Essential oil storage conditions affect the chemical composition in cultivated *Mentha spicata*

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Abstract

Spearmint (*Mentha spicata*) is widely utilized in traditional medicine and folk remedies. The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionary, chewing gum, toothpaste, and pharmaceutical industries. In this research the essential oil of air-dried samples was obtained by hydro-distillation and was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Changes in essential oil compositions were detected during storage for 3 months in refrigerator (4), freezer (−20 ℃), and at room temperature (25 ℃). Results indicated that at room temperature, the proportions of the compounds with lower boiling temperatures such as 1,8-Cineole (3.78-3.34%), limonene (7.33-6.61%), and Germacrene D (6.26-1.90%) decreased significantly. Furthermore, the essential oil composition showed the least alterations and *Mentha spicata* L. kept its primary quality when stored at low temperatures, particularly in a freezer, and Carvone showed a significant increase in all treatments.

Keywords: Industrial compounds; limonene; spearmint; temperatures

Farahbakhsh, J., Sh. Najafian, M. Hosseinifarahi, and **S. Gholipour**. 2021. 'Essential oil storage conditions affect the chemical composition in cultivated *Mentha spicata*'. *Iranian Journal of Plant Physiology,11* (2), 3617- 3624.

Introduction

Spearmint (Mentha spicata L), belongs to the family Lamiaceae. The plants of this family are a rich source of polyphenols and thus possessing strong antioxidant properties (Gulluce et al., 2007; Bimakr et al., 2011). Mentha spicata possesses several biological activities and is used in folkloric medicine as a carminative, antispasmodic, diuretic, antibacterial, antifungal, and antioxidant agent, and for treatment of colds and flu, respiratory tract problems, gastralgia, hemorrhoids, and stomachache (Leporatti and Ghedira, 2009; Tawaha et al., 2007; Tetika et al., 2013).

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, and wood (Sanchez et al., 2010). Essential oils have been used for centuries in medicine, perfumery, and cosmetics, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century, their use as aroma and flavor ingredients increased and became their major employment. Almost 3000

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Received: August, 2020
Accepted: December, 2020

different essential oils are known and 300 are used commercially in the flavor and fragrances market (Burt 2004). Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicrobial properties (Tajkarimi et al., 2010).

In addition, the composition of a particular essential oil may vary depending on the season of harvest, and the methods used to extract the oil (Demuner et al., 2011). Variation in chemical composition of essential oils, in particular, and extracts of medicinal plants may be observed due to the origin, the climatic and ecological conditions, and the developmental stage of collected plant materials (Miguel et al., 2004). There are little investigations about plant secondary metabolites storage especially essential oils as these metabolites are volatile and potentially could be subjected to different alterations by storage circumstances (Najafian, 2014; Najafian, 2016; Rowshan et al., 2013). Generally, the storage of essential oil at low temperatures prevents decreasing the concentrations of the oil components and helps maintaining the essential oil primary quality with the least alterations (Rowshan et al., 2013; Najafian, 2014; Najafian, 2016). There are a limited number of works on plant secondary metabolites storage particularly essential oils due to the volatility of these metabolites and their potential exposure to various changes by storage conditions. Therefore, the aim of the present study was to investigate the influence of storage on the chemical compositions of essential oils in Mentha spicata. In this regard the composition of Mentha spicata was measured in different time periods stored at 4 ◦C, −20 ◦C, and room temperature.

Materials and Methods

Plant collection and identification

Samples of Mentha spicata were prepared in May 2019 from plants growing in Behbahan city in the southeast of Khuzestan province of Iran. The plant materials were identified by Ahmad Hatami (faculty member at the herbarium of Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran). A voucher specimen was deposited in the herbarium of the Research Center for Agriculture and Natural Resources, Shiraz, Iran. The plants were shade-dried at room temperature for 14 days (20-25◦C). The essential oil content of all dried samples (100 g) was isolated through hydro distillation for 3 h with a Clevenger-type apparatus, based on the technique proposed by the British Pharmacopoeia. Over anhydrous sodium sulfate, the essential oils were dried and placed in firmly closed dark vials for additional examinations.

The circumstances for storing volatile oils

To study the effects of various storage circumstances on compositions of the essential oils, the prepared specimens were exposed to various storage temperatures including room temperature (25◦C), freezer (−20◦C), and refrigerator (4◦C), for 3 consecutive months until examination. The essential oils were analyzed in all storage treatments on a monthly basis. Furthermore, the fresh extracted essential oil was examined directly followed by extraction to define the exact effects of storage circumstances on compositions of essential oils over the test course.

GC analysis was carried out utilizing an Agilent gas chromatograph series 7890-A with flame ionization detector (FID). The temperatures of the injector and detector were maintained at 250 ◦C and 280 ◦C. Nitrogen was utilized at a flow rate of 1 ml/min as the carrier gas. Oven temperature program was 60-210 ◦C at the rate of 4 ◦C/min and then was programmed to 240 ◦C at the rate of 20 ◦C/min. Eventually, it was kept at this temperature for 8. 5 min. The split ratio was 1:50. GC-MS and the analysis were performed utilizing Agilent gas chromatograph armed with fused silica capillary HP-5MS column (0.25 mm × 30 mi. d; film thickness 0.25 m) coupled with a 5975-C mass spectrometer. Helium was utilized as carrier gas with an ionizing voltage of 70 eV. Interface and ion source temperatures were 280 ◦C and 230 ◦C, respectively. The mass range was within 45-550 amu. Oven temperature program was like theone described for the GC (Najafian, 2016).

Identifying the compounds

Table 1
Composition of Mentha spicata's hydro-distilled essential oils during 3-month storage at room temperature

No	Compound	RIa	After distillation (%)	After 1 month (%)	After 2 month (%)	After 3 month (%)
1	(E)-2-Hexenal	853	0.25	0.18	-	
2	Tricyclene	922	0.02	0.02	-	
3	-Thujene	925	0.02	0.02	-	
4	-Pinene	932	0.40	0.38	0.37	0.45
5	Camphene	951	0.07	0.05	0.05	
6	Sabinene	975	0.56	0.32	0.31	0.37
7	-Pinene	979	1.11± 0.005a	0.91± 0.005b	0.92± 0.005b	0.92± 0.003b
8	Myrcene	990	1.52±0.005a	1.35±0.005b	1.26±0.008c	1.35±0.005b
9	3-Octanol	995	0.27	0.25	0.26	0.33
10	p-Cymene	1026	0.05	0.03	-	
11	Limonene	1029	7.33±0.029a	6.68±0.008b	6.62±0.005c	6.61±0.008c
12	1,8-Cineole	1031	3.78±0.005a	3.46±0.035b	3.34±0.035c	3.34±0.008c
13	(E)-B-Ocimene	1047	0.27	0.12	0.06	
14	-Terpinene	1058	0.04	0.03		
15	cis-Sabinene hydrate	1070	0.05	0.05	0.08	
16	Terpinolene	1089	0.12	0.09		
17	Linalool	1098	0.23	0.25	0.29	0.31
18	1,3,8-p-Menthatriene	1113	0.07	0.04	0.10	
19	3-Octanol acetate	1123	0.09	0.09		
20	allo-Ocimene	1128	0.06	0.03		
21	Borneol	1167	0.70±0.003b	0.71±0.005b	0.80±0.003a	0.82±0.003a
22	Terpinen-4-ol	1178	0.13	0.12	0.10	
23	-Terpineol	1190	0.13	0.07	-	
24	trans-Dihydro carvone	1197	0.41	0.80	0.68	0.58
25	cis-Carveol	1232	0.21	0.07	-	0.47
26	Pulegone	1237	0.27	0.45	0.28	
27	Unknown	1239	0.32	0.15	0.19	
28	Carvone	1243	49.91±0.020d	50.05±0.008c	53.74±0.013b	56.92±0.017a
29	cis-Piperitone epoxide	1258	0.08	0.05	0.07	
30	Bornyl acetate	1287	0.24	0.10	0.06	0.19
31	Thymol	1290	0.23	0.11	0.09	0.25
32	Unknown	1294	0.23	0.44	0.39	
33	Carvacrol	1296	0.23	0.58	0.58	0.22
34	iso-Dihydro carveol acetate	1329	0.20	0.21	0.22	0.24
35	-Elemene	1338	0.24	0.12		
36	Piperitenone	1346	0.20	0.20		0.19
37	Eugenol	1361	0.22	0.32	0.21	
38	Piperitenone oxide	1370	10.69±0.003d	11.31±0.003b	11.72±0.008a	10.75±0.015c
39	-Copaene	1377	0.25	0.04	0.10	
40	-Bourbonene	1387	1.53±0.003d	1.61±0.003c	1.67±0.003a	1.62±0.005b
41	-Elemene	1393	0.50	0.55	0.45	0.34
42	(Z)-Jasmone	1396	0.08	0.46		
43	-Gurjunene	1411	0.21	0.15	0.14	0.16
44	(E)-Caryophyllene	1422	2.64±0.003a	2.65±0.005a	2.48±0.005b	2.31±0.008c
45	-Gurjunene	1430	0.16	0.21	0.22	0.21
46	Unknown	1447	0.55	0.34	0.27	
47	-Humulene	1455	1.10±0.005ab	1.13±0.005a	1.14±0.003a	0.92±0.008c
48	(E)--Farnesene	1459	1.07±0.008a	0.92±0.003b	0.80±0.005c	0.62±0.005a
49	allo-Aromadendrene	1462	0.17	0.17	0.19	0.18
50	Germacrene D	1484	6.26±0.005a	5.91±0.005b	3.37±0.005c	1.90±0.005d
51	Bicyclogermacrene	1498	0.61	0.60	0.25	
52	-Cadinene	1515	0.18	0.16	0.18	0.19
53	-Cadinene	1522	0.86±0.005c	0.98±0.003b	1.07±0.020a	0.96±0.006b
54	-Cadinene	1538	0.17	0.18	0.20	0.17
55	Spathulenol	1578	0.22±0.005d	1.07±0.020a	0.28±0.005c	0.90±0.005b
56	Caryophyllene oxide	1581	0.63±0.005c	0.63±0.003c	1.14±0.003a	0.80±0.012b
57	1,10-di-epi-Cubenol	1617	0.64±0.003c	0.70±0.005b	1.19±0.005a	0.61±0.003d
58	epi--Cadinol	1642	0.47	0.40	0.64	-
59	-Cadinol	1657	0.80±0.010d	0.95±0.008b	1.23±0.008a	0.88±0.012c
	Total		100%	100%	100%	100%

The constituents of essential oils were determined by calculating their retention indices under temperature-programmed circumstances for n-alkanes (C8–C25) and the essential oil on an HP-5 column under similar chromatographic circumstances. The singular compounds were identified by comparing their mass spectra with

Table 2
Composition of Mentha spicata's hydro-distilled essential oils during 3 months' storage at freezer temperature.

No	Compound	RI	After distillation (%)	After 1 month (%) (%)month (%)	After 2 month (%)	After 3 month (%)
1	(E)-2-Hexenal	*853*	0.25	0.17	0.15	-
2	Tricyclene	*922*	0.02	0.02	-	-
3	?Thujene	*925*	0.02	0.01	-	-
4	?Pinene	*932*	0.40	0.37	0.39	0.35
5	Camphene	*951*	0.07	0.05	0.08	0.05
6	Sabinene	*975*	0.56	0.31	0.33	0.31
7	?Pinene	*979*	1.11± 0.005a	0.90± 0.006c	0.95±	0.90± 0.005c
8	Myrcene	*990*	1.52± 0.006a	1.40± 0.005b	1.49± 0.003a	1.40± 0.014b
9	3-Octanol	*995*	0.27	0.24	0.26	0.26
10	p-Cymene	*1026*	0.05	0.01	-	-
11	Limonene	*1029*	7.33± 0.008a	6.71± 0.003c	7.11± 0.008b	6.73± 0.011c
12	1,8-Cineole	*1031*	3.78± 0.005a	3.48± 0.005d	3.69± 0.05b	3.51± 0.003c
13	(E)-?EOcimene	*1047*	0.27	0.23	0.20	0.18
14	?4Terpinene	*1058*	0.04	0.05	0.06	-
15	cis-Sabinene	*1070*	0.05	0.05	0.08	-
16	Terpinolene	*1089*	0.12	0.10	0.04	-
17	Linalool	*1098*	0.23	0.26	0.23	0.25
18	1,3,8-p-	*1113*	0.07	0.03	-	-
19	3-Octanol acetate	*1123*	0.09	0.08	0.12	-
20	allo-Ocimene	*1128*	0.06	0.04	0.08	-
21	Borneol	*1167*	0.70	0.67	0.71	0.72
22	Terpinen-4-ol	*1178*	0.13	0.11	0.13	0.09
23	?3Terpineol	*1190*	0.13	0.02	0.14	-
24	trans-Dihydro	*1197*	0.41± 0.005d	0.59± 0.008c	0.83± 0.003b	1.11± 0.008a
25	cis-Carveol	*1232*	0.21	0.04	0.41	-
26	Pulegone	*1237*	0.27	0.06	0.19	0.24
27	Unknown	*1239*	0.32	0.20	0.15	-
28	Carvone	*1243*	49.91± 0.11b	49.46± 0.14b	50.44±	49.72±
29	cis-Piperitone	*1258*	0.08	0.05	0.03	0.06
30	Bornyl acetate	*1287*	0.24	0.09	0.23	0.07
31	Thymol	*1290*	0.23	0.36	0.09	0.36
32	Unknown	*1294*	0.23	0.26	0.19	0.57
33	Carvacrol	*1296*	0.23	0.22	0.21	0.50
34	iso-Dihydro	*1329*	0.20	0.20	0.20	0.20
35	?5Elemene	*1338*	0.24	0.12	0.15	0.11
36	Piperitenone	*1346*	0.20	0.20	0.20	0.21
37	Eugenol	*1361*	0.22	0.14	0.21	0.30
38	Piperitenone oxide	*1370*	10.69± 0.005d	11.51± 0.003a	11.08±	11.42±
39	?9Copaene	*1377*	0.25	0.04	0.16	-
40	?0Bourbonene	*1387*	1.53	1.53	1.51	1.52
41	?1Elemene	*1393*	0.50	0.50	0.51	0.50
42	(Z)-Jasmone	*1396*	0.08	0.63	-	0.27
43	?3Gurjunene	*1411*	0.21	0.18	0.19	0.18
44	(E)-Caryophyllene	*1422*	2.64± 0.005c	2.73± 0.005a	2.73± 0.005a	2.69± 0.005b
45	?5Gurjunene	*1430*	0.16	0.24	0.24	0.23
46	Unknown	*1447*	0.55	0.54	0.51	0.78
47	?7Humulene	*1455*	1.10± 0.006a	1.20± 0.008b	1.20±	1.19± 0.003b
48	(E)-?EFarnesene	*1459*	1.07± 0.023a	1.02± 0.008a	1.03± 0.010a	1.00± 0.006a
49	allo-	*1462*	0.17	0.16	0.17	0.17
50	Germacrene D	*1484*	6.26± 0.005c	6.80± 0.036a	6.25± 0.063c	6.47± 0.040b
51	Bicyclogermacrene	*1498*	0.61	0.73	0.69	0.69
52	?2Cadinene	*1515*	0.18	0.16	0.16	0.16
53	?3Cadinene	*1522*	0.86± 0.003a	0.93± 0.005b	0.82± 0.015c	1.09± 0.012a
54	?4Cadinene	*1538*	0.17	0.18	0.17	0.18
55	Spathulenol	*1578*	0.22± 0.008c	1.00± 0.008a	0.26± 0.005c	0.31± 0.008b
56	Caryophyllene	*1581*	0.63± 0.005c	0.55± 0.005d	0.66±	0.71± 0.005a
57	1,10-di-epi-	*1617*	0.64± 0.003c	0.72± 0.006b	0.65± 0.005c	0.84± 0.005a
58	epi-?pCadinol	*1642*	0.47	0.39	0.40	0.53
59	?9Cadinol	*1657*	0.80± 0.003c	0.94± 0.005a	0.86±	0.89± 0.008b
	Total		100%	100%	100%	100%

reference standards or with the internal reference

Table 3
Composition of Mentha spicata's hydro-distilled essential oils during 3-month storage at Refrigerator temperature

No	Compound	RI	After	After 1 month (%)	After 2	After 3
1	(E)-2-Hexenal	*853*	0.25	0.08		-
2	Tricyclene	*922*	0.02	0.01		-
3	α-Thujene	*925*	0.02	0.01		-
4	α-Pinene	*932*	0.40	0.12	0.05	0.07
5	Camphene	*951*	0.07	0.02	-	-
6	Sabinene	*975*	0.56	0.15	-	-
7	β-Pinene	*979*	1.11± 0.003a	0.55± 0.005b	0.07±	0.43± 0.005c
8	Myrcene	*990*	1.52± 0.020a	0.88± 0.003b	0.47± 0.005c	0.52± 0.023c
9	3-Octanol	*995*	0.27	0.22	0.68	0.25
10	p-Cymene	*1026*	0.05	0.01	-	-
11	Limonene	*1029*	7.33± 0.037a	4.53± 0.008b	3.79± 0.008c	2.99± 0.008d
12	1,8-Cineole	*1031*	3.78± 0.008a	2.58± 0.008b	2.35± 0.018c	2.06± 0.005d
13	(E)-β-Ocimene	*1047*	0.27	0.13	0.15	-
14	γ-Terpinene	*1058*	0.04	0.03	-	-
15	cis-Sabinene hydrate	*1070*	0.05	0.05	0.08	-
16	Terpinolene	*1089*	0.12	0.07	0.09	-
17	Linalool	*1098*	0.23	0.28	0.26	0.28
18	1,3,8-p-	*1113*	0.07	0.03	-	-
19	3-Octanol acetate	*1123*	0.09	0.08	0.13	-
20	allo-Ocimene	*1128*	0.06	0.04		-
21	Borneol	*1167*	0.70± 0.005c	0.68± 0.003c	0.81± 0.005a	0.79± 0.005b
22	Terpinen-4-ol	*1178*	0.13	0.07	0.21	-
23	α-Terpineol	*1190*	0.13	0.01	0.08	0.17
24	trans-Dihydro	*1197*	0.41± 0.005d	0.65± 0.005b	0.92± 0.005a	0.61± 0.005c
25	cis-Carveol	*1232*	0.21	0.08	0.31	0.53
26	Pulegone	*1237*	0.27	0.09	0.26	0.31
27	Unknown	*1239*	0.32	0.36	0.22	-
28	Carvone	*1243*	49.91± 0.057d	52.05± 0. 32c	55.93±	57.47± 0.91a
29	cis-Piperitone	*1258*	0.08	0.05	0.05	-
30	Bornyl acetate	*1287*	0.24	0.04	0.25	0.15
31	Thymol	*1290*	0.23	0.37	0.19	0.20
32	Unknown	*1294*	0.23	0.26	0.20	-
33	Carvacrol	*1296*	0.23	0.27	0.20	0.22
34	iso-Dihydro carveol	*1329*	0.20	0.22	0.23	0.25
35	δ-Elemene	*1338*	0.24	0.13	0.16	-
36	Piperitenone	*1346*	0.20	0.21	0.22	0.22
37	Eugenol	*1361*	0.22	0.21	-	-
38	Piperitenone oxide	*1370*	10.69± 0.005d	12.30± 0.04b	12.59±	12.15± 0.15c
39	α-Copaene	*1377*	0.25	0.07	-	-
40	β-Bourbonene	*1387*	1.53± 0.005d	1.66± 0.011c	1.74± 0.003b	1.79± 0.003a
41	β-Elemene	*1393*	0.50	0.58	0.56	0.60
42	(Z)-Jasmone	*1396*	0.08	0.04	-	0.34
43	α-Guriunene	*1411*	0.21	0.20	0.18	0.19
44	(E)-Caryophyllene	*1422*	2.64± 0.003c	2.95± 0.003b	3.03± 0.008a	3.06± 0.017a
45	β-Guriunene	*1430*	0.16	0.28	0.27	0.27
46	Unknown	*1447*	0.55	0.43	0.52	0.34
47	α-Humulene	*1455*	1.10± 0.003c	1.29± 0.005a	1.23± 0.008b	1.24± 0.003b
48	(E)-β-Farnesene	*1459*	1.07± 0.005ab	1.05± 0.005ab	1.01± 0.003c	1.08± 0.005ab
49	allo-Aromadendrene	*1462*	0.17	0.18	0.19	0.20
50	Germacrene D	*1484*	6.26± 0.008c	6.59± 0.005a	5.28± 0.008b	5.24± 0.017c
51	Bicyclogermacrene	*1498*	0.61	0.67	0.56	0.51
52	γ-Cadinene	*1515*	0.18	0.19	0.22	0.23
53	δ-Cadinene	*1522*	0.86± 0.008d	1.20± 0.008a	1.02± 0.005c	1.07± 0.003b
54	α-Cadinene	*1538*	0.17	0.20	0.19	0.22
55	Spathulenol	*1578*	0.22± 0.005c	1.12± 0.003a	0.22± 0.005c	1.09± 0.005b
56	Caryophyllene oxide	*1581*	0.63	0.63	0.69	0.62
57	1,10-di-epi-Cubenol	*1617*	0.64± 0.005d	1.03± 0.005a	0.68± 0.005c	0.79± 0.005b
58	epi-α-Cadinol	*1642*	0.47	0.64	0.58	-
59	α-Cadinol	*1657*	0.80± 0.020d	1.11± 0.005a	0.87± 0.005c	0.99± 0.005b
	Total		100%	100%	100%	100%

mass spectra library and their retention indices were compared with reference standards or with the literature the percentages of the relative area obtained by FID were used for quantifying purpose, without using correction factors (Tables 1-3).

Statistical Analysis

This experiment was conducted based on a completely randomized block design with three replications. Findings were expressed as the average values plus standard deviations. Data analysis was conducted using one-way ANOVA by SPSS software (v. 25.0). Duncan's Multiple Range test was employed to measure the important differences ($p<0.05$) between treatments.

Results

In this work, the compositions of *Mentha spicata* hydro-distilled essential oils were defined at various storage times and temperatures (Tables 1-3). Totally, 59 ingredients were recognized and quantified in the *Mentha spicata* essential oils sampels. The monoterpenoid fraction includes 98.09% of the oil with the main components: Carvone, Piperitenone oxide, Limonene, Germacrene D, 1,8-Cineole, (*E*)-Caryophyllene and β-Bourbonene. The percentage of the recognized sesquiterpenoid constituents was relatively low (1.91%). Comparing the components of the essential oil of various storage times and temperatures, it was revealed that the quantities of main compounds were severely altered over storage at room temperature in comparison to the equivalent circumstances (Table1). Hence, our results indicated that the concentration of these constituents with a lower molecular weight decreased by extending the storage time particularly at room temperature (Table1).

One of our key findings is an increase trend in the amounts of Carvone, Piperitenone oxide, β-Bourbonene, (*E*)-Caryophyllene at room temperature by the storage time. The Carvone was 49.91% when distilling the oil, while it reached 56.92% after 3 months. The incrementing trend of this constituent was 49.91, 50.05, 53.74, and 56.92% at distillation time and after1, 2, and 3 and months of storage at room temperature, respectively. The increasing trend of this component was 14.04% after 3 months' storage at room temperature (Table 1).

The Piperitenone oxide also represented the same trend as Carvone. The quantity of Piperitenone oxide was 10.69% while extracting the oil and then it was11.31, 11.72, and 10.75% after1, 2, and 3 months storing at room temperature, respectively. β-Bourbonene was another key component representing an interesting change trend. According to Table1, at the end of the test, the β-Bourbonene's amount increased by 5.8% significantly by the storage time at room temperature.

On the other, Limonene was 7.33% directly after oil extraction. Then, it was continuously reduced to 6.61% and represented a trend of 9.6% reduction at the end of the storage course. The Limonene evolution trend after 1, 2, and 3 months after storing the essential oils was 6.68, 6.62, and 6.61%, respectively. The second constituent representing the same trend was 1,8-Cineole, which revealed a reducing trend by the storage time. The amount of this constituent was 3.78% while oil extraction, then it was 3.46, 3.34, and 3.34, after 1, 2, and 3 months, respectively. The concentration of this component reduced to 11% after 3 months of storage. Another key component representing a decresing change trend was Germacrene D. According to Table1, at the end of the test, Germacrene D's quantity severely reduced by 69%. The quantity of this compound while oil extraction was 6.26% and then decreased to 5.91, 3.37,and 1.90% after 1, 2, and 3 months, respectively. (*E*)-β-Farnesene presented the same trend by the storage course. The amount of (*E*)-β-Farnesene was 1.07% while extracting the oil and then it was 0.92, 0.80, and 0.62 after 1, 2, and 3 months, respectively.

Discussion

Monoterpenes are the main components of the plant essential oils, to which numerous medicinal herbs effects are attributed (Gherlardini et al., 2001). According to the results, it can be stated that the biosynthesis of carvone was initiated with geranyl diphosphate as a primary monoterpene substrate and continued through the aromatic limonene as an intermediate. Our findings are in agreement the former results (Rowshan et al., 2013; Najafian, 2014; Najafian, 2016) representing that the proportions of the two key compounds of thymol and carvacrol included a different progress trend overall storage

phases in comparison to their precursors (p-cymene and-terpinene), as well as in lavender and lemon balm. Although quantities of these constituents significantly increased in all treatments, the amount of their precursors decreased. Based on the obtained results, the stored and frozen thyme displayed the most important compounds as well as the best retention of thymol terpinene and carvacrol (Usaiet al., 2011). Furthermore, it was reported that, the lemon oil limonene content decreases from 67.1% to 30.7% by storing the oil at 25◦C with the captaken for 3 min daily. Nevertheless, storage at 5◦C, with the captaken for 3 min only oncea month, the minimum degradation was obtained (Sawamura et al., 2004).

In a similar study by Rowshan et al. (2013) on the Thymus daenensis'essential oil stored in a freezer, it was reported that the primary quality was preserved compared to the other two storage conditions (4◦C and −20◦C) over three months. Najafian (2014, 2016) also reported that the essential oils of Lavender and Lemonbalm were maintained in the freezer, indicating the maintenance of primary quality compared to the other two storage circumstances (−20 ◦C and 4 ◦C) over three months. Our work is also consistent with previous studies and the procedures proposed for reducing key compounds like Germandine D in the freezer and refrigerator (Rowshan et al., 2013; Najafian, 2014; Najafian, 2016).

The main process during storage of essential oil is evaporation of compounds with a lower boiling temperature, mainly of mono hydrocarbons. The best results of the main compound were obtained at 4 ℃ and -20 ℃ whereas there was a higher decrease in the oil quality during the storage period of three months in room temperature. It is concluded that the essential oil of *Mentha spicata* which was stored in freezer better kept its primary quality in comparison with room temperature and refrigerator storage. Generally, the storage of *Mentha spicata* essential oil at low temperatures prevents decrease in the concentrations of the oil components and helps maintain the essential oil primary quality with the least alterations. These findings may be extended to storage of essential oils of plants with the same chemical characteristics. Furthermore, these findings may benefit essential oil producers and consumers, who utilize these compounds in pharmaceutical and cosmetic industries. In conclusion, storage of secondary plant products especially essential oils is an interesting research area which needs further studies on essential oils components of various aromatic plants.

Acknowledgments

The authors wish to thank Fars Research Center for Agriculture and Natural Resources for financial support of this work.

References

Bimakr, M., R. A. Rahman, F. S.Taip, A. Ganjloo and **L. M. Salleh,** 2011. 'Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves'. *Food and Bioproducts Processing*, 89: 67-72.

Burt, S, 2004. 'Essential oils: their antibacterial properties and potential applications in foods-a review'. *International Journal of Food Microbiology*, 94(3): 223-253.

Demuner, A.J., L. C. Almeida Barbosa, C. Gonçalves Magalhaes, C. J. Da Silva, C. R. Ghelardini, C., N. Galeotti and **G. Mazzanti**, 2001. 'Local anaesthetic activity of monoterpenes and phenylpropanes of essential oils'. *Planta Medica*, 67(06): 564-566.

Gulluce, M., F. Sahin, M. Sokmen, H. Ozer and **D. Daferera,** 2007. 'Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. Longifolia'. *Food Chemistry*, 103: 1449-1456.

Leporatti, M. L and **K. Ghedira.** 2009. ' Comparative analysis of medicinal plants used in traditional medicine in Italy and Tunisia'. *Journal of Ethnobiology and Ethnomedicine*, 5: 31–39.

Miguel, G., M. Simoes, A. C. Figueiredo, J. G. Barroso, L. G. Pedro, L.G and **L. Carvalho.** 2004. 'Composition and antioxidant activities

of the essential oils of *Thymus caespititius*, *Thymus camphoratus* and *Thymus mastichina*'. *Food Chemistry*, 86(2): 183-188.

Najafian, SH. 2014. 'Storage conditions affect the essential oil composition of cultivated Balm Mint Herb (Lamiaceae) in Iran'. *Industrial Crops and Products*, 52: 575-581.

Najafian, SH. 2016. 'The effect of time and temperature on the shelf life of essential oils of *Lavandula officinalis*'. *Journal of Essential oil Research*, 28(5): 413-420.

Rowshan, V., A. Bahmanzadegan and **M. J. Saharkhiz.** 2013. ' Influence of storage conditions on the essential oil composition of *Thymus daenensis* Celak'. *Industrial Crops and Products*, 49: 97-101.

Sanchez, E., S.García and **N. Heredia.** 2010. ' Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae'*. *Applied and Environmental Microbiology*, 76(20): 6888-6894.

Sawamura, M., U. S. Son, H. S. Choi, M. S. Kim, N. T. L, Phi, M. Fears and **C. Kumagai.** 2004. 'Compositional changes in commercial lemon essential oil for aromatherapy'. *International Journal of Aromatherapy*, 14(1): 27-36.

Tajkarimi, M.M., S. A. Ibrahim and **D. O. Cliver.** 2010. ' Antimicrobial herb and spice compounds in food'. *Food Control*, 21(9): 1199-1218.

Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohamed and **T. El Elimat.** 2007. 'Antioxidant activity and total phenolic content of selected Jordanian plant species'. *Food Chemistry*, 104: 1372–1378**.**

Tetika, F., S. Civelek and **U. Cakilcioglu.** 2013. ' Traditional uses of some medicinal plants in Malatya (Turkey) '. *Journal of Ethnopharmacology*, 146: 331- 346.

Usai, M., M. Marchetti, M. Foddai, A. Del Caro, R. Desogus, I. Sanna and **A. Piga.** 2011. ' Influence of different stabilizing operations and storage time on the composition of essential oil of thyme (*Thymus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) '. *LWT-Food Science and Technology*, 44(1): 244-249.