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Analysis of Volatile Compounds from *Solanum betaceum* Cav. Fruits from Panama by Head-Space Micro Extraction

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Abstract: The characterization of the volatile compounds of two varieties of *Solanum betaceum* Cav. by means of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) is presented. The HS-SPME method for extraction of the volatiles compounds was optimized by using a 2^3 central composite design. Maximum extraction of volatile compounds was achieved by using a divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fiber, extraction temperature 76° C, incubation time 44 min, and extraction time of 46 min. The main types of compounds detected in both varieties are terpenoids, followed by aromatics, esters, and aldehydes. Golden-yellow cultivars contained higher levels of esters and terpenes, while the reddish-purple variety contained a significant amount of aromatic compounds. The data structure of the chemical information obtained as well as the relationship between variables was evaluated by means of principal component analysis and cluster analysis.

Keywords: *Solanum betaceum*; tree tomato; volatile aroma compounds; headspace-solid phase microextraction; gas chromatography-mass spectrometry; central composite design; multivariate studies.

1. Introduction

Solanum betaceum Cav. (Solanaceae; synonym: *Cyphomandra betacea* Cav.) [1], is a shrub native to South America (Bolivia, Ecuador and Peru). The plant is cultivated mostly in Brazil, Argentina, Mexico, Panama, Spain, India, South Africa and the United States of America. Its fruits are highly appreciated for their organoleptic and nutritional properties (very high content of vitamins, iron and low calories). In addition, tree tomato fruits have

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become an important marketable crop in these countries as well as in Colombia and New Zealand. The latter is one of main exporters to Europe, Japan and USA using the existing marketing channels developed for the kiwi fruit. Tree tomato is consumed raw (as part of salads) and after cooking (as an ingredient for different dishes and desserts). In Panama, two cultivars of this egg-shaped fruit, the reddish-purple and the golden-yellow color, are produced. In regard to the chemical composition of these fruits, limited information is available in the scientific literature [2]. In a recent study in which different wild and cultivated fruits from Panama were surveyed for their content of carotenoids, it was concluded that tree tomato was an appropriate source of lutein and zeaxanthin [3]. On the other hand, β-carotene and β-cryptoxanthin have been identified as the most abundant carotenoids in crops from Australia and Brazil [2,4]. Polyphenol delphinidin 3-rutinoside was found in high concentration in Brazilian reddish-purple variety, as well as other anthocyanins such as cyanidin 3-rutinoside and pelargonidin 3-glucoside-5- rhamnoside [4-5]. In addition, it was shown that the content of polyphenols hydroxycinnamic acids in the reddish-purple variety harvested in Ecuador was quantitatively higher than in the yellow variety [6]. Torrado et al. [7] assessed the volatile constituents responsible for the aroma of Colombian reddish-purple tree tomato after liquid extraction with a pentane-dichloromethane mixture. The main volatile compounds detected were *cis*-3-hexenol, 3-hydroxybutanoates, 3-hydroxyhexanoates, eugenol, 4-allyl-2,6- dimethoxyphenol, methyl hexanoate and *trans*-3-hexenal. However, volatile compounds found in fruits from Malaysia (by vacuum distillation and subsequent extraction of the distillate with dichloromethane) were reported to be quite different from the former, 3-hexenol, ethyl butyrate, methyl hexanoate and methyl butyrate being the major constituents detected [8]. Fruits aroma, which is one of the main organoleptic property that determines the degree of acceptance of a fruit by consumers, relies on the volatile compounds present in their pulp and skin. The type and quantity of these compounds, in turn, depend on different factors such as genetics, ripening phase, and storage conditions [9].

Solid-phase microextraction (SPME) is a solvent-free method used for the extraction of volatile compounds by means of a coated fiber, which is exposed to the headspace, or directly to the sample, causing the partition of volatiles compounds between the sample and the fiber. Separation of the extracted substances is most often achieved by chromatography after thermal desorption [10]. Different methods based on this technique have been used to assess the aroma profile of several tropical fruits, such as banana and mango [11]; native Brazilian tropical fruits such as *cajá* (*Spondias lutea*, L.), *graviola* (*Annona reticulata*, L.) [12], tamarind, yellow passion fruit [13] and watermelon [14]. The current investigation reports for the first time the volatile profile of two varieties of *Solanum betaceum* (tree tomato fruits) from Panama by using HS-SPME-GC-MS. Moreover, to the best of our knowledge, the aroma compounds of the golden-yellow variety have not been reported previously. The variables affecting the SPME procedure, concretely, extraction temperature, incubation time and extraction time were optimized using an experimental design approach in order to attain high recovery of the volatiles components. An exploration of the data structure of the chemical information obtained was carried out by means of principal component analysis (PCA) and cluster analysis (CA).

2. Material and methods

2.1. Fruit samples

Tree tomato (*Solanum betaceum* Cav.) samples with guaranteed Panamanian origin were obtained in local markets in Panama City. Two varieties at ripe stage were obtained, i.e. the reddish-purple and the golden-yellow. The taxonomic identity of the fruits was established by the botanist Alex Espinosa of CIFLORPAN, and voucher specimens Florpan 8734a (reddish-

purple tree tomato fruit) and Florpan 8734b (golden-yellow tree tomato fruit) were deposited in the Herbarium of the University of Panama (PMA). Fruits were washed thoroughly under running water, peeled and seeds removed from the pulp. Fruit pulp was then homogenized and kept frozen at−20° C until analysis.

2.2. Apparatus

Analysis of aroma compounds was carried out by using an Agilent 6890N gas chromatograph coupled to an Agilent 5975 quadrupole mass selective detector (Palo Alto, CA, USA). The separation was performed by means of a HP-5MS capillary column, 30 m length, 0.25 mm i.d. and a 0.25 µm phase thickness (Agilent Technologies, Palo Alto, CA, USA). Mass spectra libraries employed include the Registry of Mass Spectral Data with Structures, Wiley 7th edition, USA; and Flavors and Fragrances of Natural and Synthetic Compounds - Mass Spectral Data Base, USA.

2.3 SPME fibers survey

SPME fibers, SPME fiber holder, and 10 mL screw-top vials with PTFE-faced silicone septa were purchased from Supelco (Bellefonte, USA). Distinct SPME fibers were assayed for the extraction of volatile compounds of tree tomato: carboxen-polydimethylsiloxane (CAR/PDMS, 75 µm-*black hub plain*), polydimethylsiloxane-divinylbenzene (PDMS/DVB, 65 µm- *blue hub plain*), and divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS 50/30 µm-*gray hub plain*). In all cases fibers were conditioned before use following manufacturer's instructions, and reconditioned before each sampling. Extractions were done at 40° C with incubation time of 30 min, and extraction time of 15 min. Each sample was analyzed in triplicate and mean reported.

2.4. Head space solid-phase microextraction/Gas chromatography-mass spectrometry (HS-SPME-GC-MS)

For HS-SPME procedure, 1.00 ± 0.05 g of the tree tomato fruit pulp was transferred to a 10 mL vial. The fiber coating was placed to the headspace for temperature and times (incubation and extraction times) values set according to the experiment. Extractions were achieved with magnetic stirring. The fiber containing the extracted aroma compounds were then injected into the GC injector (splitless mode), and kept during 15 min for thermal desorption at 250°C. Helium was used as carrier gas with a constant flow-rate of 1 mL min^{-1}. The oven temperature was programmed at 50°C for 3 min, then raised to 200°C at 6°C min^{-1}, and finally up to 280°C at 10°C min^{-1}, where it was held for 6 min. Transfer line and ion-source temperatures were 280°C and 250°C respectively. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV. SCAN mode (40-400 *m/z*) was used for mass acquisition. The fruits compounds were identified by comparison of their retention indices (relative to n-alkanes), and by comparison with the mass spectra of the two libraries cited above.

2.5. Chemometric procedures

Two types of chemometrics techniques were employed in this work. First, the optimization of variables influencing the SPME extraction was performed by using a

multivariate experimental design, i.e. central composite design [15]. Three factors that have great impact on SPME extraction of aroma compounds were evaluated, i.e. extraction temperature, incubation time and extraction time. The second type of chemometric techniques, principal component analysis (PCA) [16] and cluster analysis (CA) [17] were applied to the chemical information available for exploring possible correlations between samples and aroma compounds in both fruits varieties. Although 70 different compounds could be detected by using the optimized method, the chemometric exploration was only carried out taking into account those compounds present in more than 80% of the samples, consequently, 25 compounds were considered for chemometric purposes. Therefore, the data matrix employed was a X_{20x25} in which the rows corresponded to the 20 samples (objects) of tree tomato analyzed and the columns were the variables (features) representing 25 compounds present in most of the samples assessed. Data vectors belonging to the samples of both types, reddish-purple and golden-yellow varieties, were analyzed using those different chemometric procedures indicated above. All chemometric techniques: central composite design, PCA and CA were performed using the statistical package Statgraphics Plus 5.1 (Statistical Graphics, Rockville, MD).

3. Results and Discussion

3.1. SPME fiber selection

The efficiency of HS-SPME for characterization of fruit aroma compounds demands the appropriate choice of the fiber in order to guarantee that a large and significant amount of the analytes can be extracted from the sample. In this study three SPME fibers, with more than one coating, were studied in order to ascertain extraction efficiencies. DVD/CAR/PDMS allowed for determination of a larger number of volatile and semi-volatile compounds when compared with CAR/PDMS or PDMS/DVB fibers. Maximum fiber efficiency relies on two main factors: the polarity of the coating used, and the volatility of the compounds [18]. Since experimental SPME parameters, i.e. extraction time, extraction temperature, and incubation time were the same for each fiber, the high extraction efficiency observed for DVD/CAR/PDMS fiber can be explained by the very similar polarity between this fiber and those compounds present in the headspace. Thus, because of the greater sensitivity and affinity for a wider range of compounds with different volatilities and physicochemical properties, further optimization of the SPME methodology was done by using this fiber.

3.2. SPME variables optimization: Central composite design

Multivariate designs, such as central composite design, have been used for optimization of the main variables that influence the SPME extraction in different fruits, such as passion fruit, tamarind, acerola, soursop, apple juice, banana, among others [13, 19-21]. Three SPME variables were optimized for extraction of tree tomato aroma volatiles: extraction temperature, incubation time and extraction time, due to all of these variables strongly determines the equilibrium, vapor pressures and extraction efficiency of volatiles in the headspace [22]. The optimization strategy consisted in the employment of an experimental design method: a three-factor central composite design (CCD) which is equivalent to a 2^3 full factorial design with two central points and six star points added. The information needed to calculate the optimal response surfaces for these variables was obtained from sixteen randomized experiments (as presented in Table 1). MS-detector peak area of all identified aroma compounds was used for optimization. Selection of the experimental lower and upper levels, for each variable tested in

this work, was done trying to include a significant range, yet maintaining parameters such as incubation and extractions times at values not larger than one hour, since more than 60 minutes is not practical for routine analysis. The ranges studied were 30-80 ºC, 15-60 min and 15-60 min, for extraction temperature, incubation time and extraction time, respectively. Once the sixteen experiments were carried out under the conditions explained in Table 1, the results obtained were used to construct the Pareto chart in order to ascertain which factors had a main influence on the response. As can be seen in Figure 1, extraction temperature is the main factor that influence extraction of aroma compounds from tree tomato fruits when utilizing SPME methodology ($p < 0.05$ for the corresponding ANOVA). Extraction time has lower influence (not statistically significant) and the remaining factors such as secondary interactions as well as incubation time presented minor influence in the total area peak. Response surface methodology (RSM) consists of a group of statistical approaches based on variance analysis and multiple regressions which allows for determination of changes in the response, variations of the factors levels, and more important, the very best value for achieving the higher yield. In Figure 2, the response surface as a function of the three variables studied is presented. Each plot highlights the way in which two factors influence the extraction process. It can be seen that augmenting extraction temperature increases SPME performance, achieving at 76° C a maximum. Increasing extraction temperature enhances the amount of volatile compounds released by the sample that reach the headspace. At low temperatures highly volatile compounds are delivered to the headspace, but less volatiles remains in the sample matrix due to different chemical interactions between aroma compounds and other compounds which constitute the fruit. Temperature increments permit less volatile substances to overcome chemical interactions and reach to the headspace. This is, the headspace/sample partition coefficient (K_{hs}) increases allowing more volatiles and semi-volatiles compounds to be extracted.

Table 1. Central composite design and chromatographic peak area response

Experiment number	Extraction Temperature (°C)	Incubation Time (min)	Extraction Time (min)	Peak Area (x 10^8)
1	80	38	38	7.5
2	80	60	60	7.6
3	80	15	15	3.5
4	30	60	15	1.0
5	55	38	38	9.1
6	55	38	15	4.3
7	55	38	38	9.1
8	30	60	60	2.2
9	30	15	60	3.4
10	80	60	15	8.6
11	55	38	60	8.4
12	30	38	38	4.1
13	55	15	38	6.3
14	30	15	15	1.1
15	55	60	38	5.6
16	80	15	60	7.1

No important decrease in the response was shown due to increments in temperature, although it is known that low-mass volatile compounds are released easer from the SPME fiber with increasing temperature affecting negatively the extraction [23], due to a decrease in the fiber/headspace diffusion constant (K_{fh}). The results from experimental design indicates

that optimal SPME response was achieved utilizing DVD/CAR/PDMS fiber when extraction temperature was 76° C, incubation time 44 min, and extraction time 46 min.

3.3. HS-SPME/GC-MS analysis of Solanum betaceum Cav. *fruits*

Headspace solid-phase microextraction (HS-SPME) is an inexpensive solvent free method, which permits a straightforward analysis of aroma compounds of *Solanum betaceum* Cav. fruits. The SPME optimized method developed in this research was employed for characterizing two varieties of the fruit: the reddish-purple and the golden-yellow varieties. Thus, twenty different tree tomato samples (ten for each variety) were analyzed for volatile compounds using the HS-SPME for extraction, under the condition optimized in the developed procedure. Separation and determination were performed by GC-MS according to the analytical parameters described in Section 2.4. The chromatographic approach used allows for an adequate separation and characterization by the mass spectra of tree tomato volatile compounds. Seventy compounds were identified in less than thirty five minutes: fifty-eight in the golden-yellow, and thirty-three in the reddish-purple variety. The aroma compounds identified and their relative amount in each variety are listed in Table 2. The main constituents of the golden-yellow variety, i.e. more than two percent, are: *α*-terpineol, methyl hexanoate, ethyl octanoate, 2,6-nonadienal, ethyl hexanoate, 1,8-cineole, naphthalene, methyl octanoate, *p*-cymenene, terpinene-4-ol, *α*-phellandren-8-ol, ethyl benzoate, methyl eugenol, decanal, and *β*-ionone. On the other hand, the reddish-purple variety is rich in naphthalene, α-phellandren-8-ol, nonanal, decanal, ethyl hexanoate, methyl hexanoate, ethanol, *β*-ionone, ethyl butanoate, and *α*-cedrol. Terpenoids (33%) and esters (32%) account for the characteristic aroma of golden-yellow variety; whereas terpenoids (30%) and aromatics (29%) contribute strongly to the organoleptic properties of the reddish-purple variety. A large amount of naphthalene (23%) was detected in the reddish-purple fruits. *α*-Terpineol, the main compound in the golden-yellow variety was absent in the reddish-purple. Aldehydes represent a very important group of volatiles identified in the golden-yellow (11%) and in the reddish-purple varieties (12%) (Table 2). The contribution of alcohols, aliphatics, lactones, and ketones to the overall aroma profile is small in the both varieties. Only methyl hexanoate, ethyl hexanoate, terpinene-4-ol, *α*-phellandren-8-ol and eugenol, identified in this study, were also found in the reddish-purple Colombian tree tomato [7]; meanwhile, four compounds found in the Panamanian variety were also present in the Malaysian fruit, i.e. ethyl butanoate, methyl hexanoate, ethyl hexanoate, and terpinene-4-ol [8]. The HS-SPME method used in this research for extraction of the aroma compounds is the main factor that explains these differences. The compounds in Colombian fruits were extracted by using solvents mixtures, whereas Malaysian tree tomatoes volatiles were isolated by distillation. It is known that solvent extraction may allow for losses of volatiles during extraction; meanwhile distillation process may not only reduce the amount of volatile compounds detected, but may induce the production of artifacts as well. These disadvantages are overcome by using the HS-SPME approach. Also, other factors such as the geographic location of cultivation and the age of the fruits analyzed account for these differences.

Table. 2. Components of *Solanum betaceum* Cav. fruits obtained by HS-SPME/GC-MS.

			Relative amount (%)	
Compounds	**RI_{ref}**	**RI_{exp}**	**Golden-yellow**	**Reddish-purple**
Ethanol	668	-	1.2	3.8
Methyl butanoate	723	710	0.3	nd
Ethyl butanoate	799	750	1.6	2.7
2-Hexenal	854	784	0.5	nd
Methyl hexanoate	910	841	8.4	4.6
Ethyl hexanoate	998	1002	5.9	5.4
1,8-Cineole	1030	1018	5.6	nd
γ-Terpinene	1074	1032	0.8	nd
p-Cymenene	1095	983	3.3	nd
Nonanal	1102	1001	nd	9.0
1,3,8-*p*-menthatriene	1115	1014	0.7	nd
Methyl octanoate	1128	1029	4.0	nd
2,6- Nonadienal	1154	1067	7.0	3.2
α-Phellandren-8-ol	1170	1088	2.8	11.4
Ethyl benzoate	1185	1092	2.6	nd
Terpinene-4-ol	1177	1103	2.9	1.6
Naphthalene	1178	1111	5.4	22.9
α-Terpineol	1190	1121	12.7	nd
Ethyl octanoate	1193	1126	7.1	nd
Decanal	1195	1137	2.2	6.0
cis-Citral	1242	1182	0.6	nd
trans-Citral	1270	1219	1.0	nd
α–Terpinen-7-al	1282	1239	0.4	nd
1-Methylnaphthalene	1312	1252	0.2	nd
Tridecane	1300	1256	nd	0.6
2-Methoxy-4-vinylphenol	1313	1273	0.5	nd
Methyl 4-methoxybenzoate	1371	1300	0.6	nd
Eugenol	1373	1325	0.4	1.2
2-Tridecenal	1311	1331	0.4	nd
Hexyl hexanoate	1381	1353	0.6	nd
β-Damascenone	1384	1359	1.3	1.3
Ethyl decanoate	1391	1367	0.4	nd
Tetradecane	1399	1374	0.2	1.1
Methyl eugenol	1410	1377	2.2	1.4
α-Cedrene	1409	1399	nd	1.5
Caryophyllene	1448	1406	0.1	nd
Thujopsene	1431	1419	nd	0.6
Geranyl acetone	1452	1433	3.9	2.8
Benzoquinone	nd	1453	nd	0.4
Cyclododecane	nd	1455	0.2	nd

β-Ionone	1494	1473	2.1	3.0
Methyl isoeugenol	1410	1481	0.5	nd
β-Cadinene	1519	1516	nd	1.0
dihydroactinidiolide	1525	1522	nd	1.0
Nerolidol	1565	1553	nd	1.0
Methoxyeugenol[a]	2222	1593	0.5	nd
α-Cedrol	1604	1603	nd	2.5
Benzophenone	1621	1622	0.2	0.8
Isoelemicin	1643	1640	0.2	nd
1-Octadecene	1786	1664	0.1	nd
γ-Dodecalactone	1685	1669	0.5	nd
Heptadecane	1700	1688	0.4	nd
δ-Decalactone	1503	1698	0.3	nd
Methyl tetradecanoate	1726	1711	1.4	nd
α-Hexylcinnamaldehyde	1749	1737	0.4	0.4
Octadecane	1800	1782	0.2	nd
Octadecanal	1818	1797	nd	0.4
Isopropyl tetradecanoate	1824	1804	nd	0.7
1-Hexadecene	1578	1853	0.2	nd
Nonadecane	1900	1871	0.3	nd
Methyl 11-hexadecenoate	nd	1874	nd	0.3
Methyl 9-hexadecenoate	1932	1875	0.6	nd
Farnesyl acetone	1921	1887	0.3	nd
Methyl hexadecanoate	1926	1890	0.9	1.6
Ethyl hexadecanoate	1991	1941	0.7	1.0
Eicosane	2000	1946	0.3	nd
Isopropyl hexadecanoate	1999	1962	0.3	nd
Methyl *cis*-9,octadecenoate	2082	2011	0.3	nd
Ethyl *cis*,*cis*-9,12-octadecadienoate	2159	2047	0.2	0.5
Ethyl *cis*,*cis*,*cis*-9,12,15-octadecatrienoate	2169	2051	1.1	3.8

Compounds are listed in order of elution from a HP-5 MS column; RI_{ref}: Literature Retention indices; RI_{exp}: Experimental Retention indices; nd: not detected; [a]Experimental retendion index calculated was not similar to that reported in literature, nevertheless MS spectra confirmed this compound; (-): could not be determined.

3.4. Multivariate studies

In order to study the data structure of the volatile compounds from both varieties of tree tomato, and the relationship between variables as well, two display chemometric techniques were applied to the X_{20x25} data matrix described in section 2.5, i.e. PCA and CA.

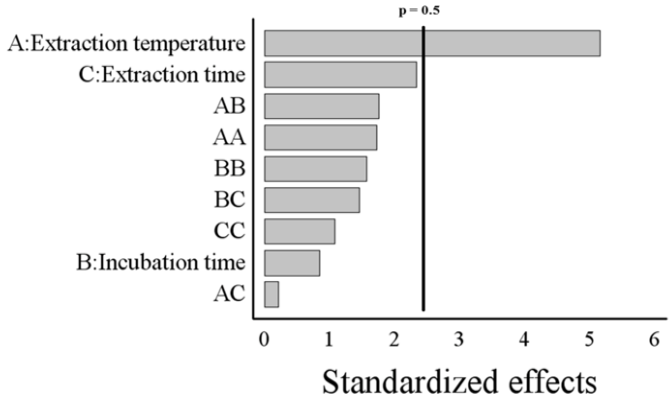

Figure 1. Pareto chart of standardized effects of the three factors analyzed by means of a 2^3 central composite design.

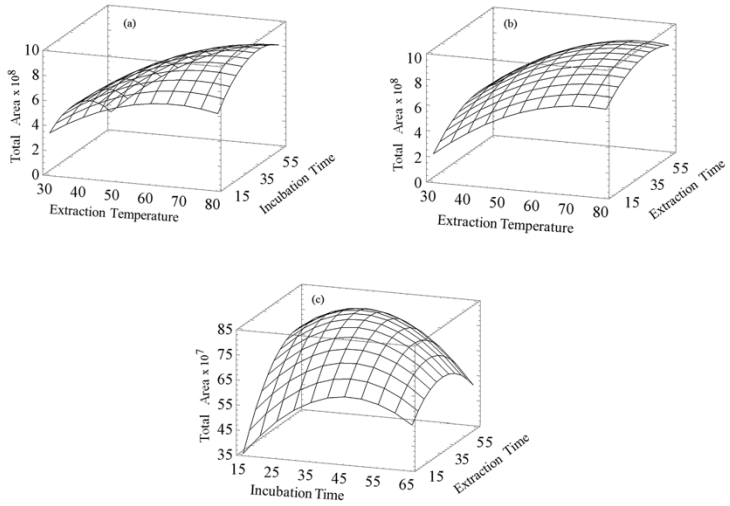

Figure 2. Response surface plot: (a) Total area *vs.* extraction temperature and incubation time on aroma volatiles. (b) Total area *vs.* temperature and extraction time. (c) Total area *vs.* extraction time and incubation time.

According to the criteria indicated above (to be detected in more than 80% of the samples) twenty five compounds were considered: benzophenone, *α*-cedrene, *α*-cedrol, *β*-damascenone, decanal, dihydroactinidiolide, ethanol, ethyl butanoate, ethyl *cis,cis,cis* 9,12,15 octadecatrienoate, ethyl hexadecanoate, ethyl hexanoate, eugenol, geranyl acetone, *α*-hexylcinnamaldehyde, *β*-ionone, methyl eugenol, methyl hexadecanoate, methyl hexanoate, naphtalene, nerolidol, 2,6 nonadienal, *α*-phellandren-8-ol, terpinen-4-ol, tetradecane and thujopsene.

3.4.1. Principal component analysis

Prior to the application of PCA, the data were autoscaled to avoid the effects produced by the variable size. PCA was performed to the autoscaled variables with the aim of studying the data structure in a reduced dimension, retaining the maximum amount of variability present in the data. From the loadings of the variables in the first three principal components, benzophenone, α-cedrene, *α*-cedrol, decanal, methyl hexadecanoate, nerolidol, *α*-

phelleanderen-8-ol, and tetradecane were the dominant features in the first principal component (PC), representing 36.4 % of the total data variability. In the second principal component (17.7 % of the total data variance), dihydroactinidiolide, ethyl butanoate, ethyl hexadecanoate, ethyl *cis,cis,cis* 9-12-15 octadecatrienoate, and methyl hexanoate were the more influential variables. For the third principal component (14.7% of total data variance) *β*-damascenone, eugenol, geranyl acetone, methyl eugenol, eugenol, naphthalene, 2,6-nonadienal, terpinen-4-ol and thujopsene were the most remarkable features. On a chemical basis, association between variables and the different PCs is unspecific: The first PC can be related with terpenoids: Carbonyl-bearing compounds due to the relevant contributions of four terpenoids (*α*-cedrene, *α*-cedrol, , nerolidol, and *α*-phelleanderen-8-ol) and two carbonyl compounds (such as decanal and benzophenone). The second PC has a clearer interpretation, it can be related with esters because the four main variables influencing this eigenvector belongs to this functional group family; and finally, the third PC is also a combined factor influenced by terpenoids, aldehydes and aromatic compounds. Score evaluation for tree tomato samples was performed by the construction of a plot in which the samples were projected in the space formed by the first three principal components, which account for the 68.8 % of the total information of the data. As can be seen in Figure 3, a natural separation between the two varieties (reddish-purple and golden-yellow) of tree tomato samples is observed, confirming that both varieties are located in different regions of the 25-dimensional space. Four samples of class 1, located in the left upper part of Figure 3, form a subset with slight different chemical characteristics, indicating an internal variability for this variety. Also, a minor overlap can be seen for both categories.

3.4.2. Cluster analysis

CA is an unsupervised procedure that allows for mapping the multidimensional space of variables to a two-dimensional tree called “dendrogram”, which is constructed on the basis of the similarity (or distance) between samples. Thus, CA was applied, in the present case, to the autoscaled data to achieve this objective.

The results obtained for the sample clustering process are presented in Figure 4 (a). As can be seen, three main clusters (I, J, and K) can be identified in the dendrogram shown. From the left, the first two clusters (clusters I and J) are made by samples of reddish-purple variety (plus one sample of class 2); while cluster K consists of samples from golden-yellow variety. In addition, in cluster I a subset composed by four samples of class 1 (previously indicated in PCA) is also revealed. Also, the small overlap between classes in the twenty five-dimensional space of the variables previously pointed out by PCA was corroborated by the level of similarity between clusters J and K, and by the presence of a sample of the variety 2 in cluster J which is composed of members of class 1. PCA and CA produced consistent results; both chemometric techniques pointed out the separation between the models for the two varieties, as well as a minor region in the multidimensional space in which both categories overlapped. In addition, a new cluster analysis, under the same conditions (Ward method and Euclidean squared distance) was carried out.

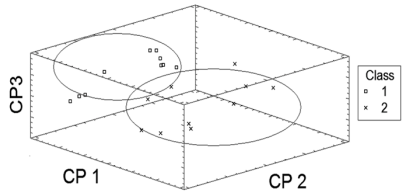

Figure 3. Eigenvector projection of the tree tomato samples in the space defined for the first three principal components. Class 1: reddish-purple variety; Class 2: yellow variety.

The dendrogram obtained, Figure 4 (b), shows the association between variables as a function of their similarity. In order to perform an interpretation based on chemical families (like in PCA), the previous dendrogram is presented in Figure 4 (c) , substituting the names of the variables by the chemical family code, i.e., terpenoids (T), Aldehydes-ketones (A), esters (E) and others (O). Similar associations than those revealed by using PCA can be confirmed by observing this dendrogram 4 (c). From left, cluster I is composed by three terpenoids plus one aromatic compound and one ester (as well as PC1 and PC3); cluster J_1 is formed mainly by terpenoids; aldehydes plus one alkane form subgroup J_2; and in cluster K, the corresponding subclusters K_1 and K_2 can be associated with esters (as PC2) and terpenoids, respectively. Terpenoids, which are the main source of volatile compounds for tree tomato, possess relations with other families such as aldehydes, alkanes, and aromatic compounds; however, esters constitute a separate factor in tree tomato volatiles profile.

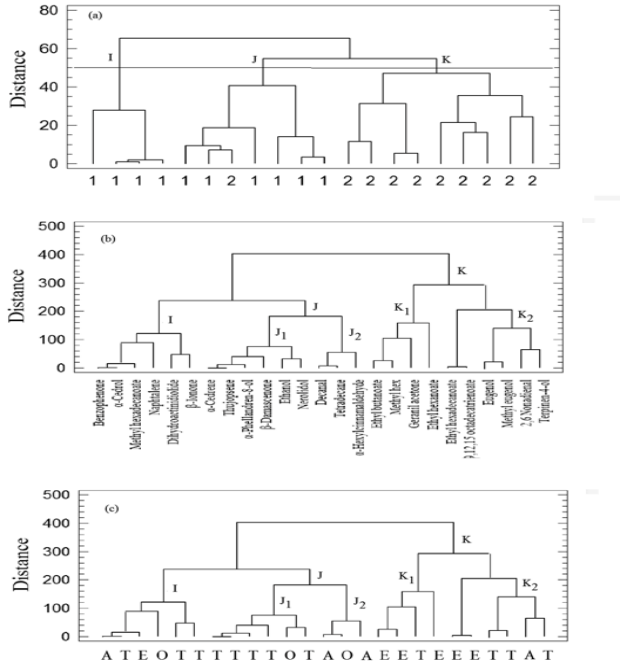

Figure 4. (a) Dendrogram of the tree tomato samples analyzed. (b) and (c) Dendrograms of variables (Ward method and Euclidean squared distance). Class 1: reddish-purple variety; Class 2: yellow variety. A: Aldehydes-Ketones; E: Esters; T: Terpenoids; O: Others.

The HS-SPME-GC-MS methodology developed in this research allowed for the identification of the volatile compounds of tree tomato fruits. The results presented in this report reveal for the first time the volatile composition of the golden-yellow variety, and the difference in the volatile aroma profile for the two most commercialized varieties of tree tomato fruits.

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