Food Research International 50 (2013) 409–419

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Investigating physicochemical, volatile and sensory parameters playing a positive or a negative role on tomato liking

Paola Piombino [a], Fiorella Sinesio [b], Elisabetta Moneta [b], Maria Cammareri [c], Alessandro Genovese [a], Maria Tiziana Lisanti [a], Maria Rita Mogno [d], Marina Peparaio [b], Pasquale Termolino [c], Luigi Moio [a], Silvana Grandillo [c,]*

[a] Department of Food Science, University of Naples Federico II, P.co Gussone, 80055 Portici, Napoli, Italy
[b] National Research Institute for Food and Nutrition (INRAN), Via Ardeatina 546, 00178 Roma, Italy
[c] Italian National Research Council, Institute of Plant Genetics (CNR-IGV), Research Division Portici, Via Università 133, 80055 Portici, Napoli, Italy
[d] Monsanto Agricoltura Italia SPA, Strada Canneto Di Rodi, 1034, Latina, Italy

ARTICLE INFO

*Article history:*
Received 4 August 2012
Accepted 23 October 2012

*Keywords:*
Consumer overall liking
Physicochemical parameters
Tomato
Sensory evaluation
Volatiles

ABSTRACT

This study aimed at providing further insights into the positive and negative drivers of tomato liking. For this purpose, 13 tomato cultivars representing different typologies were characterized for physicochemical parameters and aroma volatiles, and were assessed by a trained panel for sensory descriptors, and by Italian consumers for liking. The relationships among the different parameters and their effects on consumer liking were studied by Partial Least Squares (PLS) analysis. Among physicochemical traits and sensory descriptors, seeds, reducing sugars, firmness, thick epicarp, soluble solids, sour taste, total acidity, citrate, herbaceous aroma and brightness were found to be drivers of liking, whereas pulp thickness, humidity, fruit weight, diacetyl-like odor and mealiness showed an opposite influence. For the aroma volatiles, 2-isobutylthiazole played a key role on liking and its positive contribution seemed to be supported by (Z)-3-hexen-1-ol, but suppressed by 6-methyl-5-hepten-2-ol, especially when tomatoes had a poor volatile fraction. These results represent a contribution to the knowledge that could lead to more effective breeding strategies aimed at improving tomato sensory quality.

## 1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetables in the world, and its popularity among consumers has made this crop an important source of essential nutrients, including different antioxidant molecules (e.g. vitamin C and carotenoids) with recognized positive effects on human health (Shidfar et al., 2011). However, over the past decades consumers have started to complain about a decrease in flavor quality of modern tomato varieties. This can be considered in part as an indirect consequence of breeding programs that have traditionally focused on yield, fruit size and shelf-life traits, but it is also a consequence of commercial harvesting and post-harvest handling procedures (Krumbein, Peters, & Bruckner, 2004). In order to satisfy consumers' expectations, tomato breeders are now pursuing sensory quality as one of their major objectives. Nevertheless, the polygenic nature of most of the sensory traits (Zanor et al., 2009), the chemical complexity of liking, and the lack of efficient objective flavor selection criteria make the improvement of sensory quality still a challenging task.

Tomato fruit quality for fresh consumption depends on numerous traits relating to visual appearance, flavor and texture. While the initial consumer's choice is mainly driven by visual appearance, eating quality becomes the major influencing factor in subsequent purchases. Flavor of tomato fruits is chemically determined by a complex mixture of primary and secondary metabolites mainly including sugars, acids, minerals and volatile compounds that are measured by the taste and olfactory systems (Baldwin, Scott, Shewmakert, & Schuch, 2000). Although these chemicals are largely known, the way they integrate to produce the specific tomato flavor is not yet understood.

Several methodologies for sensory characterization have been developed (Varela & Ares, 2012). Among these techniques the classical descriptive analysis is the most powerful tool as it provides a complete description of the sensory characteristics of products, i.e. it detects differences in intensity of specific sensory attributes. Descriptive sensory analysis by trained panels, coupled with consumer tests, represents an

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*Abbreviations:* HPLC, high-performance liquid chromatography; HPLC–UV, high-performance liquid chromatography–ultraviolet detection; GC/MS, Gas chromatography–mass spectrometry; LLME, Liquid–Liquid Micro Extraction; NaOH, Sodium hydroxide; $(NH_4)_2SO_4$, Ammonium sulfate; $CH_2Cl_2$, dichloromethane; S, sulfur compounds; K, ketones; OH, alcohols; F, furans; Ald, aldehydes; Ac, acids; E, esters; Ph, phenols; CIEL*a*b*, color space; L*, lightness; CLT, Central Location Test; ANOVA, analysis of variance; SD, standard deviation; Duncan's MRT, Duncan's Multiple Range Test; PLS, Partial Least Squares; PCs, principal components; VIP, variable importance for the projection.

* Corresponding author. Tel.: +39 081 2539489, +39 081 2539026; fax: +39 081 7753579.
*E-mail address:* grandill@unina.it (S. Grandillo).

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http://dx.doi.org/10.1016/j.foodres.2012.10.033

efficient approach to describe the properties underlying tomato fruit quality for fresh consumption. However, such sensory assessment is expensive and time-consuming, and therefore there is the need to identify clear instrumental targets that could be more easily used by breeders for selection and manipulation of tomato flavor.

Several studies have attempted to establish the relationships between sensory descriptors and instrumental measurements in order to understand the contribution of individual components to tomato flavor (Carli et al., 2009; Causse, Buret, Robini, & Verschave, 2003; Zanor et al., 2009). It is generally accepted that a sufficient amount of soluble solids, mostly reducing sugars (glucose and fructose) and organic acids (citrate, malate and glutamate) in an appropriate balance of sweet and sour is a necessary, although not sufficient, condition for good flavor (Malundo, Shewfelt, & Scott, 1995; Tandon, Baldwin, Scott, & Shewfelt, 2003).

Flavor complexity is, however, determined by the olfactory system as volatiles clearly determine odor (orto-nasal) and aroma (retro-nasal) perception in tomatoes (Baldwin et al., 2000). The impact of a chemical on olfactory perception is determined by both its concentration and odor threshold in that matrix (odor units). Although over 400 aroma volatiles have been identified in tomato and tomato products (Petro-Turza, 1987), several studies have shown that only 16 aroma volatiles are present in sufficient quantities to be detected by the olfactory system, and hence are generally accepted to contribute to tomato flavor (Baldwin et al., 2000). However, minor volatiles with negative log-odor units should not be neglected as they may still contribute to the overall flavor as backgrounds notes (Baldwin et al., 2000). In addition, interactions among volatiles and also those involving the taste and olfactory systems, further complicate flavor, as specific aroma volatiles perceived by the retro-nasal olfactory system can affect the perception of sweetness or sourness and vice versa (Baldwin, Goodner, & Plotto, 2008; Tieman et al., 2012). These results underline the limitations of traditional flavor research based exclusively on odor units of individual volatiles; these models, in fact, cannot explain all the synergistic and antagonistic interactions that take place in complex foods such as tomato (Tieman et al., 2012).

A better knowledge of all the factors influencing tomato consumer's preferences is required in order to be able to improve fruit quality and to diversify this product. Preference mapping studies conducted at the European level have shown that consumer segments exist which differ in their liking of tomato varieties, and that diversification of flavor and texture is required to satisfy all consumers' expectations (Causse et al., 2010; Sinesio et al., 2010). In addition, Berna, Lammertyn, Buysens, Di Natale, and Nicolai (2005) reported that Flemish consumer segments, identified on the basis of preference differences, were highly correlated to specific aroma volatiles. Recent research conducted with a large number of heirloom varieties, using consumers in United States, confirmed that there is no “best”-tasting tomato, as preferences could be separated by age, sex, body mass and genetics; although the collected data should allow defining the parameters for a consensus best tomato in the United States (Tieman et al., 2012).

The aim of the present study was to gain further knowledge regarding key drivers of tomato liking and disliking, through the determination of physicochemical, aroma volatile and descriptive sensory profiles of tomato cultivars representing different segments. The use of a two-step regression model allowed the identification of multiple sensory and compositional parameters that could become targets for breeding strategies aimed at improving not only yield, adaptation and shelf-life traits but also sensory quality.

## 2. Materials and methods

### 2.1. Plant material

Thirteen cultivars belonging to different tomato segments were grown during Spring 2009 at Monsanto Research and Development Centre, Latina (Table 1). The local variety Principe Borghese (P.BO), famous for sun drying, was included in the experiment for its expected rich volatile profile (Lisanti, Piombino, Genovese, Pessina, & Moio, 2008). A total of 120 plants for each cultivar were grown in greenhouse, heated at minimum temperature of 8 °C with black mulching, using integrated pest management and bumble bees pollination.

Fruits were harvested over three consecutive weeks from different trusses: 2nd truss on May 18 (week 21), 3rd truss on May 25 (week 22) and 4th truss on June 3 (week 23). The harvest of May 18 was used for sensory pre-sessions (panelist agreement on descriptors and scale). The samples collected in weeks 22 and 23 were used for sensory profiling, hedonic tests and analytical measurements. Sampling was done selecting fruits at the red-ripe stage of maturity, without any visual defects or disease symptoms. Samples were harvested at the same stage of maturity with the aim of being able to analyze the relationships among physical, compositional and sensory variables. For each cultivar, fruits were pooled and then they were randomly separated into four groups and delivered to each test location within the harvesting day, at a temperature of 12 °C. For physicochemical analyses, as well as for descriptive and hedonic evaluations, after delivery, the fruits were stored in a cold room at 12 °C and were taken out to acclimatize to room temperature (22 ± 2 °C, for 12 h) prior to evaluations (which took place within 36 h from harvesting). For tomato volatile analysis, immediately after delivery, the fruits were stored at −20 °C. For the analyses, batches of fruits homogeneous for size and color were selected for each cultivar.

### 2.2. Physicochemical measurements

For each cultivar and for each harvest replicate (May 25 and June 3) two samples of at least 6 fruits each were measured. The

**Table 1**
Descriptive list of tomato cultivars used in the present study.

| Cultivar | Type | Fruit shape | Average fruit weight (g) | Company |
|---|---|---|---|---|
| Albenga (ALB) | Cuore di Bue (local variety) | Typical ribbed hearth-shape | 227 | – |
| Carlota (CAR) | Cluster | Round | 90 | Monsanto |
| Climberly (CLI) | Cluster | Round | 143 | S&G-Syngenta |
| Delizia (DEL) | Marmande | Ribbed flat-round | 259 | Clause |
| Globo (GLO) | Cluster | Round | 82 | Enza Zaden |
| Licorossa (LIC) | Large cocktail | Round | 103 | Monsanto |
| Maribel (MARI) | Cluster | Round | 99 | Enza Zaden |
| Marmandino One (MARM) | Marmande | Ribbed flat-round | 231 | Hild Samen |
| Murano-San Marzano2 (MUR) | San Marzano (local variety) | Elongated-typical San Marzano shape | 89 | La Semiorto Sementi |
| Panarea (PAN) | Cherry Truss | Round | 21 | Monsanto |
| Principe Borghese (P.BO) | Cocktail (local variety) | Cocktail high-round nippled | 35 | La Semiorto Sementi |
| Red Delight (RED) | Cocktail | Round | 51 | Sakata Seeds |
| TyTy (TYT) | Cherry Truss | Round | 28 | S&G-Syngenta |

following traits were evaluated on a single fruit basis: fruit weight (measured by technical balance, Exacta Optech), fruit firmness (measured on fruits with epicarp by a punctual deformation test using a digital Penetrometer-Fruit Pressure Tester, TR Turoni & C. Italy), and external fruit color measured with a chroma meter Minolta CR-300 (Konica Minolta, Tokyo, Japan) in order to obtain the CIEL*a*b* parameters: L* (lightness, from white to black), a* (green to red), and b* (blue to yellow). Fruit color parameters and fruit firmness were measured on two different positions of the equatorial region of the fruit, and the average of two measurements was used for the analyses.

For measurements of total acidity, pH, soluble solids content or brix, organic acids (citric and malic acids) and reducing sugars (glucose and fructose), the fruits were cut longitudinally into four wedges and a pull of 1/4 of each fruit was homogenized in Waring Blender for 1 min. A part of the homogenate was centrifuged for 10 min at 4000 rpm, and the supernatant was used for immediate measurement of total soluble solids content (°Brix), using a Refractometer (RFM81, Bellingam + Stanley, Kent, U.K.) at room temperature (22 ± 2 °C). Another part of the homogenate was placed in falcon tubes and immediately frozen and stored at −20 °C for later measurements of dry matter (to calculate humidity), total acidity, pH, glucose, fructose, citrate and malate. Dry matter content was measured by drying weighed samples at 70 ± 2 °C, and humidity was derived as 100-dry matter %. Total acidity was measured by potentiometric titration of the sample using 0.1 M NaOH (titration to pH 8.1), and was expressed as g/100 g of citric acid monohydrate; pH was measured using a digital pH meter (AMEL 23359V2.02, Milan, Italy). Citrate and malate contents were determined by HPLC–UV (Agilent 1100, California, USA) analysis. Glucose and fructose contents were measured by Ion Exchange HPLC (Dionex ICS-3000) with pulsed amperometric detector.

### 2.3. Analysis of tomato volatiles

#### 2.3.1. Liquid–liquid micro extraction (LLME)

Tomato volatiles were extracted according to the method reported by Aubert, Baumann, and Arguel (2005). After removal of crown tissues and stalks, 250 g of frozen (−20 °C) tomatoes were homogenized in a Waring blender for 2 min together with 250 mL of *n*-propyl gallate (10 mM; Fluka) as endogenous enzymes inhibitor, and 25 μL of 2-octanol (3.32 μg/mL; Sigma-Aldrich) as internal standard. The mixture was centrifuged (13,000 *g*, 5 min, 4 °C) by a refrigerated table top centrifuge Sigma 4-16K, and the supernatant was filtered through a stainless steel sieve (16 mesh). Forty milliliters of the supernatant and 12.8 g of $(NH_4)_2SO_4$ (32%; w/v; Fluka) were shaken until complete salt dissolution (15 min) and ultracentrifuged (21,000 g, 5 min, 4 °C). The supernatant was then filtered through a Waterman paper filter (grade 113v) into a 50 mL screw-capped conical centrifuge tube (polyfluor) containing a magnetic stir bar. The 50 mL of juice sample was added with 500 μL of $CH_2Cl_2$ (Sigma-Aldrich) and the mixture was extracted under magnetic stirring (60 min, 4 °C). After removal of the magnetic stir bar, the tube was sonicated for 1 min and finally centrifuged (1000 *g*, 5 min, 4 °C). The dichloromethane aromatic extract was then recovered with a syringe (500 μL) and 1.5 μL were immediately analyzed by GC/MS. Each tomato sample was extracted and analyzed in triplicate. For large-fruit cultivars (ALB, DEL, MARM) each sample was obtained by pooling half portions of three different fruits; while for small- (PAN, P.BO, TYT) and medium- (all the others) sized varieties, 10 and 6 fruits were processed for each analysis, respectively.

#### 2.3.2. Gas-chromatography/mass spectrometry (GC/MS)

The volatiles were identified with a QP-2010 quadrupole mass spectrometer coupled with a 2010AF gas chromatograph (Shimadzu, Kyoto, Japan). Electron impact mass spectra were recorded with ion-source energy of 70 eV. The GC–MS was provided with a DB-Wax silica capillary column (60 m; 0.25 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Folsom, CA 95630, USA). Volatiles were semi-quantified by calculating each response (peak area) relative to the response (peak area) of the internal standard (2-octanol), and assuming all of the response factors were 1. The chromatographic conditions and the identification procedure were the same as described by Lisanti et al. (2008). Authentic reference chemical compounds were obtained from Sigma-Aldrich (Steinheim, Germany). In a few cases the pure chemical standard was not available, and the compounds were labelled as tentative $^{(t)}$.

### 2.4. Sensory evaluations

#### 2.4.1. Descriptive analysis

Fruits, selected for uniformity of size and color, were removed of the crown tissues and stalks, and then they were washed with cold tap running water and dried with a clean towel. For sample preparation and evaluation, panelists referred to an evaluation protocol as described by Sinesio et al. (2010).

A descriptive sensory analysis was run with 8–9 professional panelists of INRAN, trained in all aspects of sensory techniques and analyses at numerous sessions over several years whose ability is routinely checked using individual control card for each assessors. The panel has a great deal of experience on tomato evaluation having been employed in several research projects on tomato (Causse et al., 2010; Sinesio, Moneta, & Peparaio, 2007; Sinesio et al., 2010). The panelists referred to a common list of 21 defined descriptors for which they had a consensus definition, including 6 visual descriptors, 3 odor (orto-nasal), 3 taste, 4 aroma (retro-nasal), 4 mouth feel and 1 aftertaste (Supplementary Table S1) (modified from Sinesio et al., 2010). Preliminary sessions (week 21), were organized for additional training during which the panelists discussed the list of descriptors in the set of products to be sure that they fully covered the span of properties in the products included in this project. In addition a pre-test session for panel calibration (determination of internal reference for each attribute) preceded the two test sessions: the panelists agreed on the level of the descriptors by tasting samples that were considered extreme for selected descriptors. For example, the cultivar ALB was the reference sample during the pre-testing sessions for its high intensity of fruity odor and aroma, fruit pulp thickness, juiciness, overall aroma (score 7 of the evaluation scale) and medium level of sweet taste (score 5). CAR was the reference for seed content, juice release, firmness and thick epicarp (score 6) while PAN for herbaceous odor and aroma, sour and salty taste (score 5). No reference sample was provided during the evaluation sessions. Inconsistency in the ratings were addressed with individual judges prior to the actual evaluation sessions. The panelists attended evaluation sessions of the 13 cultivars for two crop harvests (weeks 22 and 23). On both weeks, each panelist evaluated the complete set of tomato cultivars in duplicate. Descriptors intensity was rated using a 150 mm unstructured line scale; the left side corresponded to the lowest intensity of each attribute (0 = null) and the right side to the highest intensity (9 = strong). Each panelist received two tomatoes served as a whole fruit at room temperature and in plastic boxes closed with lids. The boxes were coded with 3-digit random numbers to not reveal the identity. Each panelist evaluated the products monadically in a different random order, 4 or 5 tomatoes per session, and three sessions were run in the same day with a break between sessions. Replications were run in two consecutive days. The trained panel worked in a sensory laboratory in individual booths, with constant conditions of light (white) and temperature (22 ± 2 °C). White unsalted bread and mineral water were provided as a palate neutralizer between the tasting of two samples. Data collection was carried out on a computer system using the FIZZ-software (Biosystemes, Counternon, France). For significantly ($P<0.05$) different descriptors, the average responses over assessors were used in the multivariate analyses.

**Table 2a**

Means and standard deviations (SD) of physicochemical traits measured on fruits of 13 cultivars (Cv) sampled in the first harvest replicate. b. Means and standard deviations (SD) of physicochemical traits measured on fruits of 13 cultivars (Cv) sampled in the second harvest replicate.

| FW (g) | | | External color | | | | | | | | | Firmness (N) | | | Humidity (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | L* | | | a* | | | b* | | | | | | | | |
| Cv[a] | Mean | (SD)[b] | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) |
| DEL | 263.2 | (98.7)a | CAR | 42.18 | (0.75)a | CAR | 24.93 | (1.17)a | CAR | 30.90 | (1.40)a | CAR | 25.19 | (5.40)a | MUR | 95.58 | (0.28)a |
| ALB | 213.0 | (48.5)b | MUR | 40.83 | (1.41)b | MUR | 24.89 | (1.72)a | MUR | 28.30 | (1.28)b | LIC | 22.86 | (3.09)ab | MARM | 95.29 | (0.18)ab |
| MARM | 205.7 | (57.4)b | ALB | 40.40 | (0.99)bc | MARM | 24.34 | (2.58)ab | DEL | 26.92 | (2.16)bc | CLI | 21.61 | (3.60)bc | ALB | 95.25 | (0.09)ab |
| CLI | 139.7 | (14.0)c | DEL | 39.82 | (1.28)bcd | DEL | 22.50 | (1.86)bc | ALB | 26.15 | (2.11)cd | GLO | 20.72 | (2.11)bc | MARI | 95.23 | (1.99)ab |
| MARI | 100.4 | (10.3)d | RED | 39.27 | (1.01)cde | TYT | 22.41 | (1.99)bc | RED | 25.70 | (1.41)cd | MARI | 18.59 | (2.89)cd | CAR | 95.05 | (0.01)abc |
| LIC | 90.4 | (13.4)d | PAN | 39.10 | (0.60)cde | LIC | 22.30 | (1.16)bc | CLI | 25.24 | (1.76)cde | PAN | 16.92 | (2.19)de | DEL | 94.92 | (0.18)abc |
| MUR | 89.8 | (25.1)d | MARM | 39.05 | (1.39)cde | ALB | 22.25 | (1.95)bcd | MARI | 24.38 | (1.55)de | TYT | 15.79 | (2.53)ef | GLO | 94.49 | (0.74)abc |
| CAR | 86.6 | (11.6)d | CLI | 38.90 | (1.10)de | RED | 21.60 | (1.59)cd | LIC | 24.32 | (1.15)de | P.BO | 15.45 | (1.86)ef | P.BO | 94.41 | (0.03)abc |
| GLO | 80.3 | (9.5)d | GLO | 38.22 | (0.53)ef | CLI | 21.30 | (1.68)cde | MARM | 24.25 | (2.19)de | RED | 15.38 | (2.46)ef | CLI | 94.34 | (0.06)abc |
| RED | 45.2 | (9.2)e | MARI | 38.21 | (0.78)ef | MARI | 19.91 | (1.81)def | PAN | 23.23 | (0.93)e | ALB | 14.25 | (2.34)ef | LIC | 94.27 | (0.49)abc |
| P.BO | 32.6 | (5.5)ef | LIC | 37.67 | (0.49)fg | P.BO | 19.63 | (1.58)ef | GLO | 23.20 | (0.91)e | MUR | 13.69 | (2.07)fg | TYT | 93.96 | (0.21)bc |
| TYT | 27.4 | (3.4)ef | P.BO | 37.63 | (1.06)fg | PAN | 19.40 | (1.35)ef | P.BO | 20.71 | (1.47)f | DEL | 10.80 | (2.15)gh | PAN | 93.86 | (0.25)bc |
| PAN | 18.8 | (4.1)f | TYT | 36.73 | (0.69)g | GLO | 17.68 | (1.46)f | TYT | 20.54 | (1.49)f | MARM | 8.02 | (2.02)h | RED | 93.57 | (0.34)c |
| F-value[c] | 96.22*** | | 13.54*** | | | 8.98*** | | | 23.88*** | | | 24.67*** | | | $2.01^{ns}$ | | |

| Soluble solids content (°Brix) | | | Glucose (g/100 g fw[d]) | | Fructose (g/100 g fw) | | pH | | Total acidity (g/100 g mhca[e] fw) | | Citric acid (g/100 g fw) | | Malic acid (g/100 g fw) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cv[a] | Mean | (SD)[b] | Cv | Mean (SD) | Cv | Mean (SD) | Cv | Mean (SD) | Cv | Mean (SD) | Cv | Mean (SD) | Cv | Mean (SD) |
| RED | 5.78 | (0.33)a | RED | 1.88 (0.07)a | RED | 2.12 (0.25)a | ALB | 4.39 (0.06)a | PAN | 0.675 (0.01)a | PAN | 0.618 (0.012)a | GLO | 0.069 (0.006)a |
| PAN | 5.52 | (0.04)ab | TYT | 1.87 (0.10)a | TYT | 1.95 (0.37)ab | P.BO | 4.33 (0.04)ab | CLI | 0.640 (0.03)a | RED | 0.588 (0.033)a | P.BO | 0.057 (0.004)ab |
| TYT | 5.24 | (0.06)bc | PAN | 1.79 (0.20)a | MARI | 1.91 (0.04)ab | MUR | 4.31 (0.01)ab | RED | 0.630 (0.04)a | CLI | 0.585 (0.021)a | DEL | 0.052 (0.014)abc |
| GLO | 5.10 | (0.20)bc | P.BO | 1.74 (0.20)a | PAN | 1.89 (0.01)ab | TYT | 4.29 (0.07)abc | LIC | 0.550 (0.04)b | LIC | 0.512 (0.035) b | MARM | 0.042 (0.005)bcd |
| LIC | 4.85 | (0.03)bcd | LIC | 1.61 (0.18)ab | P.BO | 1.87 (0.01)ab | DEL | 4.29 (0.05)abc | MARI | 0.510 (0.03)bc | MARI | 0.488 (0.014)bc | PAN | 0.034 (0.018)cde |
| P.BO | 4.74 | (0.26)cde | MARI | 1.58 (0.25)ab | LIC | 1.82 (0.28)abc | MARM | 4.24 (0.03)bcd | P.BO | 0.500 (0.01)bcd | P.BO | 0.432 (0.012)cd | ALB | 0.029 (0.007)de |
| ALB | 4.72 | (0.13)de | CLI | 1.53 (0.01)abc | CLI | 1.78 (0.20)abc | CAR | 4.22 (0.01)bcd | GLO | 0.485 (0.02)bcde | CAR | 0.420 (0.011)cd | RED | 0.028 (0.002)de |
| CLI | 4.59 | (0.01)ef | GLO | 1.52 (0.36)abc | GLO | 1.75 (0.01)abc | RED | 4.15 (0.17)cde | CAR | 0.450 (0.01)cdef | MUR | 0.416 (0.005)cd | CLI | 0.023 (0.008)de |
| MARI | 4.55 | (0.27)efg | CAR | 1.32 (0.01)bcd | DEL | 1.67 (0.14)bc | GLO | 4.12 (0.03)de | DEL | 0.445 (0.01)cdef | GLO | 0.398 (0.011)d | LIC | 0.023 (0.008)de |
| DEL | 4.50 | (0.19)efg | DEL | 1.28 (0.13)bcd | CAR | 1.44 (0.10)cd | LIC | 4.07 (0.01)ef | MUR | 0.440 (0.00)cdef | ALB | 0.388 (0.016)de | MARI | 0.021 (0.000)de |
| CAR | 4.24 | (0.01)fgh | ALB | 1.21 (0.04)bcd | MARM | 1.43 (0.13)cd | MARI | 4.07 (0.04)ef | ALB | 0.430 (0.03)def | TYT | 0.377 (0.002)de | CAR | 0.019 (0.001)e |
| MUR | 4.12 | (0.07)gh | MARM | 1.13 (0.10)cd | ALB | 1.38 (0.09)cd | PAN | 4.06 (0.00)ef | TYT | 0.415 (0.02)ef | DEL | 0.375 (0.003)de | TYT | 0.019 (0.010)e |
| MARM | 4.01 | (0.08)h | MUR | 1.06 (0.13)d | MUR | 1.16 (0.19)d | CLI | 3.98 (0.05)f | MARM | 0.390 (0.07)f | MARM | 0.318 (0.051)e | MUR | N.D.[f] N.D. N.D. |
| F-value[c] | 14.90*** | | 5.65** | | 4.67** | | 8.80*** | | 18.00*** | | 18.11*** | | 6.48** | |

[a] ALB = Albenga, CAR = Carlota, CLI = Climberly, DEL = Delizia, Globo = GLO, LIC = Licorossa, MARI = Maribel, MARM = Marmandino One, MUR = Murano, PAN = Panarea, Borghese, P. BO = Principe; RED = Red Delight, and TYT = Tyty.

[b] Means in a column with different letters are statistically different ($P<0.05$ Duncan's MRT).

[c] ***$P \leq 0.001$; **$P \leq 0.01$; ns = not significant.

[d] fw = fresh weight.

[e] mhca = monohydrate citric acid.

[f] N.D. = not detected.

**Table 2b**
Means and standard deviations (SD) of physicochemical traits measured on fruits of 13 cultivars (Cv) sampled in the second harvest replicate.

| FW (g) | | | External color | | | | | | | | | Firmness (N) | | | Humidity (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | L* | | | a* | | | b* | | | | | | | | |
| Cv[a] | Mean | (SD)[b] | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) |
| MARM | 256.7 | (62.0) a | ALB | 41.39 | (0.90) a | MUR | 26.87 | (1.31) a | ALB | 28.94 | (1.78) a | CAR | 29.36 | (3.95) a | MUR | 95.43 | (0.44) a |
| DEL | 255.2 | (43.8) a | RED | 40.36 | (1.21) ab | ALB | 24.97 | (1.91) ab | RED | 28.74 | (1.34) a | LIC | 26.04 | (6.56) a | DEL | 95.34 | (0.42) ab |
| ALB | 240.7 | (58.2) a | CAR | 39.62 | (0.97) bc | CAR | 23.30 | (0.78) bc | CAR | 28.17 | (1.24) ab | CLI | 21.58 | (6.04) b | MARI | 95.08 | (0.09) abc |
| CLI | 146.3 | (13.8) b | DEL | 38.77 | (1.11) cd | MARM | 22.67 | (1.32) bc | DEL | 27.05 | (2.27) abc | GLO | 21.51 | (4.83) b | MARM | 94.96 | (0.30) abc |
| LIC | 115.1 | (20.0) c | CLI | 38.18 | (1.06) cd | DEL | 22.14 | (1.22) c | MUR | 26.52 | (1.98) bc | TYT | 18.67 | (2.61) bc | ALB | 94.80 | (0.27) abc |
| MARI | 96.8 | (15.4) d | MUR | 38.04 | (0.92) d | MARI | 20.94 | (1.04) cd | GLO | 25.24 | (1.73) cd | RED | 17.26 | (3.74) c | CAR | 94.57 | (0.17) bcde |
| CAR | 94.0 | (22.8) d | LIC | 38.02 | (0.93) d | CLI | 18.92 | (1.08) de | LIC | 25.03 | (1.48) cd | P.BO | 17.13 | (2.60) c | CLI | 94.46 | (0.45) cde |
| MUR | 87.4 | (25.8) d | GLO | 37.82 | (0.76) d | P.BO | 18.90 | (1.41) e | MARM | 24.67 | (2.72) cd | PAN | 16.46 | (2.98) cd | P.BO | 94.43 | (0.04) cde |
| GLO | 84.4 | (17.7) d | MARM | 37.22 | (1.60) de | PAN | 18.88 | (1.37) e | MARI | 23.67 | (1.70) d | MARI | 16.27 | (4.55) cd | GLO | 94.20 | (0.04) cde |
| RED | 55.9 | (9.0) e | MARI | 36.14 | (0.98) ef | GLO | 18.00 | (1.65) e | CLI | 23.53 | (1.27) d | ALB | 15.12 | (2.37) cde | LIC | 93.71 | (0.68) def |
| P.BO | 36.7 | (5.0) f | PAN | 35.98 | (0.66) f | RED | 17.60 | (1.57) e | PAN | 21.08 | (1.13) e | DEL | 13.35 | (2.92) de | TYT | 93.67 | (0.49) ef |
| TYT | 27.6 | (4.8) fg | TYT | 35.27 | (0.64) f | LIC | 17.47 | (1.34) e | TYT | 18.47 | (1.45) f | MARM | 13.33 | (3.08) de | RED | 93.29 | (0.56) f |
| PAN | 23.0 | (4.7) g | P.BO | 33.79 | (1.03) g | TYT | 16.78 | (1.85) e | P.BO | 17.84 | (1.69) f | MUR | 11.80 | (1.83) e | PAN | 93.12 | (0.04) f |
| F-value$^{***}$ | 250.56$^{***}$ | | | 35.45$^{***}$ | | | 19.37$^{***}$ | | | 45.81$^{***}$ | | | 16.53$^{***}$ | | | 8.31$^{***}$ | |

| Soluble solids content (°Brix) | | | Glucose (g/100 g fw[c]) | | | Fructose (g/100 g fw) | | | pH | | | Total acidity (g/100 g mhca[d] fw) | | | Citric acid (gr/100 gr fw) | | | Malic acid (g/100 g fw) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cv[a] | Mean | (SD)[b] | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) | Cv | Mean | (SD) |
| PAN | 6.41 | (0.01) a | PAN | 2.19 | (0.01) a | RED | 2.23 | (0.28) a | ALB | 4.45 | (0.01) a | PAN | 0.720 | (0.00) a | PAN | 0.678 | (0.006) a | GLO | 0.078 | (0.004) a |
| RED | 6.04 | (0.06) ab | RED | 2.10 | (0.25) ab | PAN | 2.20 | (0.10) a | MUR | 4.37 | (0.04) b | RED | 0.665 | (0.06) a | RED | 0.628 | (0.057) a | DEL | 0.059 | (0.000) b |
| TYT | 5.68 | (0.02) bc | TYT | 1.99 | (0.37) abc | LIC | 2.13 | (0.50) ab | P.BO | 4.33 | (0.06) bc | CLI | 0.580 | (0.03) b | CLI | 0.537 | (0.026) b | MARM | 0.044 | (0.002) c |
| GLO | 5.50 | (0.05) bc | LIC | 1.87 | (0.28) abcd | TYT | 2.13 | (0.12) ab | CAR | 4.29 | (0.04) cd | LIC | 0.540 | (0.01) bc | LIC | 0.509 | (0.009) bc | P.BO | 0.041 | (0.004) c |
| P.BO | 5.23 | (0.13) cd | GLO | 1.70 | (0.01) bcde | GLO | 1.88 | (0.02) abc | DEL | 4.26 | (0.01) d | MARM | 0.505 | (0.05) cd | MARI | 0.452 | (0.008) cd | ALB | 0.032 | (0.005) cd |
| ALB | 5.21 | (0.05) cd | P.BO | 1.70 | (0.01) bcde | CLI | 1.75 | (0.23) abcd | MARM | 4.24 | (0.02) de | MARI | 0.490 | (0.00) cd | MARM | 0.445 | (0.006) cde | TYT | 0.023 | (0.011) de |
| LIC | 4.87 | (0.25) de | CAR | 1.56 | (0.10) cdef | P.BO | 1.68 | (0.07) bcde | GLO | 4.20 | (0.02) e | P.BO | 0.485 | (0.01) cd | P.BO | 0.435 | (0.002) de | CLI | 0.021 | (0.001) de |
| CLI | 4.82 | (0.11) de | CLI | 1.49 | (0.20) def | CAR | 1.66 | (0.03) bcde | TYT | 4.19 | (0.01) e | GLO | 0.475 | (0.01) cde | TYT | 0.416 | (0.012) def | RED | 0.021 | (0.001) de |
| MARI | 4.55 | (0.20) ef | ALB | 1.41 | (0.09) efg | ALB | 1.60 | (0.13) cde | PAN | 4.12 | (0.04) f | DEL | 0.465 | (0.02) def | GLO | 0.383 | (0.012) def | MARI | 0.018 | (0.011) e |
| MARM | 4.44 | (0.26) ef | MARI | 1.26 | (0.04) fg | MARM | 1.52 | (0.16) cde | LIC | 4.11 | (0.01) f | TYT | 0.450 | (0.00) def | CAR | 0.383 | (0.035) def | LIC | 0.016 | (0.006) e |
| CAR | 4.40 | (0.11) ef | MARM | 1.25 | (0.13) fg | MARI | 1.41 | (0.04) cde | RED | 4.10 | (0.01) f | CAR | 0.410 | (0.04) ef | MUR | 0.374 | (0.053) ef | PAN | 0.015 | (0.000) e |
| MUR | 4.15 | (0.06) f | MUR | 1.23 | (0.19) fg | DEL | 1.38 | (0.18) de | MARI | 4.09 | (0.01) f | MUR | 0.405 | (0.05) ef | DEL | 0.373 | (0.006) ef | CAR | 0.014 | (0.004) e |
| DEL | 3.98 | (0.14) f | DEL | 1.05 | (0.19) g | MUR | 1.20 | (0.16) e | CLI | 4.02 | (0.03) g | ALB | 0.395 | (0.01) f | ALB | 0.346 | (0.001) f | MUR | 0.010 | (0.000) e |
| F-value$^{***}$ | 15.54$^{***}$ | | | 7.91$^{***}$ | | | 5.78$^{**}$ | | | 39.87$^{***}$ | | | 39.87$^{***}$ | | | 22.89$^{***}$ | | | 27.67$^{***}$ | |

[a] ALB = Albenga, CAR = Carlota, CLI = Climberly, DEL = Delizia, Globo = GLO, LIC = Licorossa, MARI = Maribel , MARM = Marmandino One, MUR = Murano, PAN = Panarea, P. BO = Principe Borghese, RED = Red Delight , TYT = Tyty.

[b] Means in a column with different letters are statisitcally different (P<0.05 Duncan's MRT).

[c] fw = fresh weight.

[d] mhca = monohydrate citric acid.

$^{***}$ P<0.001.

$^{**}$ P<0.01.

**Table 3**
Consumers' overall liking.

| Cultivar | Overall-liking | | | | | |
|---|---|---|---|---|---|---|
| | μ (n = 100)[b] | | SD | 25° | 50° | 75°[c] |
| TYT | 7.1 | a | 1.55 | 6 | 7 | 8 |
| PAN | 6.7 | ab | 1.89 | 5 | 7 | 8 |
| GLO | 6.6 | ab | 1.79 | 5 | 7 | 8 |
| RED | 6.5 | ab | 1.89 | 6 | 7 | 8 |
| MARI | 6.5 | ab | 1.92 | 6 | 7 | 8 |
| CLI | 6.4 | b | 1.86 | 5 | 6 | 8 |
| LIC | 6.4 | b | 1.94 | 5 | 7 | 8 |
| P.BO | 6.3 | b | 1.91 | 5 | 7 | 8 |
| CAR | 6.2 | b | 1.98 | 6 | 6.5 | 8 |
| MUR | 5.4 | c | 2.30 | 4 | 5 | 7 |
| DEL | 5.3 | c | 2.10 | 4 | 5 | 7 |
| ALB | 5.2 | c | 2.28 | 4 | 5 | 7 |
| MARM | 5.0 | c | 2.20 | 3 | 5 | 7 |
| F-value[a] | 11.475*** | | | | | |

[a] ***$P<0.001$.
[b] Values with different letters are significantly different at $P \leq 0.05$ Duncan's MRT.
[c] 1st, 2nd and 3rd quartiles.

### 2.4.2. Hedonic test

The target group was a sample of 100 adults responsible for food shopping or sharing the responsibility with others; 47 males and 53 females, with 30% between the ages of 18–34 years, 38% between 35–55 years and 32% over 55 years. The number of consumers is consistent with estimation of the number of consumers for sensory acceptability test from Hough et al. (2006) for an alpha value of 5%, a beta value of 10%, a difference between sample means of 10% of the evaluation scale and a standard error of 0.23. The consumers were recruited in one location (Naples, Italy) from a local agency and the criteria used for participation was that they liked and ate salad tomatoes regularly (required a minimum of once a month). Moreover, they were required to have not taken part in a market research survey on tomato within the last 3 months and not be working in related industries products.

The consumer test was run as a Central Location Test (CLT). Hedonic ratings of the 13 cultivars were obtained from consumers during two tasting sessions run in two consecutive days. Tomato samples, coded according to a design balanced for order and carry-over effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989), were presented in a monadic sequential of 7 or 6 samples per session (session length 1 h). Similarly to the descriptive analysis, consumers were instructed to cut cocktail and cherry tomatoes into two half portions, from the side with the stalk to the apex and again to cut longitudinally each half into two equal parts; for larger tomatoes, each quarter was cut again along the longitudinal axis to obtain eight wedges. Then, the respondents were asked to remove any residual part of the stalk from the base of the tomato and to put in the mouth one of the tomato's wedges for overall liking evaluation, which was done on a 9-point hedonic scale anchored with "dislike extremely" (1) and "like extremely" (9), and hence with point 5 as "neither like, nor dislike".

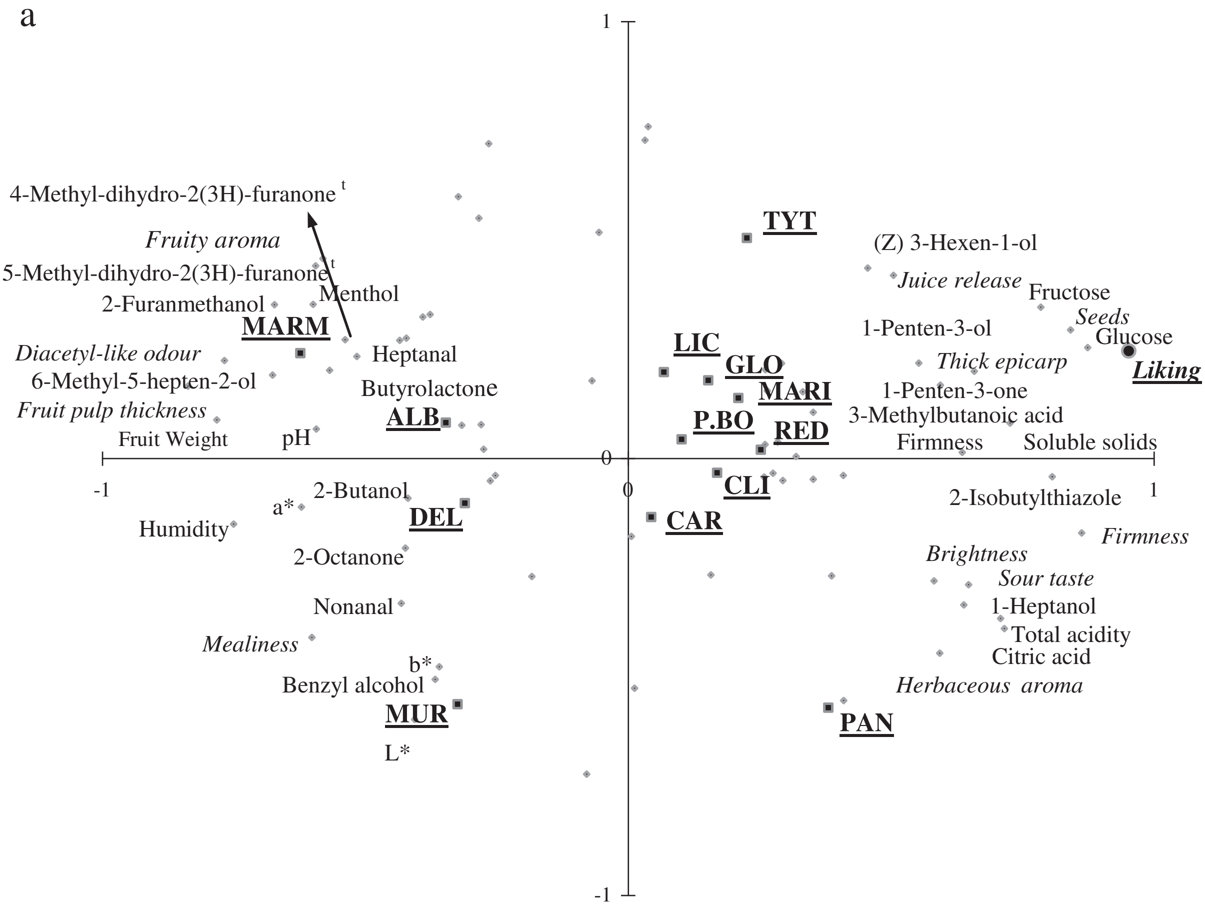

**Fig. 1.** PLS-1 loading plot (PC1 vs. PC2) for the selected GC/MS volatiles, and all sensory descriptors, physicochemical parameters (X data) versus liking (Y data). a. Harvest replicate 1, $R^2$x Cumulative = 0.356; $R^2$y Cumulative = 0.968. b. Harvest replicate 2. $R^2$x Cumulative = 0.382; $R^2$y Cumulative = 0.970. Sensory descriptors are labelled in italic. t tentative of identification (pure chemical standard was not available).

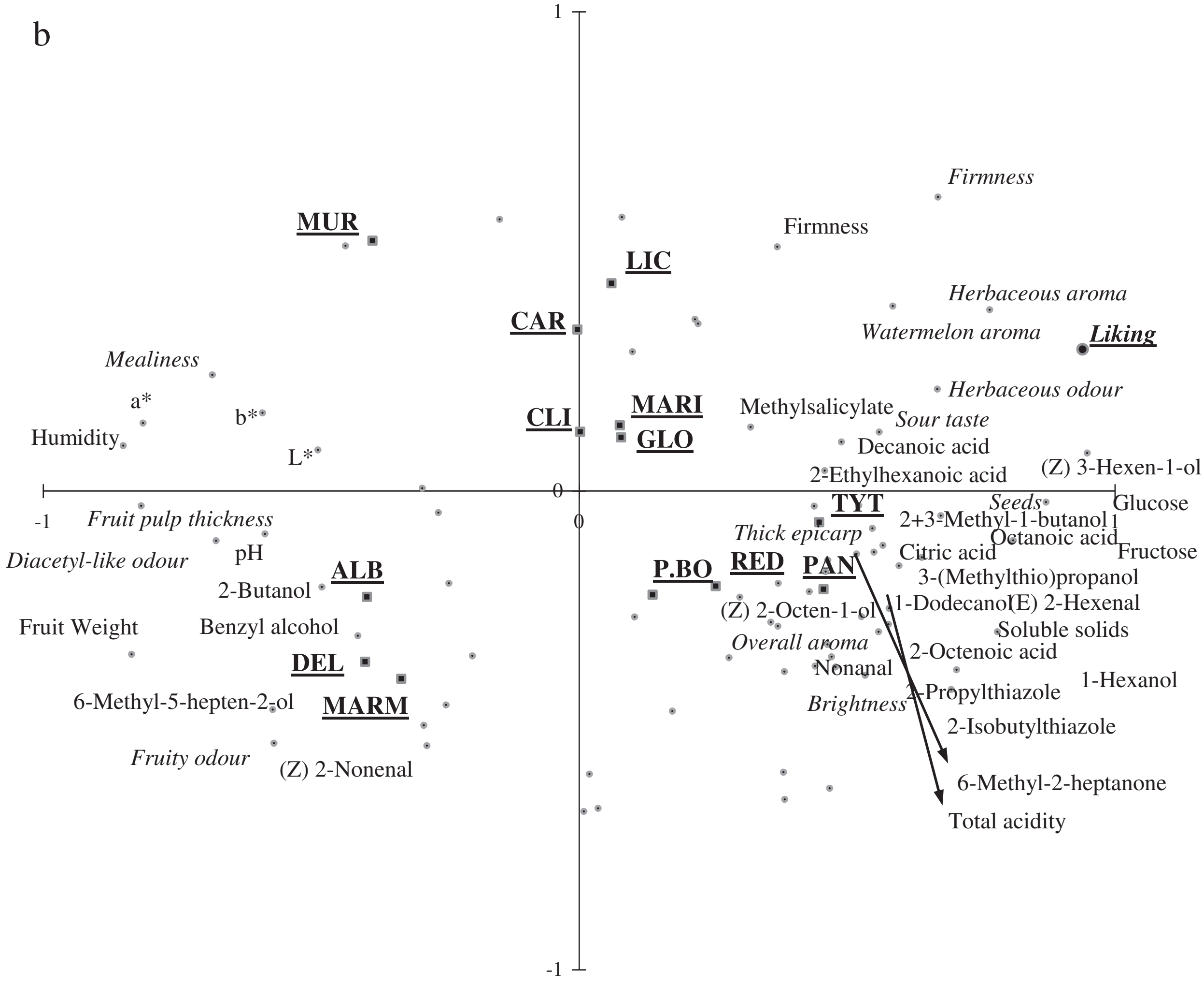

**Fig. 1** (*continued*).

### 2.5. Data analysis

Descriptive statistics, Pearson's correlation coefficients and analysis of variance (ANOVA) were calculated using XLStat 11.4 2009 (Addinsoft). Significant ($P<0.05$) correlation coefficients were considered moderate when $0.55<|r|<0.70$ and strong when $|r|\geq0.70$ (Jackson, 2012). The analysis of variance was applied for determining the significant physicochemical parameters and sensory descriptors and assessing the repeatability and the agreement within the panel. Differences among means were determined by Duncan's Multiple Range Test (MRT) ($P<0.05$).

With the aim of reducing the complexity of the data and facilitating results interpretation, multivariate modeling by PLS-1 of tomato liking (y data) on the complete set of volatiles (x data) was performed independently for each harvest replicate to identify volatiles providing higher contribution to the prediction model. The importance of the explanatory variables for the building of the t components was deduced by the variable importance for the projection (VIPs), which allows identifying the variables that are moderately ($0.8<\mathrm{VIP}<1$) or highly influential ($\mathrm{VIP}\geq1$) (Eriksson, Johansson, Kettaneh-Wold, & Wold, 2001). In order to reduce the probability of losing useful information, all volatiles having $\mathrm{VIPs}\geq0.7$ in PC1 or PC2 were selected for further elaborations (Supplementary Table S2a,b).

A further modeling with PLS-1 was explored using the selected volatiles based on VIP values, sensory descriptors and physicochemical parameters (X data) and liking (Y data). The PLS regression function of XL-Stat was used for these analyses.

Data were normalized using the 1/SD transform to remove scale effects.

## 3. Results and discussion

### 3.1. Physicochemical analyses

The ANOVA revealed significant ($P<0.01$) differences among varieties for all traits and for both harvest replicates, except for humidity in the first replicate (Table 2a, b). Trait means, averaged over the two replicates, indicated that CAR (Cluster typology) was the cultivar with the firmest fruits, as indicated by the highest firmness value (27.3 N), while MARM was the least firm one (10.7 N). The two varieties PAN (Cherry typology) and RED (Cocktail typology) had the highest soluble solids (5.97 and 5.91°Brix, respectively) and glucose (1.99 g/100 g) contents, associated with the highest values of total acidity (0.675 and 0.647 g/100 g monohydrate citric acid, respectively) and citrate content (0.648 and 0.608 g/100 g, respectively). In contrast, MUR (S. Marzano type) had the lowest soluble solids and glucose contents (4.14°Brix and 1.15 g/100 g, respectively), while ALB (Cuore di Bue typology) had the lowest total acidity (0.42 g/100 g monohydrate citric acid).

### 3.2. Sensory analysis

The results of the F-tests for the cultivar effect of the measured descriptors revealed significant differences for all descriptors and for both harvest replicates (Supplementary Table S3a, b), with a good consensus between the panelists, estimated through the "cultivar by panelist" (C × P) interaction. Some descriptors, such as "bitter aftertaste", "watermelon aroma" and "diacetyl-like aroma" registered very low mean scores for all the cultivars, but the majority of descriptsors spanned a wide range of differences on the evaluation scale. For the sake of brevity sensory differences among tomato varieties are discussed in Section 3.6 related to the multivariate analysis.

### 3.3. Consumer liking

The hedonic test revealed that on average consumers provided higher hedonic scores at 5% significant level for the cultivars TYT (Cherry), PAN (Cherry), GLO (Cluster), RED (Cocktail) and MARI (Cluster), while lower mean scores were expressed for the cultivars MUR (S. Marzano typology), DEL (Marmande), ALB (Cuore di Bue) and MARM (Marmande). However, PAN, GLO, RED and MARI were not statistically different from CLI, LIC, P.BO and CAR (only TYT presented hedonic score statistically higher than CLI, LIC, P.BO and CAR) (Table 3).

The four cultivars RED, GLO, MARI and CLI, along with other French and Dutch fresh market tomatoes, had already been evaluated in previous preference mapping studies conducted with Italian, French and Dutch consumers (Causse et al., 2010; Sinesio et al., 2010). Although those studies identified consumer segments with different preferences, the cultivar RED, alone or together with another cocktail cultivar, was the most liked genotype by the overall consumer panels. In our study, the addition of the two cherry varieties, TYT and PAN, allowed to increase the sensory space, and, consistently with previous findings, the cherry typologies received the highest "overall liking" scores (Causse et al., 2010). Also the cultivars DEL, ALB and MAR contributed to increase the sensory space, as can be seen from their opposite position on the map if compared to all the other samples (see Section 3.6, Fig. 1a, b).

### 3.4. Identification of aroma volatiles

In the set of 13 genotypes, GC/MS analysis allowed the identification of 78 and 75 different volatiles in whole fruit samples from the first and second harvest replicates, respectively (Supplementary Table S2a, b). In both cases, the most abundant classes were alcohols and aldehydes, followed by ketones, furanones/lactones, acids, sulfur compounds, esters and phenols. As specified in materials and methods, some of these compounds were tentatively identified$^{(t)}$. Among them, 3-methyl-dihydro-2(3H)furanone$^{(t)}$, 4-methyl-dihydro-2(3H)furanone$^{(t)}$ and 5-methyl-dihydro-2(3H)furanone$^{(t)}$ have chemical structures similar to other compounds previously reported as tomato's volatiles (Clarke & Bakker, 2004). Generally furanones/lactones show a range of flavor characteristics, but furan-2-ones tend to be fatty and herbaceous, especially the lower molecular weight compounds, such as those we tentatively identified (Clarke & Bakker, 2004).

In line with previous results, the richest volatile profile, including 72 compounds, was found in P.BO (Lisanti et al., 2008), whereas the lowest number of 53 volatiles was identified in CLI (data not shown).

### 3.5. Correlations between physicochemical parameters, sensory descriptors and volatiles

An overview of the Pearson correlation analysis conducted for each harvest replicate between physicochemical parameters, sensory descriptors, and a subset of 46 volatiles is shown in a heat map-format in Supplementary Fig. S1. Here we will discuss only some of the significant ($P < 0.05$) correlations averaged over the two harvest replicates.

With the exception of malate and firmness, most physicochemical traits were significantly correlated between them. Several of these correlations were expected, and were in agreement with previous findings (Carli, Barone, Fogliano, Frusciante, & Ercolano, 2011; Carli et al., 2009; Causse et al., 2003), although different trends of correlations between traits can be detected in different sets of genotypes, as it was observed in large- vs. small-fruited hybrids by Causse et al. (2003) or in cherry, beef and round tomatoes by Ursem, Tikunov, Bovy, van Berloo, and van Euwijk (2008). For example, the moderate negative correlation observed in our study between fruit weight and brix ($r = -0.58$) was found by Causse et al. (2003) only in the small-fruited hybrids; while the strong negative correlation observed between total acidity and pH ($r = -0.73$) and the strong positive correlation between $L^*$ and $b^*$ ($r = 0.93$) were found by Causse et al. (2003) only in the large-fruited hybrids. Significant strong relationships were also detected between some of the sensory descriptors. Although, differences in definition of specific descriptive notes make it difficult to compare results across different studies, still a few common trends could be found. The strongest positive correlation was reported between sweet taste and fruity aroma ($r = 0.85$); sweet taste also showed a strong positive correlation with fruity odor ($r = 0.73$), and a moderate positive with juiciness ($r = 0.63$). A positive correlation between juiciness and sweetness was also observed by Carli et al. (2009) in a set of six traditional tomato landraces, and by Causse et al. (2003) in the set of large-fruited hybrids, while in the small-fruited hybrids they found a negative correlation. Consistent with previous findings (Causse et al., 2003), a moderate negative correlation was observed between juiciness and mealiness ($r = -0.69$). This negative relationship could be explained by the fact that in mealy fruit tissue disruption occurs between cells (middle lamellae), rather than involving cells breaking across cell walls which would release cellular components, resulting in juiciness (Baldwin et al., 2000). A strong positive correlation was found between seeds and firmness ($r = 0.71$), and a moderate negative between seeds and diacetyl-like odor ($r = -0.69$); this latter descriptor had a strong negative link with firmness ($r = -0.71$), and a moderate negative with herbaceous odor ($r = -0.67$). In agreement with previous observations (Carli et al., 2009), overall a lower number of strong correlations were found between sensory descriptors and physicochemical traits.

As regards the volatiles, in line with previous studies (Tikunov et al., 2005; Zanor et al., 2009), groups of highly correlated metabolites were found (Supplementary Fig. S2). The major group was represented by lipid derivatives and correlated compounds (saturated and unsaturated C5, C6 and C7 aldehydes and alcohols). Strong correlations were also observed between guaiacol and methyl salicylate ($r = 0.91$), and between ethyl salicylate and eugenol ($r = 0.86$), all phenylpropanoid volatiles. Some other biochemically correlated odorous secondary metabolites were grouped in little clusters, including isoprenoids (6-methyl-2-heptanone, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one), lactones (4-methyl-dihydro-2(3H)-furanone$^{(t)}$, butyrolactone, 5-methyl-dihydro-2(3H)-furanone$^{(t)}$), benzenoids (benzyl alcohol, benzaldeyde, 2-phenylethanol) and thiazoles (2-sec-butylthiazole; 2-propylthiazole). Among all volatiles, 2-isobutylthiazole and 6-methyl-5-hepten-2-ol showed, in both harvest replicates, the highest number of significant correlations with physicochemical traits and sensory descriptors.

### 3.6. Effects of sensory, physicochemical and volatile data on liking

To identify the volatiles providing higher contribution to the prediction model on tomato liking a first modeling with PLS-1 was performed. A total of 43 and 45 compounds were selected for the first and second harvest replicates, respectively, with 23 volatiles

**Table 4**
Explanatory variables with VIP≥0.8 in both harvest replicates, and their effect on consumer liking.

| Variable | Replicate 1 | Replicate 2 | Effect on liking |
|---|---|---|---|
| Seeds | **1.910** | **1.233** | + |
| Glucose | **1.857** | **1.685** | + |
| Firmness (sensory) | **1.747** | **1.730** | + |
| Fructose | **1.722** | **1.523** | + |
| Fruit pulp thickness | **1.717** | **1.538** | − |
| Humidity | **1.595** | **1.586** | − |
| 2-Isobutylthiazole | **1.568** | **1.035** | + |
| Fruit weight | **1.555** | **1.837** | − |
| Diacetyl-like odor | **1.470** | **1.453** | − |
| Thick epicarp | **1.446** | *0.849* | + |
| Soluble solids content | **1.432** | **1.400** | + |
| Firmness (instrumental) | **1.344** | **1.032** | + |
| Mealiness | **1.335** | **1.234** | − |
| 6-Methyl-5-hepten-2-ol | **1.282** | **1.433** | − |
| $a^*$ | **1.243** | **1.537** | − |
| Sour taste | **1.210** | *0.971* | + |
| pH | **1.174** | **1.115** | − |
| Total acidity | **1.170** | *0.800* | + |
| Citric acid | **1.157** | **1.016** | + |
| (Z) 3-Hexen-1-ol | **1.156** | **1.966** | + |
| Brightness | **1.132** | *0.856* | + |
| $L^*$ | **1.100** | *0.988* | − |
| Herbaceous aroma | **1.083** | **1.660** | + |
| Nonanal | **1.013** | *0.973* | +/− |
| 2-Butanol | **1.007** | **1.023** | − |
| Benzyl alcohol | *0.928* | **1.051** | − |
| $b^*$ | *0.908* | **1.166** | − |

In bold VIP≥1 and in italic 0.8≤VIP<1.

**Table 5**
Peak area of 2-isobutylthiazole (n = 3 for each harvest replicate) expressed as percentage of the total volatiles peak area (n = 3 for each harvest replicate), and tomato overall liking scores in 13 tomato varieties. The genotypes are listed according to decreasing order of overall liking (average score).

| Cultivar | 2-Isobutylthiazole (peak area) | Total volatiles (∑ peak area) | 2-Isobutylthiazole/ total volatiles (%) | Tomato overall liking (average score) |
|---|---|---|---|---|
| *Replicate 1* | | | | |
| TYT[a] | 0.23 | 3.60 | 6.38 | 7.2 |
| GLO | 0.21 | 3.40 | 6.17 | 6.8 |
| RED | 0.26 | 2.53 | 10.27 | 6.8 |
| CLI | 0.21 | 2.11 | 9.25 | 6.7 |
| MARI | 0.22 | 3.02 | 7.28 | 6.7 |
| PAN | 0.39 | 4.88 | 7.99 | 6.6 |
| P BO | 0.12 | 7.16 | 1.68 | 6.4 |
| CAR | 0.04 | 2.55 | 1.57 | 6.3 |
| LIC | 0.10 | 1.62 | 6.17 | 6.3 |
| DEL | 0.06 | 2.55 | 2.35 | 5.3 |
| ALB | 0.04 | 3.30 | 1.21 | 5.0 |
| MUR | 0.02 | 3.65 | 0.55 | 5.0 |
| MARM | 0.06 | 2.57 | 2.33 | 4.6 |
| *Replicate 2* | | | | |
| TYT | 0.36 | 4.10 | 8.78 | 6.9 |
| PAN | 0.85 | 5.77 | 14.73 | 6.7 |
| LIC | 0.15 | 1.44 | 10.41 | 6.5 |
| GLO | 0.27 | 4.50 | 6.00 | 6.4 |
| MARI | 0.36 | 4.14 | 8.70 | 6.4 |
| RED | 0.53 | 3.40 | 15.59 | 6.3 |
| CAR | 0.04 | 2.84 | 1.41 | 6.2 |
| P BO | 0.31 | 4.45 | 6.97 | 6.2 |
| CLI | 0.27 | 1.88 | 14.36 | 6.1 |
| MUR | 0.03 | 3.99 | 0.75 | 5.7 |
| ALB | 0.07 | 3.22 | 2.17 | 5.5 |
| MARM | 0.08 | 4.05 | 1.98 | 5.4 |
| DEL | 0.36 | 2.60 | 13.85 | 5.2 |

[a] ALB = Albenga, CAR = Carlota, CLI = Climberly, DEL = Delizia, Globo = GLO, LIC = Licorossa, MARI = Maribel, MARM = Marmandino One, MUR = Murano, PAN = Panarea, P.BO = Principe Borghese, RED = Red Delight, and TYT = Tyty.

resulting influential variables in both replicates (Supplementary Table S2a, b). Most of these molecules directly or indirectly originate from amino acids (like methyl branched alcohols, benzenoids and S-containing compounds) and fatty acids (like C6 and correlated compounds), so that, volatile secondary metabolites deriving from these two pathways seem to have a major role on tomato liking.

The selected volatiles based on VIP values, along with sensory and physicochemical data were globally analyzed by a second PLS-1 analysis. The purpose of multivariate data modeling was to assist in the interpretation of the factors that are linked with consumer liking of the tomato cultivars and to analyze the relationships among volatiles, physicochemical parameters and sensory descriptors. Fig. 1a, b shows the correlation loading plots (PC1 *vs* PC2) for the X data and their relationship with liking (mean value) for the first and second replicates, respectively. For the first harvest replicate, the explanatory power of the independent variables of the model (cumulative $R^2X$ index) was 0.36 and the explanatory power of the model for liking (cumulative $R^2Y$) was 0.97. For the second replicate, the cumulative $R^2X$ and $R^2Y$ were 0.38 and 0.97, respectively.

The tomato samples differentiated for segments. In the first harvest replicate, samples belonging to the typologies Marmande (MARM, DEL), Cuore di Bue (ALB) and the local variety MUR were separated from the typologies Cluster (CAR, CLI, GLO, MARI), Cocktail (LIC, RED) and Cherry (TYT, PAN) and from the local variety P.BO along the PC1 (Fig. 1a). PC2 showed a few differences among the cultivars, although this dimension provided only a little contribution to overall liking. Similar results were obtained for the second harvest replicate (Fig. 1b).

In both harvest replicates, the most liked tomatoes (Cherry, Cocktail, Cluster, and the local variety P.BO) were sensory described by the analytical panel as having stronger firmness, sour taste, herbaceous aroma, brightness, as well as higher perceived content of seeds and thicker epicarp. These varieties also had higher soluble solids, reducing sugars (glucose and fructose), total acidity, citrate content, and stronger instrumentally measured firmness (Fig. 1a, b). Consistent with the Pearson's correlations reported above (Section 3.5), the perceived sour taste was strongly related to higher citrate content and total acidity, and therefore to lower pH values, as found in other studies (Causse et al., 2003; Tandon et al., 2003). In the present work, three of the less liked tomato cultivars, MARM, ALB and DEL, were perceived as having intense fruity aroma/odor, diacetyl-like odor, fruit pulp thickness and mealy texture. These cultivars also had higher fruit weight, humidity as well as $L^*$, $a^*$ and $b^*$ values. The local variety MUR was less liked mainly because of its higher mealy texture and lower firmness.

As regards the volatiles, in the first harvest replicate, six aroma compounds (mostly responsible for herbaceous/vegetal odors) resulted positively correlated to liking (Fig. 1a). In the second harvest replicate a larger number of volatiles positively linked to liking of tomato samples were identified (Fig. 1b).

The positive effect on liking of the two volatiles (Z)-3-hexen-1-ol and 2-isobutylthiazole was confirmed in both replicates (Fig. 1a, b). 2-Isobutylthiazole seems to play a primary role as aroma compound showing the highest projection on the component, well representing liking, in the first harvest replicate (Fig. 1a), and a good projection in the second harvest replicate (Fig. 1b); in the latter case, the highest projection was showed by (Z)-3-hexen-1-ol.

Already Pyne and Wick (1965) pointed out that the "green" notes of (Z)-3-hexen-1-ol contributed significantly to tomato flavor; moreover, it is well known that differences in vegetable volatiles deriving from lipids oxidation, like (Z)-3-hexen-1-ol and the C5 alcohols, depend on the tomato genotype as well as the harvest and the fruit crushing. 2-Isobutylthiazole is a heterocyclic sulfur compound deriving from aminoacid metabolism; it has been described as impact volatile compound, with a tomato green/leafy odor (Hongsoongnern & Chambers, 2008). 2-Isobutylthiazole is particularly interesting because some authors report that, differently from other volatiles, such as

(Z)-3-hexen-1-ol, its concentration depends on the genotype and it is not affected by crushing procedure, duration or oxygen exposition (Boukobza, Dunphy, & Taylor, 2001; Kazeniac & Hall, 1970).

As regards the volatiles linked to the less liked cultivars, 11 were identified in the first harvest replicate (Fig. 1a). The negative effect on liking of 2-butanol, benzyl alcohol, 6-methyl-5-hepten-2-ol together with (Z)-2-nonenal (all volatiles mostly characterized by fruity and fatty odors) was confirmed by the analyses performed on the second harvest replicate (Fig. 1b).

For both replicates, 6-methyl-5-hepten-2-ol was the volatile showing the highest projection on the negative side of the X axis, where the less preferred genotypes lay (Fig. 1a, b). 6-Methyl-5-hepten-2-ol is associated with fruity aroma, which was stronger in MAR and ALB (data not shown). 6-Methyl-5-hepten-2-ol is an open chain carotenoid derivative, and its synthesis increases during fruit ripening as a consequence of carotenoids cleavage. 6-Methyl-5-hepten-2-ol together with the corresponding keton 6-methyl-5-hepten-2-one ($VIP > 0.7$ in both harvest replicates) and 6,10-dimethyl-5,9-undecadien-2-one are associated with fruity notes, but they seem to have an important flavor effect in tomato juice and dilute paste. An increasing concentration of these compounds is involved in the development of heated paste notes in processed tomato juice. In fact, when a mixture of these 3 compounds was added, tomato juice acquired the typical flavor notes of heated, canned tomato pastes (Kazeniac & Hall, 1970). Moreover, consistent with previous findings by Sinesio et al. (2000), 6-methyl-5-hepten-2-ol showed a strong positive correlation with diacetyl-like odor.

The results obtained with our set of cultivars indicate that volatiles linked to the most liked tomatoes are mainly characterized by herbaceous/vegetable odors while those best correlated to the less liked samples are often fruity/oily (volatile descriptors are from Flavour & Fragrances, 2003–2004). The lower liking scores observed for the more fruity tomatoes seem to be in contrast with other studies that have reported fruity, floral aroma notes to be preferred in consumer panels (Baldwin, Goodner, Plotto, Pritchett, & Einstein, 2004). On the other hand, these results are in agreement with our previous research findings, which highlighted the existence among Italian consumers of segments having higher preferences for fresh tomatoes characterized by higher herbaceous and lower fruity notes (Sinesio et al., 2010). Moreover, it is also possible that in the present study the attribute “fruity” resulted to be negatively related with liking because of the simultaneous contribution of the diacetyl-like odor, weekly perceived in ALB, DEL and MARM, and for the higher mealiness and lowest firmness characterizing these three varieties.

The variables common to the two harvest replicates having high ($VIP \geq 1$) or moderate ($0.8 < VIP < 1$) importance for the prediction of liking are summarized in Table 4. Although for some variables the VIP values differ between the two harvest replicates, likely reflecting a biological variability, yet in all cases the values respect the conditions of the model we used to identify explanatory variables for tomato liking (Eriksson et al., 2001).

The main sensory drivers of liking were seeds, firmness, thick epicarp, sour taste, brightness and herbaceous aroma (Table 4). In addition, the main physicochemical parameters with a positive effect on liking were glucose, fructose, soluble solids, firmness, total acidity and citrate. However, no significant correlation between sweet taste and liking was found; this result could be due to the fact that the amount of acids present can influence the perception of sweetness (Malundo et al., 1995).

Consistent with our previous findings (Sinesio et al., 2010), a positive effect on liking was attributed to the seeds. These results could be explained with the positive correlations observed between seeds and reducing sugars (glucose and fructose) and firmness on the one hand, and the negative correlations between seeds and mealiness, on the other hand (Supplementary Fig. S1). Mealiness, in fact, had a negative impact on liking.

Among the volatiles, 2-isobutyltiazole and (Z) 3-hexen-1-ol were confirmed to be associated with overall liking. These volatiles seem to be the main responsible of the herbaceous note perceived in the tomato samples. Indeed, positive significant correlations were found between herbaceous aroma (retro-nasal) and the two volatiles 2-isobutyltiazole and (Z) 3-hexen-1-ol (Supplementary Fig. S1). The important role of (Z)-3-hexen-1-ol on consumer acceptability for fresh market tomatoes has also been reported in previous studies (Berna et al., 2005). More recently, the correlation between (Z)-3-hexen-1-ol and flavor intensity has been confirmed using transgenic plants modified to no longer express the 13-lipoxygenase (*LoxC*) gene, which codifies for the enzyme required to produce C-6 volatiles from 18:2 and 18:3 fatty acids (Tieman et al., 2012). Although, the authors did not find significant differences in preference between the control and transgenic plants assessed by consumers in United States, it might be possible that different responses would be observed testing consumers with different habits, such as those used in our study.

2-Isobutylthiazole is characterized by a tomato leaf odor and, as recently reported by Lisanti et al. (2008), several authors suggested it as a key-component of tomato aroma. It seems however that its contribution to tomato aroma can be positive or negative depending on the concentration. According to Kazeniac and Hall (1970), at concentrations ranging from 25 to 50 ppb, 2-isobutyltiazole positively contributes to fresh tomato flavor while at higher levels, its flavor becomes objectionable, described as rancid, medicinal or metallic. In order to further explore the role of 2-isobutylthiazole on tomato liking, the percentage represented by the peak area of this volatile with respect to the total volatiles area was calculated for each tomato cultivar and for both harvest replicates (Table 5). For almost all the genotypes showing a positive correlation with liking (Fig. 1a, b) and a mean liking score > 6, the 2-isobutylthiazole peak area was > 6% of the total peak area (Table 5). Exceptions to this trend were the cultivars P.BO and CAR in harvest replicate 1, and CAR and DEL in harvest replicate 2. Most likely, for P.BO the higher than average liking score (6.4) associated with a weak contribution of 2-isobuthyltiazole (1.68%) could be due to the quantitative and qualitative richness of its volatile fraction (data not shown); whereas CAR was characterized by low levels of 2-isobutylthiazole and total volatiles, in both replicates. In addition, this genotype had the lowest levels of 6-methyl-5-hepten-2-ol among all cultivars analyzed (data not shown). For harvest replicate 2, the low liking score of DEL, in spite of a strong contribution of 2-isobuthyltiazole (13.85%), could be due to a higher 6-methyl-5-hepten-2-ol content, which was 2-5 times higher than in the other 12 tomato genotypes (data not showed). Therefore, the positive effects of 2-isobuthyltiazole on liking seem to be suppressed by an increase of 6-methyl-5-hepten-2-ol content, especially when the volatile fraction of tomato is not rich.

## 4. Conclusions

In this study it was possible to observe that consumers gave higher overall liking scores to the cultivars characterized by herbaceous/green notes, higher contents of 2-isobutylthiazole and (Z) 3-hexen-1-ol, sour taste, higher citrate and reducing sugars and stronger firmness, which in our set of samples belonged to the typologies Cherry, Cocktail and Cluster. In contrast consumers gave overall lower liking scores to cultivars which were perceived as having intense fruity aroma/odor, diacetyl-like odor, fruit pulp thickness, mealy texture and lower firmness. Furthermore, our results suggest that 2-isobutylthiazole may be considered as a molecular marker of consumer liking; in fact, samples with 2-isobutylthiazole representing at least 6% of the overall tomato volatile fraction, were most liked. This positive contribution seems to be supported by (Z) 3-hexen-1-ol and suppressed by 6-methyl-5-hepten-2-ol, especially when tomatoes are characterized by a poor whole volatile fraction. However, these results should be confirmed in further investigations extended to a wider range of tomato cultivars and metabolites, including fatty acids and amino acids, as well as to a more diversified set of consumers.

In conclusion, this study represents a contribution towards the identification of key parameters underlying consumer liking for fresh market tomatoes that could lead to more effective breeding strategies aimed at improving tomato sensory quality.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2012.10.033.

## Acknowledgments

The authors would like to acknowledge the sensory panel (INRAN, Rome, Italy) and all consumers for their kind participation in the sensory or hedonic tests, Antonella Vitiello and Michele De Martino for technical assistance; Luciana Monetti and Gianni Ottaviani for excellent plant management and cultivation. This work was funded by the Italian MIUR project GenoPOM and by the EUSOL project PL 016214-2.

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