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The influence of duration of the distillation of fresh and dried flowers on the essential oil composition of lavandin cultivated in Republic of Macedonia

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Abstract

The main objective of this study was to analyze the essential oil composition of cultivated lavandin (*Lavandula x intermedia* Emeric ex Loisel., Lamiaceae) from Republic of Macedonia and to evaluate the influence of the drying time of plant material and the distillation duration on the oil composition. Four different essential oils were obtained by hydrodistillation in a Clevenger type apparatus from freshly harvested and air dried flowers of lavandin and varying in distillation time (30 min and 2 h). Fifty-six compounds were identified by GC/FID/MS representing 96.57% to 98.23% of the total oil. Prevailing constituents in all tested oils were linalool, borneol and terpinene-4-ol, present in amounts from 35.23 to 42.24%, 12.28 to 16.68% and 4.44 to 5.62%, respectively. The content of 1,8-cineole varied from 3.99% to 8.42% while that of camphor was between 5.96% and 7.04%. Linalyl acetate was present in amounts from 1.26% to 3.21%. Unexpectedly, the duration of distillation insignificantly influenced the essential oil composition. Few differences, mainly in the content of borneol (13.71% and 16.68% in the oil from fresh and dried flowers, respectively) were recorded in the essential oils obtained by 30 minutes distillation, while the content of the other constituents was almost unchanged. However, the isolated essential oils of lavandin did not comply with the international standards requirements for the lavandin essential oil composition.

Key wards: *Lavandula intermedia*, Lamiaceae, GC/FID/MS, duration of hydrodistillation, drying time

Introduction

Medicinally and commercially important *Lavandula* species are perennial shrubs growing in rocky and calcareous areas in Mediterranean basin. For commercial production of essential oil, three species are used: *L. angustifolia* Mill. (common or English lavender), *L. latifolia* Medik. (spike lavender) and *L. angustifolia x L. latifolia (L. x intermedia* Emeric ex Loisel.*)* known as lavandin. There are also several cultivars that are planted for commercial production of essential oils with similar features to lavandin, such as: Grappenhall, Provence, Grosso, Dutch Mill, Abrial and Seal (lavender production, http://www.nda.agric.za/docs/brochures/essoilslavender.pdf).

Lavender flowers and oil are often used in the households as an herbal remedy for nervous disturbance and flatulence while externally are used to cure nervous headache (Gamez et al., 1990), anxiety and mild depression (Lewis & Kowalski, 2002; Lenrner et al., 2005). The essential oil act with powerful antimicrobial properties (Sabara and Kunicka-Styczynska, 2009) and is useful in the treatment of burns, sunburns, scalds, bites, vaginal discharge and anal fissure (Shafaghat et al., 2012). It is also known as antioxidant (Economou et al., 1991), antispasmodic (Cavanagh and Wilkinson, 2002), aromatic, carminative, cholagoge, diuretic, nervine, sedative, stimulative, stomachic and tonic (Lis-Balchin and Hart, 1999; Gilani et al., 2000;

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Kalemba and Kunicka, 2003). It is used in aromatherapy as well (Sabara and Kunicka-Styczynska, 2009). Industrial application of lavender oil include production of high-quality perfumes, candles, incense sachets, potpourri, wands, pillows, flower bundles, dried arrangements, wall hangings, wreaths and (Pauls et al., 2004) is used in bath products such as soap, shampoo, bath oil, lotion and bath salt. Owing to its safety and sedative action it is incorporated extensively in cosmetic preparations (Azar et al., 2011). In food manufacturing it is added in flavoring beverages, ice-creams, candy, baked goods and chewing gum (Kim and Lee, 2002, Wogiatzi et al., 2011).

The world's production of Lavandula species is mainly directed to lavandin cultivars, which have more vigorous growth and give greater amounts of essential oil. The average annual production of lavandin essential oil riches 1200 tons traditionally produced in Europe and United States (Peterson, 2002). On the other hand, the world production of lavender (*L. angustifolia*) oil is only 200 tons per annum, predominantly emanating out of Europe (Peterson, 2002). It is well known that chemical composition of the essential oils of lavender and lavandin largely depend on the species from which it is obtained (Kustrak and Besic, 1975; Wogiatzi et al., 2011). Key differences in the composition of these oils is the low amount of camphor (less than 1%) and the absence of bornyl acetate in lavender (*L. angustifolia*) oil, while lavandin (*L. intermedia*) oil contains camphor between 10-12% and linalool up to 20% (Peterson, 2002).

The characteristic odor of lavender oil originates from the presence of linalyl acetate and according to the regulations lavender oil should not contain less than 25% of this constituent. Additionally, the content of 1,8-cineole should be less than 2.5% (Ph. Eur. 7). On the other hand, the content of these components in lavandin essential oil varies a lot depending on the characteristics of the cultivars and other factors (Wogiatzi et al., 2011).

Lavender oil can be easily isolated from the flower heads using steam distillation. The time of distillation influences the essential oil composition and its quality. If distillation time is too short, compounds with higher boiling point may be missing from the oil. When the time of distillation is too long, the oil may have unpleasant smell. According some authors, nearly 75% of the total yield comes in the first 25 minutes of distillation and gives a commercial grade of lavender oil (Pitman, 2004). Even the steam distillation is still the most preferable technique of oil isolation, during this process, molecular rearrangements, hydratation of double bonds and hydrolyzis of esters to alcohols and carboxylic acids can occur. In high-quality lavender oil the ratio between linalyl acetate and linalool should be higher than one (Porter et al., 1982; Wesolowska et al., 2010). Linalool poses sweet odor and linalyl acetate refreshing one, appreciated in many applications (Wesolowska et al., 2010). Nowadays, commercially produced lavender oil is often distilled for only 15 minutes at a very high temperature and under very high pressure (Wesolowska et al., 2010). Because of its poor quality, synthetic linalyl acetate is often added in order to make it smell like the genuine plant fragrance (Sell, 2006; Wesolowska et al., 2010).

Within the one *Lavandula* species, the essential oil composition differs significantly depending on where and under what condition the plant was grown (Guillen et al., 1996). Other variations in essential oil composition may occur depending on duration, temperature and pressure of distillation (Wesolowska et al., 2010). Therefore, the aim of the present study was to evaluate the influence of distillation time on the composition of lavandin (*L. x intermedia*) essential oil as well as to determine the differences in chemical composition of the oils isolated from fresh or dried flowers of lavandin cultivated in Republic of Macedonia. Obtained results will be evaluated according to different standards requirements.

Material and methods

Plant material

Flowering tops of cultivated lavandin were collected in a full blooming of plants during the summer 2012. A voucher specimen of the plant was deposited at the Herbarium of the Institute of Pharmacognosy and Pharmaceutical Botany at the Faculty of Pharmacy in Skopje, Republic of Macedonia (No. LI-01/012). Proper amount of freshly collected material was distilled during the same day and the rest was put on a paper sheets in a shade and left to air drying. Dried flowers were also used for isolation of essential oil.

Essential oil isolation

Essential oils were isolated by hydrodistillation in a Clevenger-type apparatus following the procedure from European Pharmacopeia (Ph. Eur. 7). Four different essential oils were isolated from fresh and dried flowers of lavandin, by distillation lasting 30 min and 2 hrs. The obtained oils were dried over anhydrous sodium sulfate and stored in refrigerator until analysis. Two repetition of distillation were performed.

GC/FID/MS analysis

Essential oil samples were analyzed on Agilent 7890A Gas Chromatography system equipped with flame ionization detector (FID) and Agilent 5975C mass spectrometer (MS) equipped with capillary flow technology which enables simultaneous analysis of the sample on both detectors. HP-5MS (30 m x 0.25 mm, film thickness 0.25 μm) capillary column was used. Operating conditions were as follows: oven temperature 60 °C (5 min), 1 °C/min to 80 °C (2 min) and 5 °C/min to 280 °C (5 min); flow rate of 1ml/min (He); injector T=260 °C; FID T= 270 °C; 1μl injection volume at split ratio 1:1.

The mass spectrometry conditions were: ionization energy 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50-500 Da. The MS was operated in scan mode. Identification of the components present in essential oils was made by comparing mass spectra of components in essential oils with those from NIST, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovat′s (retention) indices that were determined using mixture of homologous series of normal alkanes from C_9 to C_{25} in hexane, under the same above mentioned conditions.

The percentage ratio of essential oils components was computed by the normalization method of the GC/FID peak areas and average values were taken into further consideration (n=3).

Results and discussion

The results of GC/FID/MS analysis of the essential oils, isolated from fresh and dried flowers of lavandin, obtained either by hydrodistillation of 30 minutes or 2 hours duration, are presented in Table 1. Total of fifty-six compounds were identified in the analyzed samples of lavandin representing 96.57% to 98.23% of the total oil. The most abundant compound in the analyzed essential oils was linalool (35.23 to 42.24%), followed by borneol and terpinene-4-ol, presented in amounts from 12.28% to 16.68% and from 4.44% to 5.62%, respectively. The content of 1,8-cineole varied from 3.99% to 8.42% and that of camphor between 5.96% and 7.04%. In all tested samples of lavandin oil the content of linalyl acetate was very low, presented in amounts from 1.26% to 3.21%. Similar quantities of lavandulyl acetate were determined (1.53-2.42%).

From the presented results a smaller decrease in the content of linalool can be noticed when prolonged distillation procedure was applied (38.58%) compared to the 30 minutes distillation procedure (40.13%). Similar trend of decreasing was noticed in the abundance of almost all other dominant compounds. For instance camphor amount decreased from 6.62% to 5.96%, borneol from 13.71% to 12.28% and terpinene-4-ol from 5.62% to 5.24%. The 2 hrs hydrodistillation increased the amount of 1,8-cineole (from 7.17% to 7.39%), *cis*-β-ocimene (from 2.45% to 2.54%) and *trans*-β-ocimene (from 0.78% to 0.85%) (Table 1). Increased content after prolonged distillation of fresh flowers was also notice in eugenol (from 0.00% to 1.89%) and eugenol acetate (from 0.04% to 3.05%). Also, few other esterified monoterpene compounds were identified in very small quantities, linalyl and lavandulyl acetate, followed by bornyl, neryl and geranyl acetate and lavandulyl isovalerate. After all, essential oils obtained from fresh flowers by 30 minutes and 2 hours lasting distillation pro-

Table 1. Chemical composition of the essential oils isolated from lavandin (%)

				Fresh flowers		Dried flowers	
No.	KIL	KIE	Components	2 h	30 min.	2 h	30 min.
1	931	940.3	α–Thujene	-	tr	-	-
2	939	944.2	α-Pinene	0.21	0.18	0.30	-
3	953	953.7	Camphene	0.50	0.50	0.56	-
4	975	970.7	Sabinene	0.11	0.12	0.15	-
5	980	972.8	β-Pinene	0.24	0.20	0.26	0.46
6	991	984.4	Myrcene	1.01	0.93	1.09	tr
7	1007	999.1	Δ^3-Carene	0.27	0.23	0.34	-
8	1018	1004.8	α-Terpinene	tr	-	-	-
9	1025	1011.9	*p*-Cymene	0.13	0.06	0.12	-
10	1031	1015.5	β-Phellandrene	3.84	3.64	4.41	1.16
11	1033	1017.2	1,8-Cineole	7.39	7.17	8.42	3.99
12	1040	1025.4	*cis*-β-Ocimene	2.54	2.45	3.15	1.17
13	1050	1035.1	*trans*-β-Ocimene	0.85	0.78	1.11	0.42
14	1062	1044.1	γ-Terpinene	0.20	0.15	0.25	0.13
15	1088	1073.5	α –Terpinolene	0.45	0.43	0.52	0.26
16	1098	1091.8	Linalool	38.58	40.13	35.23	42.24
17	1142	1125.7	*neo*-allo-Ocimene	0.87	0.64	0.87	-

				Fresh flowers		Dried flowers	
No.	KIL	KIE	Components	2 h	30 min.	2 h	30 min.
18	1143	1139.2	Camphor	5.96	6.62	6.84	7.04
19	1150	1150.0	Hexyl isobutyrate	tr	0.07	-	-
20	1162	1159.3	Pinocarvone	-	-	0.09	0.11
21	1165	1162.9	Borneol	12.28	13.71	13.77	16.68
22	1166	1166.2	Lavandulol	1.65	1.70	1.52	1.93
23	1177	1172.5	Terpinene-4-ol	5.24	5.62	4.44	5.25
24	1183	1180.0	*p*-Cymene-8-ol	tr	-	tr	tr
25	1189	1183.3	α-Terpineol	1.24	1.25	1.35	1.35
26	1191	1186.8	Hexyl butyrate	0.48	0.55	0.46	0.59
27	1193	1195.9	*cis*-Piperitol	tr	0.05	0.08	0.10
28	1212	1205.7	*trans*-Carveol	tr	tr	0.11	0.06
29	1228	1217.7	Nerol	0.15	0.12	0.16	0.14
30	1239	1227.2	Cumic aldehyde	0.29	0.33	0.42	0.46
31	1243	1232.7	Carvone	0.14	0.20	0.25	0.29
32	1256	1245.7	Linalyl acetate	1.26	2.18	1.86	3.21
33	1285	1272.7	Bornyl acetate	0.07	0.07	0.09	0.11
34	1289	1278.5	Lavandulyl acetate	1.53	1.64	1.84	2.42
35	1290	1290.9	Thymol	tr	-	-	-
36	1331	1314.6	Hexyl tiglate	0.03	0.05	0.06	0.06
37	1356	1341.6	Eugenol	1.89	-	-	-
38	1365	1348.0	Neryl acetate	0.38	0.18	0.26	0.24
39	1380	1362.9	Daucene	0.05	0.06	0.07	0.08
40	1383	1366.3	Geranyl acetate	0.40	0.32	0.40	0.39
41	1409	1390.4	α-Gurjunene	tr	0.03	0.03	0.05
42	1418	1399.6	*trans*-Caryophyllene	0.39	0.48	0.63	0.67
43	1458	1435.4	*trans*-β-Farnesene	1.18	1.60	2.08	2.21
44	1477	1450.4	γ-Muurolene	0.04	0.06	0.08	0.08
45	1480	1462.5	Germacrene D	0.08	0.09	0.12	0.12
46	1510	1486.0	Lavandulyl isovalerate	0.49	0.61	0.75	0.80
47	1513	1493.6	γ-Cadinene	0.04	0.06	0.10	0.09
48	1524	1501.7	δ-Cadinene	tr	tr	0.08	0.06
49	1536	1505.0	Eugenol acetate	3.05	0.04	-	-
50	1564	1539.7	*trans*-Nerolidol	tr	-	tr	-
51	1574	1557.1	Germacrene-D-4-ol	0.05	-	-	tr
52	1581	1565.5	Caryophyllene oxide	0.15	0.18	0.22	0.27
53	1627	1595.5	1-epi-Cubenol	tr	tr	tr	tr
54	1641	1621.0	τ-Cadinol (epi-α–Cadinol)	0.28	0.21	0.20	0.19
55	1653	1634.9	α–Cadinol	0.13	0.07	0.08	0.07
56	1686	1661.8	α-epi-Bisabolol	1.92	2.00	1.60	1.54
			Total:	98.23	97.84	96.88	96.57

n=3; tr < 0,02%; (-) - not present; KIL - Kovats (retention) index - literature data; KIE - Kovats (retention) index experimentally determined (AMDIS)

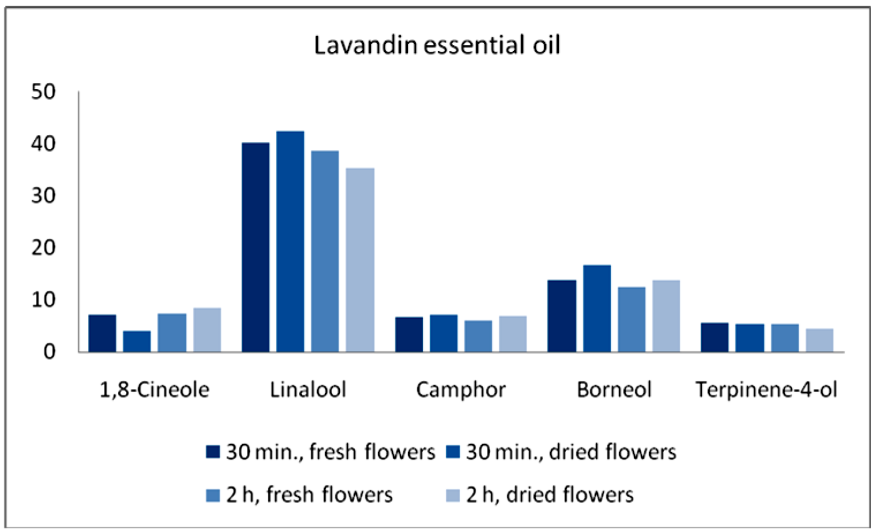

Fig. 1. The content of the main compounds in lavandin essential oil after 30 minutes and 2 hours distillation of fresh or dried lavandin flowers.

cedures showed insignificant variance in essential oil composition and the main constituents (linalool, borneol, 1,8-cineole, camphor and terpinene-4-ol) content (Fig. 1).

At the same time, there were significant differences in the essential oil composition of tested lavandin compared to various essential oils originated from diverse regions world-wide (Table 2). Higher amounts of borneol were found in our lavandin oils than spike lavender oil that shows high level of linalool, 1,8-cineole and camphor and very low level of linalyl acetate (Table 1 and 2). Analyzed lavandin essential oils were more similar to the spike lavender oil than to oils of lavandin cultivars (var. "grosso" and var. "abrial"). This findings correlate with the Renaud's report for the essential oil composition of 10 cultivars of lavandin and lavender, including *L. x intermedia* varieties stating that lavandin "grosso" oil has the highest amount of camphor (8.1%) and low amount of borneol (Renaud et al., 2001).

It is worth to be mentioned that *L. x intermedia* (*L. angustifolia x L. latifolia*) is a hybrid different from *L. x hybrida* Balb. ex Ging. (*L. dendata x L. latifolia*) that produces essential oil with different chemical composition with 1,8-cineole (up to 33.5%) and camphor (up to 12.4%) as major constituents (Tucker et al., 1993). On the other hand, Chu and Kemper used *L. x intermedia* as a synonym for *L. x hybrida* Reverchon or *L. x hybrida* Burnamii (lavandin,

Table 2. The chemical composition of various lavender and lavandin oils (%) (according to International standards)*

Constituent	*L. angustifolia* Lavender	*L. intermedia* Lavandin "Grosso"	*L. intermedia* Lavandin "Abrial"	*L. latifolia* Spike lavender
Linalool	25-38	24-35	26-38	34-50
Linalyl acetate	25-45	28-38	20-29	<1.6
1,8-Cineole	1-2	4-7	6-11	16-39
β-Caryophyllene	3-12	-	-	-
Limonene	< 1.0	0.5-1.5	0.5-1.5	0.5-3
Terpinene-4-ol	2-6	1.5-5	0.3-1	-
β-Ocimene	2.5-6	0.5-1.5	1.5-7	-
Lavandulyl acetate	3.4-6.2	1.5-3	1-2	-
Lavandulol	> 0.1	0.2-0.8	0.4-1.2	-
α-Terpineol	<2	-	-	0.2-2
Camphor	0.5-1	6-8	7-11	10-20
trans-α-Bisabolene	-	-	-	0.4-2.5
Borneol	-	1.5-3	1.5-3.5	-
Pinene	-	-	-	1-3

*data published by MacTavish and Harris, 2002

a hybrid of *L. angustifolia x L. latifolia*), different species from *L. x hybrida* Balb. ex Ging. (*L. dendata x L. latifolia)* (Chu and Kemper, 2001). Anyhow, the chemical composition of analyzed lavandin oils could not met the international standard requirements for this oil.

Wesołowska stated that the time of distillation had a significant influence on the content of lavender oil constituents (Wesołowska et al., 2010). Zhekova and Nedkov pointed out that there are divided opinions about variability of the content of the main compounds in lavender oil during the distillation process. For instance, linalyl acetate, compound used for quality assessment compound of lavender oil, could be found in large quantities in the first 15 minutes of the distillation process. The same authors reported that highest oil yield from true lavender was gained within the first 15 minutes of the distillation. The quantity of linalyl acetate was constantly increased during entire 30 minutes and the oils separate between 15 and 30 minute possessed the best smell performance. For the other varieties it was indicated that during the process of distillation the amount of linalyl acetate, linalool and β-ocimene had declined. It was also noticed that the undesirable components for the smell of lavender oil, such as borneol and terpinene-4-ol, had greater content than before (Zhekova and Nedkov, 2010). Our results showed that borneol was present in high amount in the analyzed lavandin oils in all experimental cases (12.28-16.68%) and opposite to above findings decreased with prolonged distillation of 2 h. The amount of the undesirable monoterpene alcohol, terpinene-4-ol was almost constant in all experimental conditions (4.44-5.62%).

Commercially available lavender and lavandin essential oils are produced by distillation of freshly harvested plant material (flowering tops in full blossom). Distillation process is usually carefully designed as it can increase or reduce the value of the oil (lavender production, www.nda.agric.za/docs/brochures/essoilslavender.pdf). The duration, pressure, temperature and the average speed of distillation are factors that influence the essential oil quality. On the other hand, for research and experimental purposes, the essential oils are isolated in Clevenger type apparatus often from dried plant material and then analyzed by GC-MS (Ihsan, 2008; Wesolowska et al., 2010; Azar et al., 2011; Najafian et al., 2012; Shafaghat et al., 2012). Some authors compared the essential oil composition of oils obtained from fresh and dried lavender and found significant similarities in the oils' composition. Thus, the percentages of 1,8-cineole, linalool, borneol, camphor and linalyl acetate in essential oil from fresh lavender were 18.9%, 34.2%, 12.1%, 4.6% and 3.1%, respectively and were almost identical in the oil obtained from dried lavender (20.3%, 33.0%, 11.0%, 4.7% and 3.6% for 1,8-cineole, linalool, borneol, camphor and linalyl acetate, respectively) (Ihsan, 2008).

The obtained results showed that drying of the lavandin inconsiderably influenced the essential oil composition and the whole character of the lavandin oil remained almost unaffected (Fig. 1). This finding are in accordance with data published previously for lavender oil (Ihsan, 2008).

Conclusion

Essential oil composition of cultivated lavandin (*L. x intermedia*) from Republic of Macedonia, analyzed by GC/FID/MS, showed presence of fifty-six compounds representing 96.57% to 98.23% of the total oil. The main identified constituents of the oil were linalool (35.23-42.24%), borneol (12.28-16.68%), 1,8-cineole (3.99-8.42%), camphor (5.96-7.04%) and terpinene-4-ol (4.44-5.62%), while linalyl acetate was present in very low amounts (1.53-2.42%). Regardless the duration of the distillation process (30 min and 2 hrs) and differences in the characteristics of plant material (fresh or dried flowering tops), the isolated lavandin oils did not show significant differences in their chemical composition

The chemical composition of analyzed lavandin oils did not comply with the international standard requirements.

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Резиме

Влијание на времетраењето на дестилацијата на свежи и на суви цветови врз составот на етеричното масло од култивиран лавандин од Република Македонија

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Клучни зборови: *Lavandula intermedia*, Lamiaceae, GC/FID/MS, времетраење на хидродестилација, време на сушење.

Главната цел на оваа студија беше да се анализира составот на етеричното масло од култивиран лавандин (*Lavandula x intermedia* Emeric ex Loisel., Lamiaceae) во Република Македонија и да се процени влијанието на сушењето на растителниот материјал и времетраењето на дестилацијата врз составот на маслото. Со хидродестилација на свежо набрани и исушени цветови на лавандин во Клевенџер апарат беа добиени 4 различни етерични масла во зависност од времето на дестилацијата (30 минути и 2 часа). Со GC/FID/MS анализа беа идентификувани вкупно 56 компоненти што претставува 96,57% - 98,23% од вкупното масло. Доминанти компоненти во анализираните етерични масла беа: линалол (од 35,23% до 42,24%), борнеол (од 12,28% до 16,68%) и терпинен-4-ол (од 4,44% до 5,62%). Содржината на 1,8-цинеол вариираше од 3,99% до 8,42%, додека содржината на камфор беше помеѓу 5,96% и 7,04%. Линалил ацетат беше присутен во количини од 1,26% до 3,21%. Неочекувано, времетраењето на дестилација незначително влијаеше на составот на етеричните масла. Беа забележани неколку разлики во етеричните масла добиени со 30 минутна дестилација, главно во содржината на борнеол (13,71% во масло дестилирано од свежи цветови и 16,68% во масло од суви цветови), додека содржината на другите соединенија беше речиси непроменета. Независно, хемискиот состав на изолираните етерични масла не е во согласност со меѓународните стандарди барања за составот на етеричните масла од лавандин.