Valle Estela (Orcid ID: 0000-0001-8256-0623)

Running title: Chemical composition of tasty tomato fruit of the Andean varieties

CONTRASTING METABOLIC PROFILES OF TASTY TOMATO FRUIT OF THE ANDEAN VARIETIES IN COMPARISON WITH COMMERCIAL ONES

Matilde D´Angelo[1,7], María I. Zanor[1,7], María Sance[2], Pablo R. Cortina[3], Silvana B. Boggio[1], Pablo Asprelli[2], Fernando Carrari[4], Ana N. Santiago[3], Ramón Asís[5], Iris E. Peralta[2,6], Estela M. Valle[1]*

[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), Rosario, Argentina

[2] Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina.

[3]Instituto de Investigación en Físico-Química de Córdoba (INFIQC-CONICET), Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

[4]Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (IB-INTA), and CONICET, B1712WAA Hurlingham, Argentina

[5] Facultad de Ciencias Químicas – CIBICI, Universidad Nacional de Córdoba – CONICET, Córdoba, Argentina

[6] IADIZA CCT-CONICET, Mendoza, Argentina.

[7] These authors contributed equally to this work

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.8930

***Corresponding author:**

Estela M. Valle e-mail: valle@ibr-conicet.gov.ar

ABSTRACT

BACKGROUND

Fruits of most commercial tomato (*Solanum lycopersicum* L.) cultivars are deficient in flavour. In contrast, traditional "criollo" tomato varieties are appreciated for fruit of excellent organoleptic quality. Small farmers from Argentine Andean Valleys have maintained their own tomato varieties, which were selected mainly for flavour. This work aims to correlate the chemical composition of the fruit with the sensory attributes of eight heirloom tomato varieties. The long-term goal is to identify potential candidate genes capable of altering chemicals involved in flavour.

RESULTS

Sensory analysis and metabolomics of fruit were determined. The data revealed that defined tomato aroma and sourness correlated with citrate, and several volatile organic compounds (VOC), such as α-terpineol, *p*-menth-1-en-9-al, linalool and 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (DMHEX), a novel volatile recently identified in tomato. Instead, the sensory attributes sweetness and not acidic taste correlated with characteristic tomato taste, and, besides fructose and glucose, with two VOCs, benzaldehyde, and 2-methyl-2-octen-4-one.

CONCLUSIONS

These data provide new evidences of the complex chemical combination that induced the flavour and aroma of the good-tasting "criollo" tomato fruit. That is, the compounds that correlated with defined tomato aroma and acidic taste did not correlate with sweetness, or with characteristic tomato taste.

KEYWORDS:

metabolomics profile; tomato fruit; tomato landraces; sugars; volatile compounds

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is one of the most important crops worldwide[1] and because of its high consumption dramatically contributes to human diet. Modern commercial tomato cultivars have been mainly selected for high yield, disease and pest resistances, fruit firmness, transportation tolerance and long shelf life, but are deficient in fruit taste and flavour and not well accepted by consumers[2]. Genomic analysis of 360 accessions revealed that modern tomato is narrowed in the genetic basis due to conventional breeding[3], and provides molecular insights toward further improvement.

The original place of tomato domestication has been a topic of interesting debate, and recently a two-step process in America has been supported[4]. A first selection occurred in the Andean region of Peru and Ecuador, and a second phase in Mesoamerica where native people cultivated tomatoes for consumption. Most probably from there, tomatoes were introduced in Europe in the mid-16th century[4]. Early tomato cultivation and consumption in Europe were first reported in Italy[5] and extended other countries worldwide. Modern tomato breeding generates productive commercial varieties but with flavour deficiencies that had been chemically identified[6]. In contrast, heirloom varieties are usually appreciated for the good organoleptic quality of their fruits, and recently the compounds that made the most significant contributions to flavour and consumer preferences have been recognised[6]. Andean farmers contribute to maintain the diversity of tomato varieties that adapted to specific conditions in villages situated in limited geographical areas[7]. New interests are focused in traditional tomato varieties as genetic sources of quality traits. Particular morphological and agronomical characteristics[8] as well as biochemical composition[9], and phenotypic diversity[10] have been reported in tomato landraces. Likewise, a diverse chemical composition involved in fruit taste and functional quality was found in a collection of local varieties from Valencia, a Mediterranean region of Spain[11]. More recently, another report[12] showed that Italian tomato landraces with different fruit type had significant changes in quality related to metabolites, depending on their genetic background.

In Andean valleys of Argentina, agriculture is mainly performed by small families, in which traditional varieties of vegetable landraces are highly appreciated for their flavour, colour, and aromas[13,14]. Farmers typically keep their own seed that are adapted to marginal environments, where commercial cultivars do not usually perform well[15,16]. Recently, an interesting collection of traditional tomatoes have been recovered in Argentina[14], mainly from Andean areas, and maintained in the Germplasm Bank of the National Institute of Agricultural Technology. These tomato accessions displayed significant differences regarding fruit morphological characteristics, agronomic performance, and metabolic composition[17]. Furthermore, the evaluation of hydrophilic antioxidant composition in the same tomato germplasm found an association with fruit traits, geographical origin, and altitude, and showed that landraces had the highest levels of most antioxidants in comparison with commercial varieties and wild species[18].

Previous efforts to link the chemical composition of tomato fruit with the organoleptic properties have been made. Primary metabolite contents and volatiles were assessed in fruit of a subset of tomato lines, containing marker-defined introgressions in five regions controlling fruit quality variation from the cherry tomato into large-fruited genetic backgrounds[19]. The extensive profiling, combined with the results of a trained tasting panel, allowed the identification of some metabolic quantitative trait loci (QTL), which co-localized with sensory QTL. In other studies, sensory attributes contributing to organoleptic perception, such as sweetness, saltiness, and sourness for taste showed weak connectivity among themselves[20]. The authors reported that positive and negative contributors to tomato flavour could have direct implications for crop improvement strategies. A significant correlation between chemical quality attributes of tomato fruit with sensory determinants, such as skin firmness and sweet taste was revealed[21]. Sugar content in fruit was positively correlated with the overall tomato assessment. Firmness and sweet taste were significantly correlated with organic acids and soluble solids contents. Additionally, interactions between organoleptic perception such as taste (sweetness) and retronasal olfaction are of considerable interest in the chemical senses[22]. Although sweetness of tomatoes is widely thought to result from sugars, volatiles proved to be essential contributors to sweetness[22], as it was the case of the apocarotenoid geranial which positively correlated with sweetness. These authors suggested that aroma volatiles contributed to perceived sweetness independent of sugar concentration. More recently, other authors[23] reported that fruit flavour of different tomato genotypes grown in Florida correlated with many volatiles such as acetaldehyde, which also positively correlated with perception of sweetness and sourness. They concluded that tomato flavour quality is based on a balanced volatile profile with moderate acid levels and relatively high levels of sugars[23]. Newly, results from whole-genome sequencing of an extensive tomato germplasm, including 398 modern, heirloom and wild accessions, identified candidate loci capable of altering chemicals involved in flavour that also contributes to consumer liking[6]. Summarizing, the chemical definition of the characteristic tomato taste and flavour is highly variable, depending of the combination of sugars, acids and volatile compounds of the tomato fruit from different geographical origins.

The primary goal of this work is to determine the fruit chemical composition of Andean and commercial tomato varieties and establish associations with the organoleptic properties.

MATERIALS AND METHODS

Plant material

Nine tomato cultivars (accessions numbers or name 552, 557, 569, 571, 572, 3806, 4750, M82 and Garden Peach (GPEA) were obtained from the Horticulture Germplasm Bank of La Consulta Agricultural Experimental Station of the National Institute of Agricultural Technology (INTA), Mendoza, Argentina (Table S1). Among them, five tomato accessions (#552, #557, #569, #571, #572) were recovered from Andean regions of northwestern Argentina, one accession cultivated in Mendoza (#3806), one breeding advance line (accession #4750), and two commercial varieties (M82 and GPEA). Field crop evaluation was done at the Horticultural Institute of National University of Mendoza, Argentina (S 33°0.3’; W 68°52.2’; 912 meters above sea level) during 2009.

Seedlings were grown until four true leaves in 150-mL pots and transplanted spaced 30 cm in the row and a distance of 100 cm from row to row. A randomized parcel design with three repetitions of six plants was used, and a total of 18 plants per accession were grown. Fruits from all accessions were harvested at mature red stage (firm fruit) in a sunny day between 10:00 am and 16:00 pm on March 10th 2009. Fruit ripening stage of GPEA was determined by epicarp colour change green to pale yellow and by pressing it gently. For each accession, six different fruits were harvested from six plants. Mesocarp tissue of the harvested fruits was obtained by removing the epicarp, locule tissues, and seeds, and immediately frozen in liquid nitrogen and stored at –80 ºC until analysis of the primary metabolite composition by proton nuclear magnetic spectroscopy (^{1}H-NMR). Fully ripe fruit was used for the solid phase micro-extraction (SPME) and gas chromatography mass spectrometry (GC-MS) analyses. Three biological replicates (three fruits of different plants) were used for the chemical analyses. Frozen samples were ground with liquid nitrogen using a mortar and pestle until obtaining a homogeneous and fine powder, which was processed as described below. Data of sensory attributes and quantification of soluble metabolites and volatile compounds (VOCs) were integrated to find statistically significant correlations.

Sensory Analyses

Organoleptic trials were performed by tasting panels composed of 14 semi-trained volunteers. They were women and men ranging from 20 to 50 years old including smokers and non-smokers. Each panellist evaluated four tomato varieties in a sensory evaluation session. Fully ripe fruits were harvested early in the morning and prepared for tasting panels. Tomato fruit was cut into segments and seeds were removed. The sensory panel experiment was run equivalent to the Quantitative Descriptive Analysis methodology[24] but following a more extensive training stage. Fruit attributes were: characteristic tomato taste and aroma, sweetness and sourness. Each descriptor was evaluated at five levels: 1) Characteristic taste: uncharacteristic, slightly characteristic, mildly characteristic, moderately characteristic or characteristic; 2) Sweetness: not sweet, slightly sweet, mildly sweet, sweet or very sweet; 3) Sourness: very acidic, acidic, mildly acidic, slightly acidic or no acidic; and 4) Aroma: very indefinite, slightly indefinite, mildly indefinite, moderately defined or defined tomato aroma. Tomato fruits were presented to the panellists according to a Williams Latin Square design[25]. The frequency of the evaluation obtained for the level of each descriptor in the whole taste panel (i.e., 0.5 means that 50% of the panellists evaluated a given descriptor level) was represented by radial graphic using Microsoft Excel software 2010. Radial or spider graphs as useful tool to visually compare and contrast fruit flavour attributes of different tomato accessions.

^{1}H-NMR spectroscopy

Metabolic profiles by ^{1}H-NMR spectroscopy were performed according to previous procedures[26]. Fruit powder (1 g, obtained as described above) from each sample was rapidly dissolved in 0.3 mL of cold 1 M sodium phosphate buffer (pH 7.4) prepared in D_2O (deuterated water) to obtain a mixture containing about 30% by weight of D_2O. The mixture was centrifuged at 13 500 rpm for 15 min at 4 °C and the supernatant filtered to remove any insoluble material. Internal standard [TSP: 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid] (1 mM) was added to the resulting transparent soluble fraction, and the solution was subjected to spectral analysis at 600.13 MHz on a Bruker Avance II spectrometer. Proton spectra were acquired at 298 K by adding 512 transients of 32 K data points with a relaxation delay of 5 s. A 1D-NOESY pulse sequence was utilized to remove the water signal. The 90° flip angle pulse was always ~10 μs. Proton spectra were referenced to the TSP signal (′ = 0 ppm), and their intensities were scaled to that of TSP. Spectral assignment and identification of specific metabolites was established by fitting the reference ^{1}H-NMR spectra of several compounds using the software Mixtures, developed *ad hoc* as an alternative to commercial programs[27]. Further confirmation of the assignments for some metabolites was obtained by acquisition of new spectra after addition of authentic standards.

SPME GC-MS

Tomato VOC profiles by GC-MS and the identification procedure were the same as previously described[28]. Briefly, tomato fruit powder (1.0 g obtained as described above) was placed in a polypropylene tube (15 mL) and immersed in a water bath at 35 °C for 10 min. Next, 15 μL of 2-methylcyclohexanone (internal standard dissolved in methanol at a concentration of 23 mg L^{-1}) were added to the samples in addition to one mL ethylenediaminetetraacetic acid/sodium hydroxide (EDTA/NaOH) solution and CaCl$_2$ (2.2 g). EDTA/NaOH aqueous solution was prepared by adjusting 100 mM EDTA to a pH 7.5 with NaOH. Samples were sonicated for 15 min. Then, one mL processed sample was transferred to a 10 mL screw-capped (magnetic cap) vial, fitted with a silicone septum. The vial was introduced in a Combi Pal (Varian Inc.) autosampler and conditioned 10 min at 50 °C with 500 rpm shaking speed. After that, VOCs arising from the sample headspace were extracted using a SPME fiber assembly divinylbenzene/carboxen/polydimethylsiloxane (50/30 μm, 1 cm long from Supelco Ltd., Bellefonte, PA, USA) during 35 min at 50 °C and with 250 rpm shaking speed. Absorbed VOCs were immediately desorbed at 250°C in the injection port of the GC during 1 min. Volatiles were semi-quantified by calculating peak area of each VOCs relative to peak area of the internal standard[29], and assuming all of the response factors were 1. Compound identification was based on comparison with NIST 98 mass spectral library and retention times of authentic standards.

Statistical analyses

Statistical analyses were performed using Microsoft Excel 2010. If two observations are described as different, this means that their difference was determined to be statistically significant ($P < 0.05$) by the performance of Student's *t*-tests. Principal Components Analysis (PCA) and Pearson correlation coefficients were made and calculated using the statistical software InfoStat[30]. The score and loading graphs generated by the software InfoStat were superimposed in the Fig. 2 showing the PCA. Pearson correlation coefficients to determine relationships between sensory attributes and metabolites were calculated using the embedded CORREL function in Microsoft Excel 2010. Hierarchical Clustering was made using Multiple Array Viewer (MeV) software[31]. False colour imaging was performed on the log2-transformed data. Evaluation of statistical significance of differences for primary metabolite and VOC contents was also performed using one-way analysis of variance (ANOVA) followed by

Tukey's multiple comparison test with the aid of the InfoStat 2008 for Windows[30], and for non-parametric tests, the Wilcoxon pairwise rank-sum test was used.

Heat map

Heat map of correlation analysis was performed using MeV software. Regions of red and blue indicate negative or positive correlation between traits as depicted in the reference colour bar.

RESULTS

Taste panels

The results of fruit organoleptic evaluation are shown in Fig. 1. Among Andean landraces, accession #572 was qualified the best for characteristic taste (0.71) while accessions #571, #569, #557, #552 and #3806 also have a good score (between 0.45 and 0.55). Commercial varieties M82 (control) and GPEA have lower scores for taste, and accession #4750 was considered to have moderately characteristic taste (Fig. S1). Regarding defined aroma, accession #3806 was evaluated as the most defined, while M82 (control) and GPEA have the lowest scores (Fig. 1). Concerning other qualities, the panel evaluated accessions #572 and #557 as the sweetest (Fig. 1), followed by accessions #569, #3806, #552, #571, and accession #4750, GPEA and M82 were consider slightly or not sweet (Fig. S1). In the evaluation of sourness accession #572 resulted as no acidic while accession #4750 and GPEA were consider with acidic taste (Fig. 1), and all the others were qualified as slightly to mildly acidic (Fig. S1). These data were subjected to PCA, and the two main components explained 56.6 % of the variability in the organoleptic qualities (Fig. S2). PC1 was mainly loaded by characteristic tomato taste and defined tomato aroma whereas PC2 by indefinite aroma and non-characteristic taste. The traditional landraces #552, #557, #569, #572 and #3806 were clearly separated from #571, #4750, GPEA and M82 (Fig. S2). Based on these results, it is concluded that Andean tomato landraces grouped based on better taste and aroma, with good fruit characteristics in accession #572, a cherry type, as well as accessions #557, with large pear fruit shape, and #569, an oval plum tomato. Also with oval to elongated fruits accessions #552 and #3806 were intermediate evaluated, while accession #571 was considered the most unfavourable for fruit sensorial characteristics. The other varieties, accession #4750, GPEA, and M82, have low fruit sensorial qualities, being the last one evaluated as poor in taste and aroma.

Metabolic profiling of mature tomato fruits

Fruit primary metabolite and VOC contents of nine tomato varieties were evaluated by applying two standardized methods (^{1}H-NMR and SPME GC-MS) to associate chemical composition with taste quality. A total of 26 primary metabolites of known structure were quantified in ripe fruits corresponding mainly to soluble sugars, organic acids, and amino acids (Table 1). When comparing metabolite contents of the selected varieties to the contents in the cultivar M82, it is clear that quantitative changes in sugar (D-fructose, D-glucose, and D-galactose) and organic acids (citrate, pyruvate, and trans-cynnamate) are prevalent, being changes in amino acid composition less abundant (L-Asn, L-Asp and L-Glu) or not different, such as L-Ala and L-Gln among all the varieties. The heat map of the metabolite profiles of the tomato varieties (Fig. S3) indicates contrasting composition of soluble metabolites in the two commercial varieties (M82 and GPEA) and accession #571. The tastier tomato accessions #557, #3806, and #572 showed different composition of soluble metabolites in comparison to commercial varieties, while #552 and #569 showed more similarities in the metabolic profiles to commercial varieties than to the other Andean tomato accessions.

Concerning the VOC composition, fruit of the five Andean varieties showed higher significantly different VOCs in comparison with the other tested varieties (ANOVA, $P < 0.05$), and two of the Andean varieties (accessions #569 and #571) exhibited the highest differences of VOCs (Table S2). Two of them, *trans*-2-heptenal and methyl salicylate, also showed significantly different values in fruit of most varieties when compared to M82 fruit. It is important to highlight that in accession #571, most VOCs were significantly reduced when compared to the cultivar M82. Accession #572 showed the significantly highest value of α-pinene among varieties, and compared to the cultivar M82 was ~280 times higher, while GPEA showed UNK *m/z* 161 ~120 times higher than the cultivar M82. All these data were subjected to PCA (Fig. 2). The two PCA components (PC1 and PC2) explained 52.4 % of total variability in the chemical composition reflected by soluble metabolites and VOCs. The PC1 mostly separates the soluble metabolites from most of VOCs, so the higher variability between the metabolic compositions of the different accessions is explained by these variables. The metabolic composition of accessions #552, #569, #572, #557, #3806, the cultivar M82, and GPEA is represented mostly by VOCs, while the composition of #571 and #4750 is better represented by the soluble metabolites. The compounds more related with good fruit flavour of accessions #557, #572, #569 and #3806 were different from those associated with the worst fruit flavour of accession #571. Accessions #557 and #3806 were related with the VOCs 2-ethyl-1-hexanol, benzaldehyde, UNK *m/z* 120 and *trans*-2-hexenal, while accessions #572 and #569 were related with 2-octenal, benzylnitrile, octanal, *p*-methoxytoluene and duraldehyde and the soluble metabolite sucrose. On the other hand, the worst flavour accession #571 was related with soluble metabolites L-Trp, L-Thr, L-Val, L-Asn, L-Glu, 2-oxoglutarate, ethanol, pyruvate, and L-Asp and with de VOCs: *cis,cis*-1,4-pentadiene, UNK *m/z* 57-3 and hexanal. Interestingly, there are six VOCs that negatively correlated with fruit of the accession #571, which was qualified as the worst variety in relation to characteristic aroma and taste, but positively correlated with the best-tasting fruit of accessions #569 and #572 (Table S2). These VOCs are 2,5-diterbutylbenzoquinone, β–ionone epoxide, propyl salicylate, isoamyl salicylate, 3-methylheptylacetate, and UNK *m/z* 57-2. Another VOC, α-citral, significantly and negatively correlated to #571, but positively correlated to #569. It is worth to mention that propyl salicylate, isoamyl salicylate, and 3-methylheptylacetate are novel VOCs recently identified in tomato samples[28].

Integration of sensory and chemical data

As the number of chemicals potentially influencing the taste and aroma of tomato seems to be large, we performed multivariate analysis of the data to find statistically significant correlations among the traits. The total variables integrated (146) from the fruits of the nine tomato varieties were VOCs (100), soluble metabolites (26), and sensory parameters (20). Positive (1039) and negative (269) significant ($p<0.05$) correlations were detected (Fig. S4, Table S3). The highest and significant Pearson correlation coefficients (0.99) were found among VOCs (i.e., terpinolene and linalool; the unknown compound UNK *m/z* 57-3 and *cis*,*cis*-1,4-pentadione; isoterpinolene and eugenol). Additionally, VOCs highly and significantly correlated with other volatiles (i.e., 3-methylbutanal, 2-nonen-1-ol, or the unknown compound UNK *m/z* 119). Regarding soluble metabolites, GABA, L-Thr, L-Val, and succinate showed the primary number of significant correlations (Fig. S4, Table S3).

Defined aroma and very acidic attributes significantly correlated with several VOCs (Table S3). From them, those which showed correlation coefficients higher than 0.70 were extracted from Table S3 and shown in Table 2. Characteristic taste showed no significant correlation with the compounds analysed. Nevertheless, sweetness and no acidic taste correlated with characteristic taste, which correlated, besides D-fructose and D-glucose, to a lower extent with benzaldehyde, and 2-methyl-2-octen-4-one. It is worthwhile to mention that 2-methyl-2-octen-4-one and DMHEX (Table 2) are VOCs recently identified in tomato[28]. Also, very sweet taste significantly and negatively correlated with only one VOC, methyl butanoato (Table S3),

DISCUSSION

During tomato domestication and further breeding processes, several traits have been improved such as yield, pest resistance, fruit size, and physical appearance. Nowadays, consumers demand fruit with better qualities, and modern breeders need to design strategies to improve the organoleptic properties while high yield is maintained[32]. However, breeding for sensory quality is not an easy task[33], since experiences of sensations perceived by humans are difficult to quantify. Combination of many chemical compounds more than their specific concentration may contribute to give the characteristic flavour of the tomato fruit[22,23].

Tomato landraces evaluated in this study are well adapted to high altitude environments of the Andean valleys of the northwestern Argentina (Table S1). This study reveals the chemical nature related to the characteristic flavour and aroma of good tasting tomato fruit from landraces selected over time by small farmers of Andean valleys[15]. Furthermore, the Andean farmers typically keep their own seed and cultivated varieties according their management practices. Selection had been made for culinary purposes based on fruit quality, taste, and aroma, assuring that the improved flavour is maintained. The results demonstrate that Andean tomatoes are of great importance for the study of the phenotypic and genetic diversity in traditional or “criollo” varieties, for germplasm conservation, as well as for their use in genetic improvement[16]. Important traits associated with fruit nutritional qualities and organoleptic properties are present in these accessions, which constitute interesting genetic resources to be incorporated in breeding programs. Some of the landraces adapted to the altitude preserved as well a good fruit flavour (Fig. 1, Fig. S1 and S2). The best-tasting fruit belonged to the accessions #569, #572 and #557, followed by #552 and #3806. In parallel, the soluble metabolites and VOCs composition of the red fruit were determined by metabolomic studies (Tables 1 and S2). The integration of all the data from sensory panels and metabolomics allowed to correlate each organoleptic property (sweet, sour, characteristic taste and aroma to tomato fruit) with the chemical composition (Table S3 and Fig. S4). Methyl butanoate was the only VOC that significantly and negatively correlated to sweetness. This compound is a short-chain ester and one of the primary compounds in the fresh fruit of the gooseberry that confers a green-fruity odor[34]. One significant finding is that novel VOCs recently described in tomato fruit for the first time[28] (such as propyl salicylate, α-hexylcinnamaldehyde and benzophenone, among others) correlated with a specific organoleptic property, and most of them significantly and positively correlated with sweetness (Table S3). However, there is not an overlapping pattern between VOCs that significantly correlated with sweet taste and VOCs correlating with sour taste (Table S3 and Fig. S4). The term flavour denotes the combination of taste and retronasal olfaction, which is the perception of odorants in the mouth[35]. It is worth to mention that the sense of sweet and sour, two fundamental perceptions in mammalians, are mediated by its class of taste receptor cells[36]. Moreover, attractive flavour and sweet are sensed by heterodimeric G protein-coupled receptors, while the other two essential tastes of sour and salt sensing are mediated by ion channel receptors. More recently, a potassium channel was found as a critical component of sour taste transduction[37]. The difference in taste sensing mechanism could explain that soluble metabolites, and VOCs showed not-overlap between sweet and sour tastes. In our hands, four VOCs (α-terpineol, p-menth-1-en-9-al, DMHE, and linalool) overlapped in the perception of defined tomato aroma and very acidic attribute (Table 2). From a total of 13 flavour-associated VOCs that were significantly reduced in modern varieties[6], we found that the good tasting accession #569 showed a significant higher content in *trans*-*trans*-2,4-decadienal and a significant lower content in phenylacetaldehyde when compared to cultivar M82. Additionally, *trans*-*trans*-2,4,-decadienal was found significantly increased in accessions #3806, #552 and #572, and *trans*-2-heptenal were significantly decreased in three Andean accessions (#557, #569 and #572) when compared to cultivar M82. This analysis corroborates that not necessarily the amount of VOCs is important in flavour definition but the combination of VOCs.

The soluble metabolites and VOCs detected in this work are valuable for future studies on the discovery of new metabolic pathways affecting on tomato fruit taste. Considering that genetic diversity is fundamental to improve tomato fruit quality, Andean landraces could be used to introduce new traits. The results of this study revealed a promising breeding perspective since the incorporation of Andean accessions could reinforce genetic variability, and the combination of valuable new compounds could contribute to improve the fruit quality and taste of cultivated tomatoes. Additionally, the lack of correlation between the levels of specific VOCs and the levels of their precursor metabolites indicated that the rate of volatile production is not governed by precursor supply but rather at the transcriptional or post-transcriptional level, which is in agreement with other works in this area[19,38].

Natural environmental adaptation, domestication and independent artificial selection events would have generated different genetic constitutions, confirming that traditional agricultural habitats are important reservoirs of genetic diversity.

ACKNOWLEDGEMENTS

M.D. and P.R.C. were recipients of fellowships of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). M.I.Z., S.B.B., F.C., R.A., I.E.P., and E.M.V. are career members of CONICET. This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and National Institute of Agricultural Technology (INTA).

REFERENCES

1 FAO (Food and Agriculture Organization of the United Nations). (2017). URL http://www.fao.org/faostat/en/#data/QC

2 Causse M, Friguet C, Coiret C, Lépicier M, Navez B, Lee M *et al.*, Consumer preferences for fresh tomato at the European scale: A common segmentation on taste and firmness. *J Food Sci* **75**:S531–S541 (2010).

3 Lin T, Zhu G, Zhang J, Xu X, Yu Q, Zheng Z *et al.*, Genomic analyses provide insights into the history of tomato breeding. *Nature* **46**:1220-1226 (2014).

4 Blanca J, Montero-Pau J, Sauvage C, Bauchet G, Illa E, Díez MJ *et al*., Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genomics* **16**:257 (2015).

5 Peralta IE, Spooner DM and Knaap S, Taxonomy of wild tomatoes and their relatives (*Solanum* sect. *Lycopersicoides*, sect. *Juglandifolia*, sect. *Lycopersicon*; Solanaceae). The American Society of Plant Taxonomists, Systematic Botany Monographs, USA (2008).

6 Tieman D, Zhu G, Resende MFR, Lin T, Nguyen C, Bies D *et al*., A chemical genetic roadmap to improved tomato flavor. *Science* **355**:391-394 (2017).

7 Figàs MR, Prohens J, Raigón MD, Fernández-de-Córdova P, Fita A and Soler S, Characterization of a collection of local varieties of tomato (*Solanum lycopersicum* L.) using conventional descriptors and the high-throughput phenomics tool Tomato Analyzer. *Genet Resour Crop Evol* **62**:189–204 (2015).

8 Goncalves LSA, Rodrigues R, Do Amaral Jr AT, Karasawa M and Sudre CP, Heirloom tomato gene bank: assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. *Genet Mol Res* **8**:364–374 (2009).

9 Carbonell-Barrachina AA, Agusti A and Ruiz JJ, Analysis of flavor volatile compoundsby dynamic headspace in traditional and hybrid cultivars of Spanish tomatoes. *Eur Food Res Technol* **222**:536–542 (2006).

10 Terzopoulos PJ and Bebeli PJ, Phenotypic diversity in Greek tomato (*Solanum lycopersicum* L.) landraces. *Sci Hortic* **126:**138–144 (2010).

11 Figàs MR, Prohens J, Raigón MD, Fita A, García-Martínez MD, Casanova C *et al*., Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups. *Food Chem* **187**: 517–524 (2015).

12 Baldina S, Picarella ME, Troise AD, Pucci A, Ruggieri V, Ferracane R *et al*., Metabolite profiling of Italian tomato landraces with different fruit types. *Front Plant Sci* **7**: 664 (2016).

13 Parodi L, *La agricultura aborigen argentina*. EUDEBA, Universidad de Buenos Aires, (Cuadernos de América nº 4) (1999).

14 Peralta IE, Makuch M, García Lampasona S, Occhiuto PN, Asprelli PD, Lorello IM *et al*., *Catálogo de poblaciones criollas de pimiento, tomate y zapallo colectadas en valles andinos de la Argentina*. Mendoza: Ediciones INTA. (2008).

15 Asprelli PD, Occhiuto PN, Makuch MA, Lorello IM, Togno LS, García Lampasona SC *et al*., Recolección de germoplasma criollo de especies cultivadas y su distribución en regiones andinas de Argentina. *Horticultura Argentina* **30**:30-45 (2011).

16 Asprelli PD, Lorello IM, Occhiuto PN, Togno LS, Makuch MA, García Lampasona SC *et al*., First collection of landrace vegetable crops cultivated in Valle Fértil, Argentina. *AgriScientia* **29**: 41-50 (2012).

17 Asprelli PD, Sance M, Insani EM, Asis R, Valle EM, Carrari F, *et al*., Agronomic performance and fruit nutritional quality of an Andean tomato collection. *Acta Horticulturae* **1159**:197-204 (2017).

18 Di Paola Naranjo RD, Otaiza S, Saragusti AC, Baroni V, Carranza AV, Peralta IE *et al*., (2016). Hydrophilic antioxidants from Andean Tomato Landraces assessed by their bioactivities in vitro and in vivo. *Food Chem* **206**:146–155.

19 Zanor MI, Rambla JL, Chaïb J, Steppa A, Medina A, Granell A *et al*., Metabolic characterization of loci affecting sensory attributes in tomato allows an assessment of the influence of the levels of primary metabolites and volatile organic contents. *J Exp Bot* **60**:2139–2154 (2009).

20 Carli P, Arima S, Fogliano V, Tardella L, Frusciante L and Ercolano MR, Use of network analysis to capture key traits affecting tomato organoleptic quality. *J Exp Bot* ***60***:3379-3386 (2009).

21 Kowalczyk K, Gajc-Wolska J, Radzanowska J and Marcinkowska M, Assessment of chemical composition and sensory quality of tomato fruit depending on cultivar and growing conditions. *Acta Scientiarum Polonorum* **10**:133–140 (2011).

22 Tieman D, Bliss P, McIntyre LM, Blandon-Ubeda A, Bies D, Odabasi A *et al*., The chemical interactions underlying tomato flavor preferences. *Curr Biol* **22**:1035–1039 (2012).

23 Baldwin EA, Scott JW and Bai J, Sensory and chemical flavor analyses of tomato genotypes grown in Florida during three different growing seasons in multiple years. *J Am Soc Hort Sci* **140**:490-503 (2015).

24 Stone HS and Sidel JL, *Sensory Evaluation Practices*, (3rd ed.). San Diego: Academic Press (2004).

25 MacFie HJH, Assessment of the sensory properties of food. *Nutrition Reviews* **48**:87-93 (1990).

26 Sorrequieta A, Abriata LA, Boggio SB and Valle EM, Off-the-Vine Ripening of Tomato Fruit Causes Alteration in the Primary Metabolite Composition. *Metabolites* **3**:967-978. (2013).

27 Abriata LA, Utilization of NMR spectroscopy to study biological fluids and metabolic processes: Two introductory activities. *Concepts in Magnetic Resonnance Part A* **40A**:171–178 (2012).

28 Cortina PR, Asis R, Peralta IE, Asprelli PD and Santiago AN, Determination of volatile organic compounds in Andean tomato landraces by headspace solid phase microextraction-gas chromatography-mass spectrometry. *J Braz Chem Soc* **28**:30-41 (2017).

29 Piombino P, Sinesio F, Moneta E, Cammareri M, Genovese A, Lisanti MT *et al.*, Investigating physicochemical, volatile and sensory parameters playing a positive or a negative role on tomato liking. *Food Res Int* **50**:409-419 (2013).

30 Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M and Robledo CW *InfoStat, versión 2008,* Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina (2008).

31 Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N *et al*., TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **34**:374-378 (2003).

32 Klee HJ and Tieman DM, Genetic challenges of flavor improvement in tomato. *Trends Genet* **29**:257-262 (2013).

33 Folta KM and Klee HJ, Sensory sacrifices when we mass-produce mass produce. *Horticulture Research* **3** doi:10.1038/hortres.2016.32 (2016).

34 Hempfling K, Fastowski O, Kopp M, Nikfardjam MP and Engel KH, Analysis and sensory evaluation of gooseberry (*Ribes uva crispa* L.) volatiles. *J Agric Food Chem* **61**:6240-6249 (2013).

35 Shepherd GM, The human sense of smell: are we better than we think? *PLoS Biol* **2**:e146 (2004).

36 Yarmolinsky DA, Zuker CS and Ryba NJP, Common sense about taste: from mammals to insects. *Cell* **139**:234–244 (2009).

37 Ye W, Chang RB, Bushman JD, Tu Y-H, Mulhall EM, Wilson CE *et al*., The K+ channel KIR2.1 functions in tandem with proton influx to mediate sour taste transduction. *Proc Natl Acad Sci USA* **113**:E229–E238 (2016).

38 Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD and Klee HJ, Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor

volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci USA* **103**:8287–8292 (2006).

Table 1. Soluble metabolites composition of tomato fruits from different accessions. Primary metabolite content (µmol g FW^{-1}) was determined by ^{1}H -NMR in red tomato fruit.

Metabolite	**M82**	**4750**	**3806**	**GPEA**	**552**	**557**	**569**	**571**	**572**
D-fructose	81.02 ± 8.20^{a}	$174.41\pm16.75^{a,b}$	213.15 ± 15.56^{b}	$185.77\pm7.89^{a,b}$	250.95 ± 15.31^{b}	207.44 ± 8.69^{b}	213.99 ± 14.75^{b}	260.54 ± 30.87^{b}	258.74 ± 50.82^{b}
D-glucose	88.08 ± 9.01^{a}	$215.11\pm14.42^{a,b}$	$267.28\pm28.34^{b,c}$	$233.14\pm5.90^{a,b,c}$	$281.93\pm29.92^{b,c}$	$281.38\pm24.65^{b,c}$	$269.71\pm14.35^{b,c}$	$281.10\pm24.72^{b,c}$	294.86 ± 34.10^{c}
D-galactose	4.86 ± 0.97^{a}	$22.54\pm1.28^{b,c}$	$20.74\pm6.86^{a,b,c}$	$19.30\pm5.35^{a,b,c}$	ND	$13.52\pm5.40^{a,b}$	$22.48\pm4.19^{b,c}$	35.27 ± 12.23^{c}	$19.51\pm2.63^{a,b,c}$
D-xylose	5.17 ± 1.62^{a}	$16.86\pm2.63^{a,b}$	6.27 ± 3.95^{a}	$14.71\pm4.23^{a,b}$	ND	7.82 ± 1.81^{a}	9.73 ± 0.29^{a}	23.93 ± 10.25^{b}	$16.85\pm2.66^{a,b}$
Sucrose	0.42 ± 0.24^{a}	$4.58\pm2.21^{a,b}$	$0.56\pm0.15^{a,b}$	$0.92\pm0.42^{a,b}$	13.36 ± 7.36^{b}	$7.70\pm1.99^{a,b}$	$3.71\pm1.66^{a,b}$	$1.03\pm0.21^{a,b}$	$4.55\pm2.51^{a,b}$
GABA	$2.93\pm0.89^{a,b,c}$	19.53 ± 0.94^{e}	$8.67\pm1.54^{c,d,e}$	$3.67\pm0.44^{a,b,c,d}$	1.33 ± 0.54^{a}	$10.65\pm1.63^{c,d,e}$	$1.99\pm0.78^{a,b}$	$14.79\pm4.49^{d,e}$	$4.71\pm0.59^{b,c,d}$
L-Ala	1.83 ± 0.30^{a}	3.77 ± 0.14^{a}	4.72 ± 2.05^{a}	1.99 ± 0.52^{a}	1.99 ± 0.16^{a}	3.03 ± 1.08^{a}	2.86 ± 0.49^{a}	3.73 ± 1.49^{a}	1.52 ± 0.15^{a}
L-Asn	$2.67\pm0.52^{a,b,c}$	$2.47\pm0.11^{a,b,c}$	$4.37\pm0.74^{b,c}$	$1.82\pm0.22^{a,b}$	$1.93\pm0.31^{a,b}$	$3.90\pm1.03^{a,b,c}$	1.62 ± 0.34^{a}	6.47 ± 2.00^{c}	$3.24\pm0.42^{a,b,c}$
L-Asp	5.86 ± 0.85^{a}	4.94 ± 0.23^{a}	$8.04\pm1.09^{a,b}$	4.52 ± 0.03^{a}	$8.74\pm1.41^{a,b}$	5.50 ± 0.57^{a}	5.48 ± 1.73^{a}	14.90 ± 1.72^{b}	$7.96\pm0.80^{a,b}$
L-Glu	$5.53\pm1.10^{a,b}$	$10.23\pm0.21^{a,b,c}$	$11.27\pm1.27^{b,c,d}$	$11.71\pm0.70^{b,c,d}$	$7.86\pm0.68^{a,b,c}$	$10.69\pm1.69^{a,b,c,d}$	5.28 ± 1.85^{a}	23.91 ± 3.76^{d}	$16.71\pm1.54^{c,d}$
L-Gln	3.86 ± 1.12^{a}	3.20 ± 0.21^{a}	6.96 ± 1.58^{a}	6.40 ± 1.03^{a}	5.00 ± 0.78^{a}	8.02 ± 1.53^{a}	5.31 ± 2.25^{a}	11.35 ± 3.74^{a}	8.00 ± 1.96^{a}

L-Ile	0.11±0.03[a,b]	0.53±0.03[d,e]	0.60±0.05[e]	0.17±0.03[a,b,c]	0.08±0.02[a]	0.45±0.12[c,d,e]	0.20±0.04[a,b,c,d]	0.44±0.14[c,d,e]	0.21±0.01[b,c,d,e]
L-Phe	0.75±0.15[a,b]	1.52±0.00[b,c]	1.78±0.13[c]	0.51±0.02[a]	0.48±0.09[a]	1.40±0.12[b,c]	0.47±0.10[a]	1.90±0.40[c]	0.91±0.08[a,b]
L-Thr	0.64±0.14[b,c,d]	1.02±0.10[c,d,e]	1.07±0.21[c,d,e]	0.11±0.02[a]	0.41±0.02[b,c]	1.71±0.29[d,e]	0.52±0.16[b,c]	2.42±0.70[e]	0.35±0.01[b]
L-Trp	0.15±0.02[a,b]	0.38±0.03[c]	0.27±0.01[b,c]	0.13±0.00[a]	0.19±0.03[a,b]	0.26±0.05[a,b,c]	0.18±0.02[a,b]	0.50±0.10[c]	0.25±0.03[a,b,c]
L-Val	0.14±0.01[a,b]	0.51±0.06[d]	0.27±0.07[b,c,d]	0.10±0.02[a,b]	0.09±0.02[a]	0.20±0.06[a,b,c]	0.13±0.02[a,b]	0.50±0.08[c,d]	0.19±0.02[a,b,c,d]
ethanol	1.31±0.06 [a]	3.58±0.58[c,d]	1.97±0.02[a,b]	1.07±0.14[a]	2.33±0.19 [a,b,c,d]	2.29±0.24[a,b,c]	2.93±0.57[b,c,d]	3.88±0.32[d]	1.62±0.22[a,b]
methanol	3.93±0.29[a]	9.24±0.47[b,c]	9.35±0.29[b,c]	8.14±0.28[a,b,c]	8.17±0.20[a,b,c]	9.93±0.87[b,c]	6.62±1.41[a,b]	13.28±1.76[c]	9.37±1.83[b,c]
citrate	13.80±1.52[a]	55.39±2.83[b]	31.19±1.99[a,b]	39.19±4.74[a,b]	28.20±4-48[a,b]	31.89±8.48[a,b]	23.57±2.76[a,b]	45.31±15.65[a,b]	29.83±2.94[a,b]
malate	5.20±0.15[a]	5.31±0.46[a]	4.33±1.58[a]	4.52±1.46[a]	7.06±1.41[a]	2.42±0.56[a]	5.27±1.51[a]	6.30±0.65[a]	5.54±1.45[a]
2-oxoglutarate	4.35±1.18[a]	4.75±0.42[a,b]	8.63±1.89[a,b]	5.62±0.64[a,b]	3.68±0.45[a]	6.97±1.23[a,b]	3.13±0.89[a]	16.04±4.82[b]	6.68±1.81[a,b]
succinate	0.80±0.27[a,b]	1.58±0.02[b,c]	1.75±0.19[b,c]	1.06±0.24[a,b]	0.54±0.08[a]	1.29±0.05[a,b,c]	0.52±0.15[a]	3.21±0.76[c]	1.04±0.23[a,b]
trigonelline	0.67±0.17[a]	2.20±0.73[a]	1.08±0.24[a]	1.97±0.16[a]	1.82±0.60[a]	1.54±0.51[a]	0.71±0.31[a]	2.45±0.92[a]	2.75±0.37[a]
pyruvate	2.17±0.20[a]	5.40±0.47[d,e]	2.94±0.56[a,b,c,d]	4.87±0.46[c,d,e]	2.33±0.39[a,b]	2.00±0.26[a]	2.62±0.50[a,b,c]	7.72±1.02[e]	4.71±0.84[b,c,d,e]
trans-cinnamate	0.30±0.05[a]	0.94±0.07[b]	1.34±0.19[b,c]	1.57±0.09[b,c]	0.81±0.16[b]	1.20±0.04[b,c]	0.30±0.05[a]	2.97±0.96[c]	1.09±0.15[b,c]
benzoate	0.84±0.08[a,b]	0.69±0.05[a,b]	0.77±0.09[a,b]	0.53±0.07[a]	1.08±0.07[b,c]	0.79±0.09[a,b]	1.01±0.08[b,c]	1.41±0.14[c]	1.98±0.19[b,c]

Each value represents the average of three independent biological replicates. The values followed by different letter superscripts within each row indicate that they were significantly different at a probability level of 0.05 according to ANOVA tests. FW, fresh weight; ND, not detected.

Table 2. List of compounds that significantly correlated to tomato defined aroma and sourness. The values are correlation coefficients (considering values higher than 0.70) between the listed VOC and the sensory attribute "Defined Aroma" and "Very Acidic" taste. They were extracted from Table S3.

VOC name	Correlation coefficient of defined aroma / very acidic taste	*p*
α-terpineol	0.76 / 0.92	0.017 / 0.003
DMHEX	0.76 / 0.91	0.017 / 0.004
p-menth-1-en-9-al	0.74 / 0.92	0.023 / 0.003
linalool	0.73 / 0.86	0.026 / 0.013

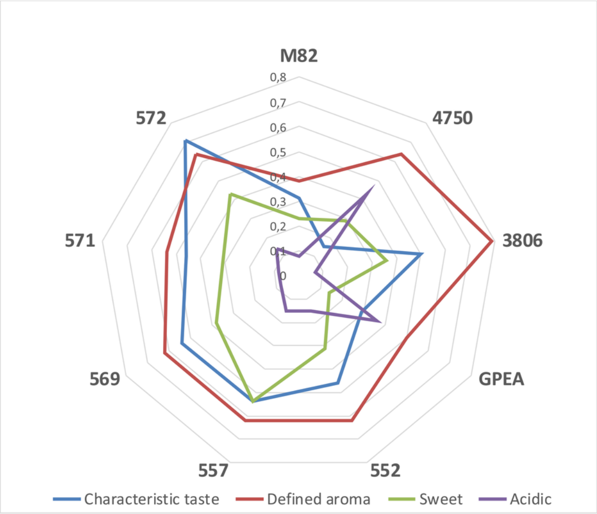

Fig. 1. **Sensory analysis of red ripe tomato fruits of accessions from Argentine Andean valleys**. Evaluation of accessions #3806, #552, #557, #569, #571, #572, #4750, cultivar M82 and GPEA for the attributes characteristic taste, defined aroma, sweet, and acidic taste. The radial graphics represent the frequency obtained for each descriptor in the whole taste panel (i.e. 0.5 means that 50% of the panellists evaluated a given descriptor level).

Fig. 1 new2.tif

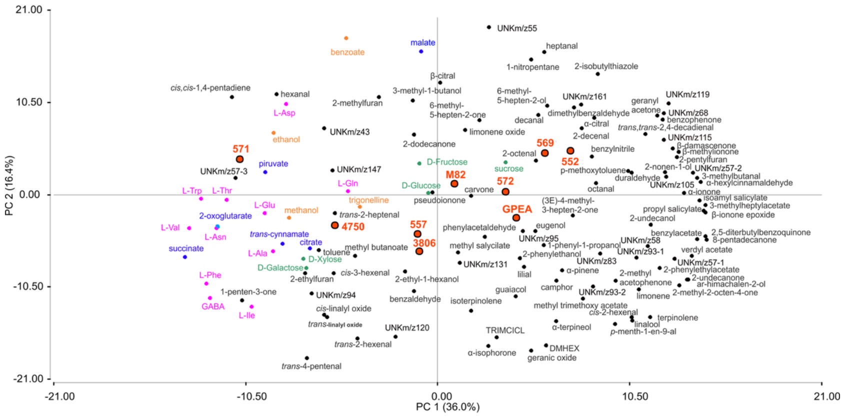

Fig. 2. Biplot of the metabolic profile of ripe red fruits of the tomato accessions #552, #557, #569, #571, #572, #3806, #4750, cultivar M82, and GPEA. Graph was obtained by superimposing score plot (tomato varieties) and loading plot (metabolite content) obtained by the software InfoStat and shows the data variability profile. Tomato accessions are indicated in red, VOCs in black, sugars in green, organic acids in blue, free amino acids in pink and other metabolites in orange.

Fig. 2 final.tif