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Evaluation of extraction yield and chemical composition of the essential oil of five commercial varieties of basil (*Ocimum basilicum L.*)

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Abstract: Basil (*Ocimum basilicum* L.) is one of the most cultivated aromatic species worldwide, being used as a spice and medicinal plant. The literature reports a wide variability in essential oil composition and its extraction yield of varieties of this species due to genetic and environmental factors. The present work aimed to evaluate the yield extraction and chemical composition of five commercial varieties of *O. basilicum* grown in Caxias do Sul, South Brazil. Ten plants of the varieties *'Italiano'*, *'Alfavaca verde'*, *'Manolo'*, *'Fraganza'*, and *'Sabory'* were cultivated in a greenhouse at the Campus-Sede of the University of Caxias do Sul. Plant material was collected, and the essential oil was extracted by hydrodistillation for 4 h using a Clevenger apparatus. Essential oil extraction yield was determined at the end of the process, and the obtained oils were sent to chromatographic analysis. Qualitative analysis was carried out by GC/MS, whereas quantitative analysis was performed by GC-FID. Regarding essential oil yield, the *'Italiano'* and *'Fraganza'* varieties had the highest extraction yields (1.00 and 0.90 % v/w, respectively), whereas the *'Manolo'* variety had the lowest yield (0.57 % v/w). Concerning the chemical composition, the essential oils from the five varieties presented the linalool chemotype, whose contents varied between 47.9 and 79.9 wt. %. The other varieties presented eugenol as the second major compound (5.5-9.1 wt. %), with exception of the oil of *'Manolo'* variety, which had estragole as the major compound (14.6 wt. %) and the absence of hydrocarbon monoterpenes in the oil. The varieties *'Alfavaca verde'* and *'Fraganza'* clustered in both the dendrogram and the PCA, probably due to the same linalool content and the higher 1,8-cineole and lower eugenol contents, whereas the varieties *'Italiano'* and *'Sabory'* clustered together, though the linalool contents in these varieties was less similar.

Keywords: Chemical analysis, Chemotype, Linalool, Terpenes.

Resumo: *O manjericão (Ocimum basilicum L.) é uma das espécies aromáticas mais cultivadas em todo o mundo, tendo uso como condimento e como planta medicinal. A literatura relata uma grande variabilidade na composição do óleo essencial e no rendimento de extração das variedades desta espécie devido a fatores genéticos e ambientais. O presente trabalho teve como objetivo avaliar o rendimento da extração e a composição do óleo essencial de cinco variedades comerciais de O. basilicum cultivadas em Caxias do Sul, Sul do Brasil. Dez plantas das variedades 'Italiano', 'Alfavaca verde', 'Manolo', 'Fraganza' e 'Sabory' foram cultivadas em uma estufa no Campus-Sede da Universidade de Caxias do Sul. O material vegetal foi coletado e o óleo essencial foi extraído por hidrodestilação por 4 h com um extrator Clevenger. O rendimento de extração do óleo essencial foi determinado ao final do processo, e os óleos obtidos foram encaminhados para análise cromatográfica. A análise qualitativa foi realizada por CG/EM, enquanto a análise quantitativa foi realizada por CG-DIC. Em relação ao rendimento de óleo essencial, as variedades 'Italiano' e 'Fraganza' apresentaram os maiores rendimentos (1,00 e 0,90 % v/m, respectivamente), enquanto a variedade 'Manolo' apresentou o menor rendimento (0,57 % v/m). Quanto à composição química, os óleos essenciais das cinco variedades apresentaram o quimiotipo linalol, cujos teores variaram entre 47,9 e 79,9 % m/m. As demais variedades apresentaram eugenol como segundo composto principal (5,5-9,1 % m/m), com exceção do óleo essencial da variedade 'Manolo', que apresentou o estragol como composto principal (14,6 % m/m) e ausência de hidrocarbonetos monoterpênicos. As variedades 'Alfavaca verde' e 'Fraganza' agruparam-se tanto no dendrograma como no PCA, provavelmente por apresentarem teores muito semelhantes linalol e os maiores teores de 1,8-cineol e menores teores de eugenol, enquanto as variedades 'Italiano' e 'Sabory' se agruparam juntos, embora o conteúdo de linalol nessas variedades fosse menos semelhante.*

Palavras-Chave: *Análise química, Linalol, Quimiotipo, Terpenos.*

1. Introduction

Ocimum basilicum L., commonly known as 'basil', is an aromatic plant from the Lamiaceae family, greatly appreciated as a spice and also used in traditional medicine as a medicinal plant [1]. Basil is a perennial shrub that grows mainly in tropical and subtropical climates worldwide [2].

Besides its uses as a spice and medicinal plant, *O. basilicum* is also an important source of essential oil, which is extracted mainly from plant leaves and flowers. Indeed, most of the therapeutic and medicinal properties of basil are regarded as being of its essential oil [3].

Basil essential oil is a complex mixture of several terpenes, obtained through steam distillation and/or hydrodistillation of the plant or its parts. The literature has reports on the biological activity of this essential oil, especially antimicrobial and antifungal activity [4].

O. basilicum is also known due to its wide distribution throughout the world. Effects of breeding and geographic isolation have generated several basil varieties whose essential oils may differ greatly in yield and chemical composition [5].

According to the literature, there are several different *O. basilicum* chemotypes, with quite variable yields. The most recurrent ones are the 'linalool', 'eugenol', 'estragole', 'methyl chavicol', and their derivatives [5-6]. Regarding essential oil yield, the literature has reports on yields as low as 0.4 % v/w, and above 1.5 % v/w, depending on both plant genetics and environmental conditions [7-8].

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This high variability in essential oil yield and composition may render it difficult to evaluate and determine the chemotype and yield of crossings of different basil plants for commercial use, regardless of the mother plants.

Thus, the objective of the present work was to evaluate the extraction yield and the chemical composition of the essential oil of five commercial varieties of *O. basilicum*.

2. Material and methods

2.1 Obtainment of plant material and essential oil extraction

Seeds of five commercial varieties of basil were supplied by Feltrin Sementes (Brazil). The following varieties were studied: *'Italiano'*, *'Alvavaca verde'*, *'Fraganza'*, *'Manolo'*, and *'Sabory'*.

The seeds were put to germinate and were grown up to the adult stage in a greenhouse located in the Campus-Sede of the University of Caxias do Sul, at the geographical coordinates 29°09'46"S and 51°08'37"W and an altitude of 817 m above sea level. The plants were grown in a greenhouse to reduce the variability of environmental factors. The plants were cultivated in 5 L pots containing Carolina Soil® substrate. The plants were fertilized with the modified Hoagland nutrient solution, prepared according to the recommendations of Smith et al. [9]. The irrigations were carried out daily, with an alternate application of tap water and nutrient solution (100 mL both).

Ten plants of each variety were grown, totaling 50 plants in the experiment. After approximately six months of cultivation (from February 2018 to August 2018), leaves from plants of each variety were collected and dried in a kiln for 72 h at 35±5 °C. After drying, the leaves of each treatment were homogenized, and three replicates were taken for essential oil extraction.

The essential oil was extracted by hydrodistillation, using a glass Clevenger apparatus with a scale; the scale had a capacity of 3.00 mL and a resolution of 0.05 mL. About 100 g of dried leaves were used in each extraction, with the exact mass measured using an AD1000 scale (Marte®, Brazil). It was used a 5 L round bottom flask with 2 L of tap water (proportion of 1:20 plant material:water). Heating was provided by an electric mantle (690 W, Quimis®, Brazil) at half-power (345 W).

The extraction was carried out for 4 h. Condenser temperature was kept at 10±2 °C using a refrigerating system. After extraction, the volume of obtained essential oil was measured using the scale of the Clevenger, and the essential oil was collected with a glass amber bottle (5 mL).

Essential oil extraction yield was measured using Equation 1, proposed by Pauletti et al. [10].

$$Y = 100 \times \frac{V}{M} \quad (1)$$

Where 'Y' is the essential oil yield (% v/m), 'V' is the volume of extracted essential oil (mL), and 'M' is the mass of plant material used in the extraction (g).

The essential oil samples were stored away from sunlight in a cold chamber (4±2 °C) and sent immediately for chromatographic analysis.

2.2 Chromatographic analysis

Essential oil samples were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) for qualitative analysis and gas chromatography with flame ionization detector (GC-FID) for quantitative analysis, according to the procedures described by Serafini et al. [11], with modifications.

GC/MS analyses were carried out using a Hewlett Packard 6890 gas chromatograph coupled to an MSD 5973 selective mass detector, equipped with HP Chemstation software and the Wiley 275 spectral library. It was used an HP-Innowax column (30 m x 250 µm), with 0.50 µm film thickness (HP, Palo Alto, USA). Temperature programming was 40 °C for 8 min, 40-180 °C at 3 °C·min^{-1}, 180-230 °C at 20 °C·min^{-1}, and keeping at 230 °C for 20 min. Interface temperature of 280 °C, split ratio 1:100, helium as carrier gas at 56 kPa and flow rate of 1.0 mL·min^{-1}, ionization energy of 70 eV. Injected sample volume of 1.0 µL, diluted in hexane (1:10).

GC-FID analyses were performed using a Hewlett Packard 6890 gas chromatograph and an HP-Innowax column (30 m x 250 µm), with 0.50 µm film thickness (HP, Palo Alto, USA). Temperature programming was the same as GC/MS analysis. Injector and detector temperature of 250 °C, split ratio 1:50, hydrogen as carrier gas at 34 kPa, and flow rate of 1.0 mL·min^{-1}. Injected sample volume of 1.0 µL, diluted in hexane (1:10).

Essential oil components were identified by comparison of the mass spectra of the peaks with the Wiley 275 library and by comparison of their linear retention indexes (LRI) with the ones reported in the NIST Webbook [12]. The LRI values were calculated using the Vand den Dool and Kratz equation, and a solution of alkanes (C9-C30) as the standard.

2.3 Experimental design and statistical analysis

The experimental design was completely randomized, with the basil variety as the factor, in three replicates for each treatment, totaling 15 replicates for the experiment.

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The results of essential oil yield underwent Levene's test (homoscedasticity) and Shapiro-Wilk test (normality of residuals), followed by analysis of variance (ANOVA) and the post hoc Tukey's multiple range test at 5 % probability ($\alpha = 0.05$).

Essential oil composition was analyzed by hierarchical cluster analysis (HCA) and principal component analysis (PCA). HCA was carried out using Ward's method and Euclidean distance. The results of each essential oil component were used as input data. The covariance matrix of the results was used to generate the PCA. The statistical analyses were performed using the Statistica 12 software (Statsoft, USA).

3. Results and discussion

3.1 Essential oil yield

The results of essential oil yield for each basil variety are presented in Table 1.

According to Table 1, it is possible to observe that the *'Italiano'* and *'Fraganza'* varieties presented higher essential oil yields (1.00 and 0.90 % v/w, respectively). On the other hand, the variety *'Manolo'* presented the lowest average yield (0.57 % v/w). The other two varieties, *'Alafavaca verde'* and *'Sabory'*, presented intermediate yields (both 0.80 % v/w), not differing statistically from the other varieties.

Table 1. Essential oil extraction yield (mean±standard deviation; % v/w) of the five basil varieties studied.

Basil variety	Extraction yield (% v/w)
'Italiano'	1.00±0.20 a
'Alfavaca verde'	0.80±0.10 ab
'Fraganza'	0.90±0.10 a
'Manolo'	0.57±0.06 b
'Sabory'	0.80±0.10 ab
F-value	5.30
p-value	0.015
Coefficient of variation (%)	14.89

Means followed by the same letter do not differ statistically by Tukey's multiple range test at 5 % probability ($\alpha = 0.05$).
Source: Authors (2021).

Serafini et al. [11] reported essential oil extraction yields in the range of 0.68-1.39 % v/w for nine basil varieties grown in South Brazil. Pirmoradi et al. [1], studying 21 accessions of two *O. basilicum* varieties, reported essential oil extraction yield ranging between 0.6 and 1.1 % v/w. Vieira and Simon [3], evaluating the essential oil yield of 15 *O. basilicum* varieties from various parts of the world and grown in the Midwestern United States, observed yields ranging between 0.54 and 1.64 % v/w.

As commented by Mandoulakani et al. [13], the essential oil yield is mostly influenced by both genetic and environmental factors. Literature reports that plants under stress from biotic and abiotic origins may increase the amounts of secondary metabolites to counteract and mitigate the stressors. Thus, drought or excess rainfall, UV radiation, and the presence of pests and herbivores may induce the plant to produce more terpenes, increasing essential oil yield [14].

Padalia et al. [8], who evaluated the effect of environmental factors and harvest time on two *O. basilicum* taxa, reported essential oil yields in the range of 0.41-0.64 % v/w for plants grown in North India. Belkamel et al. [7], testing different cut times for basil plants grown in Morocco, observed a wide variation in essential oil yield (0.57-1.27 % v/w).

It is important to observe that most secondary metabolites and, within them, the terpenes, are substances with antioxidant, antimicrobial, insecticidal, and repellent activity [15]. Considering that these compounds act mostly in plant defense against environmental threats and stressors, plants under stress are prone to produce more essential oil than the ones under milder conditions, which do not require to take active response against the stressors [13-14].

However, the five basil varieties were grown under the same environmental conditions (in a greenhouse and under the same irrigation regime), the observed differences are probably the result of genetic differences inherent to each variety. In this sense, some specific alleles may be more active, directing cell metabolism to pathways that generate specific terpenes at the expense of other photoassimilates, increasing the overall essential oil yield [13].

3.2 Chemical composition of the essential oils

The detailed chemical composition of the essential oils of each basil variety is presented in Table 2.

According to Table 2, it can be seen that linalool was the major compound in the essential oil of the five basil varieties, whose content ranged between 74.93 and 79.90 wt. %. Regarding the minor compounds, eugenol was the second most abundant compound in four of the five varieties, with contents ranging between 3.46 and 9.07 wt. %.

On the other hand, estragole was the second major compound in the essential oil of the *'Manolo'* variety, whose average content was 14.62 wt. %. However, this compound was present in much smaller amounts in the other essential oils, with contents below 1.0 wt. %.

According to Telci et al. [5], *O. basilicum* and the species from the *Ocimum* genus, in general, are characterized by the high variability in essential oil composition. Several populations have specific compositions that may be related to geographic localization, but this is not a rule [16].

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Literature reports great diversity in terms of chemotypes. The most recurrent ones are 'linalool', ''linalool-methyl chavicol' and 'linalool-eugenol, as well as their derivatives [1,3].

All of the basil varieties studied in the present work presented essential oils of the 'linalool' chemotype. Silva et al. [17], who studied three different *O. basilicum* varieties in Northeast Brazil, reported linalool as the major compound in two of the essential oils and estragole in the other. On the other hand, Serafini et al. [11], studying nine basil cultivars grown in South Brazil, reported that all had essential oils of the linalool chemotype, whose contents ranged between 46.5 and 83.6 wt. %.

On the other hand, Occhipinti et al. [16] reported a basil variety from Italy whose essential oil presented τ-cadinol as the major compound, completely unrelated to other basil cultivars reported in the literature. Smigielski et al. [18] reported methyl-eugenol as the major compound of *O. basilicum* essential oil obtained from plants grown in Poland. Raina and Gupta [19] studying the essential oil from several varieties of *Ocimum* species collected in India, commented that the composition of the oils was quite variable, with *O. basilicum* cultivars presenting distinct chemotypes, as 'linalool', 'methyl chavicol', and '(*E*)-methyl cinnamate.

Table 2. Chemical composition (mean±standard deviation; wt. %) of the leaf essential oil of the five basil varieties studied.

Compound	Calc. LRI	Lit. LRI[1]	Mass percentage (wt. %)				
			'Italiano'	*'Alfavaca verde'*	*'Fragranza'*	*'Manolo'*	*'Sabory'*
α-pinene	1023	1022	-	0.38±0.22	-	-	-
β-pinene	1108	1108	0.42±0.24	0.62±0.21	0.51±0.32	-	0.50±0.29
sabinene	1124	1120	-	0.35±0.20	-	-	-
myrcene	1170	1166	0.45±0.26	0.46±0.13	0.38±0.22	-	1.69±0.63
limonene	1203	1199	0.34±0.20	0.38±0.03	0.43±0.25	-	-
1,8-cineole	1210	1210	3.77±0.97	8.24±0.96	6.23±2.03	2.78±1.10	4.02±1.41
(Z)-β-ocimene	1259	1251	0.61±0.35	0.57±0.13	0.40±0.23	-	-
copaene	1498	1496	-	0.45±0.26	-	-	-
camphor	1530	1531	0.38±0.22	0.53±0.32	0.52±0.19	0.76±0.25	1.38±0.80
linalool	1554	1551	79.90±0.57	78.11±3.76	77.21±3.69	74.93±6.33	76.05±5.67
bornyl acetate	1594	1591	0.43±0.03	0.80±0.50	1.68±0.44	0.40±0.09	0.62±0.10
β-elemene	1598	1598	0.71±0.09	1.52±0.40	1.58±0.96	1.32±0.30	0.85±0.70
β-caryophyllene	1602	1604	0.35±0.03	0.26±0.15	0.55±0.32	-	0.94±0.54
terpinen-4-ol	1615	1612	0.73±0.14	-	0.77±0.44	-	-
estragole	1687	1683	0.30±0.17	0.32±0.18	0.23±0.39	14.62±3.36	0.94±0.54
germacrene-D	1725	1726	0.64±0.05	0.73±0.13	0.93±0.50	0.63±0.39	1.13±0.89
β-selinene	1732	1729	0.35±0.06	0.28±0.16	0.53±0.31	0.55±0.32	0.64±0.49
γ-cadinene	1772	1765	0.41±0.07	0.43±0.25	0.46±0.32	0.28±0.16	1.42±0.15
geraniol	1856	1853	1.64±0.32	-	-	-	-
eugenol	2190	2186	7.94±1.31	5.52±1.81	5.98±1.84	3.46±1.01	9.07±3.80
Hydrocarbon monoterpenes			1.82	2.77	1.72	-	2.19
Oxygenated monoterpenes			95.09	93.52	92.62	96.65	92.08
Hydrocarbon sesquiterpenes			2.46	3.22	4.06	2.78	4.98
Total identified			99.37	99.51	98.39	99.73	99.25
Not identified			0.63	0.49	1.61	0.27	0.75

[1] – NIST Webbook [12].Source: Authors (2021).

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tDespite the wide variability in essential oil composition, most basil varieties have essential oils that present linalool, eugenol, and estragole in their composition, with exception of plant populations whose geographic distribution is generally remote [16].

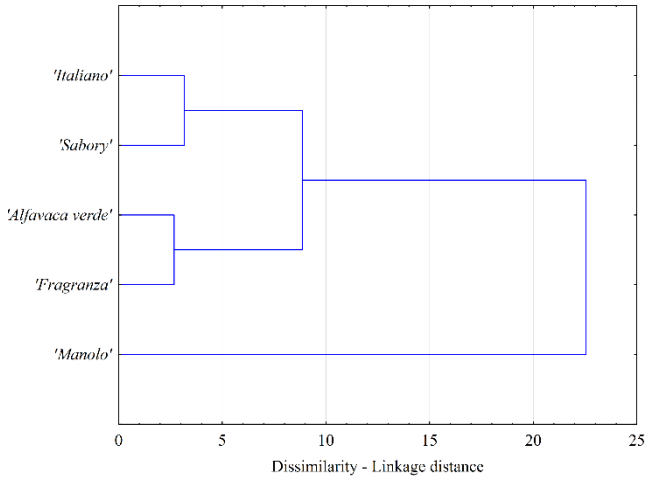
Relative to chemical classes, all varieties had essential oils with similar contents of oxygenated monoterpenes and hydrocarbon sesquiterpenes. The main difference occurred on the *'Manolo'* variety, which had no hydrocarbon monoterpenes in its essential oil.

In general, most basil essential oils are characterized by the presence of large amounts of oxygenated monoterpenes, since the major compounds (linalool, estragole, eugenol) and their derivatives are classified in this category [1,4]. However, in some varieties, the contents of sesquiterpenes may be high, contributing to 10-15 % [19] and up to 25 % of the entire essential oil composition, as reported by Telci et al. [5] for some basil varieties grown in Turkey.

An HCA was carried out to observe the separation patterns of the basil varieties as a function of the essential oil composition and to observe the degree of dissimilarity among samples. The dendrogram compiling the five basil varieties is presented in Figure 1.

According to the dendrogram (Figure 1), it is possible to observe that the *'Manolo'* variety separated first, with a large dissimilarity relative to the other varieties. By verifying the chemical composition (Table 2), it is possible to note that the essential oil of this variety had greater amounts of estragole, and the absence of monoterpenes, as well as the smallest amounts of 1,8-cineole and eugenol relative to the five studied varieties.

Figure 1. Dendrogram of the five basil varieties studied, based on the chemical composition of the essential oils obtained (Euclidean distance; Ward's method).



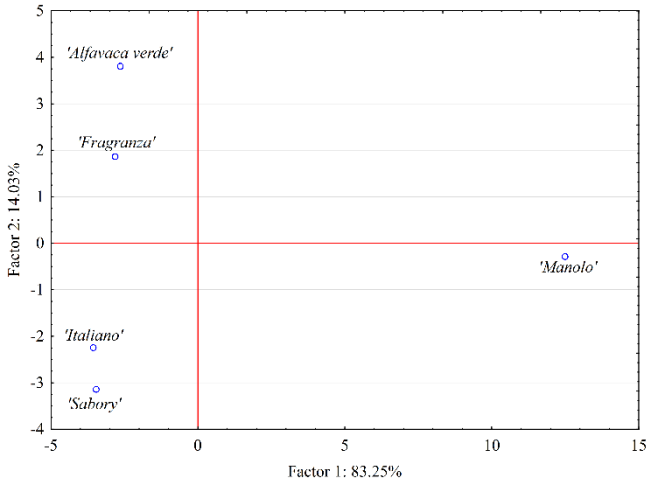
Source: Authors (2021).

The other four varieties split into two groups with similar linkage distances between them. The varieties *'Italiano'* and *'Sabory'* formed a cluster, probably due to the similar amounts of 1,8-cineole and eugenol. The varieties *'Alfavaca verde'* and *'Fraganza'* also clustered with the smallest dissimilarity among all varieties, and this occurred probably due to very similar linalool content, as well the contents of eugenol, camphor, β-elemene, γ-cadinene, and 1,8-cineole.

Tsasi et al. [2], evaluating the effect of harvesting on the composition of five varieties of *O. basilicum* grown in Greece, observed that essential oils of the same chemotype formed clusters, however, sub-clusters related to minor compounds were formed within the main clusters. Telci et al. [5] and Pirmoradi et al. [1] also observed the formation of main clusters related to essential oil chemotype; sub-clusters were formed within the main clusters and were related to the presence and content of minor compounds.

A PCA was carried out to observe the differences in essential oil composition and the similarities in terms of main factors. The PCA scatterplot of the five basil varieties distributed as a function of essential oil composition is presented in Figure 2.

Figure 2. PCA scatterplot of the five basil varieties according to the chemical composition of the essential oil.



Source: Authors (2021).

By analyzing the PCA scatterplot (Figure 2), it is possible to observe the same pattern seen in the dendrogram (Figure 1). The *'Manolo'* variety was highly distanced, as observed in the chemical composition (Table 2). The other four varieties clustered more closely, but in two different quadrants, in the same pattern observed in the dendrogram.

The table containing the factor loadings of each variable relative to each principal factor is presented in Table 3.

Verifying the factor loadings (Table 3), Factor 1 was mainly influenced by estragole content (0.816761), followed by linalool

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(0.087293) and eugenol (0.059949) contents. For Factor 2, the compounds that influenced it more were 1,8-cineole (0.409497), linalool (0.265813), and eugenol (0.210286).

This explains the high distancing of the *'Manolo'* variety in the X-axis (Factor 1) and the proximity of the other four varieties (Figure 2). Relative to Y-axis (Factor 2), the clustering of the *'Alfavaca verde'* and *'Fraganza'* varieties in the upper left quadrant was the result of higher 1,8-cineole and lower eugenol contents, whereas the clustering of the *'Italiano'* and *'Sabory'* varieties in the lower left quadrant was probably the result of lower 1,8-cineole and higher eugenol contents (Table 2).

Table 3. Variable contributions to each principal factor, based on the covariance matrix.

Variable	Factor 1 (83.25 %)	Factor 2 (14.03 %)
α-pinene	0.000026	0.001927
β-pinene	0.001039	0.000586
sabinene	0.000022	0.001635
myrcene	0.002487	0.013606
limonene	0.000306	0.002038
1,8-cineole	0.026807	0.409497
(Z)-β-ocimene	0.000606	0.002236
copaene	0.000037	0.002702
camphor	0.000006	0.005378
linalool	0.087293	0.265813
bornyl acetate	0.000803	0.009184
β-elemene	0.000177	0.015668
β-caryophyllene	0.001184	0.002708
terpinen-4-ol	0.000594	0.000038
estragole	0.816761	0.034902
germacrene-D	0.000228	0.000408
β-selinene	0.000037	0.000776
γ-cadinene	0.000746	0.008088
geraniol	0.000894	0.012525
eugenol	0.059949	0.210286

Source: Authors (2021).

Vieira and Simon [3] observed high variability in the PCA distribution of *O. basilicum* varieties; the same authors reported that linalool, 1,8-cineole, methyl chavicol, and spathulenol were the substances that influenced most the distribution and clustering of the evaluated essential oils. Maggio et al. [6] also observed a wide variation in the PCA distribution of basil cultivars relative to the chemical composition of the essential oils, however, the terpenes that had the greater influence were α-cadinol, eugenol, linalool, and methyl eugenol.

4. Conclusion

The five basil varieties presented different performances regarding essential oil yield, ranging from 0.57 % v/w (*'Manolo'* variety) to 1.00 % v/w (*'Italiano'* variety). Regarding the chemical composition, all varieties presented the linalool chemotype, however, the *'Manolo'* variety was the only one that presented high estragole contents (14.6 wt. % against 0.2-0.9 wt. % in the other four) and the absence of hydrocarbon monoterpenes. The varieties *'Alfavaca verde'* and *'Fraganza'* clustered in both the dendrogram and the PCA, probably due to the same linalool content and the higher 1,8-cineole and lower eugenol contents, whereas the varieties *'Italiano'* and *'Sabory'* clustered together, though the linalool contents in these varieties was less similar. This indicates that, despite presenting the same chemotype, the essential oil of commercial basil varieties may have a distinct chemical composition, probably due to genetic factors and the joint effect of genetics and the environment.

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