sustainability

MDPI

Article

Effect of Fertilization in Selected Phytometric Features and Contents of Bioactive Compounds in Dry Matter of Two Varieties of Basil (*Ocimum basilicum* L.)

Natalia Matłok [1,*], Józef Gorzelany [1], Agnieszka Ewa Stępień [2], Adam Figiel [3] and Maciej Balawejder [4]

1 Department of Food and Agriculture Production Engineering, Collegium of Natural Sciences, University of Rzeszow, St. Zelwerowicza 4, 35-601 Rzeszów, Poland; gorzelan@ur.edu.pl
2 Department of Dietetics, Institute of Nursing and Health Sciences, Collegium of Medical Sciences, University of Rzeszow, al/Mjr. W.Kopisto 2a, 35-310 Rzeszów, Poland; astepienurz@gmail.com
3 Institute of Agricultural Engineering, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37a, 51-630 Wroclaw, Poland; adam.figiel@up.wroc.pl
4 Department of Chemistry and Food Toxicology, Collegium of Natural Sciences, University of Rzeszow, St. Ćwiklińskiej 1a, 35-601 Rzeszów, Poland; maciejb@ur.edu.pl
* Correspondence: natalia.matlok@onet.pl

Received: 22 October 2019; Accepted: 20 November 2019; Published: 22 November 2019

Abstract: This study investigated the effects of sustainable, organic and standard mineral fertilization in selected phytometric features and contents of bioactive compounds in dry matter of two varieties of *Ocimum basilicum* L. The herbal material was first examined for its phytometric characterisation and then subjected to the combined convective pre-drying and vacuum-microwave finish drying method (CPD-VMFD). The energy consumption for the drying process of plant material in the case of CPD-VMFD is lower in comparison to the convection method (CD). The obtained dry material was assessed for determination of the colour parameters. Next, the analysis to identify the total content of polyphenols and the antioxidant properties (ABTS) was done. The dried material was subjected to head space–solid phase microextraction (HS-SPME) to determine volatile compound content. The herbal material obtained from the basil cultivated with an addition of sustainable, organic fertilizer was found to have a significantly higher content of bioactive compounds than the control, especially of polyphenols and volatile compounds like eucalyptol. Presumably, this is an effect of the elicitation process resulting from the fact that extract from common nettle was applied as an organic fertilizer component.

Keywords: herbs; sustainable fertilization; elicitation process; drying; antioxidant activity; total polyphenolic content; HS-SPME

1. Introduction

Herbs and spices are a natural source of bioactive compounds, in particular those with antioxidant properties (antioxidants) [1], that play an important role in defence mechanisms in the human body, and consequently prevent development of diseases of affluence, such as atherosclerosis, cancer, premature aging or myocardial infarction [2].

Common basil (*Ocimum basilicum* L.) is the most popular herbaceous plant and is commonly used as a culinary herb for its characteristic aroma, which influences the taste and flavour of food, and subsequently the digestion process [3]. Cultivation of numerous varieties of basil in the family of Lamiaceae [4] is widespread in Asia, Africa, South America and in the Mediterranean. In many countries it is also cultivated at home in plant pots [5]. The herb is a good source of antioxidants,

e.g., polyphenols, as well as components of essential oils that determine its health-promoting properties, such as antioxidant, antimicrobial, germicidal, antispasmodic, chemopreventive, radioprotective and antineoplastic [6–9].

Ocimum basilicum L. belongs to thermophilic plants, and the thermal conditions under which vegetation occurs are the most important factor determining the number of inflorescences, the date of flowering, as well as the content and chemical composition of the essential oil. It is also characterized by high light and soil requirements [10]. The highest yields are obtained in fertile soil, rich in nutrients, well-drained and medium-firm with regulated pH in the range of 6.5–7.2 [11,12]. Basil has high nutritional requirements, mainly with regard to nitrogen, which increases the herb's yield as well as the yield and composition of essential oil [13,14]. In addition to nitrogen, potassium and phosphorus have an impact on the quantity and quality of crops [15]. Increasing the potassium dose contributes to an increase in the concentration of phenolic compounds responsible for the antioxidant activity of basil [14]. However, the use of phosphorus increases the content of essential oil in herbs [16].

The contents of bioactive compounds in raw basil plants depends iter alia on the fertiliser and the quality of the substrate applied [14,17]. By introducing an organic fertilizer based on plant extracts it is possible to obtain a raw material with increased contents of bioactive compounds, an effect which is promoted by the phenomenon of elicitation. This process induces a defence response in a plant, and leads to a number of biochemical processes, as a result of which numerous groups of secondary metabolites are produced. Elicitors which can be used include plant extracts and chemical compounds, such as abscisic acid, methyl jasmonate and trans–anethole [18].

This study examined the impact of the plant variety and fertilizer applied on the selected phytometric parameters and contents of bioactive compounds in dry material from basil (*Ocimum basilicum* L.).

2. Materials and Methods

2.1. Plant Materials

Plant material examined in the study consisted of leaves collected from plants of two variety (*Genovese* and *Violetto*) of common basil (*Ocimum basilicum* L.), obtained from the pot experiment which was carried out.

2.2. Pot Experiment

The two-factor pot experiment involving cultivation of common basil was carried out in a greenhouse in three replications. The experiment investigated the factors of the common basil variety and the applied fertilizer (organic and mineral). The organic cultivation method involved the use of a novel, organic substrate consisting of neutral peat material with a pH of 5.5–6.5 (70% by weight), fortified with extract of common nettle (*Urtica dioica* L.) (10% by weight) and with horse manure (20% by weight). The pre-sowing soil treatment involved application of the organic controlled-release fertilizer Bioilsa N 12.5 (NaturalCrop Poland Sp. z o.o., Warszawa, Poland). The conventional cultivation method was based on the use of neutral peat substrate, which, prior to planting the seeds, was amended with mineral fertilizer (Table 1). In each replication the experimental unit comprised a surface of 0.5 m^2 and consisted of 32 plant pots, each with a volume of 0.78 dm^3; in each of these, four seeds were planted at a depth of approximately 0.5 cm. The moisture level in the substrate used in the cultivation of basil was maintained at 60% full water content (FWC). The experiment was carried out at a temperature of 25 °C. No chemical plant protection treatments were performed during the experiment. Harvesting was carried out five weeks after the seeds were planted.

Table 1. Characteristics of the methods used in cultivation of common basil (*Ocimum basilicum* L.).

Fertilization Method	Substratum Composition	Substratum				Nitrogen Fertilization		
		pH in KCl	(mg 100 g Substratum^{-1})			N Total (%)	Dose (g N Plant^{-1})	Type of Fertilizer
			P_2O_5	K_2O	Mg			
mineral	neutral peat (100%)	6.4	40.4	244.2	62.0	0.74	0.15	Ammonium nitrate
organic	neutral peat (70%), extract of common nettle (10%), horse manure (20%)	5.3	237.0	1282.0	269.0	1.18	0.15	Bioilsa N 12.5

2.3. Measurement of Phytometric Features of the Plants

During the harvesting of basil biomass, measurements were performed to assess selected morphological properties of the plants: Weight and height of the plants, as well as size and weight of single leaves randomly picked from the middle of the first, second and third branching from the growth cone. For this purpose, 20 plants were randomly selected in each experimental variant; they were cut directly above the substrate and weighed.

2.4. Methods of Drying

The samples of raw common basil, each 100 g ± 1 g, were subjected to the combined method in three technological repetitions. The combined convective pre-drying and vacuum-microwave finish drying method (CPD-VMFD) [19] involved pre-drying of the samples for 3 h in a convection dryer at a temperature and airflow of 40 °C and 0.8 m s^{-1}, respectively. Subsequently, additional drying was performed in a vacuum-microwave dryer at a power of 240 W magnetrons in a rotating drying chamber until the sample mass measurement (0.05 g accuracy) showed a weight value corresponding to the assumed final moisture content of 5% wb.

2.5. Modelling of Drying Kinetics

Modelling of the drying kinetics was performed using Table Curve 2D (Systat Software, San Jose, California, USA), which enabled fitting of the modified Page's model (Equation (1)) to experimental points. Page's model is often used to predict the decrease in the moisture ratio (MR) versus the time of drying (t) taking into account the highest values of the coefficient of determination R^2 and the lowest values of the root mean square error (RMSE) when compared with the fitting results using other drying models.

$$MR = A \cdot e^{-k \cdot t^n}. \quad (1)$$

where:

A—coefficient corresponding to the initial value of MR,
k—drying constant,
n—exponent.

The moisture ratio (MR) was expressed as the ratio of the current moisture content to the initial moisture content (Equation (2)), as measured by a gravimetric method [20].

$$MR = \frac{M}{M_0} \quad (2)$$

where:

M—current moisture content,
M_0—initial moisture content.

The value of M_0 was determined gravimetrically, taking into account the initial mass of sample and dry matter content after drying for 24 h at 60 °C in the dryer. The values of M were predicted according to the value of M_0 and the current mass of the dried samples. The value of the final moisture content was confirmed gravimetrically. The mass of the samples was determined using an analytical balance with an accuracy of 0.001 g.

2.6. Plant Extract

The weight of each sample was calculated according to the dry matter content for both the dried product and the raw material. An equivalent of 0.5 g of dry matter of each sample was then extracted by stirring with 10 ml of 70% aqueous ethanol (Chempur, Gliwice, Poland) for 30 min at room temperature [21]. All extractions were performed in triplicate.

2.7. Total Polyphenolic Content

The total polyphenolic content was determined using the Folin-Ciocalteu (Chempur, Gliwice, Poland) reagent method as described by Prior et al. [22] with some modifications. A 125 µl aliquot of the diluted extract was added to 2.375 ml deionized water and 125 µl Folin-Ciocalteu reagent (diluted 1:1 with water, acids 0.8–1.0 mol dm^{-3}). After shaking, the mixture was incubated for 3 min at room temperature. Then, 250 µl of 7% Na_2CO_3 (Chempur, Gliwice, Poland) solution was added; after mixing, the solution was stored for 30 min at room temperature in dark. The absorbance at 760 nm was measured in triplicate using a Thermo Scientific Evolution 201 spectrometer (Thermo Electron Scientific Instruments LLC, Madison, WI USA). The results were corrected for the dilution of gallic acid (mg ml^{-1}) (Sigma-Aldrich, Steinheim, Germany) as a standard.

2.8. Antioxidant Activities (ABTS Method)

The free radical scavenging activity was determined by the 2,2-azino-bis-3-ethylbenzothia zoline-6-sulfonic acid method (ABTS) according to Re et al. [23]. The absorbance was measured at 734 nm with a Thermo Scientific Evolution spectrometer (Thermo Electron Scientific Instruments LLC, Madison, WI USA) in triplicate. The results were corrected for the dilution of Trolox (mg 100 ml^{-1}) (Sigma-Aldrich, Steinheim, Germany) as a standard.

2.9. Colour Change

The basil leaf colour was measured, as defined by the Commission Internationale de l'Eclairage (CIE) L*a*b* system, being reflected in light using a Colour Ques spectrophotometer (HunterLab, Reston, VA USA), by introducing samples into a cuvette with an optical path length of 10 mm. The spectra were developed in the Easy Mach QC program (HunterLab, Reston, VA USA) for a 108° viewing angle and a D65 light source. The following colour parameters were determined: L* = brightness, a* = red and green (redness and greenness) and b* = yellow and blue (yellowness and blueness). In addition, the colour differences (DE) of the leaves subjected to drying were calculated assuming fresh material as the colour reference. The mean value of three replicates was taken as the result of the determination.

2.10. Head Space–Solid Phase Microextraction HS-SPME

The raw and the dry material were subjected to head space–solid phase microextraction analyses, with the use of 100 µm polydimethylsiloxanes (PDMS) fibre manufactured by Supelco Ltd. (Bellefonte, PA, USA). Prior to the analyses, the fibre was conditioned in accordance with the manufacturer's instruction, at 250 °C for a duration of 30 min in the injector of a gas chromatograph. The refined material, with a weight of 3 ± 0.01 g, was placed in a 100 ml glass vial and sealed with a cap provided with silicone septa. Exposition of the fibre in the headspace phase of the samples took place for 30 seconds at a temperature of 20 °C. Subsequently the fibre was transferred to the injector of the gas

chromatograph (temperature 250 °C), where the analytes were thermally desorbed for a duration of 5 minutes. The composition of the compounds desorbed from SPME fibre was examined using a gas chromatograph (GC-MS, Varian 450GC coupled with 240 MS).

2.11. Chromatographic Analysis

The composition of the desorbed compounds was examined using the gas chromatograph Varian 450 GC with the mass detector (GC-MS) Varian 240 MS (SpectraLab Scientific Inc., Markham, ON, Canada). The carrier gas applied was helium, with a flow rate of 1 ml min^{-1}; the injector temperature was 250 °C. Separation of analytes was conducted using a 30 m x 0.25 mm capillary column, with a moderately polar stationary phase HP-5 (methyl phenyl polysiloxane), with a film thickness of 0.25 µm. The column oven temperature programme at the start was at 50 °C for 5 min of isotherm, followed by an increase of temperature at the rate of 10 °C min^{-1} up to 300 °C (5 min of isotherm). The analysis was continued for a duration of 35 minutes. The compounds were identified based on NIST.08 and the Willey database.

2.12. Statistical Analysis

The significances of difference were tested using one way analysis of variance (ANOVA) and Student's test ($\alpha = 0.05$) with STATISTICA 13.1 software.

3. Results and Discussion

3.1. Phytometric Features of Plants

The fertilization applied in the experiment (Table 1) affected the selected phytometric features of common basil (*Ocimum basilicum* L.) (Table 2). The highest values of the relevant phytometric features, i.e., the weight and height of the plants as well as the weight of single leaves in the middle part of the first, second and third branching from the growth cone, were identified in the material treated with the organic fertilizer. Presumably it was the effect of an increased content of the main macronutrients (N, P, K and Mg) in the peat substrate, resulting from its fortification with horse manure containing the elements listed in the fertilizer value. The increased content of nutrients in the medium, mainly nitrogen, increased the basil herb yield [24–28]. In addition to nitrogen, potassium and phosphorus also affected the quantity and quality of the basil yield [13].

Table 2. Phytometric features of the plants of the *Genovese* and *Violetto* basil variety, treated with organic and mineral fertilizers.

Variety	Fertilization Method	Plant Height (cm)	Plant Weight (g)	Leaf Weight (g)		
				B1 **	B2 **	B3 **
Genovese	organic	20.41 ± 1.32 ^{a}	5.47 ± 0.34 ^{a}	0.38 ± 0.06 ^{a}	0.52 ± 0.06 ^{a}	0.38 ± 0.07 ^{a}
	mineral	15.93 ± 1.24 ^{b}	3.76 ± 0.78 ^{b}	0.26 ± 0.07 ^{a}	0.44 ± 0.14 ^{a}	0.19 ± 0.03 ^{b}
Violetto	organic	21.24 ± 1.57 ^{c}	5.21 ± 0.57 ^{c}	0.19 ± 0.04 ^{b}	0.20 ± 0.03 ^{c}	0.26 ± 0.04 ^{c}
	mineral	17.16 ± 1.20 ^{d}	2.85 ± 0.53 ^{d}	0.13 ± 0.02 ^{b}	0.17 ± 0.03 ^{d}	0.18 ± 0.04 ^{d}

Note: ± SD; n = 20; ^{1} statistical ($p < 0.01$) differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters. * difference at $p < 0.05$. ** B1, B2 and B3—first, second and third branches from the growth cone.

3.2. The Kinetics of Drying

The kinetics of the drying treatment (CPD 40 °C–VMFD 240 W) applied to leaves of two common basil varieties, relative to the applied fertiliser, are shown in Figure 1.

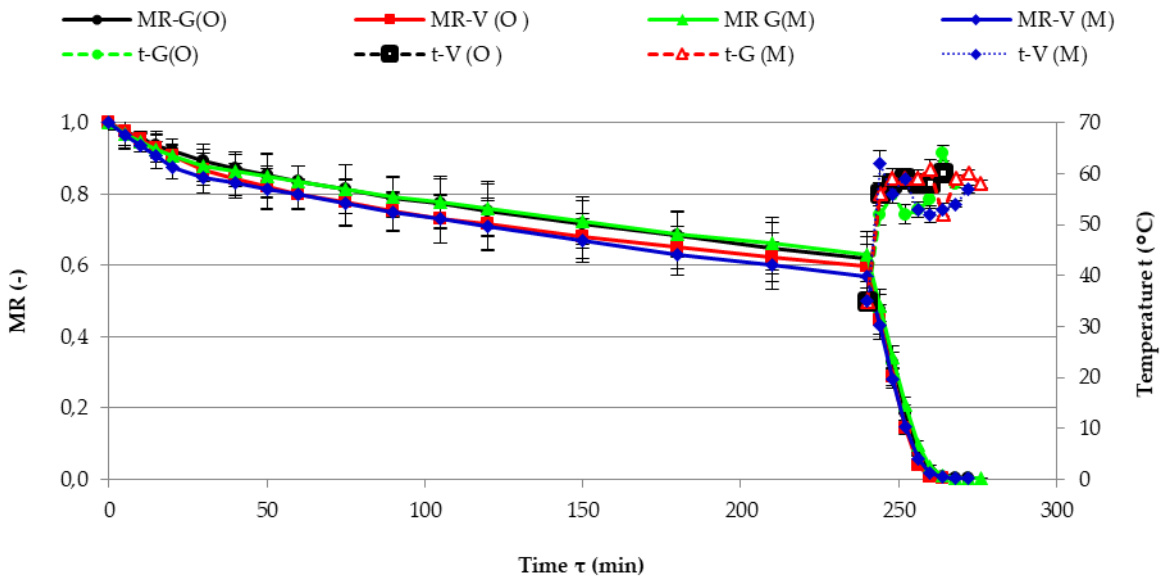

Figure 1. Water content decrease in *Ocimum basilicum* L. leaves during vacuum-microwave drying after convection pre-drying (CPD) at 40 °C followed by the vacuum-microwave finish drying method (VMFD). Note: MR = moisture ratio, V = variety *Violetto*, G = variety *Genovese*, (O) = organic fertilization, (M) = mineral fertilization, t = temperature.

The herbal material produced from common basil was subjected to combination drying (CPD 40 °C–VMFD 240 W). The method uses microwave radiation, as an additional source of heat, in order to reduce the duration of the drying process, and consequently to improve the quality of the final product [20]. The shorter drying time of the *Cistus creticus* herb by the CPD 40 °C–VMFD 240 W method compared to traditional CD convection drying at 40, 50 and 60 ° C was noted by Stępień et al. [19]. In addition, the use of the combined drying method CPD 40 °C–VMFD 240 W compared to convective CD drying at 40 ° C, the results showed reduced energy consumption by 56.7% per g of dried raw material [19]. The dry material obtained from *Genovese* basil, treated with organic and mineral fertilizer, reached the final moisture level of about 2% after 272 and 276 minutes, respectively. The same moisture was obtained in *Violetto* basil subjected to drying for a slightly shorter duration of time, i.e., 264 and 272 minutes, respectively, in the material treated with organic and mineral fertilizer. Undoubtedly, this is associated with the lower MR values achieved following CPD, respectively, amounting to 0.5915 and 0.5641 for the *Violetto* variety, compared to 0.6119 and 0.6237 for Genovese, and at the same time constituting the values of parameter A in Page's model describing the VMFD process (Table 3).

Table 3. The Page's model parameters for CPD and VMFD as well as the total drying time and final moisture content in dried leaves of *Ocimum basilicum* L.

Variety	Fertilization Method	Drying Period	Constants			Statistics		Drying Time (min)	Final Mc_{wb} (%)
			A	*k*	*n*	R^2	RMSE ‡		
Genovese	organic	CPD	1	0.01000	0.7036	0.9992	0.003543	272	2.15
		VMFD	0.6119	0.02408	1.5908	0.9981	0.011675		
	mineral	CPD	1	0.01203	0.6591	0.9977	0.005573	276	1.94
		VMFD	0.6239	0.02460	1.5534	0.9982	0.010332		
Violetto	organic	CPD	1	0.01845	0.6139	0.9966	0.007820	264	2.15
		VMFD	0.5915	0.02454	1.6536	0.9971	0.015425		
	mineral	CPD	1	0.01773	0.6266	0.9956	0.008989	272	2.15
		VMFD	0.5641	0.02691	1.5836	0.9989	0.008025		

Note: A = coefficient corresponding to the initial value of MR, k = drying constant, n = exponent, R^2 = coefficient of determination, RMSE ‡ = lowest values of the root mean square error, Mc_{wb} = final water content.

3.3. Colour

The colour of dry plant material largely depends on the occurrence of natural plant pigments, which are easily degraded during a drying process [29]; it also results from the applied drying technique and temperature. Microwave-assisted convective drying to a large extent preserves biologically active components, at the same time only slightly affecting the colour of the final product [20,30,31]. Analysis of the results (Table 4) shows that the type of fertilizer applied did not significantly affect the parameters of colour in the dry material. However, dry leaves of common basil treated with organic fertilizer were characterised by brighter colour. The values of the parameter b* also suggest that application of this fertilizer was related to the stronger light-yellow colour of the dry material from basil. Similar correlation was found in the case of change in the shades of green (parameter a*) (Table 4). Differences in the colour of the obtained dried material may result from the applied fertilization. Fertilization, especially with higher nitrogen doses, affects the content of selected assimilation dyes in leaves, mainly chlorophyll [32], which determines the colour of fresh herbs. However, the drying process significantly affects the degradation of chlorophyll dyes, which in consequence translates into the final colour of the obtained dried material. Śledź et al. [33] found a strong relationship between chlorophyll degradation and the colour factor a*. Differences in the colour of the obtained drought may also result from the degree of chlorophyll degradation during drying, which is varied for particular species and varieties of herbs [33]. Many researchers suggest that a change in the colour of herbs from yellow to green results from the applied combination drying (CPD –VMFD) [31,34].

Table 4. Assessment of colour in dry leaves of *Ocimum basilicum* L. relative to the variety and fertilizer applied.

Variety	Fertilization Method	L*	a*	b*
Genovese	organic	51.02 ± 1.40 ^{a}	−0.02 ± 0.03 ^{c}	11.63 ± 0.63 ^{f}
	mineral	50.25 ± 1.50 ^{a}	−0.06 ± 0.04 ^{c}	13.63 ± 1.74 ^{f}
Violetto	organic	45.02 ± 0.74 ^{b}	−1.99 ± 0.15 ^{d}	3.60 ± 0.46 ^{g}
	mineral	44.12 ± 0.24 ^{b}	−2.50 ± 0.13 $^{e\,**}$	6.33 ± 0.32 ^{h**}

Note: ± SD; n = 3; ^{1} statistical ($p < 0.01$) differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters. ** difference at $p < 0.05$. L* = colour brightness, a* = the proportion of green or red colour, b* = the proportion of blue or red yellow colour.

3.4. Bioactive Compounds Content

Analysis of the results related to the antioxidant potential and total polyphenol contents showed significant differences in these parameters relative to the fertilizer applied (Table 5). Presumably, this is an effect of elicitation resulting from the fact that extract from common nettle, known for its potential as an elicitor, was applied as the fertilizer in the organic cultivation. Szymanowska et al. noted a similar stimulating effect on the increase of phenolic compounds content in purple basil using jasmonic acid as the elicitor. Elicitation with jasmonic acid resulted in a 45% increase in phenolic compounds [35].

Table 5. Bioactive potential (ABTS test) and total polyphenolic content in dried basil *Genovese* and *Violetto* leaves obtained as a result of vacuum-microwave drying after convection pre-drying (CPD 40 °C–VMFD 240 W).

Variety	Fertilization Method	ABTS (Trolox mg 100 ml^{-1})	Total Phenolic Content (Gallic Acid Equivalent mg ml^{-1})
Genovese	organic	665.43 ± 0.19 ^{b}	77.57 ± 1.13 ^{d}
	mineral	231.48 ± 1.87 ^{c*}	14.68 ± 1.13 ^{e*}
Violetto	organic	579.57 ± 3.04 ^{g}	50.37 ± 1.57 ^{i}
	mineral	303.88 ± 5.11 ^{h*}	20.21 ± 1.46 ^{j*}

Note: ± SD; n = 3; ^{1} statistical ($p < 0.01$) differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters. * difference at $p < 0.05$.

3.5. Chemical Composition of Volatile Fraction HS-SPME

The SPME analysis showed the material contained compounds typically occurring in basil essential oils. The main components identified included: Eucalyptol, linalool, eugenol and methyl eugenol (Figure 2) [36]. Their contents differed depending on the substrate used and the common basil variety. Headspace analysis showed a visible tendency for increased content of eucalyptol in the case of basil treated with sustainable, organic fertilizer. This is particularly clear in the case of Genovese cultivation subjected to this type of treatment as this compound is predominant, with 32.3% content in headspace (Table 6). Eucalyptol determined the aroma (aroma threshold values 1–64 ppb) of the material, which, based on the initial organoleptic examination, was found to have the strongest fragrance. The material from basil treated with mineral fertilizer was found to predominantly contain methyl eugenol (aroma threshold values 68–85 ppm) which is characterised by lower volatility than eucalyptol and this explains the lower intensity of the aroma. These parameters clearly suggest higher quality of the herbal material produced using sustainable, organic fertilizer; this finding in combination with the biometric characteristics clearly confirms usefulness of this type of fertilizer in the production of herbal material from common basil.

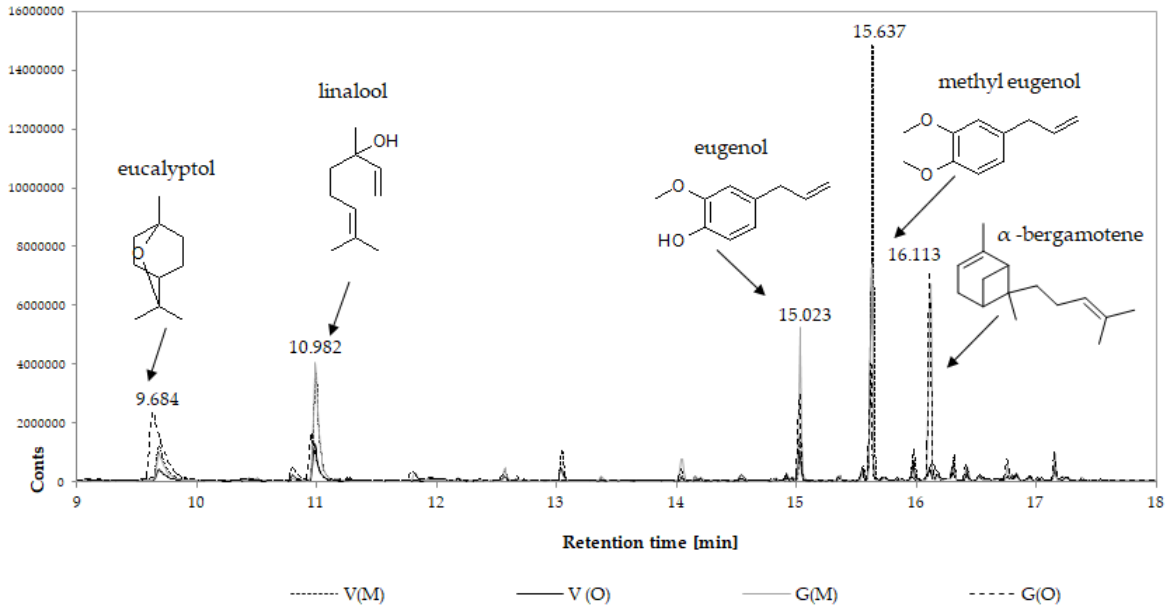

Figure 2. Chromatograms of solid phase microextraction (SPME)–gas chromatography (GC) analysis for the volatile fraction of dry material from common basil of the variety *Genovese* (G) and *Violetto* (V) treated with organic (O) and mineral (M) fertilizer.

Table 6. Chemical composition of SPME headspace fraction in dried common basil (*Ocimum basilicum* L).

No.	RT (min)	Peak Share in the Chromatogram (%)				Ordinary Substance Name	Systematic Substance Name	No. CAS
		V(O)	V(M)	G(O)	G(M)			
1	9.684	8.475	4.47	32.31	7.866	eucalyptol	4,7,7-trimethyl-8-oxabicyclo[2.2.2]octane	470–82–6
2	10.98	12.19	18.94	12.60	22.57	linalool	(±)-3,7-Dimethyl-1,6-octadien-3-ol, (±)-3,7-Dimethyl-3-hydroxy-1,6-octadiene	78–70–6
3	11.26	0.839	0.587	–	–	fenchol	1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol	1632–73–1
4	12.56	1.016	1.216	0.932	1.611	(-)-α -terpineol	2-[(1S)-4-methyl-1-cyclohex-3-enyl]propan-2-ol	10482–56–1
5	13.04	3.006	3.618	–	–	fenchyl acetate	1,3,3-trimethyl-2-norbornanyl acetate	13851–11–1
6	13.37	trace	0.340	–	0.501	carvacryl methyl ether	4-isopropyl-2-methoxy-1-methylbenzene	6379–73–3
7	14.04	trace	0.458	1.350	2.481	(−)-bornyl acetate	endo-(1S)-1,7,7-Trimethylbicyclo [2.2.1]hept-2-yl acetate	5655–61—8
8	14.92	1.134	0.330	trace	trace	α -terpinyl acetate	2-(4-methyl-3-cyclohexen-1-yl)- 2-propanyl acetate	80–26–2
9	14.96	1.134	0.310	trace	trace	(-)-α -cubebene	4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.01,5]dec-3-ene	17699–14–8
10	15.02	5.068	3.949	9.588	15.02	eugenol	2-Methoxy-4-(2-propenyl)phenol, 4-Allyl-2-methoxyphenol, 4-Allylguaiacol	97–53–0
11	15.36	trace	0.485	trace	0.506	α -copaene	1,3-dimethyl-8-(1-methyl ethyl) tricyclo(4.4.0.0.02,7-)dec-3-ene stereoisomer	3856–25–5
12	15.50	1.629	1.346	1.316	0.936	(−)-β-elemene	β-Elemene, (1S,2S,4R)-(−)-2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane, (1S,2S,4R)-1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)cyclohexane	515–13–9
13	15.63	35.25	39.17	11.27	19.39	methyl eugenol	4-Allyl-1,2-dimethoxybenzene, Eugenol methyl ether, Eugenyl methyl ether	93–15—2

Table 6. *Cont.*

No.	RT (min)	Peak Share in the Chromatogram (%)				Ordinary Substance Name	Systematic Substance Name	No. CAS
		V(O)	V(M)	G(O)	G(M)			
14	15.73	trace	0.344	trace	trace	3,5-diisopropylphenol	3,5-diisopropylphenol;Einecs 248-086-0;Phenol, 3,5-bis(1-methylethyl)-; 3,5-bis(1-methylethyl)phenol	26886–05–5
15	15.98	3.273	2.761	trace	trace	β-caryophyllene	(−)-trans-Caryophyllene, trans-(1R,9S)-8-Methylene-4,11, 11-trimethylbicyclo[7.2.0]undec-4-ene	87–44–5
16	16.11	2.045	1.446	20.34	19.34	α -bergamotene	(1R*,5R*,6R*)-2,6-dimethyl-6-(4-methylpent-3-en-1-yl) bicyclo[3.1.1]hept-2-ene	17699–05–7
17	16.11	1.100	1.373	0.802	0.497	α -guaiene	1,4-dimethyl-7-prop-1-en-2-yl-1, 2,3,4,5,6,7,8-octahydroazulene	3691–12–1
18	16.31	2.450	2.099	0.790	0.945	nerolidol	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol, 3-Hydroxy-3,7,11-trimethyl-1, 6,10-dodecatriene	7212–44—4
19	16.42	1.216	0.964	1.716	1.325	α-Humulene	α-Caryophyllene, trans,trans,trans-2, 6,6,9-Tetramethyl-1,4,8-cycloundecatriene	6753–98–6
20	17.15	2.33	1.607	2.709	2.363	γ-muurolene	(1R,4aR,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2, 3,4a,5,6,8a-hexahydro-1H-naphthalene	30021–74–0
TOTAL		**82.17**	**85.81**	**95.75**	**95.37**			

Note: RT = retention time, G = variety *Genovese*, V = variety *Violetto*, (O) = organic fertilization, (M) = mineral fertilization, no. CAS = number of chemical abstracts service.

4. Conclusions

As a result of the conducted research it was found that utilized fertilization improved the selected phytometric properties and the content of bioactive compounds in the dried two varieties of common basil (*Ocimum basilicum* L.). The use of organic peat substrate fortified with extract from common nettle and horse manure in combination with application of sustainable, organic fertiliser Bioilsa N 12.5 is a good method of common basil cultivation. It was shown that the use of sustainable, organic fertiliser allows to obtain a higher yield for common basil plants, and the drying process based on the combined method (CPD-VMFD) allows to obtain herbal products with the highest content of bioactive compounds and desirable sensory qualities (colour). Particularly notable is the increase in the antioxidant potential and polyphenol contents. Headspace analysis also shows significant differences that explain the stronger and more attractive aroma of the obtained herbal products

Author Contributions: Conceptualization, methodology and writing—original draft, N.M., M.B.; Formal analysis, J.G., A.F.; Investigation, A.E.S.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dragland, S.; Senoo, H.; Wake, K.; Holte, K.; Blomhoff, R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J. Nutr.* **2003**, *133*, 1286–1290. [CrossRef] [PubMed]
2. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. [CrossRef]
3. Nurzyńska-Wierdak, R.; Borowski, B.; Dzida, K. Yield and chemical composition of basil herb depending on cultivar and foliar feeding with nitrogen. *Acta Scientarium Pol. Hortorum Cultus* **2011**, *10*, 207–219.
4. Paton, A.; Harley, R.M.; Harley, M.M. Ocimum: An Overview of Relationships and Classification. In *Medicinal and Aromatic Plants—Industrial Profiles*; Holm, Y., Hiltunen, R., Eds.; Harwood Academic: Amsterdam, The Netherlands, 1999; pp. 1–38.
5. Makri, O.; Kintzios, S. *Ocimum* sp. (Basil): Botany, cultivation, pharmaceutical properties, and biotechnology. *J. Herbs Spices Med. Plants* **2007**, *13*, 123–150. [CrossRef]
6. Carović-Stanko, K.; Orlic, S.; Politeo, O.; Strikic, F.; Kolak, I.; Milos, M.; Satovic, Z. Composition and antibacterial activities of essentials oils of seven *Ocimum* taxa. *Food Chem.* **2010**, *119*, 196–201. [CrossRef]
7. Moghaddam, A.M.D.; Shayegh, J.; Mikaili, P.; Sharaf, J.D. Antimicrobial activity of essentials oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria. *J. Med. Plants Res.* **2011**, *5*, 3453–3456.
8. De Vincenzi, M.; Silano, M.; Maialetti, F.; Scazzocchio, B. Constituents of aromatic plants: II. Estragole. *Fitoterapia* **2000**, *71*, 725–729. [CrossRef]
9. Kaledin, V.I.; Pakharukova, M.Y.; Pivovarova, E.N.; Kropachev, K.Y.; Baginskaya, N.V.; Vasilieva, E.D.; Ilnitskaya, S.I.; Nikitenko, E.V.; Kobzev, V.F.; Merkulova, T.I. Correlation between hepatocarcinogenic effect of estragole and its influence on glucocorticoid induction of liver-specific enzymes and activities of FOXA and HNF4 transcription factors in mouse and rat liver. *Biochemistry* **2009**, *74*, 377–384. [CrossRef]
10. Rakic, Z.; Johnson, C.B. Influence of environmental factors (including UV-B radiation) on the composition of the essential oil of *Ocimum basilicum*—Sweet basil. *J. Herbs Spices Med. Plants* **2002**, *9*, 157–162. [CrossRef]
11. Biesiada, A.; Kuś, A. The effect of nitrogen fertilization and irrigation on yielding and nutritional status of sweet basil (*Ocimum basilicum* L.). *ACTA Sci. Pol. Hortic. Hortorum Cultus* **2010**, *9*, 3–12.
12. Mordalski, R.; Buchwald, W.; Kucharski, W.A.; Zalińska, H.; Forycka, A. Impact of mineral fertilization on yield of common basil (*Ocimum basilicum* L.) herb. *Post Fitoter* **2018**, *19*, 143–148. [CrossRef]
13. Nurzyńska-Wierdak, R. Sweet basil (*Ocimum basilicum* L.) flowering affected by foliar nitrogen application. *Acta Agrobot.* **2011**, *64*, 57–64. [CrossRef]

14. Nguyen, P.M.; Kwee, E.M.; Niemeyer, E.D. Potassium rate alters the antioxidant capacity and phenolic concentration of basil (*Ocimum basilicum* L.) leaves. *Food Chem.* **2010**, *123*, 1235–1241. [CrossRef]
15. Nurzyńska-Wierdak, R.; Rożek, E.; Borowski, B. Response of different basil cultivars to nitrogen and potassium fertilization: Total and mineral nitrogen content in herb. *ACTA Sci. Pol. Hortic. Hortorum Cultus* **2011**, *10*, 217–232.
16. Ramezani, S.; Rezaei, M.R.; Sotoudehnia, P. Improved growth, field and essentials oil content of basil grown under different levels of phosphorus sprays in the field. *J. Appl. Biol. Sci.* **2009**, *3*, 96–101.
17. Adhikari, B.M.; Bajracharya, A.; Shrestha, A.K. Comparison of nutritional properties of Stinging nettle (*Urtica dioica*) flour with wheat and barley flours. *Food Sci. Nutr.* **2016**, *4*, 119–124. [CrossRef]
18. Kochan, E.A.; Balcerczak, E.; Szymczyk, P.; Sienkiewicz, M.; Zielińska-Bliźniewska, H.; Szymańska, G. Abscicis acid gerulates the 3-hydroxy-3-methylglutaryl CoA reductase gene promoter and ginsenoside production in Panax quinquefolium hairy root culures. *Int. J. Mol. Sci.* **2019**, *20*. [CrossRef]
19. Stępień, A.E.; Gorzelany, J.; Matłok, N.; Lech, K.; Figiel, A. The effect of drying methods on the energy consumption, bioactive potential and colour of dried leaves of Pink Rock Rose (*Cistus creticus*). *J. Food Sci. Technol.* **2019**, *56*, 2386–2394. [CrossRef]
20. Alibas, I. Determination of drying parameters, ascorbic acid contents and color characteristics of nettle leaves during microwave-, air- and combined microwave-air-drying. *J. Food Process Eng.* **2010**, *33*, 213–233. [CrossRef]
21. Mahmoudi, H.; Aouadhi, C.; Kaddour, R.; Gruber, M.; Zargouni, H.; Zaouali, W.; Ben Hamida, N.; Ben Nasri, M.; Ouerghi, Z.; Hosni, K. Comparison of antioxidant and antimicrobial activities of two cultivated *Cistus* species from Tunisia. *J. Biosci.* **2016**, *32*, 226–237. [CrossRef]
22. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [CrossRef] [PubMed]
23. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
24. Sifola, M.I.; Barbieri, G. Growth, yield and essentials oil content of three cultivars of basil grown under different levels of nitrogen in the field. *Sci. Hortic.* **2006**, *108*, 408–413. [CrossRef]
25. Rao, E.V.S.P.; Puttana, K.; Rao, R.S.G.; Ramesh, S. Nitrogen and potassium nutrition of French basil (*Ocimum basilicum* Linn). *J. Spices Aromat. Crop.* **2007**, *16*, 99–105.
26. Sarab, D.; Badi, H.N.; Nasi, M.; Makkizadeh, M.; Midi, H. Changes in essentials oil content and yield of basil in response to different levels of nitrogen and plant density. *J. Med. Plants* **2008**, *7*, 60–70.
27. Zheljazkov, V.D.; Cantrell, C.L.; Ebelhar, M.W.; Rowe, D.; Coker, C.E. Productivity, oil content, and oil composition of sweet basil as a function of nitrogen and sulphur fertilization. *Hortic. Sci.* **2008**, *43*, 1415–1422.
28. Daneshian, A.; Gurbuz, B.; Cosge, B.; Ipek, A. Chemical components of essential oils from basil (*Ocimum basilicum* L.) grown at different nitrogen levels. *Int. J. Nat. Eng. Sci.* **2009**, *3*, 8–12.
29. Krokida, M.K.; Maroulis, Z.B.; Saravacos, G.D. The effect of the method of drying on the color of dehydrated products. *Int. J. Food Sci. Technol.* **2001**, *36*, 53–59. [CrossRef]
30. Arslan, D.; Özcan, M.M.; Okyay, M.H. Evaluation of drying methods with respect to drying parameters, some nutritional and colour characteristics of peppermint (*Mentha x piperita* L.). *Energy Convers. Manag.* **2010**, *51*, 2769–2775. [CrossRef]
31. Sarimeseli, A. Microwave drying characteristics of coriander (*Coriandrum sativum* L.) leaves. *Energy Convers. Manag.* **2011**, *52*, 1449–1453. [CrossRef]
32. Jaroszewska, A. Assimilatory pigment contentof stone fruit trees leaves in relation to irrigation and fertilization, infrastructure and ecology of rural areas. *Kom. Tech. Infrastruktury Wsi* **2011**, *6*, 155–164.
33. Śledź, M.; Witrowa-Rajchert, D. Influence of microwave-convective drying on chlorophyll content and colour of herbs. *Acta Agrophys.* **2012**, *19*, 865–876.
34. Therdthai, N.; Zhou, W. Characterization of microwave vacuum drying and hot air drying of mint leaves (Mentha cordifolia Opiz ex Fresen). *J. Food Eng.* **2009**, *91*, 482–489. [CrossRef]

35. Szymanowska, U.; Złotek, U.; Karaś, M.; Baraniak, B. Anti-inflammatory and antioxidative activity of anthocyanins from purple basil leaves induced by selected abiotic elicitors. *Food Chem.* **2015**, *172*, 71–77. [CrossRef] [PubMed]
36. Eckelmann, S. Biodiversitat der Gattung *Ocimum* L., Insbesondere der Kultursippen. Die Doktorarbeit, University of Kassel: Kassel, Germany, 2002; Volume 19, p. 236. Available online: http://www.genres.de/infos/igrreihe.htm (accessed on 21 November 2019).