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# Variability in the Chemical Composition of Essential Oil Derived from *Ocimum basilicum* L. var. Minimum over Several Months

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### Authors' contributions

This work was carried out in collaboration between all authors. Author EAVC designed the study, wrote the work protocol and wrote the first draft of the manuscript. Author KACG managed the literature search and designed the work protocol. Author JGGV made the obtaining of the essential oil. Author SGRR performed the essential oil analysis. Author YFCV performed the spectral data management. Author SMBC made and reviewed the final conclusions of the article. Author ACC advised on the final revision of the article and reading of spectral tables. All authors read and approved the final manuscript.

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## ABSTRACT

*Ocimum basilicum* L. ('licorice' variety) is a plant commonly known as albahaca or basil that is used in a variety of industries around the world. The variability in the chemical composition of the essential oil derived from this plant, gathered in different growing months, is reported herein. The aerial parts, those parts exposed to the air, of *O. basilicum* L. yielded an average of 0.3% ± 0.25% of the essential oil in the four month period of the evaluation.

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Using GC-MS analysis, 107 components were detected in the essential oil, 102 of them were identified (95.3%) and 15 showed a greater than 1% relative yield. Estragol, at 40.6%, and linalool, at 17.8%, were the most important components of the essential oil. According to the refractive index (1.5230), the essential oil could be classified into the Reunion Type. Based on the relative percentage of the two most important components, estragol and linalool, the essential oil could be classified as European Type 1, and considering the possible chemo type based on the percentage of estragol, the classification corresponded to the BA Type. This is the first time that an evaluation of the changing chemical composition of the essential oil derived from *O. basilicum* over four consecutive months has been published.

**Keywords:** *Ocimum*; basil; essential oils.

## 1. INTRODUCTION

Basil (*Ocimum basilicum*) is a plant widely used around the world for both medicinal purposes as well as in many other industries. During the last 30 years, many investigators have expressed an interest in the plant because of a growing appreciation for natural products. The family *Lamiaceae*, containing all of the species and varieties of *Ocimum* spp, contains many plants that produce essential oils.

*O. basilicum* L. is an annual aromatic plant originally from India, Iran and other regions. The plant is cultivated because of its medical, alimentary and religious uses and was one of the first plants introduced by colonists to the new world. The *Ocimum* genus includes more than 150 species and now has a wide distribution in tropical and subtropical regions [1,2]. The genus includes many aromatic plants that are important for the perfume, cosmetic, food and pharmaceutical industries.

The possible biological applications of *O. basilicum* L. depend in part on the chemical composition of its essential oil. The presence of linalool, geraniol and nerol in the oil make the production of different perfumes and soaps possible, while the presence of linalool and camphor are preferred for the production of phytomedicines [3]. Because of the importance of the specific chemical composition on the overall application of the oil, it is important to evaluate the variability in this chemical composition of different oils obtained from different months of plant growth, which was the incentive for the present investigation.

Herein, the yield and the variation of the chemical composition of the essential oil were evaluated in order to propose a classification for the variety of oil under investigation.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Fresh *Ocimum basilicum* L var. Minimum Material

The aerial parts of the plant (Fig. 1), which are the parts exposed to the air, were collected in Huaraz, Perú in each month of the study. This collection was performed as close as possible to the distillation of the essential oil. A voucher was deposited in the National Botanic Garden of Cuba with the number HFC: 087057.



**Fig. 1.** *Ocimum basilicum* L. var. minimum

### 2.2 Obtaining the Essential Oil

The essential oil was obtained using a hydro-distillation Clevenger machine [4]. The distillation of 250 g of plant material was done using 250 mL of water for two hours each month of the study to yield one batch of oil per month of gathered plant. The yield was calculated using a volume/weight ratio, and the essential oil was kept in the refrigerator until the GC-MS analysis was performed.

### 2.3 Analysis of the Essential Oil Using GC-MS

An Agilent GC 6890N machine equipped with a 5975B inert mass-selective detector and a split-splitless injector in splitless mode was used for the analysis (Agilent, Palo Alto, CA). Separations were made on an HP-5Ms fused-silica capillary column (30 m x 0.25 mm), with a film thickness of 0.25  $\mu\text{m}$  (Agilent, Avondale, PA). The GC oven temperature was kept at 60°C for 2 min and programmed to reach 200°C at a rate of 20°C/min, then to go from 200°C to 300°C at a rate of 8°C/min before holding constant at 300°C for 5 min. The injection and source temperatures were 320°C and 250°C, respectively. The MS interface temperature was 250°C. The electron ionization/mass spectrometry (EI/MS) spectrum was taken at 70 eV with an injection of 0.3  $\mu\text{L}$ . The mass spectrum was continuously acquired from 35 to 800 m/z with 3.12 scans/s in full-scan mode. Peak identification was achieved by computer matching of the mass spectra against commercial libraries (National Institute of Standards and Technology [NIST], 2011 GC/MS). The final identification was performed using the Kovats retention index in comparison with C8-C32 paraffin. Quantitative calibration mix D-2887 (cat 4-8882, Supelco, PA, USA) was used for the quantification.

### 2.4 Analysis of the Essential Oil

The determinations were made according to Miranda [5].

## 3. RESULTS AND DISCUSSION

### 3.1 Obtaining the Essential Oil

During the four month of the evaluation, the overall yield of the essential oil decreased in the last two months, particularly in January, to 50%

of the average of the measurements (0.3%; Table 1).

According to Hiltoren [6], the yield of essential oil from *O. basilicum* L. is around 0.2–1.5%. In the present investigation, the plant evaluated is *O. basilicum* L. var. minimum, and it had an average essential oil yield of 0.3%, similar to the result described by Hiltoren and Tapanes R. [6,7] reported a 1% yield of the essential oil for the Cuban species *O. basilicum* var. Lime, which was higher than the one found for the minimum variety. Khalid Mahmood [8] obtained a 0.22% yield of the essential oil for *O. sanctum* in Pakistan, which is very similar to the percent obtained in this investigation.

### 3.2 Analysis of the Obtained Essential Oil

The essential oil is pale yellow in colour and the refractive index classifications reported in the literature are described in Table 2.

The refractive index of the *O. basilicum* var. minimum essential oil is closer to that reported for the Reunion type of essential oil. Hiltoren [6] reported that Reunion type essential oils contained a lot of estragol and camphor with low quantities of linalool, 1,8-cineol and eugenol in their chemical composition. In the present investigation, the essential oils obtained contained a lot of estragol and linalool, with low quantities of 1,8-cineol, camphor and eugenol. The main component of our oils is estragol (methyl chavicol), so they resemble a Reunion-type essential oil.

### 3.3 Chemical Composition of Oils According to GC-MS

It was possible to identify 102 components from the 107 detected compounds in the oil (Fig. 2).

**Table 1. Yield of the essential oil in volume/weight for each month in the evaluation period**

Month	Date	Weight of plant (g)	Essential oil (vol.)	Yield (%)
October	25-10-16	250	0.8 ml	0.33%
November	17-11-16	250	1 ml	0.416%
December	07-12-16	250	0.7 ml	0.29%
January	18-1-17	250	0.4 ml	0.16%
Average				0.3%

**Table 2. Refractive index according to the literature (Tapanes 1985)**

Parameter	Oil in study	Literature	Literature
		European type	Reunion type
Refractive index	1.5230 $\pm$ 0.002	1,4730 a 1,4900	1,5120 a 1,5190

The GC chromatogram is very complex, with some of the important peaks having retention times of 10.68 min, 12.62 min, 15.26 min, 17.027 min, 19.38 min, 20.32 min and 24.47 min. The component with a retention time 15.26 min was the one of the higher relative percentages in the essential oil.

Estragol, at 40.6%, and linalool, at 17.8%, were the most important components. According to Hiltoren [6], the major components of the essential oils must be around 20–50% of the total yield of the oil, and the results obtained herein

correspond to this statement. Five components of the total oil could not be identified in the present investigation. Tables 3-A to E show the chemical identification by GC-MS.

According to Tables 3-A to E, the components with highest percent of yield are estragol at 40.57% and linalool at 17.82% over the four month period of the investigation, and another 13 components have yields over 1%. Figs. 3 and 4 show the mass spectra of estragol and linalool in comparison with the database used for the identification.

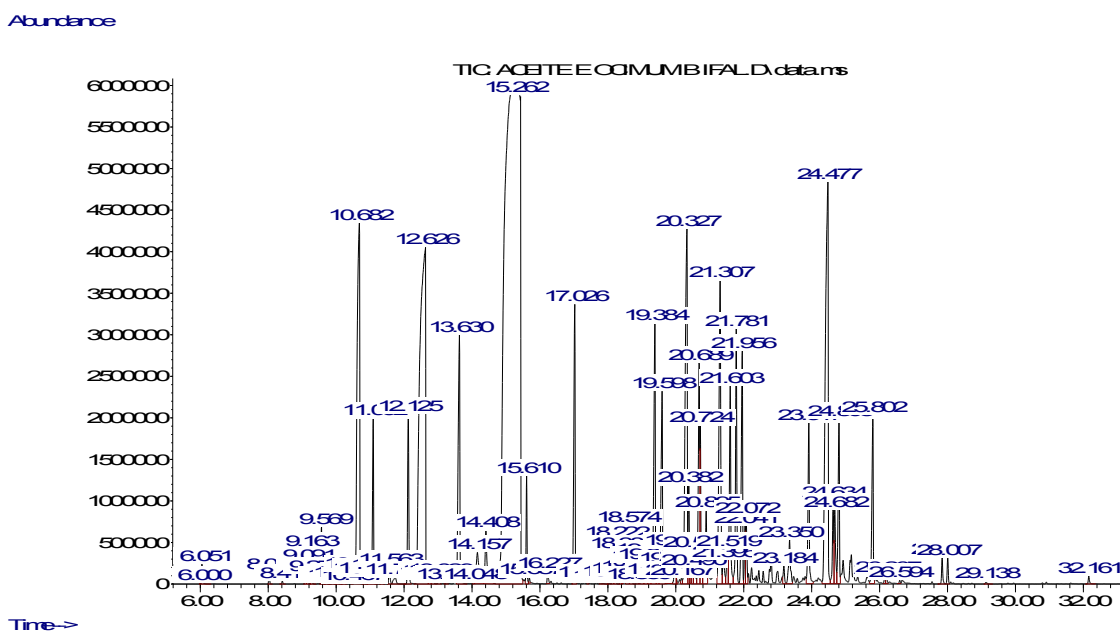


Fig. 2. Chromatogram of *O. basilicum* var. minimum essential oil

Table 3-A. Identification and quantification of the components of the essential oil derived from *O. basilicum* var. minimum

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
1	Ethyl-2-methyl-etanoate	5.981	NOT	0.002	NOT	NOT
2	2-hexenal	6.063	0.005	0.002	0.005	0.012
3	cis-3-hexenol	6.134	0.106	0.002	0.082	0.132
4	Triciclene	7.745	NOT	0.003	NOT	NOT
5	$\alpha$ -tujene	7.896	NOT	0.018	0.010	0.016
6	<b><math>\alpha</math>-pinene</b>	<b>8.067</b>	<b>0.058</b>	<b>1.029</b>	<b>0.176</b>	<b>0.235</b>
7	$\alpha$ -fenchene (H)	8.406	0.001	0.012	0.001	0.002
8	Camphene	8.444	0.016	0.246	0.028	0.040
9	<b>Sabinene</b>	<b>9.141</b>	<b>0.104</b>	<b>0.926</b>	<b>0.140</b>	<b>0.195</b>
10	<b><math>\beta</math>-pinene</b>	<b>9.218</b>	<b>0.179</b>	<b>1.715</b>	<b>0.319</b>	<b>0.419</b>
11	1-octeno-3-ol	9.342	0.061	0.130	0.077	0.122
12	3-octanona	9.495	0.002	0.018	0.018	0.031
13	<b>Mircene</b>	<b>9.622</b>	<b>0.289</b>	<b>1.722</b>	<b>0.286</b>	<b>0.387</b>
14	3-octanol	9.781	0.005	0.011	0.011	0.022
15	$\alpha$ -Felandrene	9.934	0.019	0.120	0.032	0.052

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
16	3-hexenil acetate	10.053	0.005	0.008	0.003	0.004
17	δ-3-carenene	10.092	0.009	0.082	0.010	0.017
18	α-terpinene	10.274	0.023	0.128	0.098	0.123
19	Limonene	10.54	0.020	0.145	0.129	0.050
20	<b>1,8-cineol (eucalyptol)</b>	<b>10.757</b>	<b>4.881</b>	<b>9.947</b>	<b>4.968</b>	<b>5.337</b>
21	cis-ocimene,Z-(B)-	10.846	0.042	0.110	0.015	0.024
22	fenilacetaldehyde	11.016	0.050	0.005	0.008	0.013
23	<b>trans-ocimene-(E)-B</b>	<b>11.147</b>	<b>0.921</b>	<b>2.479</b>	<b>0.354</b>	<b>0.476</b>
24	γ-terpinene	11.393	0.059	0.197	0.179	0.248
25	Cis- sabinenehidrate	11.598	0.113	0.123	0.030	0.040

**Table 3-B. Identification and quantification of the components of the essential oil derived from *O. basilicum* var. minimum**

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
26	n-octanol	11.766	0.101	0.132	0.141	0.184
27	<b>Terpinolene</b>	<b>12.157</b>	<b>1.103</b>	<b>1.353</b>	<b>0.316</b>	<b>0.442</b>
28	Cis-epoxiocimene	12.275	0.003	0.011	0.022	0.029
29	<b>Linalool</b>	<b>12.647</b>	<b>10.056</b>	<b>12.526</b>	<b>24.061</b>	<b>24.668</b>
30	Octenilacetate	12.784	0.029	0.043	0.042	0.077
31	Fenchol	12.888	0.047	0.082	0.047	0.056
32	Cis-p-ment-2-en-1-ol	13.057	0.051	0.036	0.036	0.065
33	1,3,8,-p-mentatriene	13.227	0.006	0.007	0.076	0.171
34	4-acetyl-1-methylciclohexene	13.282	0.013	0.031	0.113	0.004
35	Trans-p-2-menten-1-ol	13.501	0.016	0.009	0.019	0.043
36	Trans-epoxiocimene	13.528	0.016	0.026	0.007	0.02
37	<b>L-canfor</b>	<b>13.640</b>	<b>2.199</b>	<b>1.698</b>	<b>0.469</b>	<b>0.648</b>
38	Pinocarvona	14.079	0.011	0.008	0.002	0.010
39	Borneol +α -terpineol	14.151	0.608	0.524	0.282	0.523
40	Terpinen-4-ol	14.432	0.630	0.333	1.409	1.887
41	<b>Estragol(methylchavicol)</b>	<b>15.279</b>	<b>40.219</b>	<b>44.338</b>	<b>41.644</b>	<b>36.080</b>
42	Fenchilacetate	15.585	0.524	0.432	0.564	0.708
43	Chavicol	16.239	0.086	0.054	0.492	0.566
44	<b>α -bornilacetate</b>	<b>17.030</b>	<b>2.019</b>	<b>1.717</b>	<b>1.204</b>	<b>1.470</b>
45	Cis-Methylcinamate	17.440	0.020	0.006	0.007	0.016
46	Exo-2-hydroxicineol acetate	18.244	0.255	0.039	0.089	0.123
47	α-cubebene	18.406	0.176	0.130	0.087	0.086
48	Eugenol	18.592	0.376	0.304	0.170	0.251
49	NI	18.699	0.077	0.019	0.012	0.031
50	p-Eugenol	18.883	0.015	0.008	0.018	0.008

**Table 3-C. Identification and quantification of the components of the essential oil derived from *O. basilicum* var. minimum**

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
51	α-copaene	19.037	0.126	0.079	0.160	0.220
52	trans-methylcinamate	19.166	0.208	0.160	0.035	0.122
53	β-elemenene or bourbonene	19.221	0.171	0.108	0.026	0.158
54	β-cubebene or germacrene D	19.323	0.063	0.022	0.052	0.121
55	<b>β-elemenene</b>	<b>19.390</b>	<b>1.988</b>	<b>1.276</b>	<b>0.796</b>	<b>1.581</b>
56	α-cubebene	19.469	0.047	0.018	0.033	0.057
57	Cis-jasmonate	19.502	0.023	0.011	0.062	0.041
58	<b>metileugenol</b>	<b>19.596</b>	<b>1.463</b>	<b>0.676</b>	<b>0.384</b>	<b>0.251</b>
59	α -pactchulene	19.813	0.034	0.021	0.003	0.018
60	α -bergamotene	19.850	0.124	0.071	0.083	0.093
61	β-cariofilene	19.995	0.250	0.127	0.180	0.200

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
62	$\alpha$ -amorfene	20.032	0.023	0.012	0.002	0.027
63	$\alpha$ -longipinene	20.137	0.029	0.011	0.021	0.024
64	Germacrene D	20.191	0.038	0.014	0.023	0.040
65	<b><math>\alpha</math>-bergamotene</b>	<b>20.319</b>	<b>3.885</b>	<b>2.315</b>	<b>2.382</b>	<b>2.667</b>
66	$\alpha$ -guaiaene	20.385	0.551	0.403	0.615	0.689
67	Cis-B-farnesene	20.434	0.101	0.041	0.052	0.068
68	Aromadendrene	20.522	0.254	0.044	0.047	0.054
69	$\Gamma$ -cadinene	20.555	0.122	0.134	0.206	0.108
70	<b>Trans-B-farnesene</b>	<b>20.692</b>	<b>1.563</b>	<b>1.489</b>	<b>0.788</b>	<b>1.032</b>
71	$\alpha$ -humulene	20.749	0.738	0.038	0.617	0.596
72	Epi-biciclosesquifellandrene	20.910	0.536	0.249	0.422	0.483
73	<b>Germacrene D</b>	<b>21.301</b>	<b>3.016</b>	<b>1.469</b>	<b>2.783</b>	<b>2.901</b>
74	B-selinene	21.404	0.237	0.095	0.092	0.074
75	Valencene	21.541	0.280	0.103	0.095	0.080

**Table 3-D. Identification and quantification of the components of the essential oil derived from *O. basilicum* var. minimum**

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
76	<b>Biciclogermacrene</b>	<b>21.607</b>	<b>1.467</b>	<b>0.617</b>	<b>1.748</b>	<b>1.823</b>
77	<b><math>\delta</math>-guaiaene</b>	<b>21.781</b>	<b>1.710</b>	<b>0.832</b>	<b>1.575</b>	<b>1.451</b>
78	<b><math>\alpha</math>-amorphene</b>	<b>21.950</b>	<b>1.677</b>	<b>0.721</b>	<b>1.099</b>	<b>1.250</b>
79	Epi- $\alpha$ -selinene	22.044	0.304	0.136	0.055	0.072
80	B-sesquifelandrene	22.093	0.457	0.180	0.350	0.423
81	Trans- $\alpha$ -Bergamotene	22.166	0.055	0.013	0.050	0.033
82	Trans- $\Gamma$ -bisabolene	22.251	0.118	0.037	0.022	0.043
83	Oxaciclodec-10-en-ona	22.344	0.014	0.069	0.024	0.019
84	$\alpha$ -cadinene	22.394	0.034	0.017	0.021	0.012
85	Germacrene B	22.472	0.105	0.027	0.030	0.042
86	Nerolidol	22.832	0.216	0.139	0.273	0.342
87	Lineal alcohol C13-C15	23.018	0.130	0.044	0.012	0.024
88	NI	23.164	0.046	0.018	0.013	0.008
89	Espatulenol	23.208	0.131	0.032	0.062	0.089
90	Cis-Methyl-p-metoxicinamate	23.378	0.421	0.276	0.091	0.120
91	NI	23.927	1.165	0.369	0.634	0.726
92	<b>Epi-biciclosesquifelandrene</b>	<b>24.438</b>	<b>5.897</b>	<b>2.385</b>	<b>3.936</b>	<b>3.845</b>
93	B-eudesmol	24.624	0.549	0.225	0.260	0.309
94	T-muurolol	24.674	0.542	0.228	0.332	0.347
95	<b><math>\gamma</math>-gurjunene</b>	<b>24.795</b>	<b>1.156</b>	<b>0.536</b>	<b>0.233</b>	<b>0.260</b>
96	Trans Methyl-p-metoxicinamate	24.953	0.245	0.184	0.062	0.067
97	Anymol	25.156	0.129	0.050	0.105	0.139
98	$\alpha$ -bisabolol,	25.189	0.187	0.049	0.052	0.079
99	<b>Farnesol</b>	<b>25.809</b>	<b>1.192</b>	<b>0.606</b>	<b>0.018</b>	<b>0.022</b>
100	Cis,trans-farnesal	26.176	0.040	0.023	0.044	0.019

**Table 3-E. Identification and quantification of the components of the essential oil derived from *O. basilicum* var. minimum**

No	Compound	Rt (min)	Oct %	Nov %	Dec %	Jan %
101	NI	26.219	0.051	0.007	0.014	0.008
102	Bencilbenzoate	26.59	0.021	0.004	0.024	0.091
103	Farnesilacetate 3	27.873	0.162	0.058	0.078	0.032
104	NI	28.036	0.156	0.063	0.006	0.087
105	Farnesilacetona C	29.176	0.017	0.004	0.006	0.007
106	Fitol	32.16	0.052	0.060	0.343	0.347
107	Neofitadienol 2,6,10-trimetil,14-etilene-14-pentadecene	32.16			0.030	0.011

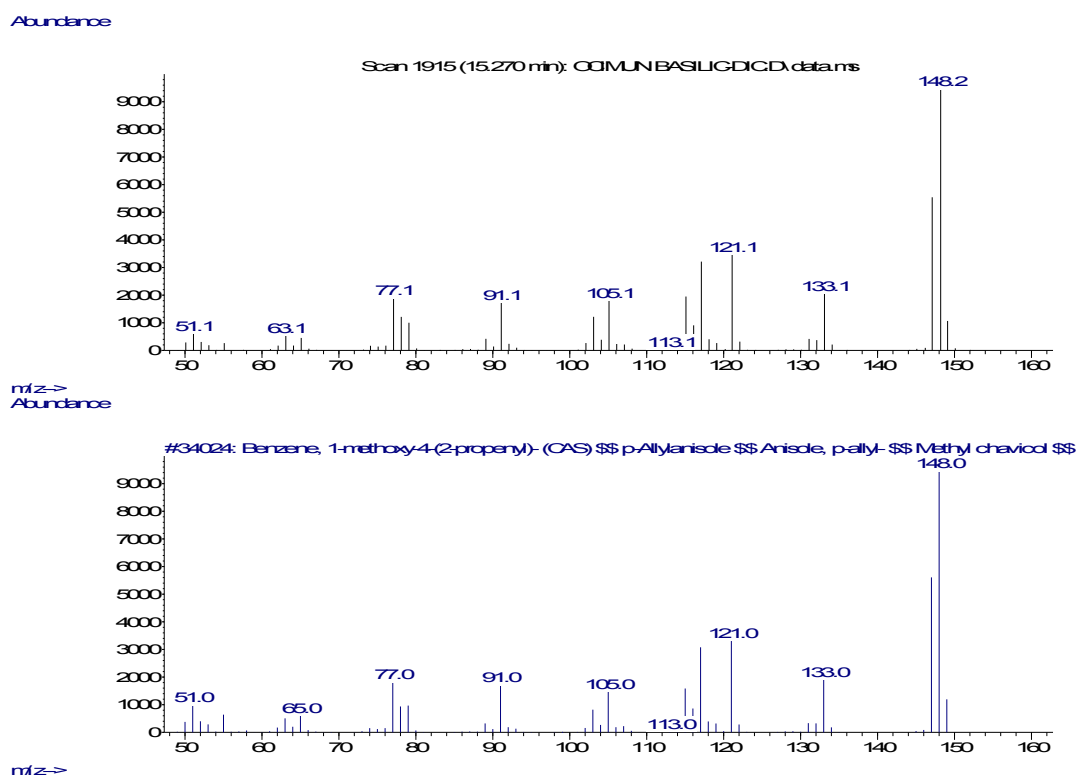


Fig. 3. The mass spectra for estragol (a) as obtained in the study and (b) compared to the XXX database

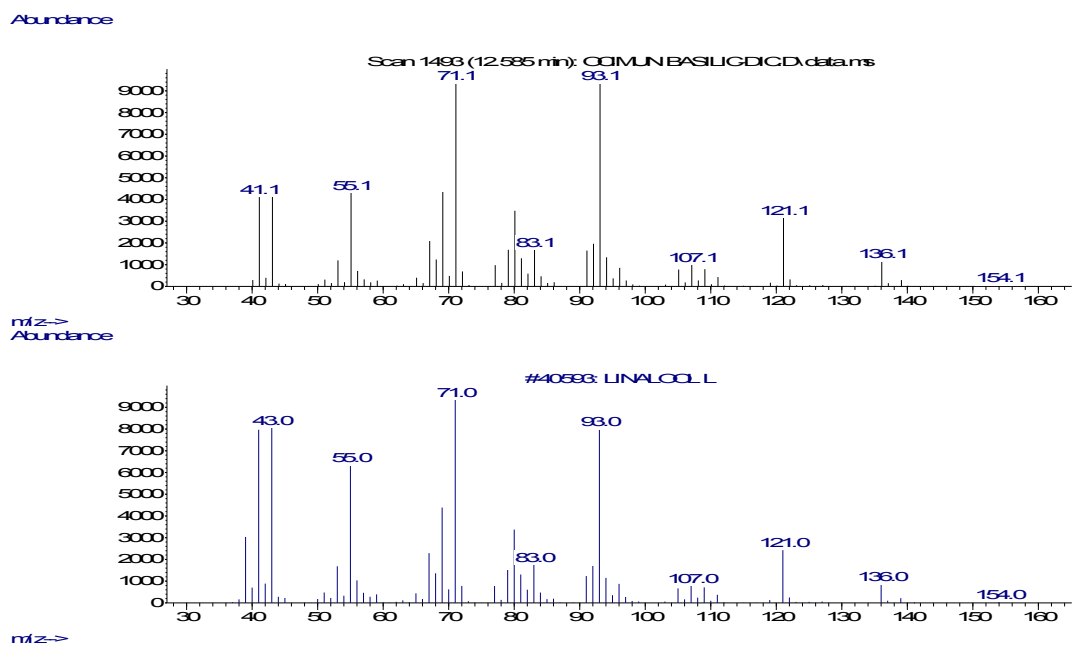


Fig. 4. The mass spectra for linalool (a) as obtained in the study and (b) compared to the XXX database



The results obtained herein are quite different from the literature. Murillo E. et al. [9] identified 25 components in the essential oil from this plant, (methyl cinamate as the main component), Rivas K. et al. [10] identified 14 components (isoestragol as the main component), Saldarriaga L [11] identified 60 components (linalool as the main component), Cardoso Ugarte G.A. [12] reported in a review article, that the main component of the essential oil depends on the region where the plant grew and Telci et al. [13] confirmed this same idea.

Fig. 5 compares the relative composition of the main three components of the essential oil in each of the four months under evaluation. December and January are the best months to obtain highest yields of both estragol (41.6%) and linalool (24.06%), although November produced the highest overall percent of estragol alone (44.3%) even though the yield of linalool was not very high, at only 12.5%. Meanwhile 1,8-cineole was found at lower than 10% yield in every one of the months under evaluation.

Additionally, in Fig. 6 it is possible to compare the average variability in yield of the six main

components of the extract. The graphic shows that the highest components are estragol (40.6%), linalool (17.8%) and 1,8-cineole (6.3%).

### 3.4 Classification of the Essential Oil Derived from *O. basilicum* var. Minimum

According to the chemical composition and the origin of the plant, an essential oil can be classified as: (1) European Type (methyl chavicol), (2) Reunion Type (camphor), (3) Methyl cinamate Type or (4) Eugenol Type Guenther [14]. Hiltoren [5] proposed the following four categories according the chemical composition of the essential oil. Type A is the most common essential oil from basil and contains linalool and methyl chavicol (estragol) as the main components. Cineol and eugenol have to be present, but camphor and methyl cinamate are present in only low quantities. Type B consists of camphor-type oils that have to be rich in camphor with low quantities of  $\alpha$ -pinene, cineol, linalool and methyl chavicol (estragol). Type C oils contain between 15–75% of methyl cinamate, and Type D oils contain between 30–80% of eugenol.

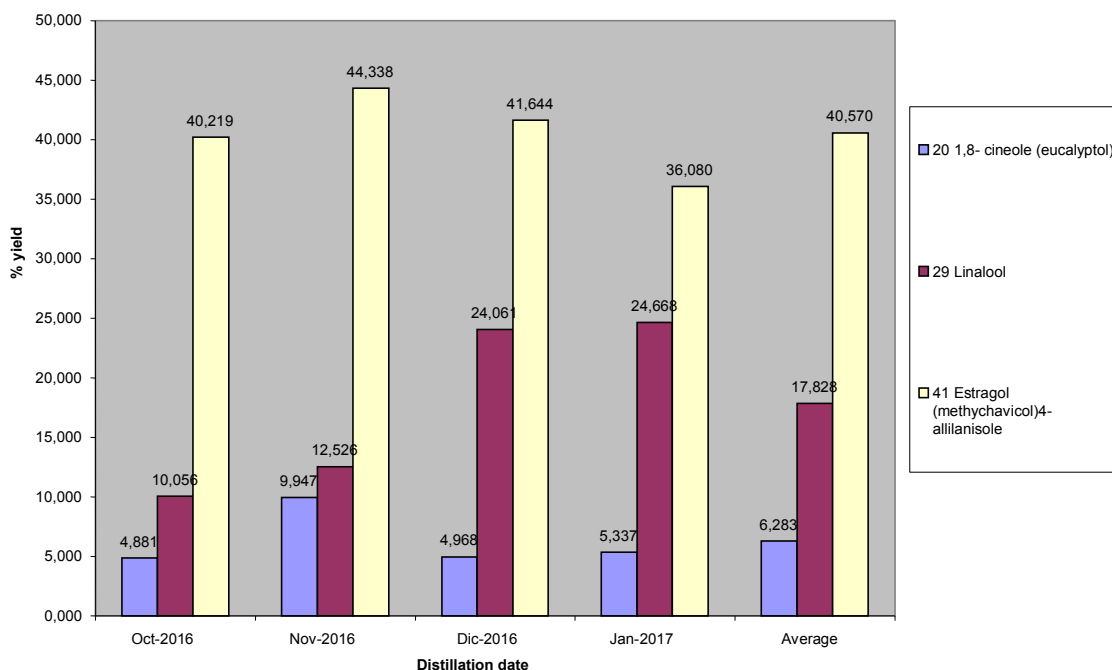
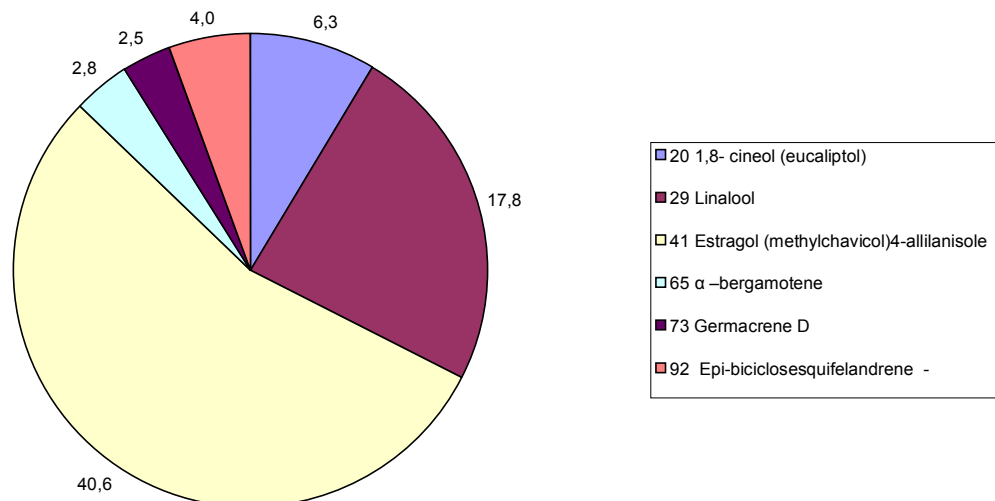


Fig. 5. Comparison of yield between the three main components of the essential oil over time



Average yield variability of the 6 main components



**Fig. 6. Average yield variability of the six main components of the essential oil**

For the essential oil obtained herein, according to the refractive index (1.5230), it could be classified as the Reunion Type. According to the relative percentage of the two most important components, methyl chavicol (estragol; 40.6%) and linalool (17.82%), the essential oil could be classified as European Type I [6] and considering the chemo type, the classification falls under the BA Type according to Sánchez [15].

#### 4. CONCLUSIONS

The essential oil made from the aerial parts of *O. Basilicum* L. var. minimum is obtained in an average yield of  $0.3\% \pm 0.02\%$  over the four months of the evaluation period. In total, 107 components were detected in the essential oil using GC-MS analysis, and 102 of them were identified, with 15 of them present in a greater than 1% yield. The main components of the essential oil are estragol (methyl chavicol), present at 40.6%, and linalool, present at 17.8%.

According to the refractive index (1.5230), the essential oil could be classified as the Reunion Type. According to the relative percent of the two main important components, estragol (40.6%) and linalool (17.82%), the essential oil could be classify as European Type I and considering the

chemo type, the classification falls under the BA Type.

For the first time, the evaluation of the variability in the chemical composition of essential oils made from plants collected over time is reported.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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