Chemical Composition of Essential Oil from Fresh *Salvia Rosmarinus* from Jordan at Different Drying Methods

Areej M. Al-Ghoul[1*], Sara Alwardat[1], Abeer R. Abdelhalim[2]

[1]*Department of Chesmistry, Faculty of Science, Yarmouk University, Jordan*
Email: Areejm@yu.edu.jo, Areej_alghoul@yahoo.com
[2]*Faculty of Science, Taibah University, AlmadinaAlmonawara, Saudi Arabia*

Abstract. The composition of essential oil is affected by different drying methods. The present study aimed to evaluate the effect of drying methods (shade-drying (SD), oven-drying(OD), and freeze-drying (FD)) on the essential oils composition of *Salvia rosmarinus*at. The extracted *S. rosmarinus*essential oil samples subjected to different drying methods were extracted by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Eighty-three compounds were tentatively identified, with 1,8-cineole, α-pinene, α-fenchene, and bornyl being the major components. The shade-dried method best maintained the composition of rosemary essential oil and showed the best retention of α-Pinene, 1,8-Cineole, the most important compounds, as well as α-terpineol and safranal. The oven-dried method displayed the worst results, with a significant loss of 37 compounds. However, oven-drying produces a significant increase in the concentrations of borneol, iso-menthol, α-terpineol, camphor, and bornyl acetate.

Keywords: Essential Oil Composition, Freeze-Drying, Oven-Drying, *Salvia Rosmarinus,* Shade-Drying.

1. INTRODUCTION

Salvia rosmarinus Spenn (synonym: *Rosmarinus officinalis* L.), which is known traditionally as rosemary, is an aromatic shrub nativeto the Mediterranean, belonging to the Lamiaceae family [1]. It grows wild in Asia, Eurasia, Australia, and South Africa [2]. In Jordan, *S. rosmarinus* has been used by local people as an anti-inflammatory and for the treatment of kidney stones, rheumatism, and colic. [3]

The essential oil extracted from *S. rosmarinus*is commonlyused in the perfume industry and asa flavoring agent [4]. In addition, it was found to have the ability to release the symptoms caused by respiratory disorders, to motivate hair growth, to decrease stress and mental alertness, and it has been reported that it can be used to curerheumatoid disease [5-7]. It has been found that *S. rosmarinus*can be used to improve memory [8-10]. Moreover, *S. rosmarinus*can be used as an antibacterial agent, and in the monitoring of the propagation of tumor cells [11-15]

The presence of high amounts of water in the fresh herbs may lead to severe deterioration caused by microbial growth and biochemical reactions. The most common method to dry herbs is hot air-drying, but it can cause thermal damage to the essential oil composition. [16]. The typical composition of rosemary essential oils contained varied levels of α-pinene, 1,8- cineole, and camphor [17]. Different studies have been reported to survey the compositions of the essential oil extracted from the rosemary plant from different areas. A study on the essential oil from Morocco indicated that1,8-cineole (51.1%), α-pinene (16.2%), and camphor (16.8%) were the main components of its oil [18]. Another study on the essential oil from Buenos Aires indicated that α-pinene (31.2%), 1,8-cineole (21.6%), camphor (7%), and camphene (5%) were the main components of its oil [19]. Another study on the essential oil from the same plant from Cordoba province showed the oil contained a high concentration of camphor (35.7%), verbenone (26.6%), and β- caryophyllene (15.8%) [20]. However, a limited number of studies have been conducted to study the chemical diversity of Jordanian *S. rosmarinus*. by using different drying methods. The present study aimed to evaluate the composition of the essential oil extracted from fresh *S. Rosmarinus* and the composition after being subjected to different drying methods (shade-drying, oven-drying at 80°C, freeze-drying). *S. rosmarinus* essential oil from all samples was extracted by hydrodistillation and analyzed by using gas chromatography-mass spectrometry (GC-MS).

2. MATERIAL AND METHODS

2.1. Sample Preparation

The plant materials of *S. rosmarinus* were collected in June 2023 from the western region situated in Irbid City, Jordan. The specimens were recognized by Mohamed Al-Jawarneh from Al-Yarmouk University. A voucher plant specimen was deposited in the Yarmouk University Herbarium.

2.2. Drying Techniques and Equipment

Considering the fresh (FR) and the three types of drying methods (shade-drying (SD), oven-drying at 80°C (OD), and freeze-drying (FD) were used in this experiment, plant samples were divided into five groups to ensure the uniformity of plant materials in the treatments. The shade-drying method was done by spreading the fresh flowering aerial parts of *S. rosmarinus* in the shade under natural airflow and ambient temperature (mean value of 22°C) for about two weeks. In the case of the oven-drying method, the samples were dried using the oven at a temperature of about 80°C. To prepare the freeze-drying samples, the samples were placed in a freeze-dryer for two weeks at −52°C.

2.3. Extraction and Analysis of Oil

One hundred grams of fresh leaves of *S. rosmarinus* were subjected to hydrodistillation using a Clevenger apparatus. 100 g of dried material was hydrodistilled in clevenger for 3 hr. The oils extracted from the plant were stored in a refrigerator at 4 ± 1°C.

2.4. Gas Chromatography-Mass Spectrometry

Chemical analysis of the essential oils was performed by using Gas chromatography-mass spectrometry (Chromatic Crystal 9000 GC-MS, Yoshkar Ola, Russia) covered with a CR-5 MS column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 μm film thicknesses). In the MS detector, the electron ionization mode was operated at 70 eV. The temperature column was programmed from 40°C for 1 min (isothermal) to 280°C at a constant rate of 3°C/min. Helium was used as a carrier gas (1.0 mL/min). The relative peak areas were used to calculate the relative percent concentrations of the detected compounds. The chemical constituents of the essential oils were identified by comparing their calculated Kovats retention index (KI) (relative to n-alkanes C8-C30), matching their recorded mass spectra with the built-in library spectra (NIST, Gaithersburg, MD, USA, and Wiley Co., Hoboken, NJ, USA) and by mass spectrum matching to authentic standards.

3. RESULTS AND DISCUSSIONS

3.1 Essential Oil Composition

Table 1summarizes the GC/MS analysis results of the essential oils obtained from the plant material after being subjected to different drying methods. The analysis resulted in the identification of 83 constituents in the different hydro-distilled oils. In total, 78, 69, 41, and 68 components were identified in the essential oils in FR, SD, OD, and FD samples, respectively. Distillation of the fresh plant and samples dried by different methods resulted in both increased and reduced essential oil content depending on the drying method. based on their chemical structures, the identified compounds were grouped into seven main groups. These were oxygenated monoterpene, hydrocarbon monoterpene, oxygenated sesquiterpene, hydrocarbon sesquiterpene, aliphatic compound, hydrocarbon diterpene, and oxygenated diterpene.

The major components of the fresh sample of rosemary were1,8-cineole (37.62), α-pinene (31.3), α-fenchene (6.97), and bornyl (3.46). Most of the compounds originally found in fresh rosemary essential oil were significantly reduced during oven drying.

Increasing the drying temperature significantly decreased the essential oil content from 78 compounds to 41 in the oven-dried sample as well as the concentration indicating the worst results, with a significant loss of 37 compounds. For example, the concentration of α-pinene was 37.62 % in fresh rosemary samples and its concentration decreased to 4.23 % after this thermal process. α-pinene and sabinene seemed to have more affinity to the water fraction contained and thus were lost with the water during the drying process. On the other hand, the concentration of bornyl, safranal, α-terpineol, and camphor increased after this thermal process. The concentration of camphor and bornyl was shown to be increased with increasing temperature in a study by Zheljazkov *et al* [21].

Shade-dried best maintained the composition of rosemary essential oil and showed the best retention of α-Pinene, 1,8-Cineole, the most important compounds, as well as α-terpineol and safranal.

Compared to the fresh samples, the concentration of the oxygenated monoterpene was significantly increased in the cases of SD, OD, and FD where the contents were obtained to be 50.85%, 75.74%, 6.85%, 61.65%, and 88.39%, respectively. On the other hand, opposite results were obtained in the case of the hydrocarbon monoterpene of the essential oils of the plant material dried at SD, OD, and FD obtained to be 6.29%, 41.33%, 36.89%, and 9.76% respectively when comparing to the fresh sample (47.71%).

Table 1. The GC/MS analysis of the hydrodistillation obtained from *Salvia rosmarinus*at different drying methods.

No.	KI	Name of compound	% of Composition			
			Fresh	Dry	Oven	Frozen
1	902	Butyl propanoate	0.01	-	-	-
2	915	Artemisia triene	0.19	0.05	-	0.14
3	921	α-Thujene	0.41	0.08	-	0.10
4	929	α-Pinene	31.3	31.07	4.23	27.40

5	942	α-Fenchene	6.97	5.34	1.80	4.38
6	964	(2-methyl-(3E)-Octen-5-yne	0.07	0.01	-	-
7	969	Sabinene	2.29	0.61	-	0.63
8	972	β-Pinene	0.32	0.15	-	0.17
9	977	3-Octanone	0.06	0.01	0.02	0.05
10	984	trans-Isolimonene	1.37	1.07	0.58	0.99
11	999	α- Phellandrene	0.23	0.18	-	0.19
12	1010	δ-3-Carene	0.93	0.70	0.34	0.81
13	1018	ρ-Cymene	1.79	0.8	1.79	0.68
14	1028	1,8-Cineole	37.62	36.07	38.07	35.48
15	1032	(Z)-β-Ocimene	0.05	0.07	-	0.05
16	1052	γ-Terpinene	1.09	0.61	0.68	0.67
17	1061	cis-Sabinene hydrate	0.12	0.01	0.03	0.01
18	1079	ρ-Mentha-2,4(8)-diene	0.77	0.44	0.36	0.51
19	1081	meta-Cymenene	-	0.08	-	0.14
20	1091	6,7-Epoxymyrcene	0.09	0.10	-	0.10
21	1094	Linalool	1.30	0.86	1.95	0.98
22	1097	n-Nonanal	0.02	0.02	0.04	0.02
23	1110	endo-Fenchol	0.10	0.11	0.10	0.12
24	1115	cis-ρ-Menth-2-en-1-ol	-	-	0.07	-
25	1116	α-Campholenal	0.08	0.08	-	0.05
26	1124	allo-Ocimene	0.04	0.05	-	0.04
27	1130	trans- Pinocarveol	-	-	0.05	-
28	1138	Camphor	0.03	11.33	22.23	13.08
29	1139	trans-α-Necrodol	-	-	-	0.03
30	1143	Camphene hydrate	0.06	0.08	0.17	0.06
31	1149	Menthone	0.03	0.03	-	0.04
32	1150	Pinocarvone	0.12	0.10	0.16	0.12
33	1153	3-Thujanol	0.02	0.02	-	0.03
34	1162	Borneol	3.46	2.63	7.26	3.15
35	1164	cis-Pinocamphone	0.04	0.03	0.05	0.03
36	1170	Terpinen-4-ol	-	-	2.70	-
37	1172	iso-Menthol	1.37	0.94	-	1.08
38	1176	meta-Cymen-8-ol	-	-	0.12	-
39	1177	5-methylene-2,3,4,4-tetramethyl-Cyclopent-2-enone	0.05	0.06	-	0.06
40	1186	α-Terpineol	2.29	2.20	5.92	2.64
41	1188	trans-ρ-Mentha-1(7),8-dien-2-ol	0.14	0.11	-	0.15
42	1196	Safranal	2.63	2.39	8.97	3.82

43	1197	n-Dodecane	-	-	0.14	-
44	1209	trans-Piperitol	0.02	0.02	0.02	0.03
45	1218	endo-Fenchyl acetate	0.01	-	-	-
46	1228	cis-Carveol	0.03	0.04	0.03	0.04
47	1234	Ascaridole	0.01	0.01	-	0.01
48	1241	Carvotanacetone	0.01	0.01	0.02	0.01
49	1243	Geraniol	0.01	0.03	0.01	0.05
50	1255	Piperitone	-	0.04	0.06	0.04
51	1257	Geranial	0.02	0.01	-	0.01
52	1274	Bornyl acetate	0.99	0.19	0.26	0.18
53	1282	2-ethyl-endo Fenchol	-	0.04	0.05	-
54	1289	Thymol	0.04	0.04	0.05	0.07
55	1303	ρ-Cymen-7-ol	0.01	0.01	-	0.02
56	1323	Piperitenone	0.07	0.03	0.03	0.04
57	1339	Eugenol	0.02	0.01	0.01	0.03
58	1361	Longicyclene	-	0.01	-	0.01
59	1366	α-Ylangene	0.07	0.05	0.021	0.06
60	1382	Thujic acid	0.03	0.07	-	0.04
61	1408	(Z)- Caryophyllene	0.31	0.22	0.18	0.30
62	1428	β-Gurjunene	0.01	0.01	-	0.01
63	1443	α-Humulene	0.20	0.14	0.11	0.20
64	1464	cis-Cadina-1(6),4-diene	0.04	0.03	-	0.03
65	1484	γ-Himachalene	0.02	-	-	-
66	1488	α- Amorphene	0.01	-	-	-
67	1495	n-Pentadecane	0.01	0.01	0.18	0.01
68	1498	Bicyclogermacrene	0.01	0.01	-	0.01
69	1501	α-Muurolene	0.04	0.03	-	0.04
70	1508	Epizonarene	0.08	0.06	0.04	0.08
71	1566	Caryophyllene oxide	0.05	0.03	-	0.04
72	1593	Humulene epoxide II	0.04	0.03	-	0.05
73	1616	Junenol	0.01	0.02	-	0.05
74	1627	epi-α-Muurolol	0.02	0.01	-	0.02
75	1640	Vulgarone B	0.02	0.03	-	0.07
76	1651	3-Thujopsanone	0.01	0.01	-	0.03
77	1671	α- Bisabolol	0.01	0.01	-	0.03
78	1830	Cryptomeridiol	0.01	-	-	-
79	1840	(E)-Pseudoisoeugenyl 2-methylbutyrate	0.03	0.01	-	-
80	1913	Procerin	0.01	-	-	-
81	2195	1-Docosene	0.01	-	0.02	0.01

82	2379	Methyl labdanolate	0.17	0.04	0.68	0.15
83	2894	Nonacosane	0.01	-	-	-
		Oxygenated monoterpene	50.85	57.74	61.65	88.39
		Hydrocarbon monoterpene	47.71	41.33	36.89	9.76
		Oxygenated sesquiterpene	0.22	0.17	0.39	-
		Hydrocarbon sesquiterpene	0.78	0.59	0.79	0.35
		Aliphatic compound	0.22	0.05	0.11	0.70
		Hydrocarbon diterpene	0.13	-	-	-
		Oxygenated diterpene	0.20	0.09	0.16	0.75
		Other	-		-	0.02
		Total percentage	100	99.97	99.99	99.97

CONCLUSIONS

This study investigatedthe essential oils extracted from *Salvia rosmarinus*at different drying methods (fresh (FR), shade-drying SD, oven-drying(OD), and frozen-drying (FD)).The researchers found the essential oil composition of rosemary depended on the processing drying method. Eighty-three compounds were tentatively identified, with 1,8-cineole, α-pinene, α-fenchene, and bornyl being the major components. Shade-drying is the best method to maintain the composition of rosemary essential oil while oven-drying is the worst. However, oven-drying produces a significant increase in the concentrations of borneol, iso-menthol, α-terpineol, camphor, and bornyl acetate.

REFERENCES

[1]. Abdelhalim, A. and Hanrahan, J. Biologically active compounds from Lamiaceae family: Central nervous system effects. *Studies in Natural Products Chemistry.* 2021; 68: 255-315.

[2]. Shu Y, Chen Y, Qin K, et al. Effect of different drying methods on the essential oils of mint (Mentha haplocalyx). *Nat Prod Commun*. 2013; 8(10):1479–1480.

[3]. Abdelhalim,A., Aburjai, T., Hanrahan, J., and Abdel-Halim, A. Medicinal plants used by traditional healers in Jordan, the Tafila region. *Pharmacognosy magazine*. 2017:13, 95-101.

[4]. Erkan N, Ayranci G, Ayranci E. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid, and sesamol. Food Chem. 2008; 110:76-82.

[5]. Oluwatuyi M., Kaatz, G., and Gibbons, S. *Phytochemistry.* 2004; 65: 3249–54.

[6]. Zhang A, Sun, H., and Wang, X. *European Journal of Medicinal Chemistry.* 2013; 63: 570–577.

[7]. Rahila, M. P., Surendra, N. B., Laxmana, N. N., Pushpadass, H. A., Manjunatha, M., and Franklin, M. E. Rosemary (Rosmarinus officinalis Linn.) extract: A source of natural antioxidants for imparting autoxidative and thermal stability to ghee. *Journal of Food Processing and Preservation.* 2018; 42 134-143.

[8]. Abdelhalim, A., Karim, K., Chebib, M., Aburjai, T., Khan, I., Johnston, G., and Hanrahan, J. Antidepressant, Anxiolytic, and Antinociceptive Activities of Constituents from Rosmarinus Officinalis. *J Pharm Sci*. 2015; 18(4): 448 – 459.

[9]. Perry, E.K., Pickering, A.T., Wang, W.W., Houghton, P., and Perry, N. Medicinal Plants and Alzheimer's Disease: Integrating Ethnobotanical and Contemporary Scientific Evidence. *The Journal of Alternative and Complementary Medicine*. 1998; 4: 419-428.

[10]. Ozarowski, M., Mikolajczak, P.L., Bogacz, A. ,Gryszczynska, A., Kujawska, M., Jodynis-Liebert, J. Rosmarinus officinalis L. leaf extract improves memory impairment and affects acetylcholinesterase and butyrylcholinesterase activities in rat brains. *Fitoterapia*. 2013; 91:261-271.

[11]. Wang, W., Li, N., Luo, M., Zu, Y., and Efferth, T. Antibacterial activity and anticancer activity of Rosmarinus officinalis l. essential oil compared to that of its main components. *Molecules*. 2012; 17(3): 2704-2713.

[12]. Jordán, M. J., Lax, V., Rota, M. C., Lorán, S., and Sotomayor, J. A. Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of Rosmarinus officinalis L essential oil and its polyphenolic extract. *J. Ind. Crops Prod*. 2013a ; 48: 144–152.

[13]. Barrera, C.A., and Acosta G. E. Actividad antibacteriana y determinación de la composición química de los aceites esenciales de romero (Rosmarinus officinalis), tomillo (Thymus vulgaris) y cúrcuma (Curcuma longa) de Colombia. *Rev. Cuba. Plantas Med*. 2013; 18(2): 237-246.

[14]. Kontogianni, V. G., Tomic, G., Nikolic, I., Nerantzaki, A. A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I. P., and Tzakos A.G. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chem*. 2013; 136(1): 120–129.

[15]. Saleem, N., Abdelhalim, A., Alghoul, A., and. Abdel-Rhman, S. In-vitro antimicrobial activity of Acacia farnesiana and Rosmarinus officinalis crude extract and isolated compounds against food borne pathogens. *IJMSSSR*. 2023; 5: 420-426.

[16]. Alghoul, A. M., Al-Qataisheh, B. K., and Abdelhalim, A. R., Essential Oil composition of the flowering aerial parts of Fresh and air-dried *Scolymus hispanicus* L. from Jordan. *World Journal of Pharmacy and Pharmaceutical Sciences.* 2023; 12 (12)

[17]. Jordan, M. J., Lax, V., Rota, M. C., Lorán S, and Sotomayor, J.A. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control*. 2013; 30 (2): 463–468.

[18]. Annemer, S., Farah, A., Stambouli, H., Assouguem, A., Almutairi, M.H., Sayed, A.A., Peluso, I., Bouayoun, T., Talaat Nouh, N.A., and El Ouali Lalami, A. Chemometric investigation and antimicrobial activity of *Salvia rosmarinus* Spenn. *Essential Oils Molecules*. 2022; 27(9): 2914.

[19]. Ojeda-Sana, A. M., van Baren, C. M., Elechosa, M. A., Juárez, M. A., and Moreno, S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. *Food Control*. 2013; 31(1): 189- 195.

[20]. Olmedo, R., Asensio, C., and Grosso, N. Thermal stability and antioxidant activity of essential oils from aromatic plants farmed in Argentina. *Ind. Crops. Prod*. 2015; 69: 21-28.

[21]. Valtcho, D., Zheljazkov, T., Astatkie, I., and Zhalnov, T. D. Method for Attaining Rosemary Essential Oil with Differential Composition from Dried or Fresh Material. *Journal of Oleo Science*. 2015; 65(5): 485-496.

DOI: https://doi.org/10.15379/ijmst.v10i2.3333