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Volatile Compounds and Total Phenolic Content of *Perilla frutescens* at Microgreens and Mature Stages

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Abstract: Microgreens are considered products of high biological value because they contain natural and beneficial metabolites and antioxidants in high amounts; also, consumers appreciate them very much for their aromas. In this work, we focused our attention on the volatile organic compounds (VOCs) emitted from whole fresh leaves of two Chinese basil varieties (*Perilla frutescens* var. *frutescens* and var. *crispa*) at the microgreens stage; to show that the emission is microgreens specific we tested whether this capacity remains during subsequent growth of the plants. We found differences between the VOCs produced by the leaves of the two varieties at the microgreens stage and significantly reduced emission after development (additional four weeks of growth) particularly for the green variety (var. *frutescens*). The main volatiles emitted by whole leaves were D-Limonene for the red variety (*crispa*) and 2-Hexanoylfuran for the green one. In addition, the total phenolic content (TPC) and antioxidant power increase in adult leaves. These results clearly indicate that the particular smell of microgreens Perilla leaves depends on the specific variety and is not related to the amount of total phenols or antioxidant capacity of the leaves.

Keywords: VOCs; Chinese basil; shiso; antioxidant power; aromatic profile

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1. Introduction

Microgreens are young vegetable seedlings harvested generally after the complete development of the cotyledons and/or the formation of the first leaves; they are considered innovative and emerging foods. In recent years they have aroused interest from consumers thanks to their sensory and visual attributes, as well as nutritional due to the high contents of bioactive molecules, such as polyphenols, carotenoids, vitamins and other antioxidant compounds [1–4]. In addition, they add colors and flavor to foods. Microgreens is a marketing term used to describe a product category distinct from sprouts that have no specific legal definition.

Perilla frutescens (L.) Britt, commonly called perilla, perilla mint, or Chinese basil in western countries, zisu in China and shiso in Japan [5], is an annual herbaceous plant of the Lamiaceae family widely cultivated in Asia and used as an edible vegetable for its pleasant taste and as a traditional medicinal plant for its many health benefits, as well as for coloring and the cosmetic industry (skin creams, soaps, etc.) [5–7]. In fact, recent pharmacological studies have shown that the leaves of *P. frutescens* carry rich bioactive components, like phenolics, flavonoids, anthocyanins, tannins, and exhibits a variety of activities, including antioxidant, antiallergy, anti-inflammation, antitumor, and antibacterial activities [8–12] and its essential oil is promising for the treatment of disorders caused by depression [13].

P. frutescens var. *frutescens* and var. *crispa*, which are characterized by green and red leaves, respectively, have recently gained an increasingly broad acceptance as a novel crop so that they were recently studied by Rouphael et al. [14] to test the effects of moderate salt stress on the content of bioactive compounds and secondary metabolites.

Given also the increasing interest in microgreens, the aim of the present work was essential to evaluate the aromatic profile of intact leaves of *P. frutescens* var. *frutescens* and var. *crispa* at the microgreens stage and at an adult stage (after 4 weeks of further growth) to show if the VOCs emission is a peculiar characteristic of the microgreens and the differences between the two varieties To our knowledge, VOCs produced by the whole (intact, not processed or dehydrated or subject to the extraction of essential oils) *Perilla* leaves are being analyzed for the first time to represent the odor perceived by consumers of microgreens before chewing.

2. Materials and Methods

2.1. Plant Culture Conditions

Microgreens of *P. frutescens* var. *crispa* and var. *frutescens* (Figures 1 and 2) were obtained from the Ortogourmet company (Laterza, Taranto, Italy) where microgreens were grown in greenhouse leaving seeds germinate in brown peat (23 °C, 95% RH) in presence of half-strength Hoagland nutrient solution. The seeds come from the Chiltern Seeds Company (Wallingford, England, UK). It took about 20 days from sowing to the microgreens stage, at an optimum temperature of 30–32 °C. The microgreens were subsequently divided and planted singularly in 3 L pots, diameter 13 cm, height 13 cm in a mix of turf:vermiculite 3:1 plus slow-release N/P/K fertilizer, and placed in the greenhouse where the plants were periodically irrigated. The average temperature was 27 °C (minimum 22 °C, maximum 32 °C).

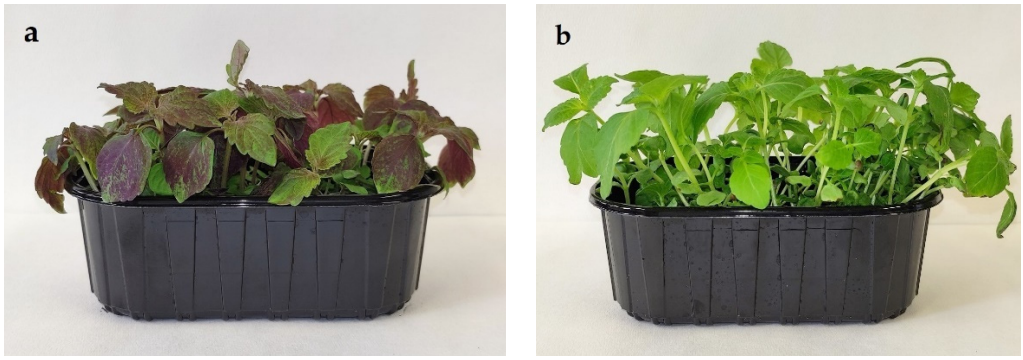

Figure 1. Trays of microgreens of *P. frutescens* var. *crispa* (**a**), and var. *frutescens* (**b**).

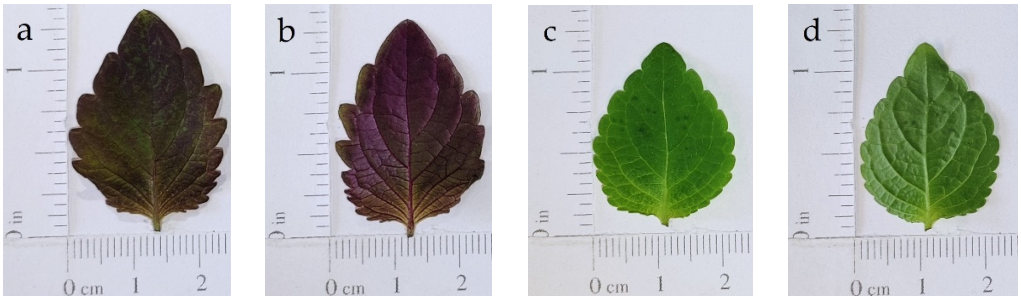

Figure 2. Leaves of microgreens of *P. frutescens* var. *crispa* (**a**,**b**), and var. *frutescens* (**c**,**d**). Upper leaf pages are shown in (**a**,**c**), lower leaf pages in (**b**,**d**), respectively.

Fresh *P. frutescens* leaves were analyzed at the microgreens stage and after further 4 weeks of growth (as representative of an adult stage). The dry weight was determined by placing the leaves in an oven at a temperature of 105 °C until constant weight.

2.2. Analysis of Volatile Organic Compounds

The analyses were carried out by solid-phase microextraction (SPME) methodology essentially as described by Negro et al. [15]. The analyses were repeated three times and the leaves were taken from 4–5 plants for microgreens and 1–2 plants for the 4-week stage. Approximately 1 g (FW) of leaves was sealed into 20 mL SPME vials (Agilent Technologies, Palo Alto, CA, USA) by metal screw-caps with pre-notched Teflon silicone septa. The vials were then placed at 40 °C for 10 min in a thermostatically controlled bath to allow the evaporation of the compounds; hereafter, an SPME syringe was inserted and the fiber (50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane, Supelco/Merck KGaA, Darmstadt, Germany), which was previously conditioned for 5 min at 235 °C in the gas chromatograph injector, was exposed for 10 min to absorb the volatile compounds. Subsequently, the fiber was inserted into the injector port of a gas chromatograph with a mass spectrometry detector (Agilent 7890B coupled with MS single quadrupole Agilent 5977A) and the desorption of the volatile compounds was performed at 235 °C for 4 min. At this point, the chromatographic run was started with an Agilent HP-5 ms column (30 m × 0.25 mm, 0.25 µm) (which temperature was raised from 60 °C to 230 °C with a constant increase of 3 °C/minute) with helium (purity > 99.999%) and a constant flow of 1.0 mL/min. Compounds were identified by library search and analytical standards if available. The mass spectrum of an unknown compound was searched in the data processing system [16]. Substances with a score above 800, both in terms of identity and purity, were considered to be identified after comparing the detected compound with the one in the NIST Computational Chemistry Comparison and Benchmark database [16]. Retention Index (RI) was obtained essentially as reported by Zhao et al. [17] employing as reference the retention times of a series of C_8–C_{20} alkanes separated under the GC-MS conditions mentioned above, and applying the following formula:

$$\mathrm{RI} = 100 \times n + \frac{100\ (t_\mathrm{a} - t_\mathrm{n})}{t_{\mathrm{n}+1} - t_\mathrm{n}} \quad (1)$$

where, t_a is the retention time of the unknown peak a; t_n the retention time of *n*-alkane C_n; and $t_{\mathrm{n}+1}$ the retention time of *n*-alkane C_{n+1}; *n* = carbon number of the alkane which elutes before the unknown peak a.

The semi-quantitative analysis of volatile compounds was carried out as reported by Zhao et al. [17] with some modifications. The compound 1,7,7-Trimethylbiciclo [2.2.1] 2-Heptanone was chosen as internal standard; 2 µL of a solution 1.25 µg/mL of internal standard in hexane were added to the samples. The calculation of the amount of VOCs was determined with the following formula: Qc = (Qs × Ac)/As where Qc is the amount of VOC in the sample, Qs the amount of standard, Ac is the peak area of the VOC in the sample and As the peak area of the standard.

2.3. Total Phenols Determination

For phenols, determination samples were finely powdered with mortar and pestle in presence of liquid nitrogen and subjected to extraction in a ratio of 1:20 FW/V with a solution of methanol:water (75:25) acidified with formic acid 0.1% for 20 min in constant agitation in an ultrasonic bath. Then the extract was centrifuged, and extraction was repeated on the pellet. The total phenolic content (TPC) was determined using the spectrophotometric Folin-Ciocalteau method [18] measuring the absorbance, after a 1:10 dilution, with a JASCO (Tokyo, Japan) V-550 UV/VIS spectrophotometer at a wavelength of 765 nm; data were expressed as gallic acid equivalent (GAE)·per mg/g dry weight (DW).

2.4. Antioxidant Activity Determination

The evaluation of the antioxidant activity was carried out by testing three aspects: scavenger, reducing and quenching capacity.

DPPH Assay. Antioxidant activity was determined in vitro by evaluation of the free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) (DPPH

assay) [19]. Inhibition of free radical DPPH$^{\bullet}$ was expressed as Trolox (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) equivalents (TE) per g DW.

Ferric Reducing Antioxidant Power (FRAP). The ferric reducing ability was determined by the FRAP method [20]. The absorption of the reaction mixture was measured at 593 nm using Perkin Elmer (Waltham, MA, USA) 2030 Multilabel reader Victor X5 after 3 min of incubation at 37 °C. The samples were measured in triplicate, and the FRAP was expressed as Trolox equivalents (TE)/g DW.

Superoxide anion scavenging activity assay. The assay was carried out according to Beauchamp and Fridovich [21]. The photo-induced reactions were performed using fluorescent lamps (200 W at 1 m). All samples were measured in triplicate, and the superoxide anion scavenging activity was expressed as g DW corresponding to half-maximal inhibitory concentration (IC_{50}).

2.5. Statistics

All data were reported as the mean ± standard deviation (SD), with at least three replications for each sample. Statistical evaluation was conducted by Duncan's test to discriminate among the mean values. All statistical analyses were performed using the software Statistica (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Phenolic Content and Antioxidant Activity

We measured the total phenolic content (TPC) in the leaves of Chinese red and green basil at the microgreen stage and after further four weeks to give more information concerning the secondary metabolites produced by young *P. frutescens* plantlets; Table 1 shows that, despite the modest values, Chinese red basil leaves contain a slightly higher level of TPC than the green variety at both stages. However, total phenols increase after 4 weeks of growth from the microgreens stage.

Table 1. Total phenolic content of red (var. *crispa*) and green (var. *frutescens*) Chinese basil at the microgreen stage and after further 4 weeks of growth.

	P. frutescens* var. *crispa	***P. frutescens* var. *frutescens***
	(mg/g DW)	**(mg/g DW)**
Microgreens stage	15.42 ± 0.51 b	9.94 ± 0.59 b
4 weeks stage	20.40 ± 0.35 a	15.47 ± 0.15 a

In the same column, different letters correspond to statistically different means (Duncan's test, $n = 3$, $p < 0.05$).

To evaluate the antioxidant activity of the *P. frutescens* we employed three different tests (DPPH, FRAP and superoxide anion scavenging activity assay) and data, expressed as Trolox equivalent (TE)/g DW or as g DW corresponding to IC_{50}, were included in Table 2. Both red and green Chinese basil adult leaves showed a slightly higher antioxidant activity than microgreens leaves but this was statistically confirmed only by FRAP and superoxide anion scavenging activity test results (Table 2).

Table 2. Antioxidant activity of the *P. frutescens* var. *crispa* (red) and var. *frutescens* (green) expressed as Trolox equivalent (TE µmol/g DW) for DPPH and FRAP, and as IC_{50} (g DW) for the superoxide anion scavenging activity assay.

	P. frutescens* var. *crispa			***P. frutescens* var. *frutescens***		
	DPPH TE (µmol/g DW)	**FRAP TE (µmol/g DW)**	**Superoxide Anion IC_{50} (g DW)**	**DPPH TE (µmol/g DW)**	**FRAP TE (µmol/g DW)**	**Superoxide Anion IC_{50} (g DW)**
Microgreens stage	0.81 ± 0.16 b	4.12 ± 0.7 a	9.1 ± 0.2 a	0.62 ± 0.08 b	3.62 ± 0.5 a	12.2 ± 0.3 a
4 weeks stage	1.18 ± 0.12 a	5.53 ± 0.8 a	7.2 ± 0.2 b	0.89 ± 0.08 a	4.56 ± 0.5 a	8.1 ± 0.2 b

In the same column different letters correspond to statistically different means (Duncan's test, $n = 3$, $p < 0.05$).

3.2. Volatile Organic Compounds Emitted by the Two Varieties of Chinese Basil

The VOCs released by the leaves of Chinese red basil leaves at the two growth stages are listed in Table 3 where their amounts are shown as area % of the peaks obtained after GC and as values in ng/g FW obtained through a semi-quantitative procedure.

Table 3. Volatile organic compounds produced at microgreen stage by Chinese red basil (var. *crispa*) and their semi-quantitative determination (ng/g FW).

No.	R.I.	Compound Name	Peak Area %		ng/g FW	
			Microgreen Stage	4 Weeks Stage	Microgreen Stage	4 Weeks Stage
1	1009	3-Carene	6.02	3.98	14.26	5.06
2	1018	p-Mentha-1(7),8-diene	3.61	2.57	8.54	3.27
3	1021	unknown	0.69	0.65	1.62	0.82
4	1025	unknown	1.76	3.11	4.16	3.95
5	1027	unknown	0.66		1.55	
6	1033	D-Limonene	51.14	59.63	121.11	75.82
7	1045	unknown	0.55		1.31	
8	1143	unknown	13.13	1.82	31.09	2.31
9	1218	unknown	2.11		5.00	
10	1258	unknown	0.52	1.62	1.24	2.06
11	1285	α-Perilla aldehyde	3.89	3.21	9.20	4.08
12	1305	unknown	0.81		1.92	
13	1358	unknown	0.52		1.23	
14	1385	unknown	0.66		1.56	
15	1392	Isocaryophyllene	3.85	19.36	9.11	24.62
16	1401	unknown	1.25	1.15	2.97	1.47
17	1418	2-Allyl-1.4-dimethoxybenzene	6.86	1.16	16.25	1.48
18	1452	*cis*-Methyl isoeugenol	1.33	1.74	3.14	2.21
19	1537	unknown	0.66		1.57	
		Total			236.83	127.16

At the microgreens stage, the compound present in greater quantity is D-Limonene, about 121 ng/g FW, followed by a not identified compound (No. 8), approximately 32 ng/g, then 2-Allyl-1,4-dimethoxybenzene (16.2 ng/g), 3-Carene (14.3 ng/g), α-Perilla aldehyde (9.2 ng/g), Isocaryophyllene (9.1 ng/g) and p-Mentha-1(7),8-diene (8.5 ng/g), for a total of around 237 ng/g FW of leaf tissue.

After 4 weeks of further growth, the total amount of VOCs drops by approximately half (to 127 ng/g FW); all single volatile compounds also decrease significantly with the exception of Isocaryophyllene (about 25 ng/g) which becomes the second most abundant compound after D-Limonene (about 76 ng/g) reaching almost 20% of the total compared to the 59.63% of D-Limonene.

The GC/MS chromatogram of Figure 3 shows graphically the previous data as the peaks representing the more abundant volatiles appear higher at the microgreens stage than at 4 weeks stage, except for the Isocaryophyllene peak (compound No. 15).

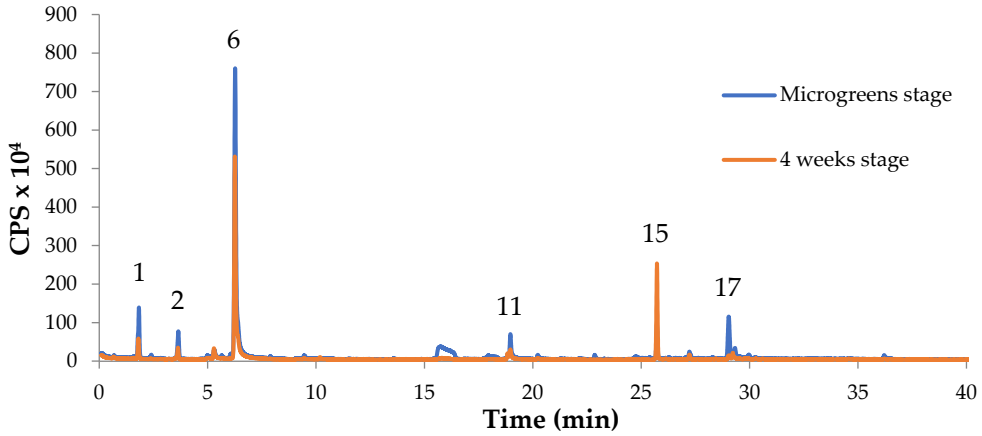

Figure 3. Comparison of VOCs of Chinese red basil (var. *crispa*) at microgreens stage and after further 4 weeks. The numbers of the peaks correspond to the volatile compounds identified (see Table 3).

The leaves of Chinese green basil (var. *frutescens*) microgreens produce fewer VOCs but in larger quantities; in fact, as shown in Table 4, we have detected a total of approximately 1135 ng/g FW of volatiles among which are present, in descending order of quantity, 639.1 ng/g FW of 2-Hexanoylfuran, 154.8 ng/g of Perillene, 68 ng/g of an unknown compound (No. 6), 57.1 ng/g of β-Caryophyllene and 55.6 ng/g of *trans*-Methyl-Isoeugenol. Surprisingly, after 4 weeks of further growth, we were unable to identify VOCs emitted by Chinese green basil leaves above the instrument detection threshold (data not shown). This means that the leaves of *P. frutescens* var. *frutescens* lose their ability to produce volatile compounds during growth.

Table 4. Volatile organic compounds produced at microgreen stage by Chinese green basil (var. *frutescens*) and their semi-quantitative determination (ng/g FW).

No.	R.I.	Compound Name	Peak Area %	ng/g FW
1	985	unknown	1.99	22.58
2	1102	Perillene	13.63	154.81
3	1225	unknown	0.84	9.52
4	1265	2-Hexanoylfuran	56.27	639.10
5	1289	unknown	1.47	16.67
6	1369	unknown	5.99	68.01
7	1385	unknown	1.16	13.19
8	1410	Methyl-eugenol	1.98	22.54
9	1417	β-Caryophyllene	5.03	57.08
10	1421	unknown	1.56	17.68
11	1456	*trans*-Methyl-Isoeugenol	4.90	55.62
12	1491	Farnesene	0.97	11.05
13	1493	*cis*-Methyl-Isoeugenol	2.26	25.70
14	1531	*cis*-Calamenene	0.80	9.09
15	1668	unknown	0.59	6.67
16	1674	Cadalene	0.57	6.48
		Total		1135.80

Thus, the two Chinese basil varieties, red and green, behave differently during the growth after the microgreens stage not only in quantitative but also in qualitative terms

with regards to the production of volatile compounds. In fact, only one of the main volatile compounds is common between the two varieties at the microgreens stage, the *cis*-Methyl isoeugenol.

Figure 4 confirms graphically that the volatile profile of Chinese green basil is drastically reduced at a level close to zero after 4 weeks of growth.

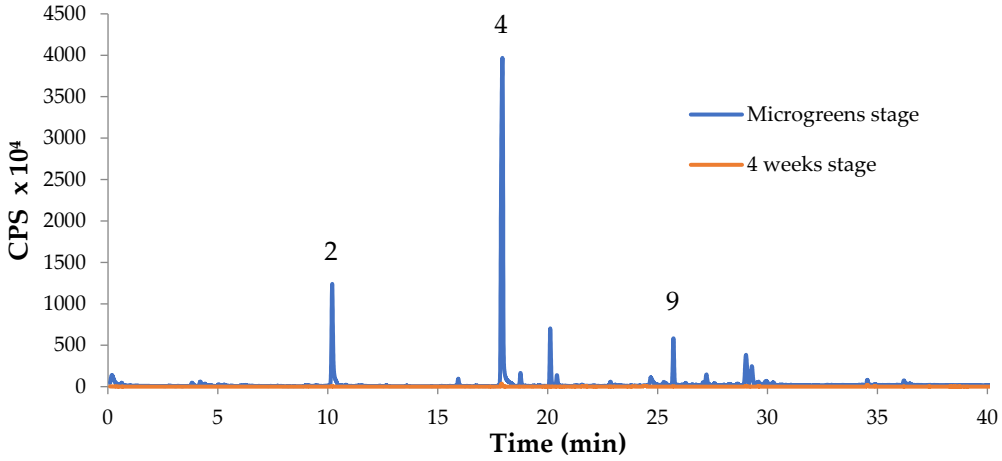

Figure 4. Comparison of VOCs of Chinese green basil (var. *frutescens*) at microgreens stage and after 4 weeks of further growth. The numbers of the peaks correspond to the volatile compounds identified (see Table 4).

As summarized in Tables 5 and 6, the two Chinese basils produce volatiles that results in a different perception of their odor/aroma. The Chinese red basil leaves emit mainly citrusy, spicy and woody odors and flavors, while the leaves of Chinese green basil produce compounds that tend to have fruity, sweet, spicy and herbaceous odors and flavors.

Table 5. Perception of the aromatic compounds of Chinese red basil.

Compound No.	Name	Percept: Odor/Flavor
1	3-Carene	Odor: lemon, resin [22] Odor Type: citrus; citrus terpenic herbal pine solvent resinous phenolic cypress medicinal woody [23] Flavor Type: citrus; citrus pine terpenic herbal resinous tropical peppery juniper wasabi [23]
2	p-Mentha-1(7),8-diene	Not found
6	D-Limonene	Odor: lemon, orange [22], mint [24] Odor Type: citrus; citrus orange fresh sweet [23] Flavor Type: citrus; sweet orange citrus terpenic [23]
11	α-Perilla aldehyde	Odor: spice [22] Odor Type: herbal; fresh green herbal grassy sweet minty cumin [23] Flavor Type: spicy; woody, spicy, waxy, sweet, citrus, lime and aldehydic [23]
15	Isocaryophyllene	Odor: wood [22] Odor Type: woody; woody spicy [23]
17	2-Allyl-1,4-dimethoxybenzene	Not found, but probably similar to Methyleugenol (4-Allyl-1,2-dimethoxybenzene)
18	*cis*- Methyl isoeugenol	Odor Type: spicy; spicy clove blossom carnation woody [23] Flavor Type: spicy; spicy warm clove resinous galanga smoky woody powdery [23]

Table 6. Perception of the aromatic compounds of Chinese green basil.

Compound No.	Name	Percepts: Odor/Flavor
2	Perillene	Odor: woody [23]; woody, flowery, citrus [24]
4	2-Hexanoylfuran	Odor Type: fruity; sweet fruity ketonic green apricot peach [23] Flavor Type: fruity; sweet fruity green waxy beany [23]
8	Methyl Eugenol (4-Allyl-1,2-dimethoxybenzene)	Odor: clove, spice [22] Odor Type: spicy; sweet fresh warm spicy clove carnation cinnamon [23] Flavor Type: spicy; spicy cinnamon clove fresh peppery woody [23]
9	β-Caryophyllene	Odor: wood, spice [22] Odor Type: spicy; sweet woody spicy clove dry [23] Flavor Type: spicy; spicy clove woody nut skin powdery peppery [23]
11	*trans*-Methyl-Isoeugenol	Odor Type: spicy; spicy clove blossom carnation woody [23] Flavor Type: spicy; spicy clove resinous galanga smoky woody powdery [23]
12	Farnesene	Odor: wood citrus sweet [22] Odor Type: woody; citrus herbal lavender bergamot myrrh neroli green [23] Flavor Type: green; fresh green vegetable celery hay fatty tropical fruity [23]
13	*cis*-Methyl-Isoeugenol	Odor Type: spicy; spicy clove blossom carnation woody [23] Flavor Type: spicy; spicy clove resinous galanga smoky woody powdery [23]
14	*cis*-Calamenene	Odor: herb, spice [22,23]. Herbal, spicy and savory [25]. Fresh, minty [26]
16	Cadalene	Not found

4. Discussion

Total phenolic content in our samples is low if compared with data obtained by Ahmed and Tavaszi- Sarosi [7] or Radacsi et al. [27], up to approximately 1/10. Indeed, among ten accessions of *P. frutescens* Ahmed and Tavaszi-Sarosi [7] detected a total polyphenol content between 137.5 and 234.2 mg GAE/g DW, while Radacsi et al. [27] found values between 84.7 and 204.3 mg GAE/g DW in leaves of five different accessions of *P. frutescens* analyzing them in two consecutive years. Additionally, recent publications have indicated values around 200 mg GAE/g DW for both varieties (green and red) of *P. frutescens* [28] and even values of more than 400 mg GAE/g DW in leaves of an unspecified Chinese basil variety [29].

The explanation for the low content of total phenols is that both at the microgreens stage and during the next four weeks the plants are not subject to particular stress so that the young *Perilla* leaves even compared to *Pelargonium* flowers [15] contain 1/10 of the total phenols (for DW) of the latter. Instead, the increase in TPC values in *Perilla* leaves during plant growth could be explained considering that the plants, after the microgreens stage, come out of a protected growth phase into a transplanted greenhouse environment with natural cycles of light and temperature, and periodical irrigation; therefore, leaves of mature Perilla plants are somehow "hardened" in comparison to microgreens. Thus, the increase in TPCs is related to a different developmental stage and different growth conditions as for example recently demonstrated by other authors for *Amaranthus caudatus* [30] or for African Cabbage [31].

According to the correlation between the level of phenolic compounds and antioxidant capacity [32], the low TPC content is probably the reason for the low levels of antioxidant capacity which can hardly be directly compared with other authors due to the lack of uniformity in the unit of measurement used to express the data, e.g., for the DPPH assay, from % [28] down to µg of Trolox/mg DW [29] or the IC_{50} [33].

Concerning the volatiles emitted by fresh Chinese basil leaves we have found a total of 19 compounds for *crispa* variety (Table 3) and 16 compounds for the *frutescens* variety (Table 4) but only the main seven and nine volatiles were identified, respectively. For the Chinese red basil (var. *crispa*) at the microgreens stage, the main volatile compounds were D-Limonene (51%), followed by 2-Allyl-1,4-dimethoxybenzene (7%) and 3-Carene (6%)

(Table 3); Chinese green basil (var *frutescens*) microgreens emitted mainly 2-Hexanoylfuran (56%), Perillene (14%), β-Caryophyllene (5%) and *trans*-Methyl-isoeugenol (4.9%) (Table 4). It is important to note the peculiarity that the microgreens leaves of the two varieties have in common the emission of only one compound *cis*-Methyl-isoeugenol which represents a very small proportion of the total compounds emitted in both varieties, 2.3% for the green variety and 1.3% for the red one. In addition, at the microgreens stage, Chinese green basil produces VOCs almost four times as much (1135.80 vs. 236.83 ng/g FW).

Thus, the two varieties not only have a very distinct emission profile but also behave very differently during growth after the microgreens stage as leaves of the *Perilla frutescens* var. *frutescens* practically do not emit VOCs at 4 weeks stage (Figure 4). A reduction in the emission of compounds also occurs in Chinese red basil leaves, but the reduction is just under 50%, with D-Limonene, the most abundant compound, increasing from 51% to almost 60% in relation to the total volatile compounds emitted. (Table 3).

D-Limonene is one of the most common monoterpenes found in plants [34] main components of the essential oils present in citrus peels [35] invoking a sweet, orange, citrus and terpy flavor [23]. It is also widely used as a flavoring agent and adjuvant in the food industry, especially for beverages and cosmetics [36]. The major volatile produced by Chinese green basil is instead 2-Hexanoylfuran, which is characterized by a sweet, fruity, ketonic green apricot peach odor and flavors, followed by Perillene with a woody floral and citrus odor. So, *Perilla* microgreens leaves have a mild spicy odor somewhat more citrusy and minty for the *crispa* variety and more floral and with hints of peach and apricot for Chinese green basil (var. *frutescens*).

In the literature, most studies have focused on VOCs produced by *Perilla* essential oils or on leaf extract or crushed/powdered leaves; therefore, a comparison concerning volatiles emitted by whole *Perilla* leaves is practically impossible.

In fact, VOCs include all volatile compounds produced from plants, whether processed or unprocessed organs, extracts obtained with different solvents, plant parts homogenized as such or pulverized after dehydration up to essential oils extracted by distillation. This is confusing, of course, and makes also proper comparisons difficult unless a similar methodology has been used. In nature, VOCs emitted by plants, or their organs are chemical environmental mediators serving as informative signals and defense chemicals, largely compounds with reduced molecular mass which allows significant release into the air, without a distinctive smell to humans, [37–39].

So, volatile compounds produced by essential oils, or "volatile oil compounds", do not fit into the above definition. A review of Ahmed [6] well described that the volatiles detected after the extraction of *Perilla* essential oil by various methods turn out to be more than 180. This confirms the enormous chemotypic variability that exists within the species and within VOCs. In addition, Tian et al. [40] collected *Perilla frutescens* samples from 11 different areas in China and found 119 different components present in a highly variable manner, with Limonene and 2-Hexanoylfuran (the main volatile compounds for the two varieties of *Perilla frutescens* analyzed in this work) found in only five and one of the 11 sampling sites, respectively. Similar data were recently collected by Ahmed and Al-Zubaidy [41] who individuated 63 components after GC/MS of essential oils extracted from 12 *Perilla frutescens* accessions.

On the other hand, volatile compounds of freeze-dried powdered *Perilla* leaves were investigated by Rouphael et al. [14] who found *Perillaldehyde* (41.6%) in *P. frutescens* var. *crispa* and Perilla ketone (51.5%) in *P. frutescens* var. *frutescens* as main components, while Benzaldehyde (26.7%) and cis-Jasmone (21.2%) were the second most abundant compounds in the red and green perilla, respectively. Benzaldehyde, Linalool and Caryophyllene were detected in both varieties and Perillene was specifically found exclusively in the green variety, the latter in accordance with our results (Table 4). A similar analysis was carried out by Chen et al. [42] crushing fresh and dried leaves of wild *Perilla frutescens* (L.) Britt. var. *acuta* (Thunb.) Kudo; after GC/MS they identified 23 volatile components of which the main ones were β-Caryophyllene (24.2–24.2%), Thujopsene (20.8–13.0%), Perillaldehyde

(15.1–14.2%) and (Z)-β-Farnesene (10.9–3.3%), of which only Thujopsene was not identified as VOCs in Tables 3 and 4. Additionally, Lee et al. [30] have identified a total of 142 volatile compounds from dried, roasted and then *P. frutescens* var. *acuta* Kudo; among them, Methyl benzoate and Limonene were predominant in terms of relative concentration at different roasting times suggesting that complex reactions take place during dehydration and roasting of the leaves.

Concerning attempts to characterize the odor or flavor of *Perilla frutescens* leaves, we found only two interesting articles, one paper analyzed the supernatant of finely cut leaves shaken with 10% NaCl employing a trained panel expressing a sensory evaluation; Perillaldehyde, Neral, Geranial, Eucalyptol, Methyl salicylate gave high positive correlation with the aromatic, fresh, perilla-like, green and minty-cool attributes [43]. The second paper of Laureati et al. [44] evidenced that panelists described infusions of green or red *Perilla* leaves as having a grassy and floral or an astringent and pungent odor, respectively, with green *Perilla* determining a higher level of odor intensity.

Regarding the reduction in the emission of volatile compounds from microgreens to adult leaves it can be explained either in terms of a transition from a juvenile leaf structure, and therefore, more "delicate" structure, to an adult organ characterized by a greater thickening of epidermis and cuticle, an increased presence of lignin, and fine stomatal control, as well as, hypothetically, to developmental stages for which VOCs may have a different role; for example, D-Limonene has antifeedant and antifungal properties and it is an attractant for pollinators [34], while Perillene is produced by a flower of several plant species and act as allomone/pheromone for Hymenoptera [45]. Of course, this second hypothesis requires further investigations that will be carried out starting from the data obtained in the present work.

5. Conclusions

The results presented show that the aroma profile of both Perilla varieties is higher at the microgreens stage than at the later adult stage (with green Chinese basil emitting practically no VOCs after 4 weeks) and that this profile is significantly different: the red variety produces a citrusy, spicy and woody odor and the green variety a fruity, sweet, spicy and herbaceous aroma at the microgreens stage. Thus, the different volatiles emitted differentiate the two varieties and justify the appreciation of both types of microgreens by consumers in Italy and Europe. This also confirms that microgreens are not overestimated in terms of their nutraceutical and hedonistic value even though both total phenolic and antioxidant power is higher at the adult stage.

Finally, it should be noted that this work represents the first analysis of VOCs emitted by whole Perilla leaves; in fact, *Perilla* VOCs have been studied extensively but starting from essential oils or dehydrated or homogenized organs, so as to simultaneously detect both VOCs produced following exposure of essential oils to air after cell rupture, and metabolites produced by contact between precursors and enzymes, as well as compounds generated in response to wounding/chewing. In the future, we intend to test whether the identified VOCs are constitutive or induced by particular conditions, e.g., different growing temperatures or lighting conditions so as to possibly further improve the *Perilla* microgreens product.

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