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The influence of distillation time and the flowering phenophase on quantity and quality of the essential oil of *Lavandula angustifolia* cv. 'Codreanca'

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Abstract

Lavandula angustifolia is an important species appreciated due to its cosmetically and therapeutically properties. The studied material comes from an ecological crop system situated in the ecological reservation of Legii village. Plants were harvested in 2016 in two different phenophases and were distilled for five different time period. Chromatographic analysis was made in order to achieve the chemical profile of the oil. Important concentrations of volatile compounds in essential lavender oil were found. By distillation of V1 with two hours in addition gave an additional amount of oil compared to V2 and V3. The quantity of oil doubles, from 30 minutes to 60 minutes distillation, while for 90 minutes respectively 120 minute, it increases with less than 20 %.

Key words: *chemical profile, camphor, distillation process, essential oil*

1. Introduction

The *Lavandula* genus comprise 39 species, many hybrids and about 400 varieties (ABDELKADER & al. [1]). These plants of the *Lamiaceae* family have simple, or with 4 planners stems with gland secreting volatile oils. The fragrance of plants is primarily due to the presence of monoterpenoids that are synthesized in aerial parts of plants, mainly in flowers (HASSANPOURAGHDAM & al. [2]). Essential oils obtained from different species of the genus *Lavandula* have been used both cosmetically and therapeutically for centuries with the most commonly used species being *L. angustifolia, L. latifolia, L. stoechas and L. x intermedia* (CAVANAGH & WILKINSON, [3]). Lavender oil is easily extracted from the flower inflorescences using steam distillation. When the distillation period is too short, higher boiling point compounds may be lacking and if it's too long, the smell of the oil may be unpleasant (WESOŁOWSKA & al. [4]). Steam distillation is the most common and inexpensive method to produce essential lavender oil. During this process molecular rearrangement, hydrolysis of double bonds and de-esterification of esters to alcohols and carboxylic acids may occur. Linalool and linalyl acetate are involved in determining the fragrance of the oil and the quantities may differ depending on different distillation times and can influence the odour of the oil (BOWELS, [5]). HERRAIZ-PENÂALVER & al., [6] states that the most appreciated lavender oils for cosmetic industry have a high content of linalyl acetate, linalool and low camphor content, while, hose with a high concentration of camphor are more appreciated for aromatherapy. Lavender oil has sedative, calming, relaxing, antidepressant, anti-inflammatory,

antimicrobial, analgesic, anti-fungal, tonic, antiseptic, healing and regenerating properties, therefore, it is recommended to treat insomnia, agitation, stress, nervous tension, headache, rheumatic pain, muscle cramps, dermatological disorders (acne, dermatitis, psoriasis, scars). (BUCHBAUER & al., 1991 [7]; HANAMANTHAGOUDA & al. 2010, MAHBOUBI AND FEIZABADI 2009, ROTA & al. 2004, M., SOYOLU & al. 2006). *Lavandula angustifolia* oil with a high content of linalool and linalyl acetate and very low camphor content is the most pleasant fragrance, while the oils obtained from other species have a less pleasant aroma due to the higher content of terpene and especially camphor (LYNAM & SMITH [21]). The highest content of linalool (28-30%), linalyl acetate (12-17%) and α-terpineol (7-11%) is contained by *Lavandula angustifolia* Mill. In the ISTIS catalog, since the 1992 homologation in Romania, *Lavandula angustifolia* Mill., "Codreanca", is described as a drought-resistant variety with an oil content of 1.40-1.48%, cultivated on the Legii farm.

Both the chemical composition and the percentage distribution of these components differ depending on several factors including the species or the method of distillation. For example, *Lavandula latifolia* may have a camphor percentage of 13% or even up to 25% (TISSERAND & YOUNG [13]), while *Lavandula angustifolia* shows only traces (according to STAS, 0-0.2%).

Other factors influencing the chemical composition of lavender oil are the pedoclimatic conditions, the phenophase of the material subjected to distillation, the environmental conditions (temperature, humidity), the moment of the day when harvesting (in the morning until 10 o'clock is the most favorable moment, because the plant has the highest concentration of volatile oil), the time elapsed from the harvesting till distillation, the way of preserving the plant material during this time period, but also the process and the technology used for distillation (material from which the plant is made, distillation time, pressure, temperature) (ZAGORCHEVA & al., [14]; ANGIONI & al., [15]; MUÑOZ-BERTOMEU & al., [16]).

The purpose of the experiment was to identify the optimal phenophase for harvesting lavender inflorescences for the distillation of essential oil, given that the different phenophases of lavender inflorescences can cause differences in the aromatic and chemical profile of the essential oil.

2. Materials and Methods

The research field was located in the village Legii, in Cluj County and it is part of the Legii ecological Reservation. The coordinates of this reservation are: 46 ° 51'21 "N 24 ° 02'58" E. The plantation of *Lavandula angustifolia* cultivar 'Codreanca' spreads over 2,8 ha, and has been set up in 2016 in an ecological system. The analyzed plant material comes from the harvest of 2016. Three samples of essential oil were selected: V_1 - essential oil sample from the harvest of June-July 2016. At the harvesting time, the plants achieved full flowering phenophase and distilled for four hours. V_2 sample is obtained from plants reaching full flowering phenophase harvested also in June-July 2016, distilled for 1.5 hours. The third sample V_3 is represented by the essential oil also coming from the June-July 2016 harvest, with the difference that the plants were at the end-of-blossoming phenophase. Both V_2 and V_3 samples are made from lavender essential oil obtained by distilling the plants for 1.5 hours to determine the chemical profile, respectively up to two hours, to determine the yield. In order to identify the importance of lavender plant phenophase and the distillation time period on the chemical composition of the essential oil, V_1, V_2 and V_3 chemical profiles obtained from the chromatographic analysis combined with mass spectrometry and flame ionization detector (GC-FID -MS), were presented in Table 1. These analyses were performed at the Bul National Laboratory in Bulgaria accredited and recognized in the EU. The results were statistically interpreted using variation analysis and Duncan Test.

The Gas Cromatographic analysis has been used also by other autors that have analyzed the oil obtained by steam distillation from Millefolii flos, Salviae herba, Carvi fructus, Foeniculi fructus and Thymi herba (GRIGORE et al., [23]).

3. Results and discussion

Following GC-FID –MS analysis, important concentrations of volatile compounds in essential lavender oil were found. The most important are Linalool which registered 41.44% for V2 and 43.48 for V3, much heigher values then those obtained from dried inflorescences (30,39%) by CARMEN et al., 2016 [22], while, after a 4 hour distilation, the value is smaller (37.7% - V_3).

Table 1: Chromatographic profile of lavender essential oil

No.	**Chemical composition of Lavander oil**	**V_1 full flowering distilled for 4 hours %**	**V_2 full flowering distilled for 1.5 hours %**	**V_3 end-of-blossoming distilled for 1.5 hours %**
1	α-Pinene	0.21	0.18	0.21
2	Camphene	0.25	0.13	0.19
3	β-Pinene	0.16	0.10	0.15
4	B-Myrcene	2.07	1.10	1.08
5	Limonene	1.03	0.58	0.77
6	Cis- β-Ocimene+ β-phellandrene	9.71	7.43	8.18
7	1,8-cineole	1.54	1.06	1.39
8	Trans-β-ocimene+3-octanone	4.74	4.20	3.33
9	1-octen-3-yl-acetate	1.23	1.13	0.91
10	1-octen-3-ol	0.44	0.58	0.67
11	Hexyl butyrate	0.21	0.11	0.22
12	Linalool	37.70	41.44	43.48
13	Camphor	0.21	0.14	0.16
14	Linalyl acetat	12.68	21.48	18.74
15	Terpinen-4-ol	2.03	2.86	2.73
16	Lavandulyl acetat	2.91	2.63	2.53
17	Lavandulol	0.83	1.02	0.93
18	α –Terpineol	3.85	2.19	2.53
19	Borneol	0.89	0.56	0.75
20	β –caryophyllene	3.90	3.36	3.21

Linalyl acetat was found in 21.48% (V2), 18.74% (V3) respectively 12.68 % for V_1, being a lower concentration compared to the results of the same authors (23.60%). The same analysis showed concentrations of camphor 0.14% (V2), 0.16% (V3), 0.21 (V_1) borneol (0.56% - V_2, 0.75% - V_3 respectively 0.89% - M_1). Similar concentrations of linalool were registered (39-43%), camphor 5.9-14.3% by KARA & BAYDAR, [17] and linalyl acetate (18.2%) SAEIDNIA & al., [18], K., SEIDLER-KÀOĪYKOWSKA & al, [19]. Positive correlations were found between linalool and β-pinene, geraniol and camphor and β-pinene, camphor and β-pinene. At the same time, cymene-o correlated with linalool, geraniol and β-pinene showed negative results. Also, regarding the cultivation system, geraniol, camphor and cymene-o registered higher concentrations in organic crop while linalool and β-pinene registered higher values in an conventional crop (SEIDLER-KÀOĪYKOWSKA & al., [19]).

In figure 1 are presented the quantities obtained by distillation process for each variant (V1: full flowering phenophase distilled for 4 hours; V2: full flowering phenophase, distilled

for 1.5 hours; V3: end-of-blossoming phenophase distilled for 1.5 hours). As the chart shows, the higher quantity of essential oil was obtained from V1 for all the time period of distillation. But, the total amount of oil was obtained only after 4 hours of distillation. In the case of the other two variants, V2 and V3, even the quantity of oil vas smaller for 30 minutes, 60 minutes or 90 minutes, at the end of the 120 minutes period, the distillation process ended. At this point were obtained 1620 ml essential oil from V2 and 1580 ml essential oil for V3. The quantity of oil get's double, from 30 minutes to 60 minutes distillation, while for 90 minutes respectively 120 minute, it increases with 20% respectively approximate 13-15%.

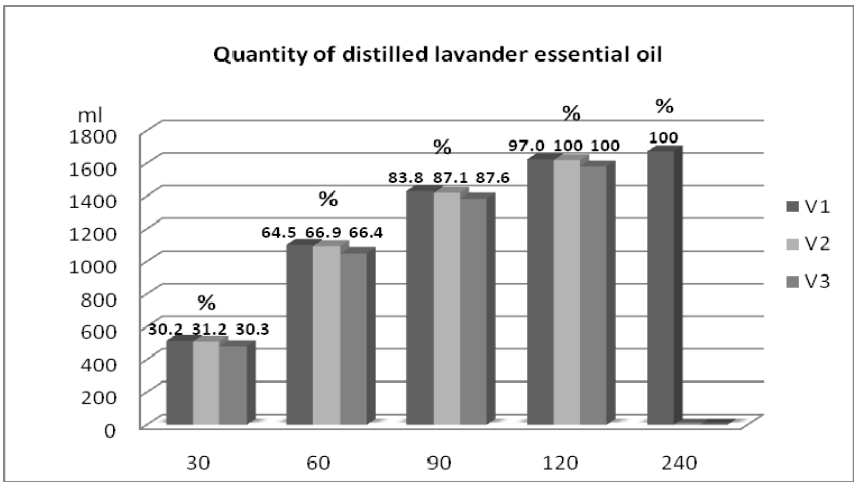

Figure 1. The amount of essential oil obtained during a determined distillation period

Analyzing the data in Table 2, which follows the effect of interaction between the sample and the distillation period of the essential oil can be observed the following: in the case of Variant 3 (V3), regardless the period of the time of distillation, provides an very significantly negative differences totaling 1576 ml essential oil. At the same time, V1 shows an increase in the amount of oil (very significant and distinct significant differences) regardless the control of the experience to which reference is made.

Table 2: The effect of interaction between the variant and the distillation period of the essential oil

Sample	Distillation period (minutes)	Quantity of essential oil			Significance of differences	Quantity of essential oil		Significance of differences
		Absolute value	Relative value %	Differences Ct_1		Relative value %	Difference Ct_2	
V_1	30	514.9	101.1	5.45	**	102.8	14.13	***
V_3		478.0	93.8	-31.50	ooo	101.7	8.08	**
V_2 (Ct_1)		509.5	100.0	-	Ct	95.4	-22.82	ooo
V_1	60	1099.0	100.7	8.0	**	101.8	19.17	***
V_3		1049.0	96.2	-41.5	ooo	101.0	11.17	***
V_2 (Ct_1)		1091.0	100.0	-	Ct	97.2	-30.33	ooo
V_1	90	1429.0	100.7	10.5	***	11.3	18.33	***
V_3		1384.5	97.6	34.0	ooo	100.6	7.83	**
V_2 (Ct_1)		1418.5	100.0	-	Ct	98.1	-26.17	ooo
V_1	120	1624.0	99.7	-4.5	o	100.9	14.33	***
V_3		1576.5	96.8	-52.0	ooo	101.2	18.83	***
V_2 (Ct_1)		1628.5	100.0	-	Ct	97.9	33.17	ooo
V_1	240	1674.5	1674.5	1674.4	***	300.0	1116.2	***
V_3		0.1	100.0	0.0	-	0.0	-558.13	ooo
V_2 (Ct_1)		0.1	100.0	-	Ct	0.0	-558.13	ooo
Average of the experience Ct_2		**103.84**						

LDS 5% 4.70
LDS 1% 6.38
LDS 0.1% 8.55

If the average of the experience is considered a control variant, V2 recorded negative differences for all the distillation period times applied in the experience.

Following the reciprocal action between the extraction time and the variant used on the oil yield, shown in Table 3, can be observed that, regardless the variant used, the best yield was obtained on the sample one (V1) and the extraction time which recorded the best values statistically ensured are after 1.5 hours up to 4 hours, with very significantly positive differences from the average of experience, considered to be the control variant. For variants two (V2) and three (V3), the best yield, regardless of the control to which reference is made, is given by distillation during 1 hour to 2 hours, reaching very significant positive differences.

Table 3: The effect of interaction between the extraction period and the variant on the yielding of the essential oil of lavender

Distillation period (minutes)	Sample	Yielding of the essential oil			Significance of differences	Yielding of the essential oil		Significance of differences
		Absolute value	Relative value %	Differences (Ct_1)		Relative value %	Difference Ct_2	
30	V_1	30.19	40.2	-44.89	ooo	40.2	-44.89	ooo
60		64.47	85.9	-10.61	ooo	85.9	-10.61	ooo
90		83.69	100.0	-	Ct	111.5	8.61	***
120		97.01	129.2	21.93	***	129.2	21.93	***
240		100.05	133.3	24.97	***	133.3	24.97	***
30	V_2 (Ct_1)	31.94	54.8	-25.68	ooo	54.8	-25.68	ooo
60		66.45	116.8	9.58	***	116.8	9.58	***
90		87.11	100.0	-	Ct	153.2	30.24	***
120		99.5	175.0	42.63	***	175.0	42.63	***
240		0.1	0.2	-56.77	ooo	0.2	-56.77	ooo
30	V_3	30.35	54.0	-25.84	ooo	54.0	-25.84	ooo
60		66.35	118.1	10.16	***	118.1	10.16	***
90		87.65	100.0	-	Ct	156.0	31.46	***
120		96.5	171.7	40.31	***	171.7	40.31	***
240		0.1	0.2	-56.09	ooo	0.2	-56.09	ooo
Average of the experience Ct_2		**62.712**						

LDS 5% 2.81
LDS 1% 3.95
LDS 0.1% 5.58

After analyzing the amount of essential oil obtained during the determined distillation periods, respectively the yield (Table 3), it can therefore be ascertained that the distillation of V1 with two hours in addition gave an additional amount of oil compared to V2 and V3.

Table 4. Synthesis of results on the amount of linalool based on phenophases

Variant	Phenophases	Chemical compound	Statistical Significance
		Content of linalool %	
V_2	full flowering distilled for 1.5 hours	41,44	B
V_3	end-of-blossoming distilled for 1.5 hours	43,52	A
Content of camphor%			
V_2	full flowering distilled for 1.5 hours	0,14	A
V_3	end-of-blossoming distilled for 1.5 hours	0,16	A
Content of linalyl acetate %			
V_2	full flowering distilled for 1.5 hours	21,49	A
V_3	end-of-blossoming distilled for 1.5 hours	18,85	B

Although the prolongation of the distillation time up to four hours translates into a superior yield of lavender essential oil, the best values statistically ensured are after 1.5 to four hours. Depending on the flowering phenophase, the linalool content varies between variants V2 and V3 and by comparing the quantities of essential oil obtained, the values reached are statistically ensured and processed reaching an increase of 1.04% (Table 4).

Regarding the content of camphor, when comparing the results obtained, can be noticed that even the difference between the two variants is very little, they are statistically ensured and the values are smaller than the maximum quantity included in STAS.

In Table 5, the influence of the flowering phenophase on the linalyl acetate content can be observed. Using the Duncan test to compare the significance of 97% -DS 0.32% between the two samples, the V2 and V3 values are significant, the V2 sample (the full blossoming phenophase) being the most valuable.

4. Conclusion

By comparing the chemical profiles of V2 and V3 by applying the Duncan test, may find that the content of camphor does not provide statistically ensured differences regaring the influence of flowering phenophases.

Although the end-of-blossoming (V3) provides a greater amount of linalool compared to the full blossom, makes it detrimental to linalyl acetate.

It can therefore be concluded that the flowering phenophases influence its chemical profile of the essential oil, in the case of linalool and linalyl acetate content reaching statistically ensured differences. The analysis thus confirms, at least in part, the literature (M., JORDAN & al., [20]).

The distillation time and blossoming period of lavander plants affects both the quantity of the essential oil and also the chemical composition of the essential oil in different proportions without omitting the influence they can exert from these points view, and other factors such as particular climate conditions, selected lavender variety, ambient temperature, air humidity and distillation temperature, etc.

From the perspective of the amount of essential oil obtained, it can be seen that distillation for 1.5 to 4 hours produces an important quantitative increase, however, from the chemical and oil quality point of view, the prolongation of the distillation time is not necessary desirable.

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