Influence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.)

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S u m m a r y

The influence of the time of distillation on the content and composition of the essential oil isolated from lavender (*Lavandula angustifolia* Mill.) by steam distillation was investigated. The maximum essential oil percentage (2%) was obtained after 2 hours of distillation, while the minimum essential oil percentage (1%) was obtained after 40 minutes of distillation. The chemical composition of the isolated oil was determined by GC-MS. Linalool (28.78–30.68%), linalyl acetate (12.35–17.67%) and α-terpineol (7.57–11.49%) were the major components of the analysed oil.

Key words: *lavender, steam distillation, essential oil, linalool, linalyl acetate, α-terpineol, GC, MS*

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill.) is a strongly aromatic shrub with pale green, narrow, linear leaves and violet-blue flowers. Native to the low mountains of the Mediterranean region, now is cultivated all over the world for its essential oil [1-2].

The herb has been popular for centuries as spasmolytic, carminative, stomachic and diuretic. Its powerful antiseptic properties are able to kill many frequent bacteria such as typhoid, diphtheria, streptococcus and pneumococcus [3]. The fragrant leaves and flowers are used fresh in salads and dishes, dried are used as a tea, for calming baths and as an insect repellent in linen cupboards [4]. Lavender yields a highly effective essential oil that is widely used in soaps making, high-quality perfumes, creams and other cosmetics. It is used in food industry as flavouring agent in lavender jelly, cookies, ice cream, honey and chewing gum [5]. Lavender oil has been also reported as antifungal agent against *Aspergillus nidulans* and *Trichophyton mentagrophytes* [6-7].

Lavender oil is easily extracted from the flower heads using steam distillation. If the time of distillation is too short, higher boiling point compounds may be lacking. When the time of distillation is too long, the oil may have unpleasant smell. According to Pitman [8], nearly 75% of the total oil yield comes in the first 25 minutes of distillation, to give a commercial grade lavender oil. Obtaining of the other molecules, like coumarins, take another 50–80 minutes of distillation.

Steam distillation is a simple method of universal application and the inexpensive equipment [9]. Unfortunately, during this process molecular rearrangements, hydrolysis of double bonds and de-esterification of esters to alcohols and carboxylic acids may occurs. In case of lavender oil, the levels of linalool and linalyl acetate are key determinants of the overall fragrance of the oil. Linalool is sweeter, but the odour of its ester, linalyl acetate is more refreshing. The linalool to linalyl acetate ratio may change in different distillation times and may affect the final odour of the oil [10].

According to literature data [11-12], in high quality lavender oil, the ratio of linalyl acetate to linalool should be higher than one.

Commercially produced lavender oil is often distilled for only 15 minutes at a very high temperature and under very high pressure. Although the oil is easily sold, it is of very poor quality. In order to make it smell like the genuine plant fragrance, synthetic linalyl acetate is often added [13].

The aim of this study was to evaluate the influence of steam distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.), cultivated in the Department of Vegetable Crops, West Pomeranian University of Technology, Szczecin, Poland.

MATERIAL AND METHODS

Chemicals

Dichloromethane (pure p.a.) was purchased from Chempur and used as received.

Plant

The research material was produced at the Horticultural Experiment Station (Dołuje, near Szczecin), which belongs to the Department of Vegetable Crops of the West Pomeranian University of Technology, Szczecin.

Lavender flowers (*Lavandula angustifolia* Mill.) were collected in full bloom, in July 2009, dried at 30°C and stored in paper bags. At the time of harvest, plants were 3 years of age.

Isolation of the essential oil

The essential oil of *Lavandula angustifolia* Mill., cultivated at the Horticultural Experiment Station of West Pomeranian University of Technology, was obtained by steam distillation.

Three portions of dried lavender flowers, each weighting 5 g, were weighted and stored in a dark place.

One portion of lavender flowers was placed in round bottom flask containing 500 ml of water and distilled for:

a) experiment 1–40 minutes;

b) experiment 2–1 hour;

c) experiment 3–2 hours.

Steam was generated separately in a steam boiler and was passed through the distillation flask. The distillate was saturated with NaCl and transferred to a separator funnel where was extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Essential oil yields were 0.05 g; 1% (a), 0.09 g; 1.8% (b), 0.1 g; 2% (c) on the dry weight basis, respectively. Two repetitions of distillation were done.

GC-MS analysis

Gas chromatography-mass spectrometry analysis (GC-MS) was performed using an HP 6890 gas chromatograph coupled with HP 5973 Network Mass Selective Detector operating at 70eV electron impact mode. Compounds were separated on 30 m long capillary column (HP-5MS), 0.25 mm in diameter (methylsiloxane modified with phenyl groups) in the 0.25 μm active phase layer.

The GC oven temperature was programmed from 45°C to 200°C (kept constant for 10 minutes) at a rate of 5°C/min and next to 250°C (kept constant for 20 minutes) at a rate of 5°C/min.

Flow rate of helium was 2.0 mL/min at 2.6 psi. The volume of sample injected was 4 μL and split injection was used (split ratio: 5.4:1).

The injector temperature was 250°C, the detector temperature was 280°C, the ion source temperature was 230°C , the solvent delay was 3 minutes and the mass scan range was *m/z* 29 – 800.

The oil samples were injected in dichloromethane.

Compound identification

Components of the essential oil were identified using an automated library search that compares unknowns with a library of standard spectra such as Wiley NBS75K.L and NIST/EPA/NIH Mass Spectral Library (2002 version).

Statistical analysis

Several results of the study (tab. 4) were subjected to an analysis of variance which was performed with Program AWAR (Department of Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy). Means were separated by the Tukey's test at $p=0.05$.

RESULTS AND DISCUSION

According to cited literature [8, 10-13] essential oils must be distilled in proper time to release all their active constituents. Distillation can determine the value of the oil, or destroy the value of the oil.

Results showed that the time of steam distillation of lavender flowers had a significant effect on the essential oil content and composition.

The minimum essential oil percentage (1%) was obtained after 40 minutes of distillation. The results of GC-MS analysis are given in table 1.

Table 1.

Composition of the steam distilled (40 min) essential oil of *Lavandula angustifolia* Mill.

peak	Rt (min)	component	area %
1	11.30	1-octen-3-ol	0.55
2	11.55	3-octanone	0.08
3	12.97	eucalyptol (1,8-cineole)	0.38
4	13.18	trans-β-ocimene	0.10

5	13.27	4-methyl-4-vinylbutyrolactone	0.27
6	13.51	cis-β-ocimene	0.06
7	14.36	cis-linalool oxide (furan type)	5.45
8	14.86	trans-linalool oxide (furan type)	5.12
9	**15.40**	linalool	29.92
10	15.56	1-octenyl acetate	1.35
11	16.27	trans-p-mentha-2,8-dien-1-ol	0.09
12	16.42	trans-verbenol	0.83
13	16.59	camphor	0.63
14	16.82	nerol oxide	0.18
15	17.12	pinocarvone	0.29
16	17.24	borneol	3.66
17	17.33	epoxylinalool	0.52
18	17.61	1-terpinen-4-ol	4.00
19	17.86	cryptone	2.98
20	**18.06**	**α-terpineol**	**11.49**
21	18.16	myrtenal	0.74
22	18.40	2,6-dimethyl-3,5,7-octatriene-2-ol	0.73
23	18.50	chrysanthenone	0.39
24	18.75	trans-carveol	0.53
25	19.01	cis-geraniol	2.42
26	19.37	cumic aldehyde	0.64
27	19.47	(+)-carvone	0.40
28	**19.82**	**linalyl acetate**	**12.35**
29	20.70	lavandulyl acetate	2.39
30	21.02	2,3,4,6-tetramethylphenol	0.16
31	21.37	eucarvone	0.19
32	22.66	nerol acetate	0.98
33	22.92	2,6-dimethyl-1,7-octadiene-3,6-diol	0.16
34	23.17	geraniol acetate	1.85
35	24.21	α-santalene	0.24
36	24.27	caryophyllene	0.23
37	24.60	bergamotene	0.04
38	25.04	(Z)-β-farnesene	0.06
39	25.81	β-cubebene	0.17
40	26.59	γ-cadinene	0.07
42	28.30	caryophyllene oxide	1.40
43	28.90	bisabolene epoxide	0.06

44	28.98	cadinadiene-1,4	0.06
45	29.56	(+)-epi-bicyclosesquiphellandrene	0.46
46	29.94	α-selinene	0.12
identified			94.79
unidentified			5.21

A total of 46 compounds representing 94.79% of the oil were identified. Linalool was the main constituent of volatile oil (29.92%), followed by linalyl acetate (12.35%) and α-terpineol (11.49%).

The higher content of α-terpineol as well as 1-terpinen-4-ol (4.00%) and low content of linalyl acetate, may be explained by molecular rearrangement and hydrolysis, which may occur during distillation.

Geraniol and various terpineol isomers can by produced from the linalyl acetate during the process of distillation and may change the smell of the lavender oil into geranium or tea-tree oil [10].

The oil content after 1 hour of distillation was found to be larger (1.8%). Forty-seven components representing 97.44% of the oil were identified (tab. 2).

Table 2.

Composition of the steam distilled (1h) essential oil of *Lavandula angustifolia* Mill.

peak	Rt (min)	component	area %
1	5.45	3-methyl-2-butenal	0.07
2	10.46	5,5-dimethyl-2(5H)-furanone	0.06
3	11.31	1-octen-3-ol	0.67
4	11.54	3-octanone	0.24
5	11.69	β-myrcene	0.38
6	12.76	o-cymene	0.13
7	12.90	limonene	0.22
8	12.98	eucalyptol (1,8-cineole)	0.99
9	13.18	trans-β-ocimene	0.98
10	13.27	4-methyl-4-vinylbutyrolactone	0.32
11	13.51	cis-β-ocimene	0.51
12	14.34	cis-linalool oxide (furan type)	5.35
13	14.84	trans-linalool oxide (furan type)	4.87
14	**15.33**	**linalool**	**30.68**
15	15.54	1-octenyl acetate	2.97
16	15.85	2-cyclohexen-1-ol	0.44
17	16.10	1,3,8-p-menthatriene	0.50
18	16.39	trans-verbenol	0.61

19	16.57	camphor	0.51
20	16.82	nerol oxide	0.18
21	17.11	pinocarvone	0.27
22	17.22	borneol	2.42
23	17.43	epoxylinalool	0.63
24	17.56	1-terpinen-4-ol	2.66
25	17.68	p-cymen-8-ol	0.30
26	17.83	cryptone	2.07
27	**17.99**	**α-terpineol**	**7.57**
28	18.13	myrtenal	0.23
29	18.48	chrysanthenone	0.28
30	18.76	trans-carveol	0.20
31	19.02	cis-geraniol	0.51
32	19.35	cumic aldehyde	0.52
33	19.46	(+)-carvone	0.28
34	**19.82**	**linalyl acetate**	**17.67**
35	20.71	lavandulyl acetate	3.23
36	22.67	nerol acetate	1.38
37	23.17	geraniol acetate	2.44
38	24.22	α-santalene	0.63
39	24.28	caryophyllene	0.68
40	25.04	β-farnesene	0.15
41	25.82	β-cubebene	0.24
42	26.59	γ-cadinene	0.13
43	28.30	caryophyllene oxide	1.40
44	28.99	α-cubebene	0.06
45	29.56	(+)-epi-bicyclosesquiphellandrene	0.62
46	29.78	ledol	0.07
47	30.54	α-farnesene	0.12
identified			97.44
unidentified			2.56

The major constituents (>2.0%) of the oil were linalool (30.68%), linalyl acetate (17.67%), α-terpineol (7.57%), cis-linalool oxide (5.35%), trans-linalool oxide (4.87%), lavandulyl acetate (3.23%), 1-octenyl acetate (2.97%), 1-terpinen-4-ol (2.66%), borneol (2.42%), geraniol acetate (2.44%) and cryptone (2.07%). Limonene (0.22%) was detected only in this oil.

The higher yield (2%) of the lavender oil was obtained after 2 hours of distillation.

In table 3 there listed the identified constituents of the isolated oil.

Table 3.

Composition of the steam distilled (2h) essential oil of *Lavandula angustifolia* Mill.

peak	Rt (min)	component	area %
1	10.44	5,5-dimethyl-2(5H)-furanone	0.15
2	11.31	1-octen-3-ol	0.74
3	11.54	3-octanone	0.12
4	12.98	eucalyptol (1,8-cineole)	0.57
5	13.18	trans-β-ocimene	0.12
6	13.29	4-methyl-4-vinylbutyrolactone	0.84
7	14.37	cis-linalool oxide (furan type)	5.78
8	14.86	trans-linalool oxide (furan type)	5.29
9	**15.40**	**linalool**	**28.78**
10	15.56	1-octenyl acetate	1.32
11	16.43	trans-verbenol	0.87
12	16.59	camphor	0.59
13	16.82	nerol oxide	0.30
14	17.12	pinocarvone	0.32
15	17.24	borneol	3.37
16	17.48	epoxylinalool	1.21
17	17.59	1-terpinen-4-ol	2.84
18	17.73	p-cymen-8-ol	0.47
19	17.86	cryptone	2.69
20	**18.04**	**α-terpineol**	**7.77**
21	18.16	myrtenal	0.70
22	18.41	2,6-dimethyl-3,5,7-octatriene-2-ol	0.58
23	18.50	chrysanthenone	0.32
24	18.75	trans-carveol	0.48
25	19.00	cis-geraniol	1.82
26	19.36	cumic aldehyde	0.82
27	19.47	(+)-carvone	0.33
28	**19.83**	**linalyl acetate**	**12.76**
29	20.71	lavandulyl acetate	2.76
30	21.38	eucarvone	0.21
31	22.67	nerol acetate	0.95
32	23.18	geraniol acetate	1.69
33	24.22	α-santalene	0.24
34	24.28	caryophyllene	0.28

35	24.60	cis,trans-α-farnesene	0.09
36	24.68	coumarin	0.15
37	25.83	β-cubebene	0.16
38	25.89	hydroxy-α-terpenyl acetate	0.14
39	26.59	γ-cadinene	0.11
40	28.32	caryophyllene oxide	1.85
41	28.91	bisabolene epoxide	0.12
42	29.56	(+)-epi-bicyclosesquiphellandrene	0.72
43	30.55	α-farnesene	0.40
identified			91.92
unidentified			8.18

The results of GC-MS analysis revealed 43 components representing 91.92% of total oil. Linalool was predominated component (28.78%). Six ester components (linalyl acetate, lavandulyl acetate, geraniol acetate, 1-octenyl acetate, nerol acetate and hydroxy-α-terpenyl acetate) were identified (12.76, 2.76, 1.69, 1.32, 0.95 and 0.14%, respectively).

Only two monoterpene hydrocarbons (trans-β-ocimene and camphor) were identified.

In the fraction of sesquiterpenes, three were identified as caryophyllene oxide (1.85%), caryophyllene (0.28%) and α-santalene (0.24%).

Monoterpene ketones: cryptone (2.69%), pinocarvone (0.32%), coumarin (0.15%), 3-octanone (0.12%), as well as monoterpene aldehyde (cumic aldehyde – 0.82%) and monoterpene ether (eucalyptol – 0.57%) were also found in the oil.

In general, we were able to identify 46 components in the oil obtained after 40 minutes of distillation, 47 components in the oil obtained after 1 hour of distillation and 43 components in the oil obtained after 2 hours of distillation.

The medicinal and olfactory properties of *Lavandulae aetheroleum* are mainly attributed to the proportional composition of linalool, linalyl acetate, eucalyptol (1,8-cineole), β-ocimene, 1-terpinen-4-ol and camphor [14-15].

The best quality oil used in perfumery contains high amounts of linalool and linalyl acetate and trace amounts of camphor. The quality of medicinally-utilized oil is determined by the proportion of monoterpenes with desired biological activity [16].

The lavender oil obtained after 40 minutes of steam distillation is probably good for medicinal purposes due to high content of camphor (0.63%), α-terpineol (11.49%) and 1-terpinen-4-ol (monoterpenes with antibacterial properties), as well as linalool (29.92%) and linalyl acetate (12.35%) which have sedative and local anaesthetic effects [17-19].

The good quality oil with low content of camphor (0.51%) and high percentages of linalool (30.68%) and linalyl acetate (17.67%) which may be suitable for perfumery was obtained after 1 hour of steam distillation.

The essential oil obtained after 2 hours of steam distillation is good enough to

be used in medicine, due to the presence of antimicrobial and antibacterial agents such as caryophyllene oxide (1.85%), α-terpineol (7.77%), 1-terpinen-4-ol (2.84%) and eucalyptol (0.57%), as well as linalool (28.78%), which also has antibacterial, antifungal, and insecticidal activity [20-21].

The coumarin (0.15%), which was detected only in this oil, has anti-inflammatory, antifungal and antiviral activity [22-25].

Linalool oxide (a mixture of the cis and trans forms), which was detected in the all oil samples, is used in perfumery (for lavender notes) and for reconstitution of essential oils [26-27].

All the studied oil samples contained less linalyl acetate than the range of 25–45%; less trans-β-ocimene than the range of between 2 and 6% and less cis-β-ocimene than the range of between 4 and 10% called for by ISO Standard 3515 [28]. They also contained higher level of α-terpineol (7.57–11.49%), than called for in the specification (0–1%).

In the lavender oil obtained after 2 hours of steam distillation, cis-β-ocimene was not detected.

Part of the results of the present study were imported into the statistical analysis to prove the significance of the effect of distillation time on the content of the main constituents of the essential oil of *Lavandula angustifolia* Mill. (tab. 4).

Table 4.

Statistical analysis of the content of some constituents of essential oil of *Lavandula angustifolia* Mill. according to distillation time

essential oil constituent (*factor I*)	distillation time (*factor II*)			
	40 minutes	1 hour	2 hours	Mean
linalool	29.92	**30.68**	28.78	29.79
linalyl acetate	12.35	**17.67**	12.76	14.26
lavandulyl acetate	2.39	**3.23**	2.76	2.79
1-terpinen-4-ol	**4.00**	2.66	2.84	3.17
α-terpineol	**11.49**	7.57	7.77	8.94
camphor	0.63	0.51	0.59	0.58
caryophyllene	0.23	**0.68**	0.28	0.40
caryophyllene oxide	1.40	1.40	**1.85**	1.55
eucalyptol (1,8-cineole)	0.38	**0.99**	0.57	0.65
3-octanone	0.08	0.24	0.12	0.15
trans-ß-ocimene	0.10	**0.98**	0.12	0.40
cis-ß-ocimene	0.06	**0.51**	– *	0.19
Mean	5.25	**5.59**	4.87	5.24
$LSD_{\alpha=0.05}$ for factor I		0.296		
$LSD_{\alpha=0.05}$ for factor II		0.104		
$LSD_{\alpha=0.05}$ for interaction I×II		0.360		

* – not found

On the basis of the obtained data it was proved that there were significant differences between the content of the main constituents of the essential oil. Significantly the highest content of the analysed constituents was noted for linalool (29.27%), while significantly lower for caryophyllene (0.40%), trans-β-ocimene (0.40%), cis-β-ocimene (0.19%) and 3-octanone (0.15%), between which there were no significant differences found. There were also no significant differences detected between the content of caryophyllene, trans-β-ocimene, and eucalyptol (0.65%) and camphor (0.58%). The differences between the content of the constituents of the highest participation in lavender oil (linalool, linalyl acetate, α-terpineol, 1-terpinen-4-ol, lavandulyl acetate, caryophyllene oxide), were statistically significant.

It was proved that the time of distillation had a significant influence on the content of mentioned above lavender oil constituents. The highest amount of these compounds was determined after 1 hour of distillation, lower – after 40 minutes and the least – after 2 hours of distillation. Moreover, comparing the three times of distillation it was found that significantly the highest content of the most of statistically analysed constituents (except 1-terpinen-4-ol, α-terpineol and caryophyllene oxide) was noted after 1 hour. In case of such constituents as camphor and 3-octanone, time of distillation had no significant effect on their content in lavender oil.

CONCLUSIONS

1. The largest yield of the lavender oil (2%) was obtained after 2 hours of steam distillation.
2. The highest concentration of linalool (30.68%) as well as linalyl acetate (17.67%) were found after 1 hour of distillation.
3. The highest amount of α-terpineol (11.49%) was found after 40 minutes of distillation.
4. The lowest concentration of linalyl acetate (12.35%) was observed after 40 minutes of distillation.
5. The coumarin (0.15%) was found only in oil obtained after 2 hours of distillation.
6. Statistical analysis of the obtained results showed significant differences between the content of the main constituents of the lavender oil. Moreover, it was proved that the distillation time had a significant effect on the amounts of these constituents.

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WPŁYW CZASU DESTYLACJI NA ZAWARTOŚĆ I SKŁAD OLEJKU ETERYCZNEGO WYIZOLOWANEGO Z LAWENDY (*LAVANDULA ANGUSTIFOLIA* MILL.)

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S t r e s z c z e n i e

Zbadano wpływ czasu destylacji na zawartość i skład olejku eterycznego wyizolowanego z lawendy (*Lavandula angustifolia* Mill.) na drodze destylacji z parą wodną. Maksymalną zawartość olejku (2%) uzyskano, prowadząc destylację przez 2 godziny, najniższą (1%), prowadząc destylację przez 40 minut. Dominującymi składnikamii wydzielonego olejku były linalol (28,78–30,68%), octan linalilu (12,35–17,67%) oraz α-terpineol (7,57–11,49%).

Słowa kluczowe: *lawenda, destylacja z parą wodną, olejek eteryczny, linalol, octan linalilu, α-terpineol, GC, MS*