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A R T I C L E

HS-SPME Comparative Analysis of Genotypic Diversity in the Volatile Fraction and Aroma-Contributing Compounds of *Capsicum* Fruits from the *annuum–chinense–frutescens* Complex

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Volatile constituents of ripe fruits of 16 *Capsicum* accessions from the *annuum–chinense–frutescens* complex, with different aroma impressions and geographical origins, were isolated by headspace-solid phase microextraction (HS-SPME) and analyzed by gas chromatography–olfactometry–mass spectrometry (GC-sniffing port-MS). More than 300 individual compounds could be detected in the studied genotypes; most of them could be identified by comparing mass spectra and retention times with authentic reference substances or literature data. Esters and terpenoids were the main groups, although other minor compounds, such as nitrogen and sulfur compounds, phenol derivatives, norcarotenoids, lipoxygenase derivatives, carbonyls, alcohols, and other hydrocarbons, were also identified. The sniffing test revealed that the diversity of aromas found among the studied cultivars is due to qualitative and quantitative differences of, at least, 23 odor-contributing volatiles (OCVs). *C. chinense*, and *C. frutescens* accessions, with fruity/exotic aromas, were characterized by a high contribution of several esters and ionones and a low or nil contribution of green/vegetable OCVs. Different combinations of fruity/exotic and green/vegetable OCVs would explain the range of aroma impressions found among *C. annuum* accessions. Implications of these findings for breeding and phylogeny studies in *Capsicum* are also discussed.

KEYWORDS: *Capsicum* peppers; *annuum–chinense–frutescens* complex; cultivar diversity; volatile fraction; odor-contributing volatiles; quality breeding

INTRODUCTION

Fruits from the cultivated forms of the genus *Capsicum* are some of the most popular plant foods in the world, and there is a plethora of dishes, pastes, and sauces to which peppers contribute their particular color and flavor (*1*). Their double use, not only as vegetable but also as a spice, and their introduction and adaptation to many different regions and human taste preferences have contributed to such diversity of uses. Furthermore, *Capsicum* peppers can be utilized at both ripe and unripe stages, although the former is preferred by European consumers (*2*).

Among the species of *Capsicum* utilized by humans, *C. annuum* L. or common pepper is the most popular, genetically diverse, and economically important species, and its cultivars are grown worldwide (*1*, *3*). In addition, *C. annuum* is phylogenetically related to other two species, namely, *C. chinense* Jacq. and *C. frutescens* L., which are also utilized profusely in many regions of the world. *C. chinense*, to which varietal types such as Havanero

and Scotch Bonnet belong, is very popular in Mexico, the Caribbean region, and India (introduced by the Portuguese from Brazil centuries ago), whereas *C. frutescens* is grown in Asia, Africa, Latin America, and the United States, Tabasco being the most representative type (*1*, *3*). The three species make up a botanical group called the *annuum–chinense–frutescens* complex and can be intercrossed, giving rise to fertile hybrids (*4*). In fact, breeders have exploited for decades both *C. chinense* and *C. frutescens* as sources of variation in the genetic improvement of *C. annuum*, mostly for resistances to pests and diseases (*5–8*).

Two main factors contribute to the flavor perception of the fruits of *Capsicum*: pungency and aroma. Pungency is the best known flavor-related trait in peppers, and it has been studied for decades from many points of view (e.g., food technology, chemistry, genetics, and breeding) (*9*, *10*). By contrast, the range of studies about the volatile fraction is much lower. Since the first studies about the volatile fraction of peppers, which allowed discovering the bell pepper pyrazine (2-methoxy-3-isobutylpyrazine) as the most relevant volatile compound for the aroma in peppers (*11*), many volatiles have been reported in the

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Table 1. Name, Varietal Type, Origin, and Fruit Traits of the Evaluated *Capsicum* Accessions

accession (abbreviation)	varietal type	geographical origin (provider)	weight (g)	length (cm)	diameter (cm)	color
C. annuum						
Aci Sivri (ACI)	cayenne (large)	Turkey	7−12	14−19	1−2	red
Bierzo (BIE)	bell (triangular)	Spain (Cons. Reg. "IGP[a] Pimiento Asado del Bierzo")	61−140	7−12	5−7	red
Chile Arbol (ARB)	cayenne (small)	Mexico/southern USA	2−6	6−8.5	0.5−1.2	red
Di Senise (SEN)	cubanelle (small)	Italy (S. Lanteri, "IGP Peperone di Senise")	28−49	12−16	3.5−4.5	red
Doux d'Alger (DOU)	cubanelle (large)	France (A. M. Daubèze, INRA-GEVES, Cavaillon)	83−132	16−20	4−5.5	red
Jalapeno M (JAL)	jalapeno	Mexico/southern USA	6−17	4.5−6.5	1.5−3	red
Numex Big Jim (NUM)	NuMex	southern USA	27−71	11−17	3−5.5	red
Piparra (PIP)	cheese pimento	Spain (COMAV)	29−46	3−5	3−5	yellow
Piquillo Lodosa (PIQ)	piquillo	Spain (J. I. Macua, "DOP[a] Piquillo de Lodosa")	20−33	7−9	3−4.5	red
Poblano L (POB)	ancho/poblano	Mexico/southern USA	30−51	6−8	4−5	brown
Serrano (SER)	serrano	Mexico/southern USA	3−6	4−5	1−1.5	red
Valenciano (VAL)	bell (blocky)	Spain (COMAV)	83−203	6−10	6−8	red
C. chinense						
ECU-994 (994)	farolillo	Ecuador (COMAV)	4.5−7.5	3.5−5	1−2	red
C. frutescens						
Laotian (LAO)	Asian hot pepper	Laos (Reimer seeds)	0.6−2	2.2−3.5	0.6−1	red
Pebrera (PEB)	bird pepper	Spain (local market at Valencia)	0.2−0.5	1.5−2.2	0.5−0.7	red
Tabasco (TAB)	tabasco	USA (Reimer Seeds)	0.3−0.8	2−2.5	0.5−0.7	red

[a] IGP, Indication of Protected Origin; DOP, Denomination of Protected Origin. These marks are guaranteed by the European Union and were created to promote the authenticity and genuine characteristics of certain food and agricultural products across the EU.

volatile fraction of fresh and processed peppers. However, no genetic diversity was considered in most studies as they only included one cultivar (*12−16*), frequently from the Bell varietal type. Other experiments included very few cultivars (*17*), mostly limited to the same varietal type (*18*). As a consequence, the volatile fraction has been studied for only a reduced part of the diversity that can be found among *Capsicum* genotypes. Therefore, many *Capsicum* volatiles may still remain unidentified.

Additionally, most of these experiments were limited to a mere quantification of the identified volatiles and no sensorial tests were performed. Thus, with very few exceptions (see, e.g., refs *13* and *17*), the real contribution of individual compounds in the overall aroma has not been established in depth. This information is particularly relevant for plant breeders as breeding programs might be based mistakenly on the improvement of quantitatively important compounds but with low or no sensorial contribution, whereas other minor compounds, with a higher contribution due to a much lower odor threshold, might be not considered.

Finally, depending on the extraction method, the pattern of volatiles may be biased at both levels, qualitative and quantitative. Thus, for example, simultaneous distillation extraction (SDE) technique, which has been utilized in many of the previous studies on the volatile fraction of peppers, requires high temperatures and usually large fruit samples (*19*). Consequently, several volatiles identified on the basis of this extraction method might be thermal artifacts and, in addition, both the content and flavor contributions of many volatiles might be also overestimated. In this respect, a less time-consuming and more accurate protocol based on the headspace isolation technique (HS), performed at relatively low temperatures, combined with the solvent-free solid phase microextraction (SPME) (*20, 21*) has become more popular in recent years. This protocol should provide a more realistic knowledge of the compounds that make up the volatile fraction of a particular food and their contribution to aroma.

Within the pepper-breeding program at the COMAV Institute, in this work we have performed a comparative analysis of the volatile fraction of ripe fruits from the three species of the *annuum−chinense−frutescens* complex. Due to their importance for flavor, total capsaicinoids were also quantified. Quantitative data were complemented by sniffing port analyses to establish the sensorial effect of individual volatiles. The evaluated collection encompassed a wide range of varietal types of *C. annuum*, the most diverse and economically important species. Several accessions of *C. chinense* and *C. frutescens* were also included as external controls and to determine the possibilities of both species as sources of variation for the genetic improvement of the aroma of *C. annuum*. To our knowledge, this is the first comprehensive study of the genotypic diversity in volatile compounds in fruits from the three mentioned species and their effect in the aroma. Some preliminary results of part of this work were reported previously (*22*).

MATERIALS AND METHODS

Plant Material. A total of 16 *Capsicum* accessions, corresponding to *C. annuum*, *C. chinense*, and *C. frutescens* (**Table 1**), were grown in Valencia, Spain, under greenhouse conditions during the spring−summer growing season in 2005 (temperature range from 20 to 35 °C). These accessions encompassed a wide range of varietal types, geographical origins, and fruit traits (**Table 1**). The highest number of accessions (*12*) corresponded to *C. annuum*, because it is the most variable and economically important species and, therefore, the one on which breeding efforts should be focused. For comparison purposes we consider that one accession of *C. chinense*, a species too exotic and difficult to grow successfully in our Mediterranean conditions, was sufficient, whereas a higher number of *C. frutescens* accessions (three) was included in the experiment as it is fully adapted to cultivation in Spain and utilized in Spanish traditional cuisine and, consequently, more interesting for Spanish growers. Fully ripe fruits from each accession were harvested at the same time in July 2005 in Valencia, 14 weeks after transplanting, and sent immediately to the Technische Universität München at Weihenstephan for their analysis. Analyses were performed in less than 2 days and, therefore, the period between harvesting and sample analyses was not more than 3 days.

Preparation of Samples and Extraction of Volatiles. Five samples per accession were prepared for analysis. Each sample was prepared by slicing a 3 g sample from fruits of three different plants (1 g each) in 3 mm length pieces. Thus, a total of 15 plants per accession were sampled in the present analyses. Isolation of volatiles was performed by means of headspace solid phase microextraction (HS-SPME) technique. Each sample was placed into a 20 mL headspace vial, sealed with a septum (Butyl/PTFE gray, AChroma, Müllheim, Germany) and an aluminum cap. Equilibration was achieved by heating the vial for 30 min in a water bath (40 °C). The SPME holder and fiber (gray, divinylbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS, 50/30 μm) were purchased from Supelco (Bellefonte, PA). Prior to the first analysis, the SPME fiber was

conditioned for 1 h at 270 °C in the injector port of a gas chromatograph. For adsorption of volatiles the fiber was exposed to the headspace of sample vials during 40 min at 40 °C. For thermal desorption the needle was inserted into the injection port (250 °C) of the GC-MS system for 30 s (splitless mode). Prior to the next analysis, the fiber was reconditioned for 15 min at 250 °C in the injector port of another gas chromatograph to ensure no carry-over of compounds from the previous sample.

Analysis of Volatiles. A SiChromat II gas chromatograph (Siemens, Karlsruhe, Germany) was directly coupled through a Life-T-effluent splitter (1:1) to a MAT 8222 mass spectrometer (Finnigan MAT, Bremen, Germany) and to a sniffing port rinsed with humidified air to prevent dehydration of the nasal membranes of panelists. An SPB5 (Supelco) fused silica capillary column (5% phenyl−95% dimethylpolysiloxane as stationary phase, 30 m length, 0.53 mm internal diameter, film thickness = 1.5 μm) was used. Helium (Linde, Munich, Germany) was utilized as carrier gas at a flow of 3 mL min^{-1} (split ratio 1:8). The column was programmed from 100 °C at a rate of 5 °C min^{-1} to 250 °C. Because of the high number of volatiles found in our analyses, with different polarities and volatilities, it was not possible to find suitable (not interfering) standards and to determine response factors. Consequently, and considering that our main objective was to compare the volatile fractions of different cultivars, quantitative data were obtained from the total ion current chromatograms by integration of the GC peak areas. In this respect, due to the lack of correction of response factors, direct comparisons between GC peak areas of different volatiles or groups of volatiles must be interpreted with caution. Linear retention indices (RI) of individual volatiles were calculated according to the method of Van der Dool and Kratz (*23*). The transfer line was maintained at 220 °C. Detection by the mass spectrometer was performed in the electron impact (EI) mode (70 eV ionization energy). Acquisition was performed in scanning mode (mass range m/z 35−350 amu).

Chemicals. Identification of volatiles was performed by comparison of mass spectra and corresponding RI values with authentic reference substances (R) or tentatively by comparison with literature data (L) or by comparison with the NIST Library (MS) as indicated in **Tables 3−5**. Reference substances were analyzed under the same gas chromatographic conditions as the *Capsicum* samples, and their mass spectra and linear RI values were recorded in a data compilation (Kollmannsberger, 1986−2005). If available, reference substances were purchased from commercial sources (Merck, Darmstadt, Germany; Fluka, Deisenhofen, Germany; Sigma-Aldrich, Taufkirchen, Germany; EGA-Chemie, Steinheim, Germany; Riedel-de-Haen, Seelze, Germany; Roth, Karlsruhe, Germany). Others were gifts from flavor companies (Haarmann & Reimer, Holzminden, Germany; Dragoco, Holzminden, Germany; Silesia, Neuss, Germany; Firmenich, Genf, Switzerland; Oril SA, Paris, France). Some of the sesquiterpenes were isolated from essential oils with known composition. Ectocarpene was a gift of Prof. L. Jaenicke (Köln, Germany).

Esters, which were not in our reference substance collection, were synthesized by acid-catalyzed esterification. The corresponding acid (acetic acid, propionic acid, butanoic acid, pentanoic acid, hexanoic acid, 2-methylpropanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, 3-methylpentanoic acid, 4-methylpentanoic acid, 5-methylhexanoic acid, 1 mmol each) was mixed with the requested alcohol (methanol, ethanol, propanol, butanol, pentanol, hexanol, 2-methylpropanol, 3-methylbutanol, 4-methylpentanol, 1 mmol each). One milliliter of H_2SO_4 (10%) was added, and the mixtures were allowed to stand for 3 days at 70 °C. After neutralization with NaHCO$_3$ (10%), the synthesized compounds were extracted with pentane dichloromethane (2:1) and analyzed.

4-Methyl-2-pentenoates were synthesized by transesterification of methyl 4-methyl-2-*E*-pentenoate (20 mg, 97%, Alfa Aesar, Karlsruhe, Germany; RI 922, m/e 41, 69, 97, 128, 73, 113, 53, 81, 59) with a mixture of hexanol, 3-methylbutanol, and 4-methylpentanol (30 mg each), containing 10% KOH. After 2 h of incubation at 60 °C, the resulting ester mixture was isolated with diethyl ether and analyzed: 3-methylbutyl 4-methyl-2-pentenoate (RI 1259, m/e 97, 114, 115, 70, 43, 41, 55, 59, 141, 184), 4-methylpentyl 4-methyl-2-pentenoate (RI 1359, m/e 115, 114, 97, 43, 56, 69, 84, 59, 155, 198), and hexyl 4-methyl-2-pentenoate (RI 1394, m/e 115, 114, 97, 43, 41, 56, 69, 59, 155, 198).

8-Methyl-6-nonenoates were synthesized by acid-catalyzed esterification of the corresponding acid with a mixture of 2-methylpropanol,

3-methylbutanol, and 4-methylpentanol in 0.3 mL of H_2SO_4 (10%) for 2 h at 70 °C and subsequent extraction with diethyl ether to give the products 2-methylpropyl 8-methyl-6-nonenoate (RI 1489, m/e 57, 69, 153, 152, 137, 109, 97, 115, 226), 3-methylbutyl 8-methyl-6-nonenoate (RI 1589, m/e 71, 69, 43, 152, 137, 109, 97, 115, 240), and 4-methylpentyl 8-methyl-6-nonenoate (RI 1685, m/e 43, 85, 69, 152, 137, 109, 97, 115, 254).

8-Methyl-6-nonenoic acid (RI 1306, m/e 69, 55, 56, 41, 95, 97, 115, 137, 170, 152) was liberated from commercially available capsaicin (10 mg, 97%, Fluka) by hydrolysis in KOH (2 days, 95 °C), subsequent addition of H_2SO_4, extraction with diethyl ether, and concentration of the ether extract on a Vigreux column (40 °C), as described by Kollmannsberger (*24*).

Analysis of Capsaicinoids. Four gram samples of each *Capsicum* accession were prepared as described above. Ten milliliters of acetone was added to each sample, and the mixture was stored for 3 days at room temperature. The supernatant was used for gas chromatographic analysis. An HP 5890 series II GC, equipped with a DB5 column (30 m length, 0.25 mm internal diameter, film thickness = 0.25 μm, carrier gas helium at a flow rate of 1 mL/min, split 1:14, injector and detector, 260 °C), was used. The column was held at 100 °C for 5 min and then programmed at a rate of 5 °C min^{-1} to 260 °C, as described by Kollmannsberger (*24*). For quantification, peak areas of all separated capsaicinoids were compared to a calibration curve obtained with pure capsaicin (97%, Fluka).

Statistical Procedures. To assess significant differences between cultivars for content in capsaicinoids and volatiles, a Duncan's multiple-range test was performed. Thus, for total volatiles and each individual volatile, a Duncan's test (significance level of $P < 0.05$) was performed on the basis of GC peak areas (five data per cultivar). The same analysis was done for total capsaicinoids on the basis of their concentration (μg g^{-1}). Hierarchical cluster analysis of *Capsicum* genotypes was carried out employing unweighted pair group method with arithmetic mean (UPGMA) on the basis of the quantified GC peak areas of identified compounds. The similarity matrix was based on Euclidean distances. NTSYSpc 2.0 software was utilized for this analysis.

Sensory Studies. A panel of four experienced assessors (two male, two female), with years of experience in organoleptic analysis of spices and food, performed all of the sensorial analyses of the experiment, which took place before midday (between 10:00 and 11:30 a.m.). For training purposes, to adapt their sensory expertise to *Capsicum* smellings, the panel judged the aroma of cuttings from fresh ripe fruits of the 16 pepper accessions (**Table 1**). For a complete training and an accurate description of the aroma of each cultivar, each panelist repeated this session twice on different days. After the aromas had been profiled with free descriptor terms (free-choice profiling), 12 adequate odor descriptors were selected to describe the overall aroma of accessions: earthy, exotic, fruity, fuel, green, paprika, peasy, pungent-hot pepper, sulfurous, sweet, unripe nut, and vegetable. Additionally, the intensity of the whole aroma was judged on a 4-point scale (1 = weak, 2 = moderate, 3 = strong, and 4 = very strong). Sniffing analyses of GC eluates were made by the same experienced panel and were usually stopped after 22 min (RI $\geq$ 1600). Only compounds detected by, at least, two panelists were considered to contribute significantly to the aroma of the corresponding sample (odor-contributing volatiles, OCVs). Panelists described the aroma of individual compounds at the sniffing analyses on the basis of the above-mentioned descriptors, complemented by some descriptors previously reported in peppers (e.g., cucumber-like, floral, musty) (*13*, *17*).

RESULTS AND DISCUSSION

Flavor Differences among Cultivars. A high degree of variation was found for flavor traits in the studied collection. Thus, total capsaicinoids ranged from <0.001 mg g^{-1} in sweet genotypes of *C. annuum* to >1 mg g^{-1} in *C. chinense* and *C. frutescens* accessions, confirming the usually pungent nature of both species (*1*). With regard to aroma, a preliminary sensorial test revealed a wide range of odor impressions among the studied 16 accessions, in concordance with the genotypic diversity encompassed in this collection (**Table 2**). Additionally, a high degree of variation was found for total volatile content (expressed as GC peak area), ranging from 32 × 10^8 to 6879 × 10^8 (**Table 2**). This

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Table 2. Total Capsaicinoid Content, Total Volatile Content, and Overall Aroma Description of the Evaluated *Capsicum* Accessions

accession (abbreviation)	total capsaicinoids[a] (μg g^{-1})	total volatiles[a] (peak area $\times 10^8$)	aroma description/intensity[b]
C. annuum			
Aci Sivri (ACI)	385 e	4332 e	fruity, sweet, paprika, vegetable/3
Bierzo (BIE)	<1 a	39 a	earthy, green, paprika/1
Chile Arbol (ARB)	724 f	1788 c	fruity, sweet, peach, paprika/2.5
Di Senise (SEN)	<1 a	41 a	sweet, vegetable, paprika/1
Doux d'Alger (DOU)	<1 a	32 a	green, earthy, paprika/1
Jalapeno M (JAL)	142 d	183 b	green, earthy, vegetable, paprika/2.5
Numex Big Jim (NUM)	85 c	272 b	sweet, exotic, earthy, paprika/2
Piparra (PIP)	3 b	253 b	earthy, paprika, fuel/1.5
Piquillo Lodosa (PIQ)	<1 a	49 a	earthy, green, paprika/1
Poblano L (POB)	<1 a	40 a	earthy, green, paprika, sulfurous/2
Serrano (SER)	540 ef	1640 c	green, peasy, paprika, fruity/3
Valenciano (VAL)	<1 a	61 a	green, vegetable, unripe nut/1
C. chinense			
ECU-994 (994)	1641 gh	6879 f	sweet, fruity, exotic, spicy/4
C. frutescens			
Laotian (LAO)	1120 g	4404 e	fruity, sweet, exotic, paprika/ 3.5
Pebrera (PEB)	1802 h	3392 d	fruity, sweet, exotic, paprika/3
Tabasco (TAB)	3255 l	3525 d	sweet, fruity, pungent, spicy/ 3.5

[a] Mean corresponding to $n = 5$ samples. Means followed by different letters in the same column are significantly different ($P < 0.05$) by Duncan's test. [b] On the basis of the odor impressions of four panelists. Aroma intensity is described on a 1–4 scale (1, low; 2, moderate; 3, intense; 4, very intense).

trait was correlated with aroma intensity. *C. frutescens* and *C. chinense* accessions were mainly characterized by exotic, fruity, and/or sweet notes (**Table 2**). Furthermore, the aromas of these accessions were the most intense of the whole collection. By contrast, a range of aroma profiles and intensities were found within *C. annuum*. Thus, we found accessions with weak and green-earthy vegetable aroma such as Bierzo, Doux d'Alger, Piquillo, or Peperone di Senise and others such as Chile de Arbol, which showed odor impression and intensity similar to those of *C. chinense* and *C. frutescens* accessions (**Table 2**). The rest of the *C. annuum* accessions showed different combinations of intensity and aroma profiles like the Turkish cayenne Aci Sivri, with an intense mixture of fruity and vegetable-like notes, or Jalapeno, with a green-vegetable aroma of moderate intensity. The earthy-green notes of Valenciano, Piparra, and Poblano possessed a strange odor impression, described as sulfurous, ressembling fuel or unripe nuts. Serrano showed a distinctive peasy note (**Table 2**).

Identification of Volatiles. More than 300 individual compounds could be detected in the studied collection, and quantitative and qualitative differences were found among the studied accessions for most compounds. Such diversity of compounds is considerably higher than reported previously in fruits of *Capsicum* and confirms the importance of genotypic diversity to study the composition of fruits. In fact, many of the identified compounds (111) have not been previously reported in *Capsicum* fruits, even considering unripe or processed peppers (e.g., canned, ground powder, smoked powder, oleoresin). Esters (**Table 3**) and terpenoids (**Table 4**) were found to be the most abundant volatiles, followed by other minor compounds such as nitrogen and sulfur compounds, phenol derivatives, norcarotenoids, alcohols, lipoxygenase cleavage products, furans, ketones, and aromatic and aliphatic hydrocarbons (**Table 5**).

A total of 107 esters were found in the volatile fraction of the analyzed peppers (**Table 3**). Although this was the most abundant group, genotypes without esters can be found within *C. annuum* such as Bierzo, Doux d'Alger, Piparra, Piquillo, Poblano, Peperone di Senise, and Valenciano. *C. chinense* and *C. frutescens* accessions were characterized by high levels and diverse patterns of these volatiles (**Table 3**), which usually provide the aroma of foods with fruity notes. A total of 69 saturated esters were identified in our analyses and, according to GC peak areas, the

highest levels corresponded to 2-methylpropanoate, 2-methylbutanoate, 3-methylbutanoate, and 4-methylpentanoate subgroups (**Table 3**). *C. chinense* and *C. frutescens* accessions showed the highest levels and the most diverse profiles of these compounds, whereas the contrary was true for Jalapeno and NuMex. Aci Sivri and Chile de Arbol, followed by Serrano, showed intermediate levels and a pattern more similar to *C. chinense* and *C. frutescens* (**Table 3**). Unsaturated esters followed saturated esters in terms of qualitative contribution to the volatile fraction. Again, *C. chinense* and *C. frutescens* accessions showed the most diverse patterns and the highest contents, followed by Aci Sivri, Chile de Arbol, and Serrano, whereas Jalapeno and NuMex had very low amounts of these compounds (**Table 3**). (*Z*)-3-Hexenyl 2-methylbutanoate, (*Z*)-3-hexenyl 3-methylbutanoate, and (*Z*)-3-hexenyl 4-methylpentanoate were found, in general, to be the most abundant esters with unsaturated alkyl part. Additionally, relatively high levels of 6-methyl-4-heptenyl esters were found specifically in *C. chinense* and, to a lesser extent, in Tabasco (**Table 3**). Among esters with an unsaturated acyl part, relatively high amounts of 3-methylbutyl 8-methyl-6-nonenoate and 4-methylpentyl 8-methyl-6-nonenoate were found in Aci Sivri, *C. chinense*, Laotian, and Pebrera (**Table 3**). Finally, several odoriferous methyl and ethyl esters were identified in the fruits of the *C. chinense* accession, ethyl 4-methylpentanoate being the most abundant compound in this group. Traces and low levels of this compound and ethyl 2-methylbutanoate were also found in the volatile fraction of Aci Sivri, Chile de Arbol, Serrano, and Tabasco (**Table 3**).

A total of 74 terpenoids were found in the volatile fraction of the studied collection, mainly sesquiterpene hydrocarbons (48), followed by monoterpenoids (17) and oxygenated sesquiterpenes (9). As reported for esters, *C. chinense* and *C. frutescens* accessions showed the highest levels and most diverse patterns of these compounds, with the only exception of Laotian, whereas the contrary was found in *C. annuum* accessions Bierzo, Doux d'Alger, Piquillo, Poblano, Peperone di Senise, and Valenciano (**Table 4**). Aci Sivri, Chile de Arbol, and Serrano, among *C. annuum* accessions, showed levels and patterns of sesquiterpenes similar to *C. chinense* and *C. frutescens* (**Table 4**). α-Copaene and several himachalenes were found to be the most common sesquiterpenes as they could be identified in many of the studied

Table 3. Individual Esters and Their Average GC Peak Areas ($\times 10^8$)[a] Identified in the Fruits of the Studied Accessions of *C. annuum*, *C. chinense*, and *C. frutescens*

compound[b]	RI[c]	ID[d]	ACI	ARB	JAL	NUM	SER	994	LAO	PEB	TAB
saturated esters											
butanoates											
3-methylbutyl butanoate	1052	R		tr[e]					1.5b	0.5a	
pentyl butanoate	1088	R		tr					1.2a	0.6a	
4-methylpentyl butanoate	1153	R	1.4a	12.1b			3.1a	2.1a	120.7d	61.4c	1.1a
hexyl butanoate	1189	R	0.1a	0.4b				0.3b	4.5d	3.7c	0.6b
4-methylhexyl butanoate[f]	1260	MS	0.1a	0.3a					6.0c	2.6b	
2-methylpropanoates											
3-methylbutyl 2-methylpropanoate	1009	R		tr					1.5b	0.1a	
pentyl 2-methylpropanoate	1044	R		tr				0.4a	3.0c	1.2b	
4-methylpentyl 2-methylpropanoate	1109	R	19.9b	31.7b	0.5a	1.9a	14.39b	163.9d	245.5e	117.6c	17.2b
hexyl 2-methylpropanoate	1144	R	0.2a	0.5a			tr	18.7e	12.8d	8.1c	3.7b
5-methylhexyl 2-methylpropanoate[f]	1208	MS							0.7a	tr	
4-methylhexyl 2-methylpropanoate[f]	1216	MS	0.7b	1.0b			0.3a	2.6c	24.5e	8.1d	3.7c
heptyl 2-methylpropanoate	1244	R		tr			tr	9.8c	1.0a	0.4a	3.1b
methyloctyl 2-methylpropanoate*[f]	1389	MS						2.6a			
pentanoates											
3-methylbutyl pentanoate[f]	1150	R		tr					0.9b	0.1a	
pentyl pentanoate	1187	R		tr				0.9a	2.1b	0.6a	
4-methylpentyl pentanoate	1251	R	26.0c	14.1b	0.2a	0.9a	9.1b	100.5f	186.1 g	77.3e	50.8d
hexyl pentanoate	1287	R		tr				4.7b	2.9a	1.7a	4.7b
4-methylhexyl pentanoate[f]	1359	MS		tr		0.1a			4.8c	1.7b	
2-methylbutanoates											
2-methylpropyl 2-methylbutanoate	1003	R							0.4b		0.1a
3-methylbutyl 2-methylbutanoate	1099	R	0.7a	2.5b			tr	0.7a	26.1c	4.7b	0.6a
pentyl 2-methylbutanoate	1136	R	0.7a	2.2a			tr	18.6b	39.5c	16.6b	1.5a
4-methylpentyl 2-methylbutanoate	1201	R	164.4c	176.0c	15.4a	20.6a	115.4b	365.7e	333.0e	232.4d	186.6c
hexyl 2-methylbutanoate	1236	R	1.4ab	3.9b	0.1a	0.5ab	0.9ab	201.8f	60.5d	41.1c	153.6e
5-methylhexyl 2-methylbutanoate[f]	1299	MS		tr				0.7a	2.4b	0.5a	2.7b
4-methylhexyl 2-methylbutanoate[f]	1307	MS	4.0b	4.8b	0.7a	0.6a	1.6a	11.8c	86.0e	32.4d	28.6d
heptyl 2-methylbutanoate	1336	R		tr				26.4b	1.6a	0.5a	27.5b
methyloctyl 2-methylbutanoate*[f]	1480	MS						2.8a			
3-methylbutanoates											
2-methylpropyl 3-methylbutanoate	1004	R						0.2a		0.2a	
3-methylbutyl 3-methylbutanoate	1103	R	0.7a	0.7a				3.9b	4.8b	0.8a	0.8a
pentyl 3-methylbutanoate	1139	R	0.7a	1.1a				68.4d	7.4c	1.6ab	1.9b
4-methylpentyl 3-methylbutanoate[f]	1205	R	185.6e	42.2c	7.3a	4.1a	32.1b	1062.0 g	222.3f	84.2d	188.5e
hexyl 3-methylbutanoate	1240	R	1.1a	1a	tr		0.2a	223.2c	11.8b	3.7a	160.4c
5-methylhexyl 3-methylbutanoate[f]	1302	MS						2.4a			2.5a
4-methylhexyl 3-methylbutanoate[f]	1310	MS	4.3b	0.8a	0.3a		0.2a	30.8d	11.9c	1.7a	30.8d
heptyl 3-methylbutanoate	1340	R		tr				66.5c	0.4a		19.6b
methyloctyl 3-methylbutanoate*[f]	1482	MS						6.4a			
hexanoates											
pentyl hexanoate[f]	1286	R		tr					1.2b	0.4a	
4-methylpentyl hexanoate	1350	R	5.9b	4.9b	0.1a	0.3a	1.1a		79.9d	27.4c	
hexyl hexanoate	1385	R		tr					2.6a		4.3a
4-methylpentanoates											
2-methylpropyl 4-methylpentanoate[f]	1112	R						0.2a			0.1a
3-methylbutyl 4-methylpentanoate[f]	1213	R	0.7a	0.4a				6.8b	7.7b	0.8a	21.8c
pentyl 4-methylpentanoate	1251	R	1.5a	0.6a				82.1d	18.4c	4.0b	119.5e
4-methylpentyl 4-methylpentanoate	1313	R	207.7c	43.4b	6.8a	5.9a	41.2b	362.4e	288.7d	178.0c	361.5e
hexyl 4-methylpentanoate	1350	R			0.7a	0.4a		169.7c	17.6b	5.4a	286.7d
5-methylhexyl 4-methylpentanoate	1412	MS									10.1a
4-methylhexyl 4-methylpentanoate	1420	MS	2.2b	0.3a	0.3a		0.2a	4.7c	9.0d	2.3b	81.1e
heptyl 4-methylpentanoate	1449	MS						7.8a			74.7b
methyloctyl 4-methylpentanoate*[f]	1588	MS						1.7a			
4-methylhexanoates											
3-methylbutyl 4-methylhexanoate[f]	1319	MS		tr			tr	0.3a			2.6b
pentyl 4-methylhexanoate[f]	1358	MS									8.9a
4-methylpentyl 4-methylhexanoate[f]	1419	MS	2.9ab	0.4a	tr		tr	5.4b	12.9c	2.9ab	124.4d
hexyl 4-methylhexanoate[f]	1455	MS									51.8a
4-methylhexyl 4-methylhexanoate[f]	1526	MS									2.4a
5-methylhexanoates											
3-methylbutyl 5-methylhexanoate[f]	1310	R		tr				0.2a			
4-methylpentyl 5-methylhexanoate[f]	1411	R	2.1b	0.4a			tr	1.4ab	6.8c	0.5a	5.0c
hexyl 5-methylhexanoate[f]	1449	R						4.2b			1.6a

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Table 3. Continued

compound[b]	RI[c]	ID[d]	ACI	ARB	JAL	NUM	SER	994	LAO	PEB	TAB
7-methyloctanoates											
2-methylpropyl 7-methyloctanoate[f]	1410	MS							3.2a		
3-methylbutyl 7-methyloctanoate[f]	1509	MS	3.1b	0.3a	0.1a				10.1c	0.2a	
pentyl 7-methyloctanoate[f]	1546	MS							2.9a		
4-methylpentyl 7-methyloctanoate[f]	1609	MS	9.1b	0.8a	0.8a	0.4a	0.4a		10.1b	0.9a	
hexyl 7-methyloctanoate[f]	1645	MS	0.7a						1.7b	0.2a	
8-methylnonanoates											
2-methylpropyl 8-methylnonanoate[f]	1509	MS	0.4a	0.1a					9.6b	0.9a	
3-methylbutyl 8-methylnonanoate[f]	1609	MS	5.5c	0.7ab	0.1a	0.2a	0.2a		17.6d	1.6b	
pentyl 8-methylnonanoate[f]	1646	MS	1.4b	0.4a					6.4c	0.9ab	
4-methylpentyl 8-methylnonanoate[f]	1709	MS	30.6d	1.8ab	1.1a	0.7a	0.4a	3.6b	35.7d	6.3c	0.7a
hexyl 8-methylnonanoate[f]	1745	MS						0.4a	0.5a	0.2a	
9-methyldecanoates											
3-methylbutyl 9-methyldecanoate[f]	1708	MS	0.5a						1.5b		
pentyl 9-methyldecanoate[f]	1746	MS	0.1a						0.4a		
4-methylpentyl 9-methyldecanoate[f]	1809	MS	0.8a						0.7a		
unsaturated esters											
esters with unsaturated alkyl part											
(*Z*)-3-hexenyl 2-methylpropanoate	1140	R					0.1a	1.8b	11.9d	3.9c	
pentenyl ?-methylbutanoate 1*	1146	MS						1.6a			
pentenyl ?-methylbutanoate 2*	1150	MS						2.8a			
(*Z*)-3-hexenyl butanoate	1182	R							1.7b	0.8a	
methylpentenyl ?-methylbutanoate*	1193	MS	0.5a	0.7a			0.4a	4.4b	7.9c	6.8c	0.8a
(*Z*)-3-hexenyl 2-methylbutanoate	1231	R		4.1b	0.2a	0.3a	3.1b	30.1c	81.1e	42.9d	7.8b
(*Z*)-3-hexenyl 3-methylbutanoate	1235	R	5.1b	0.5a	0.1a	0.1a		88.8d	17.8c	6.5b	8.9b
pentenyl 4-methylpentanoate*[f]	1259	MS						11.3a			
(*Z*)-3-hexenyl pentanoate	1282	R	3.9b				0.1a	0.7a	3.5b	1.0a	
6-methyl-4-heptenyl 2-methylpropanoate[f]	1290	MS						24.2b			1.9a
methylpentenyl 4-methylpentanoate*[f]	1307	MS	0.8a					5.4b		1.3a	8.5b
(*Z*)-3-hexenyl 4-methylpentanoate[f]	1346	MS	0.1a	1.1a		0.2a	0.6a	79.7d	28.2c	5.7b	88.5e
(*Z*)-3-hexenyl hexanoate	1381	R	4.4b						3.0ab	1.3a	
6-methyl-4-heptenyl 2methylbutanoate[f]	1381	MS						55.7b			5.1a
6-methyl-4-heptenyl 3-methylbutanoate[f]	1386	MS						189.0b			14.7a
6-methyl-4-heptenyl pentanoate[f]	1432	MS						2.9a			2.2a
6-methyl-4-heptenyl 4-methylpentanoate[f]	1439	MS						41.4a			60.7a
(*Z*)-3-hexenyl 4-methylhexanoate[f]	1451	MS									4.8a
esters with unsaturated acyl part											
3-methylbutyl 4-methyl-2-pentenoate[f]	1260	R	tr						2.1b	tr	1.0a
4-methylpentyl ?-methyl-?-butenoate*[f]	1285	MS						13.6a			
4-methylpentyl 2-methyl-2-butenoate[f]	1294	MS	tr	tr			tr	20.8c	5.0b	2.5a	4.4b
pentyl 4-methyl-2-pentenoate[f]	1295	MS						tr	tr	tr	1.1a
4-methylpentyl 4-methyl-2-pentenoate[f]	1359	R	2.1a	tr			tr	32.1c	2.7a	1.3a	21.3b
hexyl 4-methyl-2-pentenoate[f]	1394	R						2.9a			15.7b
2-methylpropyl 8-methyl-6-nonenoate[f]	1491	R						7.4a	64.4c	17.9b	
3-methylbutyl 8-methyl-6-nonenoate[f]	1590	R	58.7c	1.7a		0.9a	tr	3.0a	169.5d	36.8b	
pentyl 8-methyl-6-nonenoate[f]	1629	MS						2.9a	9.9b	3.1a	
4-methylpentyl 8-methyl-6-nonenoate[f]	1691	R	57.3c	1.1a		1.2a		40.3bc	63.6d	25.2b	4.2a
hexyl 8-methyl-6-nonenoate[f]	1728	MS						5.2b	tr	0.9a	
methyl and ethyl esters											
methyl 2-methylbutanoate[f]	771	R						tr			
methyl 3-methylbutanoate[f]	771	R						0.2a			
ethyl 2-methylbutanoate[f]	843	R		tr				tr			tr
ethyl 3-methylbutanoate[f]	844	R						0.1a			
methyl 4-methylpentanoate[f]	888	R						tr			
ethyl 3-methylpentanoate[f]	953	R						tr			
ethyl 4-methylpentanoate[f]	960	R	0.1a	0.1a			0.2a	0.4b			0.5b
esters of secondary alcohols											
3-methyl-2-butyl 3-methylbutanoate[f]	1057	MS									1.0a
3-methyl-2-butyl 4-methylpentanoate[f]	1163	MS									3.1a

[a] Mean peak area, *n* = 5. Means followed by different letters in the same row are significantly different ($P < 0.05$) by Duncan's test. [b] * indicates position of double bond (?) and/or methyl branching not exactly determined and, therefore, these compounds were tentatively identified. [c] Linear retention indices on a DB5 column. [d] The reliability of the identification proposal is indicated by the following: R, by comparison of mass spectra and RIs with authentic reference substances; MS, by mass spectrum and comparison of RI with homologues. MS-labeled compounds should be considered as tentatively identified. [e] tr, traces (GC peak area $<0.1 \times 10^8$). [f] New constituents reported for the first time in *Capsicum*.

Capsicum accessions. α-, β-, and γ-himachalenes were predominant in pungent genotypes (**Table 4**). Sativene and cyclosativene as well as derivatives of germacrene A such as (*E*)-β-elemene, some selinenes, 5-epi-aristolochene, and 4-epi-eremophilene were

Table 4. Individual Sesquiterpenes and Monoterpenes and Their Average GC Peak Areas ($\times 10^8$)[a] Identified in the Fruits of the Studied Accessions of *C. annuum*, *C. chinense*, and *C. frutescens*

compound	RI[b]	ID[c]	ACI	ARB	BIE	DOU	JAL	NUM	PIP	PIQ	POB	SEN	SER	VAL	994	LAO	PEB	TAB
sesquiterpenes																		
nonoxygenated																		
α-cubebene	1371	R					tr[d]								140.3b			34.4a
α-longipinene	1383	R	82.2d	6.3b			1.9a	tr				tr	1.8a			20.1c	22.8c	tr
α-ylangene[e]	1397	R	23.3c	59.5d									11.1b	0.5a	tr		93.5e	14.6b
cyclosativene[e]	1398	L	tr		0.5a		0.9a	1.2a	2.5b		0.6a	0.8a		tr				
α-copaene	1402	R	111.1e		4.6a		5.8a	11.6b	30.3d	3.0a	8.8ab	20.2c		2.3a	155.3f			46.4c
iso-β-elemene	1409	R				tr		2.6b	0.6a				1.1a	0.5a				
longicyclene	1410	R	28.0c	tr			0.2a									8.0b		
β-cubebene	1413	R													136.0c	4.0a		40.3b
(E)-β-elemene	1415	R			7.6c	2.4b	2.4b	32.1e	6.4c		0.3a		19.2d	5.0c		tr		
β-bourbonene[e]	1416	R													8.6a			
sativene[e]	1423	L	tr		tr		tr		tr		tr	tr		tr				
(Z)-α-bergamotene	1430	R											tr					
β-longipinene	1436	R		11.6a													tr	
β-ylangene[e]	1449	R		5.3a									tr				5.0a	
α-cedrene	1449	R		tr	tr		tr			tr	tr							
(E)-β-caryophyllene	1453	R	tr						tr				tr	0.5a	29.2b			tr
(E)-α-bergamotene[e]	1455	R					1.1a						4.1b					
himachala-2,4-diene	1458	MS	tr	6.3a			tr						tr				16.5b	
β-copaene[e]	1460	R	tr						1.1a		0.3a	tr			12.0c	2.2a		4.9b
(E)-β-farnesene	1464	R		tr													20.3a	
thujopsene[e]	1468	R					tr				tr							
himachala-1,3-diene[e]	1478	MS	tr	1.9a									4.1b			tr	2.5a	
α-himachalene	1487	R	195.1 g	44.5d			2.1a	0.4a	tr			tr	14.0b		60.5e	48.2d	101.0f	22.8c
α-humulene	1487	R													tr			
(Z)-muurola-4(14),5-diene[e]	1494	L													tr			tr
ar-curcumene[e]	1498	R					tr		tr									
selina-4,11-diene	1502	R			0.5a	0.4a	tr	2.6b	3.2b					0.5a				
γ-muurolene	1503	R	tr							t		tr			72.2b			18.4a
(E)-cadina-1(6),4-diene[e]	1507	L							tr						tr			14.3a
germacrene D	1513	R										tr			154.4b			48.5a
γ-himachalene	1515	R	414.2d	699.4e			2.4a	0.5a	3.2a			tr	268.6c		112.3b	101.8b	899.4f	244.5c
5-epi-aristolochene[e]	1519	MS			0.5a	4.7 cd	3.2c	10.8e	6.4d		tr			1.8b		tr		
4-epi-eremophilene	1524	MS			0.5a	2.4b	tr	8.1d	4.4c				5.2c	1.8b		tr		
β-bisabolene[e]	1525	R					1.3a									tr		
α-muurolene	1525	R													32.4b			7.6a
bicyclogermacrene	1529	R													tr			
α-selinene	1530	R			0.5a		tr	5.5c	1.1a				3.0b	0.9a		tr		
δ-amorphene[e]	1534	R													14.0a			
β-himachalene	1535	R	112.9e	31.9c			1.3a	0.2a	tr				11.1b			22.8bc	73.3d	14.6b
γ-cadinene	1542	R													29.2a			tr
himachala-3(12),4-diene	1543	MS		3.6a									tr			14.0b		
δ-cadinene	1547	R					tr		tr						97.1b			33.8a
E-calamenene[e]	1553	R													tr			tr
7-epi-α-selinene[e]	1553	R							tr									
(E)-cadina-1,4-diene	1561	R													25.9a			tr
α-cadinene[e]	1565	R													tr			tr
ar-himachalene[e]	1571	R	tr														tr	
α-calacorene	1573	R													tr			tr
oxygenated																		
epi-cubebol[e]	1525	R													tr			
cubebol	1545	R													tr			
(E)-nerolidol	1573	R		tr			tr						tr		tr	tr	tr	
dendrolasin[e]	1587	L			tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr		tr
epi-α-cubenol	1659	R													tr			
epi-α-cadinol	1670	R													tr			
epi-α-muurolol[e]	1671	R													tr			
α-muurolol[e]	1674	R													tr			
α-cadinol	1684	R													tr			
monoterpenes																		
tricyclene	939	R	0.8c	0.6bc		0.2a	0.5b	0.2a	0.3ab	0.1a	0.5b	0.2a	0.5b	0.3ab		0.4ab	0.5b	
α-pinene	948	R	1.1c	0.4a	0.3a		0.7b	0.2a	1.2c	0.8b			0.4a			0.1a	0.1a	

Table 4. Continued

compound	RI[b]	ID[c]	ACI	ARB	BIE	DOU	JAL	NUM	PIP	PIQ	POB	SEN	SER	VAL	994	LAO	PEB	TAB
camphene	967	R	0.3a						0.1a				0.1a					
myrcene	992	R	0.2a	0.5b			0.8c	0.4b	1.1d				0.7c		0.1a	0.3a	0.5b	0.1a
β-pinene	997	R	1.2c	0.4ab			0.2a		0.6b	1.0c			0.4ab	0.1a				
p-cymene	1035	R	1.3c	1.1c	0.4a	0.1a	0.2a	0.2a	1.8d	1.2c	tr	0.8b	1.3c		tr			
(Z)-β-ocimene	1037	R						0.9b	0.3a					0.2a	0.4a	2.1c	0.7b	0.1a
limonene	1041	R	10.4c	10.3c	1.3a	0.2a	0.3a	0.4a	12.5c	6.4c	0.2a	0.8a	3.9ab	0.1a	0.2a	0.3a	0.3a	0.1a
1,8-cineole	1045	R	tr	0.4b	0.1a		tr		0.1a			0.3ab	0.2a		tr	0.1a	0.1a	
(E)-β-ocimene	1048	R	2.9a	1.5a	1.7a			54.6d	34.8c		0.3a	3.3a	0.4a		10.4b	145.0e	43.6d	1.0a
γ-terpinene	1069	R	1.3bc	1.5c			0.1a		1.2bc	1.0b		0.1a	0.7b					
allo-ocimene	1133	L	tr					1.4b	0.3a		0.1a				tr	1.4b	0.4a	
neo-allo-ocimene[e]	1148	L	tr					2.3c	0.8b		0.2a				0.1a	2.5c	0.7b	
linalool	1102	R	0.6ab	0.9b	0.1a		1.2c	1.5c	1.2c	0.8b	0.2a	0.5ab	0.9b		0.2a			
perillene[e]	1108	R	0.4ab	0.3a	0.1a		0.7b	0.3a	0.8b	0.2a		0.1a	0.1a	0.2a	0.7b	0.1a		0.1a
(E)-limonene oxide[e]	1152	R	1.0b	1.6c					0.6ab	0.3a			0.7ab					
α-terpineol	1206	R						0.1a										

[a] Mean peak area, n = 5. Means followed by different letters in the same row are significantly different ($P < 0.05$) by Duncan's test. [b] Linear retention indices on a DB5 column. [c] The reliability of the identification proposal is indicated by the following: R, by comparison of mass spectra and RIs with authentic reference substances; MS, by mass spectrum and comparison of RI with homologues; L, by comparison of mass spectra and RIs with literature (35, 36). MS- and L-labeled compounds should be considered as tentatively identified. [d] tr, traces (GC peak area <0.1 × 10^{8}) or not quantified because of incomplete separation from a larger compound. [e] New constituents reported for the first time in Capsicum.

typical of *C. annuum* accessions, whereas bicyclogermacrene was exclusive to *C. chinense* and previously unknown in this species (*18*) (**Table 4**). Moreover, in coincidence with Pino et al. (*16*, *18*), *C. chinense* was characterized by high amounts of α-, β-, and γ-himachalene, germacrene D, and several germacrene-derived products such as α- and β-cubebene and γ- and δ-cadinene, although remarkable amounts of α- and β-copaene and γ-muurolene, not reported previously in this species, were also found (**Table 4**). *C. frutescens* and *C. annuum* accessions Aci Sivri, Chile de Arbol, and Serrano showed, in general, also high levels of the himachalene family, particularly γ-himachalene. Remarkable amounts of α-ylangene were also found in these accessions, with the only exception of Laotian (**Table 4**). Relatively high amounts of cubebenes and copaenes were also found in Tabasco type. Oxygenated sesquiterpenes were found at only traces and mainly in *C. chinense*, although (*E*)-nerolidol and dendrolasin were found in the rest of the accessions (**Table 4**).

The most diverse patterns in monoterpenes were found in Aci Sivri and Piparra (**Table 4**). Limonene and (*E*)-β-ocimene, an acyclic monoterpene, could be considered, in general, as the most abundant monoterpenes. Relatively high levels of the former were found in Aci Sivri, Chile de Arbol, and Piparra, whereas the highest levels of the latter were found in NuMex, Piparra, and *C. frutescens* accessions Laotian and Pebrera (**Table 4**).

A total of seven nitrogen and sulfur compounds were found in the volatile fraction of the studied accessions (**Table 5**). Apart from Chile de Arbol and Serrano, most accessions with high levels of esters and terpenoids showed a low content of these compounds and vice versa. Generally, *N*-methylpyrrole and 3-isobutyl-2-methoxypyrazine, followed by dimethyl sulfide and 2-heptanethiol, were found to be the most common and abundant volatiles in this group. High levels of *N*-methylpyrrole were found in Chile de Arbol, NuMex, Piquillo, and Valenciano (**Table 5**). The highest level of 3-isobutyl-2-methoxypyrazine was found in Serrano peppers. Remarkable levels could also be detected in other *C. annuum* cultivars such as Chile de Arbol and Jalapeno, whereas the rest of the accessions showed comparatively lower levels (**Table 5**). *C. chinense* was the only genotype in which this pyrazine was not detected at the GC-MS analysis. The lack of this volatile might be a trait linked to red-fruited genotypes of

C. chinense as Pino et al. (*18*) also reported this fact in red havaneros. 2-Heptanethiol was identified in all *C. annuum* accessions, with Valenciano having the highest content, whereas this mercaptan was found, at most, in traces in *C. chinense* and *C. frutescens* accessions (**Table 5**).

Among phenol derivatives, methyl salicylate was found to be the most common and abundant compound, Piquillo and Aci Sivri being the only accessions in which this phenolic ester was not found. *C. frutescens* accessions showed the highest levels of this compound, confirming its relevance in the volatile fraction of this species (*25*). Lower levels were also found in Chile de Arbol, Jalapeno, NuMex, and Serrano (**Table 5**).

Several norcarotenoids were found among the studied accessions. The most abundant compounds were four sensorially important volatiles of the ionone family: α-ionone, β-ionone, 7,8-dehydro-α-ionone, and 7,8-dehydro-β-ionone (**Table 5**). Among these typical carotenoid degradation products with sweet/floral/fruity notes (*22*), β-ionone was the most abundant and common. Aci Sivri, *C. chinense*, and *C. frutescens* accessions Laotian and Pebrera contained the highest levels of these compounds. Lower amounts of norcarotenoids were found in Chile de Arbol and NuMex, and very low levels were found in the rest of the accessions, including *C. frutescens* accession Tabasco (**Table 5**).

Several alcohols, lipoxygenase cleavage products, furans, ketones, and aromatic and unsaturated hydrocarbons were also found as minor compounds (**Table 5**). Alcohols were the largest group, ethanol and 4-methylpentan-1-ol being the most abundant ones. Some methyl-branched secondary alcohols were also found in Aci Sivri, *C. chinense*, and Tabasco (**Table 5**). The highest levels and the most diverse patterns of alcohols were mainly found in accessions rich in esters such as *C. chinense*, *C. frutescens*, Aci Sivri, and Chile de Arbol (**Table 5**). In this respect, ester biosynthesis is considered to be limited by alcohol concentration, and it has been demonstrated that the availability of alcohol can modify the content of esters in specific cultivars (*26*, *27*), which could explain our findings. Lipoxygenase cleavage volatiles, which usually contribute to flavor with green notes (*13*), were found in most genotypes, although at low levels. Relatively high levels of 1-penten-3-ol, hexanal, (*E*)-2-hexenal, (*E*)-2-nonenal,

Table 5. Nitrogen and Sulfur Compounds, Phenol Derivatives, Norcarotenoids, Alcohols, Lipoxygenase Derivatives, Furans, Ketones, and Other Hydrocarbons and Their Average GC Peak Areas $(\times10^8)^a$ Identified in the Fruits of the Studied Accessions of *C. annuum*, *C. chinense*, and *C. frutescens*

compound	RI[b]	ID[c]	ACI	ARB	BIE	DOU	JAL	NUM	PIP	PIQ	POB	SEN	SER	VAL	994	LAO	PEB	TAB
nitrogen and sulfur compounds																		
dimethyl sulfide	560	R	0.5ab	0.3a		0.4ab	0.3a	1.6c	0.2a	0.6ab	0.4ab	0.7b	0.2a	1.8c	0.5ab	0.3a	1.7c	0.2a
N-methylpyrrole	745	R	0.8a	4.5c	0.9a	1.2a	1.6ab	3.9c	2.8b	4.3c	1.3ab	0.8a	0.9a	8.5d	1.0a	0.8a	1.2a	0.7a
2-heptanethiol	970	L	tr[d]	tr	0.2a	tr	0.3a	0.2a	0.5b	0.2a	0.1a	0.3a	tr	1.3c	tr			tr
2-heptyl-methylsulfide[f]	1074	MS					0.2a						tr					tr
2-pentylthiophene	1170	MS	tr												tr			0.1a
3-sec-butyl-2-methoxypyrazine	1177	R																tr
3-isobutyl-2-methoxypyrazine	1187	R	1.0ab	1.9b	tr	tr	2.1b	tr	0.5a	0.2a	0.2a	tr	10.9c	tr		0.4a	0.4a	0.5a
phenol derivatives																		
anisole	933	R							0.2a						0.2a			
dimethylanisole[f]	1073	MS			tr	tr			tr	tr	tr	tr						
guajacol	1102	MS		0.3a				0.1a			0.1a		tr			0.2a	0.1a	0.4a
methyl salicylate	1212	R		3.3ab	0.1a	0.2a	3.1ab	5.9b	tr		0.7a	0.1a	5.6b	0.3a	1.5a	47.4 cd	41.6c	50.6d
ethyl salicylate	1285	R																tr
norcarotenoids																		
6-methyl-5-hepten-2-one	987	R		0.3a			tr	0.2a	tr	tr		0.1a		0.3a	0.1a	tr	tr	
β-cyclocitral	1237	R		tr				tr							0.1a	0.7b	1.0b	
megastigmatriene[f]	1275	MS	0.5a															
dihydroedulan[f]	1313	MS					tr							0.3a				
tetramethyl-tetrahydro-benzopyrane[e,f]	1371	R	8.0b	0.1a				0.3a					0.1a		0.6a	1.0a	0.5a	
7,8-dehydro-α-ionone[f]	1435	R	4.8c	0.5a				0.1a							3.2b	2.0ab	1.4ab	
α-ionone	1443	R	6.4c	0.5a				0.2a							2.9b	4.8bc	3.7b	0.3a
7,8-dehydro-β-ionone	1457	R	18.1d	0.9a				0.8a							5.2c	6.2c	3.1b	
geranylacetone	1459	R												tr				
β-ionone	1504	R	45.2c	5.7b			0.1a	1.2a	0.1a		tr	tr	0.8a	tr	52.0d	64.4e	40.5c	1.3a
alcohols																		
ethanol	527	R	12.8c	2.8ab	0.7a		1.5ab	4.6b	1.2ab	2.2ab	2.1ab	0.8a	4.7b		1.6ab	1.6ab	1.7ab	2.6ab
3-methyl-2-butanol[f]	680	R																0.3a
3-methyl-1-butanol	723	R	0.2ab	0.4b				0.1a		0.1a			0.2ab		0.1a	0.3ab	0.5b	0.1a
1-pentanol	758	R	0.5b	0.1a			tr						0.1a		0.4b	0.1a	0.2a	0.1a
2-penten-1-ol	764	MS	0.4b	0.1a											0.5b	0.3ab		
4-methylpentan-1-ol	830	R	2.2b	2.2b			0.1a						0.5a		19.2e	7.4c	14.4d	1.5b
(Z)-3-hexenol	852	R	0.1a										tr		1.6c	0.3a	0.7b	0.1a
4-methyl-3-penten-1-ol[f]	854	MS	0.2a	tr											0.6b	0.2a	0.7b	0.1a
(E)-2-hexenol	857	R													tr	tr		
1-hexanol	858	R	0.6b	0.2a			0.1a						0.3a		2.2c	0.2a	1.6c	0.6b
4-methylhexan-1-ol	940	MS													tr	tr	tr	tr
1-heptanol	965	R													tr	tr	tr	tr
6-methyl-4-hepten-1-ol[f]	1015	MS													1,0a			tr
benzyl alcohol[f]	1042	R													tr	tr		
7-methyl-2-octanol[f]	1061	MS	0.1a															0.2a
?-methyl-2-nonanol[f]	1141	MS	1.1a															
8-methyl-2-nonanol[f]	1162	MS	1.2a															
?-methyl-2-nonanol[f]	1178	MS													9.2a			
lipoxygenase cleavage products																		
1-penten-3-ol	677	R	0.9b	0.2a	tr		0.5ab	0.1a	0.1a	0.1a	0.1a	0.1a	0.2a	tr	0.1a	0.1a	0.4ab	tr
hexanal	802	R	1.5a	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr				
(E)-2-hexenal	844	R	0.1a						tr	tr	tr				tr	tr		
nonanal	1105	R		tr			tr										tr	tr
(Z)-2-nonenal	1150	R	tr	tr	tr				tr	tr								
(E,Z)-2,6-nonadienal	1155	R	0.2a	tr	tr	tr	tr	tr	tr				tr	tr		tr		
(E)-2-nonenal	1162	R	0.3a	tr		tr		tr					tr	tr		tr	tr	tr
furans																		
2-methylfuran	613	R	tr	tr			tr		tr	tr	tr	tr	tr	tr			tr	
2-ethylfuran	708	R	tr	tr			tr	tr	tr		tr		tr	tr	tr	tr	tr	
2-pentylfuran	997	R	1.7b	0.3a	0.1a	tr	0.3a	0.2a	0.6a	0.2a	0.2a	0.2a	0.4a	0.1a	tr	tr	0.1a	tr
ketones and methylketones																		
3-methyl-2-butanone	653	R						tr	tr				tr			tr		1.3a
3-pentanone	693	R	3.7c	0.5ab	0.2a	0.1a	1.4b	0.9b	0.4ab	0.3a	0.3a	0.2a	0.4ab	0.2a	tr	tr	0.1a	tr
2-heptanone	894	R	tr							tr		tr						
7-methyl-2-octanone[f]	1054	MS			tr	tr			tr							tr		0.6a
8-methyl-2-nonanone[f]	1156	MS	0.1a			tr					tr							1.1b
9-methyl-2-decanone[f]	1258	MS																2.3a
2-undecanone[f]	1294	R		tr	tr		tr			tr	tr			tr				

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Table 5. Continued

															accession		
compound	RI[b]	ID[c]	ACI	ARB	BIE	DOU	JAL	NUM	PIP	PIQ	POB	SEN	SER	VAL	994	LAO	PEB	TAB
hydrocarbons																		
toluol	781	R	6.3b	6.2b	5.8b	1.5a	17.2d	11.7c	1.8a	4.7b	6.6b	2.1a	3.2ab	9.8c	1.3a	0.9a	1.3a	1.8a
styrol	904	R	0.9b	1.6bc	0.3a	0.2a	0.7b	0.7b	2.2c	0.9b	0.4ab	2.2c	2.2c	0.6b	1.2b	0.7b	0.8b	0.1a
ectocarpene[f]	1168	R	tr	tr											0.1a	0.3b	0.2b	0.2b

[a] Mean peak area, *n* = 5. Means followed by different letters in the same row are significantly different (*P* < 0.05) by Duncan's test. [b] Linear retention indices on a DB5 column. [c] The reliability of the identification proposal is indicated by the following: R, by comparison of mass spectra and RIs with authentic reference substances; MS, by mass spectrum and comparison of RI with homologues; L, by comparison of mass spectra and RIs with literature (*14*). MS- and L-labeled compounds should be considered as tentatively identified. [d] tr, traces (GC peak area <0.1 × 10^{8}). [e] 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-(5*H*)-1-benzopyrane. [f] New constituents reported for the first time in *Capsicum.*

and (*E,Z*)-2,6-nonadienal were mainly found in Aci Sivri, whereas only low levels or traces were found in the rest of the accessions (**Table 5**). Although furans appeared in most accessions, only 2-pentylfuran was found at levels high enough to be quantified, particularly in Aci Sivri (**Table 5**). The same was found for ketones and methylketones, with Tabasco showing the highest content of these volatiles. The most abundant and common ketone was 3-pentanone (**Table 5**).

A butenyl-cycloheptadiene hydrocarbon, the known algal pheromone ectocarpene (*28*), was identified for the first time in *Capsicum.* It was found only in *C. chinense, C. frutescens,* Aci Sivri, and Chile de Arbol (**Table 5**). Because of great similarities in the mass spectrum, we first associated the structure of 1-(*E,Z,Z*)-3,5,8-undecatetraene with this compound (*22, 24*). The occurrence of this undecatetraene was previously described in *Capsicum* powder (*29*). By comparison with the authentic reference compounds (kindly provided by Prof. L. Jaenicke), this misinterpretation could be corrected, confirming the identity of this hydrocarbon as ectocarpene.

Finally, 33 aliphatic hydrocarbons (C12−C21 alkanes and 2-methyl- and 3-methyl-branched alkanes and alkenes), 44 aromatic hydrocarbons (namely, substituted benzols, such as toluol and styrol), other methyl- and ethyl-substituted homologues, and some naphthalene derivatives could also be identified. High levels of aliphatic hydrocarbons were found in *C. chinense* and *C. frutescens* cultivars and the most pungent *C. annuum* cultivars, such as Aci Sivri, Chile de Arbol, and Serrano. Especially the methyl-branched ones are thought to be related to capsaicin biosynthesis. The highest content of aromatic hydrocarbons was found in Serrano, whereas the lowest contents were found in *C. frutescens* samples. *C. chinense* was rich in naphthalene derivatives. However, these aromatic compounds can originate in carotenoid degradation or from environmental contamination (*11*). As their origin is doubtful and they did not contribute to flavor, no detailed data are shown.

The cluster analysis based on the patterns of volatiles showed that *C. chinense* and *C. frutescens* accessions separated from the *C. annuum* group (**Figure 1**). This is in coincidence with their evolutionary/phylogenetic relationship. Thus, although the three studied species share a common genetic pool and ancestors, *C. chinense* and *C. frutescens* arose in the Amazonian Basin, whereas *C. annuum* was domesticated separately in Mexico (*1, 3*). For that reason, *C. chinense* and *C. frutescens* could share a larger proportion of their genomes, which would explain the similarities found between their volatile fractions. Moreover, *C. annuum* accessions Chile de Arbol and Aci Sivri, with the volatile patterns most similar to those of *C. chinense* and *C. frutescens,* were basal to the rest of the *C. annuum* accessions in the dendrogram (**Figure 1**). In this respect, both accessions have been found to be phylogenetically close to these species in preliminary experiments performed with morphological descriptors and AFLP
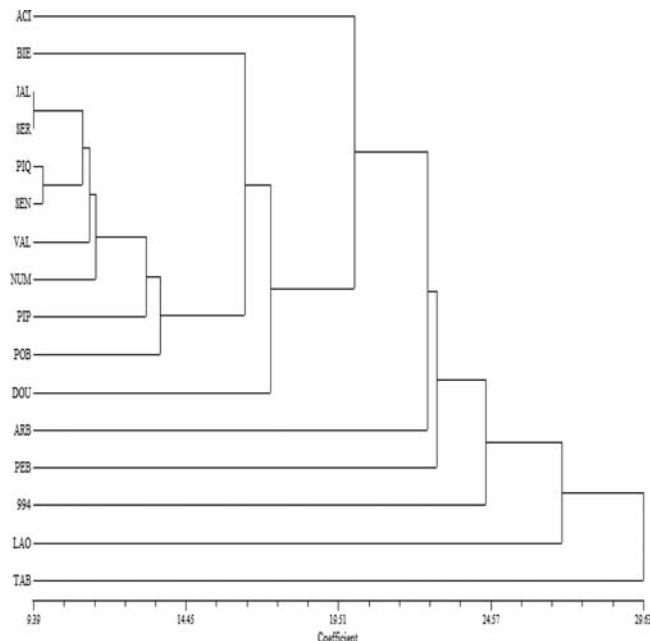

Figure 1. Dendrogram obtained from cluster analysis (UPGMA method) based on amounts (peak areas) of the identified volatiles.

molecular markers (Rodríguez-Burruezo, unpublished data). Therefore, in our opinion, volatile compounds, the synthesis of which involves a lot of enzyme-catalyzed cyclization, isomerization, reduction, and oxidation reactions (*27*), could be helpful as fingerprints in distinguishing chemotypes of *Capsicum* and considered as complementary traits in studies of phylogeny, as reported in other organisms (*30, 31*).

Sensorial Analysis. Despite the high number of volatiles identified in the studied accessions, the sniffing test revealed that differences in the aroma among fully ripe fruits of the *annuum−chinense−frutescens* complex can be explained on the basis of qualitative and quantitative differences of, at least, 23 OCVs (**Table 6**). Comparatively, this number is higher than reported by Luning et al. (*13*) in fully ripe fruits of bell peppers involving two different extraction methods and larger samples (45−250 g).

Several esters, mainly ethyl 2-/3-methylbutanoate, ethyl 3-/4-methylpentanoate, hexyl 2-/3-methylbutanoate, 4-methylpentyl 3-methylbutanoate, and 4-methylpentyl 4-methylpentanoate, were recognized during sniffing analyses of some of the accessions. Despite their comparatively lower content, ethyl esters can contribute to the whole aroma of several accessions, because of their low odor threshold (*32*). Their flavor impressions were defined by panelists as sweet and/or fruity, with an additional reminiscence of exotic fruits in the case of some ethyl esters. The highest sensory impressions of these esters were found in

Table 6. Odor Quality[a] and Intensity[b] of Odor-Contributing Volatiles Detected by the Sniffing Port Panel

compound	aroma description	ACI	ARB	BIE	DOU	JAL	NUM	PIP	PIQ	POB	SEN	SER	VAL	994	LAO	PEB	TAB
ethyl 2/3-methylbutanoate	fruity, sweet	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	+
ethyl 3-methylpentanoate	fruity, exotic	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
ethyl 4-methylpentanoate	fruity, exotic	+	+	−	−	−	−	−	−	−	−	++	−	+++	−	−	+++
hexyl 2/3-methylbutanoate	fruity	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
4-methylpentyl 3-methylbutanoate	fruity, peach	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
4-methylpentyl 4-methylpentanoate	soapy, weak fruity	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
α-pinene	pine wood	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
1,8-cineole	eucalyptus	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−
linalool	citrus, fruity, floral	−	+	−	−	+	+	+	+	−	−	+	−	−	−	−	−
2-heptanethiol	paprika, green, kerosene-like	+	+	++	+	++	++	+++	++	++	++	+	+++	+	−	−	+
3-isobutyl-2-methoxypyrazine	paprika, green, earthy	++	+++	+	+	+++	+	++	+	+	+	++++	+	−	++	++	++
methyl salicylate	green, sweet, phenolic	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++	++
α-ionone	floral	++	+	−	−	−	+	−	−	−	−	−	−	++	++	++	+
β-ionone	fruity, floral	+++	++	−	−	+	+	+	−	−	−	+	−	+++	+++	+++	+
7,8-dehydro-β-ionone	fruity, floral	++	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
hexanal	green	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
(Z)-2-nonenal	green, cucumber	+	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−
(E,Z)-2,6-nonadienal	cucumber	++	+	+	+	+	+	+	−	−	+	+	+	−	+	−	−
(E)-2-nonenal	cucumber, musty	++	+	−	+	−	+	−	−	+	−	+	−	−	+	+	+
ectocarpene	green, sweet	+	+	−	−	−	−	−	−	−	−	−	−	+	++	++	++
sotolone, RI 1106	soup seasoning, spicy	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
unidentified, RI 1020, MG 140	sweet, alcoholic	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	++
unidentified, RI 1078	passion fruit	−	+	−	−	−	−	−	−	−	−	+	−	++	−	−	+

[a] Odor impressions of four trained panelists during GC-MS-sniffing analysis. [b] −, +, ++, +++, and ++++ indicate no, low, moderate, high, and very high intensity, respectively.

C. chinense and *C. frutescens* accession Tabasco. Among *C. annuum* accessions, only Aci Sivri, Chile de Arbol, and Serrano showed levels high enough to contribute to the overall aroma (**Table 6**).

Monoterpenes like α-pinene (pine wood-like), 1,8-cineole (eucalyptus-like), and linalool (citrus, fruity, floral) were detected by panelists in only a few *C. annuum* accessions (**Table 6**). These compounds had no sensorial relevance for the aroma of *C. chinense* and *C. frutescens* fruits.

Among nitrogen and sulfur compounds, 3-isobutyl-2-methoxypyrazine and 2-heptanethiol contributed to the aroma of most cultivars (**Table 6**). A powerful characteristic of green bell peppers, aroma was found for the bell pepper pyrazine. Serrano showed the highest odor impressions of this volatile compound, although its odor was even recognized in accessions in which it was found in traces (e.g., Bierzo, Doux d'Alger) (**Table 5**), confirming that it is one of the most common OCVs in *Capsicum* peppers (*9*, *11*, *12*). *C. chinense* accession was the only genotype in which it was not detected. 2-Heptanethiol was described as green, paprika-like, and kerosene-like during the sniffing runs. This description might be similar to the sulfury, onion/vegetable-like odor impressions reported by Simian et al. (*14*). Its aroma was recorded in most of the studied accessions, although it had no relevance for the aroma of *C. frutescens* accessions Laotian and Pebrera (**Table 6**).

Although methyl salicylate could be identified in most of the samples, its characteristic sweet-green mixed smelling (*25*) was only recognized during sniffing analyses in *C. frutescens* accessions, in which the highest levels of this phenolic ester were found (**Table 5**). α-Ionone, β-ionone, and 7,8-dehydro-β-ionone contributed significantly with their floral-fruity smell to the aroma of Aci Sivri, *C. chinense*, and *C. frutescens* accessions Laotian and Pebrera (**Table 6**). α- and β-ionone also contributed slightly to the aroma of Chile de Arbol and other *C. annuum* samples.

Several aldehydes such as hexanal, (Z)-2-nonenal, (E,Z)-2,6-nonadienal, and (E)-2-nonenal, typical lipoxygenase cleavage products, were detected at the sniffing analyses (**Table 6**). Despite the low levels detected in the GC-MS analyses, the green cucumber odor of (Z)-2-nonenal and (E,Z)-2,6-nonadienal was perceived in the eluates of most *C. annuum* accessions (**Table 6**).

The aroma of Aci Sivri, *C. chinense*, and *C. frutescens* accessions was also influenced by the green-sweet impression of ectocarpene. In addition, two unidentified compounds contributed uniquely to the aroma of *C. chinense* and *C. frutescens* accession Tabasco (**Table 6**). Their odors were described as "sweet, alcoholic" (molecular weight 140, possible two isomers) and "passion fruit" (no peak recorded). These compounds are still under investigation to establish their chemical nature. Finally, sotolone was tentatively identified according to its typical odor impression at the corresponding RI (**Table 6**). Due to its low concentration in the headspace, no complete mass spectrum of this compound could be recorded.

The diversity of aromas found among *C. annuum* accessions depends on the qualitative and quantitative differences of 16 volatiles, many of them detected in only Aci Sivri and Chile de Arbol. By contrast, the aroma of several accessions such as, among others, Doux d'Alger or Valenciano, is due to very few OCVs (**Table 6**). Fruity notes recognized in Aci Sivri, Chile de Arbol, NuMex, and Serrano were due to a considerable contribution of esters and ionones. Additionally, the remarkable effect of nonenals, the bell pepper pyrazine, and 2-heptanethiol in Aci Sivri and Serrano would explain the vegetable-like and green-peasy notes of their respective overall aromas. Most of the rest of *C. annuum* accessions were characterized by weak-moderate green, vegetable, and/or earthy aromas. The predominance of 3-isobutyl-2-methoxypyrazine, 2-heptanethiol, and nonenals and the lack of esters could explain such aromas. A low contribution of the mentioned compounds is the reason for the weak aroma perceived in *C. annuum* accessions such as Doux d'Alger, Bierzo, and Senise. Comparatively, the contributions of several esters, ionones, ectocarpene, and the two unidentified volatiles with exotic notes, together with the lack of green-vegetable OCVs, are responsible for the powerful exotic-fruity aroma of *C. chinense*. The aromas of *C. frutescens* fruits, also described as sweet-fruity but less intense than *C. chinense*, with paprika-like notes in Laotian and Pebrera, could be explained by a lower

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contribution of esters and the remarkable effect of the bell pepper pyrazine and methyl salicylate. In the case of Tabasco, a high content of esters in combination with a lack of aroma contributing ionones leads to a different overall impression. The high capsaicinoid content in this cultivar could cause a pungent/irritating perception in the nose, which was described as pungent or typical of hot pepper.

Our results indicate that breeding efforts for the genetic improvement of aroma in peppers of the *annuum*−*chinense*−*frutescens* complex may be less complicated than expected at first. Previous experiments have demonstrated that OCVs can be inherited, like many other traits, from parents to their offspring in different tissues of plants such as, among others, pepino (*Solanum muricatum* L.) or citrus (*33*, *34*). In addition, the low number of compounds that contribute to the aromatic differences among *Capsicum* genotypes should simplify breeding programs after crossing pairs of cultivars with complementary OCV patterns. In this respect, although some *C. annuum* accessions contain high levels of volatiles with fruity notes, *C. frutescens* and, particularly, *C. chinense* were found to be comparatively more interesting sources of variation for these aroma profiles, with low or no contribution of green OCVs. On the contrary, if breeders are more interested in the increase of the green-bell pepper aroma quality, *C. annuum* accessions such as Serrano or Jalapeno should be utilized in breeding programs. Furthermore, the observed lack of esters in some *C. annuum* accessions or the bell pepper pyrazine in our *C. chinense* accession offers breeders the opportunity to study the inheritance of these compounds and/or to search for molecular markers linked to these compounds in future experiments.

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