



# Evaluation of the volatile fraction, pungency and extractable color of different Italian *Capsicum annuum* cultivars designed for food industry

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## Abstract

The volatile fraction investigated by means of HS-SPME/GC–MS technique, the extractable color in terms of ASTA units, and the pungency measured on the Schoville scale of different experimental *Capsicum annuum* powders, designed for food industry, were evaluated in this study. In particular, six *Capsicum annuum* cultivars were included; fresh fruits were cultivated, harvested, dried and grinded to obtain different chilli powder samples that were analyzed and compared with an additional sample purchased from the market. Extractable color ranged between  $36.9 \pm 1.5$  and  $144.2 \pm 0.8$  ASTA units, while pungency was from  $999 \pm 42$  to  $47,814 \pm 971$  SHU units, showing an inverse trend between these two parameters. The volatile fraction of samples resulted to be composed of 64 volatiles belonging to different chemical classes among which terpenes and pyrazines were the most represented ones. The differences and similarities among the chilli pepper varieties were visualized by means of Principal Component Analysis (PCA) as by Heat Map analysis.

**Keywords** *Capsicum annuum* · Chilli pepper powder · Volatile profile · Extractable color · Pungency

## Introduction

Chilli Pepper belongs to the genus *Capsicum* that includes approximately 200 wild and 5 domesticated species, diffused in many countries all over the World. The most diffused and cultivated species is *Capsicum annuum* L. to which several different cultivars belong such as Jalapeño and Bell cultivars [1].

Thanks to their aroma, color and hotness, Chilli Peppers are consumed as vegetable ingredient, food or spice. Hotness is commonly expressed as Scoville Heat Units (SHU), where 16 SHU are equivalent to 1 ppm of capsaicin and

capsaicin-like compounds. Furthermore, SHU is a scale based on the human perception of the pungency and it results associated with a universal scale arranged by the American Spice Trade Association, which expresses a measure directly related to capsaicinoids amount in ASTA units [2]. Generally, pungency in Chilli Pepper is provided by capsaicin and dihydrocapsaicin, which are responsible for approximately 80–90% of the spiciness [3].

The fruits of the *Capsicum* genus have been used also as natural food coloring; for this reason, extractable color measurement of pepper may represent a quality evaluation for the food industry, as it gives an idea of the total pigment content [4]. The pigments responsible for the color are indeed carotenoids, mainly capxanthin and also other carotenoids such as capsorubin, zeaxanthin, lutein, cryptoxanthin and alpha and beta-carotene [5]. Extractable color of chilli peppers, expressed as total pigment content, is commonly measured with the American Spice Trade Association (ASTA) method 20.1 based on absorbance at 460 nm of a solution of chilli peppers in acetone [6].

Apart from pungency and color, one of the most important factors affecting chilli pepper quality is represented by its aroma. The first volatile compound representative for

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pepper aroma was found so far in bell pepper and identified as 3-isobutyl-2-methoxypyrazine, but many other volatiles belonging to different chemical classes were detected in the volatile fraction of different chilli pepper cultivars as 2,3-butanedione with a characteristic caramel note, 1-penten-3-one which is associated with pungent and spicy sensation, hexanal responsible for grassy and herbal flavor, 3-carene characteristic of red bell pepper, ocimene and (*E*)-2-hexenal with sweet note, octanal with fruity notes, etc. [7]. The total volatile concentration changes during ripening: an increase of carbonyl compounds, in particular of (*Z*)- and (*E*)-2-hexenal, and of monoterpene hydrocarbons was noted [8], while pyrazines content may reduce during maturation, especially when the color of the fresh fruit changed from green to red, while at the same time other volatiles increased their concentration, as 2,3-butanedione, 3-carene and linalool [7].

The powder derived from milled dried fruits of chilli pepper is widely used in domestic cooking as well as by the food industry as an ingredient for pasta sauces, soups and bakery products. The present study deals with the characterization of some *Capsicum annuum* cultivars cultivated in Emilia Romagna region (Italy) and designed for food industry uses. To this purpose, pungency and color were determined and the volatile profile of dried chilli pepper milled to powder was analyzed by means of HS-SPME/GC-MS technique. The final aim of the work is to assess the high quality of the considered experimental cultivars and guarantee the products for industrial applications.

## Materials and methods

### Chemicals

Acetone, acetonitrile, ethanol, capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and 3-octanol were purchased from Sigma-Aldrich (St. Louis Missouri, United States).

### Sampling

Six different cultivars belonging to *Capsicum annuum* species were cultivated and harvested at the experimental farm named “Azienda Agraria Sperimentale Stuard” located in Parma, Emilia Romagna region, Italy. The growing cycle was of 153 days (from May to October). Among these cultivars, only one, Serrano, is commercial; while the other five cultivars were obtained by the Azienda Agraria Sperimentale Stuard as part of their chilli pepper breeding program. One cultivar, herein used to obtain sample “G”, was named Super Mario.

Fresh fruits were randomly collected at maturity from the different plants of the six cultivars, discarding the fruits evidently damaged. All the selected fruits were immediately stored at  $-20\text{ }^{\circ}\text{C}$ .

Chilli pepper samples were in part used to obtain powder: the fruits were washed with cold water and first dried at  $75\text{ }^{\circ}\text{C}$  for 1 h, and then the temperature was decreased at  $70\text{ }^{\circ}\text{C}$  for 2 h and after that at  $65\text{ }^{\circ}\text{C}$  for 21 h. The dried fruits were finally milled to powder, after having removed seeds and peduncles. From the six cultivars considered (Table 1), eight powder samples were designed to satisfy the industrial use for sauce preparations [9] and obtained as follows. Samples “A” and “B” corresponded to the same non-commercial cultivar collected at early and regular harvesting times, respectively; this sampling was performed in order to evaluate the characteristics of the experimental cultivar used, cultivated from the Azienda Agraria Sperimentale Stuard farm for the first time. Samples “C”, “E”, “F” and “G” (Super Mario) corresponded to 4 different experimental cultivars, while sample “D” was the commercial cultivar Serrano, taken as reference. Sample identified with letter “H” was a mix of *Capsicum* powder consisted in the 33% of sample G and the 67% of sample D. All the cultivars have been chosen to satisfy the food industry needs in terms of pungency, color, and adaptability to mechanical harvesting. Sample “I” is a commercial powder purchased from the market, its

**Table 1** Characteristics of the cultivars considered

Cultivar/sample	Plant height (cm)	Plant width (cm)	Fruit position	Fruit length (cm)	Fruit width (cm)	Fruit shape	Dry matter %
A	65	35	Erect	3	1.5	Round	86.0±3.3
C	70	50	Pendent	8	1.5	Elongated	97.5±0.7
Serrano-D	68	28	Pendent	8.5	1.6	Elongated	80.0±7.8
E	40	25	Erect	4.5	1.2	Elongated	90.1±0.1
F	60	30	Erect	4.5	1.2	Elongated	88.7±1.9
Super Mario-G	60	30	Erect	2.5	1	Mildly elongated	73.8±9.4

All have red mature fruit color

origin was India, the color was “brilliant red” and the pungency was “strongly hot and pungent” (label indications).

### Evaluation of the extractable color by the ASTA method

The extractable color was determined on the powder samples applying the ASTA 20 method [10]. Briefly, 0.1 g of chilli pepper powder was extracted with 20 ml of acetone on a stirrer at 25 °C for 3 h. The sample was then filtered and diluted 1:5 with pure acetone. All the extractions were performed in double. The absorbance at 460 nm of the resulting solution was measured using a Perkin Elmer UV/VIS Spectrometer, Lambda Bio20 (Waltham, Massachusetts, United States) at 25 °C using pure acetone as reference and performing a scan between 550 and 400 nm.

ASTA 20 units were determined by the following expression:

ASTA 20 units

$$= \left( \text{absorption of extract at 460 nm} \times 16.4 \times I_f \right) / \left( \text{sample amount in g} \right)$$

$I_f$  is a correction factor for the apparatus, calculated on the basis of the absorbance of a standard solution of potassium dichromate, ammonium sulfate and cobalt sulfate, while 16.4 is the extinction molar coefficient useful to convert color units in ASTA units.

### Evaluation of pungency by HPLC–UV method

To evaluate pungency, capsacinoids were extracted and quantified by HPLC–UV technique following the protocols of Perucka and Oleszek (2000) and of Barbero et al. 2008 with some modifications [11, 12]. Briefly, 0.5 g of each dried chilli pepper powder was extracted with 12 ml of acetonitrile for 4 h at 70 °C. The sample was filtered and transferred into a HPLC vial. All the analyses were repeated twice.

The HPLC analyses were carried out on a Waters Alliance 2695 Separation module (Milford, Massachusetts, United States) equipped with a Waters 2487 Dual Lambda Absorbance Detector (Milford, Massachusetts, United States). A C18 Phenomenex Jupiter (5 µm, 300 Å, 250 × 2.00 mm) column was used (Torrance, California, United States). Water (A) and Methanol (B), both acidified with the 0.2% of Trifluoroacetic Acid, were used as eluents. The total flow was maintained at 0.25 ml/min; the gradient started from 40% of B, then it increased to 85% of B in 16 min and then to 99% of B in 20 min. These conditions were maintained for 6 min (20–26 min) to flashing the column; after that, the initial conditions were re-established. The total run time was 40 min, the column oven was set at 40 °C and the injection volume was 10 µl. The UV detector was set at 280 nm.

Capsaicinoids, in particular of capsaicin, dihydrocapsaicin and nordihydrocapsaicin, were quantified on the basis of a calibration curve (calibration range 100–1000 ppm).

The results were expressed as Scoville Heat Unit (SHU), multiplying the capsaicin content obtained from the interpolation of the peak areas with the calibration curve with the right coefficient, applying the following formula:

$$\text{SHU} = [C + \text{DHC}] * 16 + n - \text{DHC} * 9.3,$$

where C and DHC were capsaicin and dihydrocapsaicin concentration in ppm,  $n$ -DHC is nordihydrocapsaicin concentration in ppm.

### Characterization of the aromatic profile of fresh peppers and paprika powders

The characterization of the aromatic profile of chilli pepper powders was performed by head-space solid-phase micro-extraction (HS-SPME) technique coupled by GC–MS analyses, following the protocol of Cirlini et al. 2012 with slight modifications [13]. 1 g of chilli powder was placed in a 30-ml vial adding 3-octanol (20 ppm) as internal standard. Headspace extraction was performed for 45 min at 40 °C using a silica fiber coated with 50/30 m of Divinylbenzene–Carboxen–Polydimethylsiloxane (DVB/Carboxen/PDMS) (Supelco, Bellefonte, PA, USA). The desorption of volatiles was performed into the GC–MS injector (230 °C for 2 min). All the analyses were performed in triplicate on a Trace 1300 gas chromatograph (Thermo Fisher, Waltham, Massachusetts, United States) coupled with an ISQ single quadrupole mass spectrometer (Thermo Fisher, Waltham, Massachusetts, United States) equipped with an electronic impact (EI) source. Separation was achieved on a SUPEL-COWAX 10 capillary column (Supelco, 30 m × 0.25 mm, f.t. 0.25 m) (Supelco, Bellefonte, PA, USA). Helium was the carrier gas (flow of 1 mL/min). The oven temperature was programmed from 50 °C, with a hold time of 3 min, to 200 °C at a rate of 5 °C/min, with a final holding time of 12 min (total run time = 45 min). The transfer line was maintained at 230 °C. Signal acquisition was performed in full scan mode (mass range  $m/z$  41–500  $m/z$ ).

Peak identification was performed by the comparison of registered mass spectra with those present in the instrument library (NIST 14). Furthermore, LRIs were calculated for each detected signal on two different stationary phase columns, SUPEL-COWAX 10 capillary column and BP5MS (SGE Analytical Science, Milton Keynes, UK, 30 m × 0.25 mm, f.t. 0.25 µm), according to retention times of a C8–C20 alkane standard solution using the same GC conditions applied for sample analyses.

## Statistical data treatment

The IBM SPSS software was applied for all data elaboration (version 23.0, Chicago, IL, USA). A two-tale Pearson correlation analysis was performed to verify the association between color intensity and pungency. Significant differences of each mean value calculated for color intensity, pungency and volatiles amount were investigated by one-way analysis of variance (ANOVA) adopting Tukey test and the results were considered different when  $p < 0.05$ . A principal component analysis (PCA, covariance method) was applied to the data obtained from the investigation of volatile compounds, to clarify the relations between the different cultivars considered in this study. Finally, a Heat Map analysis (variable scaling mode: z-score; clustering method: single linkage; distance method: euclidean) was performed to highlight and better explain relations between classes of volatiles and samples.

## Results and discussion

### Extractable color

Color determination, in terms of ASTA units, was performed on dried chilli pepper powder samples to determine the quality of the products. In fact, ASTA units can be considered a quality parameter representing the total pigment concentration and, as a consequence, the total carotenoid amount of the tested sample, since carotenoids are indeed the compounds responsible of pepper color [14].

Samples A, B, and E, pertaining to two different cultivars presented higher amounts of extractable color ( $144.23 \pm 0.78$ ,  $117.86 \pm 0.53$ ,  $107.91 \pm 0.40$  ASTA units) compared to the other samples tested; while two cultivars, G and H, showed low ASTA unit values ( $48.56 \pm 1.82$  and  $53.19 \pm 1.52$  ASTA units, respectively). The commercial sample from India, sample I, presented the lowest content of colorant compounds (Table 2).

Values obtained from the analyzed Italian cultivars were lower than those of paprika from Spain reported by Gómez-Ladrón de Guevara et al. in 1996 who obtained an extractable color amount of around 200 ASTA units for all the tested varieties; these differences are probably linked to the

**Table 2** Extractable color content (ASTA units), pungency (SHU units) and volatile amounts (ppm) of different paprika samples

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H	Sample I
Extractable color (ASTA units)	$144.2 \pm 0.8a$	$117.9 \pm 0.5b$	$67.3 \pm 1.4e$	$59.6 \pm 2.8f$	$107.9 \pm 0.4c$	$81.9 \pm 1.7d$	$48.6 \pm 1.8g$	$53.2 \pm 1.5g$	$36.9 \pm 1.5h$
Pungency (SHU units)	$7317 \pm 118f$	$6986 \pm 530f$	$31,392 \pm 327b$	$14,516 \pm 75d$	$999 \pm 42g$	$9844 \pm 770e$	$47,814 \pm 971a$	$26,341 \pm 142c$	N.d
Volatile class (ppm)									
Terpenes and derivatives	$3.03 \pm 2.13b$	$2.67 \pm 1.41b$	$9.70 \pm 0.30a$	$7.38 \pm 1.58a$	$2.61 \pm 0.79b$	$2.16 \pm 0.46b$	$7.50 \pm 4.81a,b$	$3.59 \pm 0.46b$	$4.06 \pm 0.61b$
Alcohols	$1.81 \pm 1.73a$	$1.13 \pm 0.91a$	$3.18 \pm 2.51a$	$2.89 \pm 2.22a$	$0.60 \pm 0.17a$	$0.39 \pm 0.04a$	$2.08 \pm 1.33a$	$0.47 \pm 0.05a$	$0.44 \pm 0.02a$
Aldehydes	$1.01 \pm 0.75b$	$0.94 \pm 0.50b$	$3.84 \pm 2.03b$	$2.01 \pm 0.36b$	$0.52 \pm 0.10c$	$1.23 \pm 0.14b$	$4.07 \pm 2.76a$	$1.32 \pm 0.08b$	$0.44 \pm 0.11c$
Hydrocarbons	$1.30 \pm 0.95b$	$0.88 \pm 0.47b$	$4.91 \pm 0.72a$	$4.69 \pm 0.48a$	$0.45 \pm 0.11b$	$0.65 \pm 0.07b$	$3.99 \pm 2.54a,b$	$3.70 \pm 0.83a$	$1.74 \pm 0.40b$
Pyrazines	$14.20 \pm 10.34a$	$13.16 \pm 6.16a$	$1.85 \pm 0.10b$	$6.78 \pm 8.10a$	$1.99 \pm 0.36b$	$0.59 \pm 0.09b$	$0.79 \pm 0.38b$	$7.29 \pm 1.24a$	$1.65 \pm 0.13b$
Crotenoid derivatives	$0.28 \pm 0.18a$	$0.27 \pm 0.12a$	$0.46 \pm 0.06a$	$0.43 \pm 0.04a$	$0.19 \pm 0.04a$	$0.20 \pm 0.00a$	$0.74 \pm 0.42a$	$0.43 \pm 0.07a$	$0.56 \pm 0.08a$
Ketones	$1.73 \pm 1.21a$	$1.43 \pm 0.52a$	$1.72 \pm 0.64a$	$2.08 \pm 1.43a$	$0.72 \pm 0.13a$	$0.84 \pm 0.01a$	$2.11 \pm 1.03a$	$1.95 \pm 0.16a$	$1.07 \pm 0.00a$
Esters	$0.37 \pm 0.13c$	$0.39 \pm 0.16c$	$5.97 \pm 0.41a$	$3.49 \pm 2.42a$	$0.20 \pm 0.00c$	$0.86 \pm 0.06b$	$2.35 \pm 1.49b$	$1.00 \pm 0.02b$	$0.21 \pm 0.03c$
Miscellaneous comp.	$0.64 \pm 0.43b$	$0.47 \pm 0.24b$	$1.59 \pm 0.63b$	$0.70 \pm 0.02b$	$0.37 \pm 0.07b$	$1.21 \pm 0.06b$	$3.32 \pm 1.43a$	$0.67 \pm 0.02b$	$0.47 \pm 0.04b$
Sum	$24.37 \pm 17.87b$	$21.35 \pm 10.48b$	$33.21 \pm 6.26a$	$30.44 \pm 8.54a$	$7.67 \pm 1.80c$	$8.13 \pm 0.72c$	$26.94 \pm 16.47a$	$20.43 \pm 2.93a$	$10.65 \pm 0.94c$

One-way analysis of variance (ANOVA), Tukey's test ( $p < 0.05$ ): letters a, b, c, etc. mark significant differences/analogies among the samples  
N.d. not determined

variety and the different climatic conditions during growing and harvesting. The colorant content may also depend on the drying process applied. Indeed, it was demonstrated that the temperature applied during the drying step could significantly influence the extractable color: Vega-Gálvez et al. showed a decrease in ASTA unit values when a temperature of 90 °C was used on red peppers; while using 70 °C, the highest colorant content was obtained [15]. In our case, the drying temperature was set at 70–75 °C for all the samples considered in this study, so the difference observed between samples can be ascribed to the cultivar.

## Pungency

The pungency of the analyzed chilli pepper powder samples, as measured by HPLC–UV, is reported in SHU units as this scale remains the most widely used and the best indicator for this property.

Data reported in Table 2 show an inverse trend between the extractable color amount and pungency. Among all the Italian cultivars analyzed, the sample that presented the highest pungency value was the G ( $47,814 \pm 971$  SHU units) in which the colorant content was the lowest ( $48.6 \pm 1.8$  ASTA units), while sample E showed the lowest pungency value with  $999 \pm 42$  SHU units with a medium–high amount of colorants ( $107.9 \pm 0.4$  ASTA units).

According to Weiss 2002, chilli peppers can be distinguished into five pungency classes from non-pungent (0–700 SHU) to very highly pungent (more than 80,000 SHU); on the basis of the results obtained, it is possible to identify three different groups of pungency among the considered samples: sample E is mildly pungent, samples A, B, D and F are moderately pungent, and C, G and H are highly pungent [16].

We tested the possible association between color intensity and pungency applying a Pearson correlation test to the obtained data. Results showed an inverse relation among ASTA units and SHU units ( $r = -0.747$ ,  $p = 0.03$ ); so in sample with a higher coloring compound amount, the pungency is lower and vice versa.

## Aromatic profile

The volatile profile of analysed samples resulted characterized by a total of 64 different gas-chromatographic signals, identified both by comparison of the registered mass spectra with those present in the instrument libraries (NIST) as by calculation of LRIs (Linear Retention Indexes) as shown in Table 3. All the volatiles were semi-quantified using an internal standard (3-octanol), and data were used for statistical elaborations.

The 64 identified and quantified compounds were classified in different chemical classes: terpenes and derivatives

(20), pyrazines (4), alcohols (8), aldehydes (4), ketones (6), esters (5), hydrocarbons (6), carotenoid derivatives (4) and miscellaneous compounds (7). The two classes mainly represented in the volatile fraction of chilli pepper samples were terpenes and derivatives (12–38% of the total volatile amount, samples A and B and I, respectively) and pyrazines (3–62% of total volatile amount, samples G and B, respectively), followed by esters, hydrocarbons, aldehydes, ketones, alcohols (and carotenoid derivatives (Table 2). The experimental cultivar C and the commercial one Serrano (sample D) had the highest quantity of volatile compounds, while E and F samples showed the lowest values. Sample H, composed by a mixture of Serrano cultivar (67%) and sample G (33%), showed a lower value of volatiles with respect to its corresponding starting samples. Data obtained for the sample purchased from the market coming from India were close to those of the experimental E and F powders.

The class of terpenes and derivatives showed the largest diversity of compounds, as reported in a study conducted on fresh ripened *Capsicum annum* and its spice in 2017 [17]. Terpenes were higher in sample C ( $9.70 \pm 0.30$  ppm). This category of volatiles was mainly represented by monoterpenes that can be generated during the heating step [18]; even if some of them as  $\alpha$ -copaene and caryophyllene were detected also in the volatile fraction of fresh chilli pepper [19]. Other compounds as  $\alpha$ -pinene,  $\beta$ -myrcene, limonene,  $\gamma$ -terpinene, linalool and nerolidol were found in the aromatic fraction of paprika samples produced in Hungary [20]. Among terpenes and derivatives, the most representative in terms of quantity were D-limonene and (+)-aromadendrene.

Among pyrazines, associated with nutty and green aromatic notes, 2-isobutyl-3-methoxy-pyrazine, the typical compound responsible of the flavor of fresh pepper, was found in our samples even if in lower amounts as compared to those of the other 3 detected pyrazines. In fact, 2-isobutyl-3-methoxy-pyrazine amount ranged around 0.1 ppm, and this low quantity is probably due to a loss of this compound during fresh pepper processing to produce paprika, as reported so far by Mateo, et al. [21]. Indeed, 2-isobutyl-3-methoxy-pyrazine is very common in fresh pepper especially when the fruit is still unripe and it was demonstrated that its content decreases during fruit ripening [7]. In the majority of the analyzed samples, the most abundant pyrazine was the 2,3,5,6-tetramethyl pyrazine. The highest amount of all these compounds was found in A and B samples, around 14 ppm, representing about the 60% of the total volatile content; while the lowest were calculated for G and F samples (about 0.6–0.7 ppm and 3–7% of the total volatile content).

Different alcohols, aldehydes and ketones were identified and quantified in the volatile fractions of our chilli pepper powder samples. The higher values of these three volatile categories were found in samples C, D and G. The presence of these molecules was associated with oxidative

**Table 3** Identification of volatile compounds, with relative aromatic notes, calculated LRIs, identification methods, and references

Chemical class, compound name	Odor type	LRI-wax	LRI-BP5	Identification method	References
<b>Terpenes and derivatives</b>					
$\alpha$ -Pinene	Herbal	1026	924	MS + LRI	[8, 20]
$\beta$ -Pinene	Herbal	1111	974	MS + LRI	[8, 13]
Sabinene	Woody	1125	975	MS + LRI	[8]
$\beta$ -Myrcene	Spicy	1168	989	MS + LRI	[8, 17]
D-Limonene	Citrus	1202	1032	MS + LRI	[8, 17]
$\gamma$ -Terpinene	Terpenic	1249	1061	MS + LRI	[17]
Trans- $\beta$ -Ocimene	Floral	1256	1045	MS + LRI	[8, 17]
p-Cymene	Terpenic	1276	1023	MS + LRI	[17, 26]
Sulcatone	Citrus	1345	983	MS	
Thujone (F)	Thujonic	1432	1105	MS + LRI	[13]
$\alpha$ -Copaene	Woody	1487	1342	MS + LRI	[17, 19]
Camphor	Camphoreous	1524	1270	MS + LRI	[27]
Linalool	Floral	1551	1102	MS + LRI	[8, 17]
Caryophyllene	Spicy	1602	1410	MS + LRI	[19]
$\alpha$ -Himachalene		1651	1442	MS + LRI	[8]
$\alpha$ -Gurjunene		1706	N.d.	MS	[28]
$\beta$ -Himachalene		1721	N.d.	MS + LRI	[8, 20]
(+)-Aromadendrene	Woody	1735	1491	MS + LRI	[8, 20]
Geranyl acetone	Floral	1869	1449	MS + LRI	
Trans-Nerolidol	Floral	2049	N.d.	MS + LRI	[20]
<b>Pyrazines</b>					
Trimethyl pyrazine	Nutty	1409	997	MS + LRI	[17]
2,3,5,6-Tetramethylpyrazine	Nutty	1481	1090	MS + LRI	[17]
2,3,5-Trimethyl-6-ethylpyrazine	Nutty	1517	n.d.	MS	
2-Isobutyl-3-methoxypyrazine	Green	1530	1178	MS + LRI	[8, 17]
<b>Alcohols</b>					
3-Methylbutanol	Fermented	1210	1210	MS + LRI	[17]
4-Methyl-1-pentanol	Nutty	1317	N.d.	MS + LRI	[19, 20]
1-Hexanol	Herbal	1356	862	MS + LRI	[8, 17]
1-Octen-3-ol	Earthy	1454	982	MS + LRI	[8]
2,3-Butanediol	Creamy	1548	796	MS + LRI	[29]
2-Furanmethanol	Bready	1674	n.d.	MS + LRI	[30]
Benzyl alcohol	Floral	1896	n.d.	MS + LRI	[27]
2-Phenyl ethanol	Floral	1932	1117	MS + LRI	[17]
<b>Aldehydes</b>					
2-Methyl propanal	Spicy	822	528	MS + LRI	[17, 21]
3-Methyl butanal	Aldehydic	903	625	MS + LRI	[17, 21]
Hexanal	Green	1091	805	MS + LRI	[8, 17]
Benzaldehyde	Fruity	1535	965	MS + LRI	[8, 17]
<b>Ketones</b>					
3-Octanone	Herbal	1261	987	MS + LRI	[27]
3-Hydroxy-2-butanone	Buttery	1295	710	MS + LRI	[17]
2-Furanyl ethanone	Balsamic	1517	912	MS	
$\gamma$ -Butyrolactone	Creamy	1648	N.d.	MS + LRI	[17]
4-Ketoisophorone	Musty	1711	N.d.	MS + LRI	[17]
1-(1H-pyrrol-2-yl) ethanone	Musty	1993	1073	MS	
<b>Esters</b>					
Hexyl isobutanoate	Green	1301	1159	MS + LRI	[19]
3-Octadecyl acetate	Herbal	1341	n.d.	MS	
2-Methy-hexyl-butanoate	Green	1384	1199	MS + LRI	[19]

**Table 3** (continued)

Chemical class, compound name	Odor type	LRI-wax	LRI-BP5	Identification method	References
Hexyl-pentanoate	Green	1521	1307	MS + LRI	[19]
Methyl salicylate	Minty	1800	1196	MS + LRI	[17, 26]
Hydrocarbons					
2,2,4,6,6-Pentamethyl heptane		941	984	MS	
2-Methyl-tridecane		1351	1332	MS + LRI	[19]
2-Methyl-tetradecane		1450	1465	MS + LRI	[20]
Pentadecane	Waxy	1494	1500	MS + LRI	[8, 20]
Hexadecane	Waxy	1594	1601	MS + LRI	[8]
Heptadecane		1700	1707	MS + LRI	[8, 20]
Carotenoid derivatives					
Safranal	Herbal	1659	1203	MS	
Dihydro- $\beta$ -ionone	Woody	1849	n.d.	MS + LRI	[20]
$\beta$ -Ionone	Floral	1957	1484	MS + LRI	[8, 20]
5,6-Epoxy- $\beta$ -ionone	Fruity	2012	n.d.	MS + LRI	[17]
Others					
Dimethyl sulfide	Solforous	780	740	MS + LRI	[17]
1-Methyl-1H-pyrrole	Woody	1148	1150	MS + LRI	[8, 20]
“Camphor like”		1211			
2-Pentyl-furan	Fruity	1236	991	MS + LRI	[8, 17]
Acetic acid	Acidic	1466	710	MS + LRI	[17]
Guaiacol	Phenolic	1883	N.d.	MS	
Dihydroactinidiolide	Fruity	2344	N.d.	MS	

enzymatic degradation of unsaturated fatty acids as linoleic and linolenic acids. In particular, hexanol and hexanal, detected in our samples, may have been originated by the action of lipxygenases starting from linoleic acid [22]. Among alcohols, 3-methylbutanol and 2-phenylethanol were detected in all the analyzed samples. As indicated by other authors, the presence of these two alcohols may be related to a fermentative degradation of amino acids, in particular 3-methylbutanol can be formed from isoleucine, while 2-phenylethanol derives from phenylalanine [17]. 2-furanmethanol, associated with bread aromatic notes, pertained to the sub-category of furans, can be formed during the heating processes by Maillard reaction [23]. To produce chilli pepper powder, all the samples considered in this study were subjected to different heating steps at a temperature ranged between 65° and 75 °C. This kind of processing may give rise to Maillard and Strecker reactions. In particular, compounds considered markers for Strecker degradations in paprika are some aldehydes found in our samples: 2-methylpropanal and 3-methylbutanal. These compounds are generated during the drying step of the manufacturing of paprika powder as demonstrated by Rainer Cremer and Eichner [24].

Six different ketones were identified and quantified in the volatile fraction of the samples, in particular two with buttery and musty notes, 3-hydroxy-2-butanone and

4-ketoisophorone, already identified in *Capsicum* aromatic profile [17].

Four carotenoid derivatives were found in different amounts in the samples analyzed: sample C was the richest in these compounds ( $5.97 \pm 0.41$  ppm). The molecule that most represented this class of volatiles was indeed  $\beta$ -ionone and its derivatives, dihydro- $\beta$ -ionone and 5,6-epoxy- $\beta$ -ionone.  $\beta$ -ionone can be generated during heating of the fresh chilli pepper and in particular its formation resulted linked to  $\beta$ -carotene degradation as indicated by Rainer Cremer and Eichner in 2000 [24].

Small amounts of hydrocarbons were also detected in the different samples considered in this study (1–2 ppm). The presence of esters, even if low in comparison to all the other chemical classes, contributed to green and herbal aromatic notes. Hexyl isobutanoate found in our samples was detected also in green peppers [8], while methyl salicylate, a phenol derivative, is not characteristic of *Capsicum annuum* specie because its presence was observed also in *Capsicum chinense* fresh fruit volatile fraction [25].

Among the category named as “miscellaneous compounds”, the presence of a sulfur compound, dimethyl sulfide, was of particular interest. This molecule, associated with sulfurous aromatic notes, can be formed starting from methionine via Strecker degradation reaction or via the hydrolysis of S-methylmethionine [24].

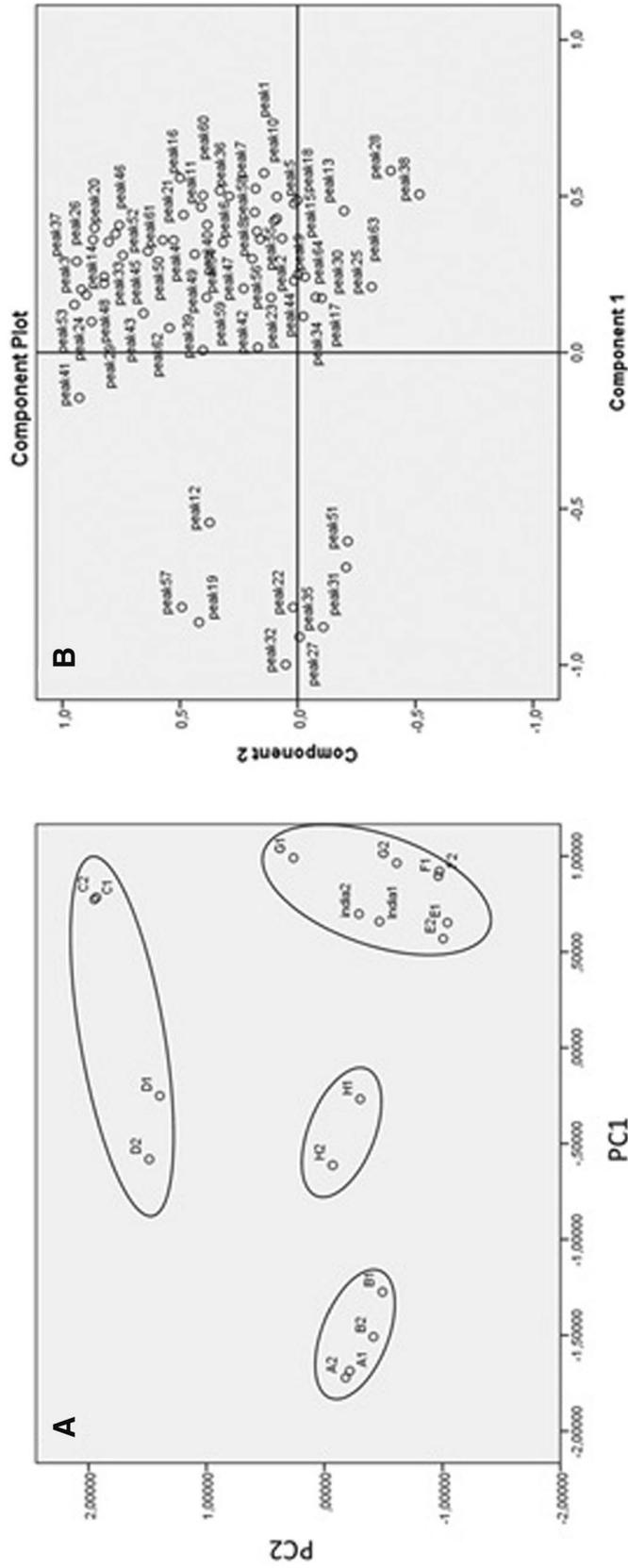
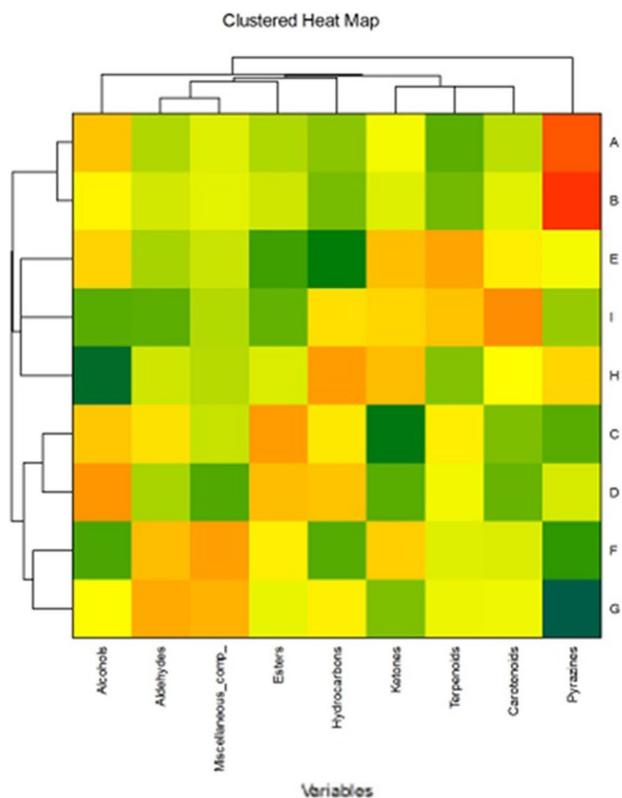


Fig. 1 Scatter plot of the scores from PC1 and PC2 obtained using volatile compound concentrations as variables (a) and loadings of the 64 variables (peaks) utilized for PCA analysis of chili pepper powder samples (b)



**Fig. 2** Heat Map analysis performed using volatile concentrations obtained for different chilli pepper powder samples

To elucidate possible similarities or differences between the different cultivars analyzed, from the aromatic point of view, all the results obtained from HS-SPME/GC-MS analyses were used as variables in an unsupervised pattern recognition method as principal component analysis (PCA), carried out applying the covariance matrix to the extraction of two components that explained together the 94% of the total variance; component 1 explained the 74% of the variance while component 2 explained the last 20%.

The loading plot (Fig. 1a) showed that chilli pepper powder samples A and B, pertaining to the same pepper cultivar, early and at maturity harvested, respectively, were shown to be different from the other samples on the basis of component 1 (PC1). These products were mainly characterized by three variables as 2,3,5,6-tetramethylpyrazine (peak32), trimethyl pyrazine (peak27), 2,3,5-trimethyl-6-ethylpyrazine (peak35) (Fig. 1b) and, in particular, the amount of these compounds was higher in these samples as compared to the other cultivars considered, as previously described.

The higher amount of esters, in particular hexyl-pentanoate (peak 37), 2-methy-hexyl-butanoate (peak 26) and hexyl isobutanoate (peak 20), but also of 3-methyl butanal (peak 3), 2-pentyl-furan (peak 14) and (+)-aromadendrene (peak 53) (Fig. 1b) discriminated samples C and D, similar to each other, from all the other cultivars.

The most discriminative volatiles characterizing the cluster formed by samples E, F, G and I (India) were mainly represented by some terpenes and derivatives as camphor (peak 38), thujone (peak 28) and trans-nerolidol (peak 63), and also by 1-hexanol (peak 25), while sample H resulted characterized by a different content of linalool (peak 42), pentadecane (peak 34), caryophyllene (peak 44) and sulcatone (peak 23) (Fig. 1b) as compared to the other samples considered.

To better highlight the different distribution of volatiles into samples, a Heat Map analysis was performed (Fig. 2). The map is in strong agreement with the previous described results. In particular, the highest clusterization distance was obtained for samples A and B compared to F and G, mainly based on pyrazines content (higher in A and B) and aldehydes and miscellaneous compounds (higher in F and G). In consideration of the different chemical origin of pyrazines compared to other volatile classes, these compounds are classified separately from the others and provide a relevant contribution to sample differentiation.

## Conclusions

In this study, the composition of the volatile fraction of different cultivars of *Capsicum annuum* was characterized, and at the same time, the pungency and the color content were evaluated. All the obtained data suggested that the composition could be related to the cultivar used to produce chilli pepper powder.

The extractable color of the chilli pepper powder samples considered in this study ranged from  $36.9 \pm 1.5$  to  $144.2 \pm 0.8$  ASTA unites, while three pungency categories were identified: mild, moderate and high. Moreover, an inverse relation between color intensity and pungency was found.

The volatile fraction of powder samples was shown to be composed of 64 different compounds pertaining to different chemical classes, mainly generated from the thermal degradation of carotenoids by the Maillard and Strecker reactions. The volatile amount was strongly linked and influenced by the cultivar as demonstrated by PCA and Heat map analyses.

This study may represent a useful approach to characterize new pepper varieties used to produce spicy chilli powder or paprika and to identify cultivars with desirable volatile content, pungency and color, suitable for the industrial choice and use.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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