,8-dihydro- α -ionol	2721	B	34.20 $\pm$ 2.04	n.c.	153.10 $\pm$ 8.33	n.c.	Unknown	*
3-hydroxy-5,6-epoxy- β -ionol	2762	B	5.85 $\pm$ 0.11	n.c.	12.00 $\pm$ 0.29	n.c.	Unknown	*
Vomifoliol	3129	B	52.28 $\pm$ 2.66	n.c.	112.24 $\pm$ 6.63	n.c.	Unknown	*
Total			210.91		648.10			
<i>Terpenes</i>								
Linalool	1532	A	18.35 $\pm$ 0.41	3.1	28.39 $\pm$ 0.63	4.7	6	*
Nerol	1778	A	1.24 $\pm$ 0.05	< 1	3.16 $\pm$ 0.02	< 1	300	
3,7-dimethyl-1,5-octadien-3,7-diol	1925	B	3.17 $\pm$ 0.17	n.c.	2.13 $\pm$ 0.16	n.c.	Unknown	
8-hydroxy-dihydrolinalool	2180	B	2.92 $\pm$ 0.22	n.c.	8.55 $\pm$ 0.38	n.c.	Unknown	
8-hydroxy-6,7-dihydrolinalool	2242	B	1.73 $\pm$ 0.17	n.c.	13.62 $\pm$ 1.30	n.c.	Unknown	*
E-8-hydroxy-linalool	2279	B	11.91 $\pm$ 0.32	n.c.	32.28 $\pm$ 1.03	n.c.	Unknown	*
Total			39.32		88.13			
<i>Higher alcohols</i>								
2-methyl-1-butanol	1194	A	133.48 $\pm$ 4.79	n.c.	21.84 $\pm$ 0.67	n.c.	Unknown	***
1-pentanol	1274	A	2.83 $\pm$ 0.09	< 1	3.04 $\pm$ 0.13	< 1	4000	
1-hexanol	1339	A	5.86 $\pm$ 0.02	< 1	5.99 $\pm$ 0.09	< 1	2500	
Z-3-hexen-1-ol	1367	A	4.23 $\pm$ 0.39	< 1	4.97 $\pm$ 0.17	< 1	70	
6-methyl-5-hepten-2-ol	1448	B	12.43 $\pm$ 0.32	< 1	6.55 $\pm$ 0.06	< 1	2000	*
Benzyl alcohol	1843	A	154.84 $\pm$ 13.02	< 1	45.54 $\pm$ 3.54	< 1	10,000	*
2-Phenyl ethanol	1877	A	140.89 $\pm$ 13.30	2.1	98.28 $\pm$ 7.58	1.5	68	*
Total			454.56		186.21			
<i>Volatile phenols</i>								
Guaiacol	1827	A	21.37 $\pm$ 0.21	7.1	5.37 $\pm$ 0.16	1.8	3	*
Eugenol	2129	A	53.59 $\pm$ 4.51	8.9	28.29 $\pm$ 1.99	4.7	6	*
4-Vinylguaiacol	2157	B	2.41 $\pm$ 0.12	< 1	2.05 $\pm$ 0.19	< 1	3	
Total			77.37		35.71			
<i>Aldehydes</i>								
Benzaldehyde	1495	A	8.57 $\pm$ 0.58	< 1	7.06 $\pm$ 0.09	< 1	350	
Total			8.57		7.06			
<i>Esters</i>								
Methylsalicylate	1745	B	25.51 $\pm$ 1.14	n.c.	12.52 $\pm$ 0.70	n.c.	unknown	*
Total			25.51		12.52			

Table 3 continued

Aroma compounds	RI ^a	ID ^b	Alida	OAV ^d for Alida	Merve	OAV for Merve	Odor threshold (µg/kg of water)	<i>p</i> ^c
General total			816.24		977.73			

^aRI: Linear retention index based on a series of n-hydrocarbons for DB-WAX

^bID: A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; B, identity tentatively assigned by comparing mass spectra, and LRI

^cn.c., not calculated; no odor threshold available

^dOdor activity value = average concentration of samples/odor threshold in water

^e* *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001

Conclusion

Although a number of studies aimed at identifying the volatile components of fresh tomatoes have been conducted, few have focused on the components linked to glycosides and their possible effect on final taste. In this study, 5,6-epoxy-β-ionone and 3-hydroxy-β-ionone have been reported for the first time as part of the free aroma of both tomato cultivars. Among aldehydes, the C6 aldehydes (i.e., hexanal and (Z)-3-hexenal) and C12 aldehydes (i.e., (E,Z)-2,4-decadienal and (E,E)-2,4-decadienal) could be important flavor compounds in the Alida and Merve cultivars based on their OAVs. In addition, Alida contained volatile phenols (i.e., guaiacol and eugenol) at levels capable of impacting tomato flavor. When the odor activity values of both varieties are taken into consideration, Merve has been appeared to have a fresh and green note than Alida. In terms of consumer preferences, this is one of the desirable characteristics of tomatoes. A high flavor potential occurring as glycosidically bound aroma compounds was observed in both cultivars. The total amounts of bound C₁₃-norisoprenoids and volatile phenols significantly exceeded those of their free counterparts. Terpenoids, except for geranial, were not detected in free form but were present in glycosidic form. Moreover, quantitative differences in bound aroma compounds were observed between the two cultivars. This situation has been obviously observed when the total amounts of norisoprenoids and terpenes are taken into consideration. Norisoprenoids and terpenes are the most important groups in terms of odor potential in aroma compounds. From this point of view, Merve has a strong odor potency compared to Alida. Acid or enzyme-mediated hydrolysis of glycosides has been known to lead to the liberation of flavor compounds, such as norisoprenoids, terpenoids and volatile phenols. 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 using of the enzyme in processed tomato products by industrial. Further sensory work will have to be done to link the

analytical differences between the free volatiles of both cultivars to consumer preferences and assess the potential effect of the released aglycons on the final flavor of the tomato fruit.

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