

Full Paper

Changes in essential oil content and composition of leaf and leaf powder of *Rosmarinus officinalis* cv. CIM-Hariyali during storage

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Abstract: To see the changes in the essential oil content and composition of rosemary (*Rosmarinus officinalis* L. cv. CIM-Hariyali) during postharvest storage, freshly harvested leaves were kept under sun and shade conditions for one month and one year respectively. In addition to this, since most of the spices are marketed also in powdered form, leaves powder of rosemary was also kept for nine months. The essential oil content was found to vary non-significantly in stored leaves (from 1.05% to 1.3%; fresh weight basis). In contrast, leaves stored in the powdered form for nine months showed sharp decrease in essential oil content (from 2.7% to 1.1%; dry weight basis). GC and GC-MS analysis of the oils revealed the presence of camphor (23.1-35.8%), 1,8-cineole (21.4-31.6%) and α -pinene (6.7-15.6%) as major constituents. The leaves stored in powdered form contained higher percentages of oxygenated monoterpenoids (71.7-83.7%) compared to those in leaves that were kept in the shade (63.3-70.5%) and in the sun (65.7-67.4%). The study suggested that rosemary leaves should be dried in the shade and stored as such for better yield of quality essential oil.

Keywords: essential oil, rosemary, *Rosmarinus officinalis* cv. CIM-Hariyali

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.; Lamiaceae) is a spice and medicinal herb widely used around the world. It is mainly produced in Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa [1]. Rosemary is used since antiquity in food, cosmetics, medicinal and pharmaceutical products [2]. The leaves of rosemary are used in foodstuffs, especially for the control of microbial infections [3-5]. The leaves are also reported to be an antioxidant due to the presence of rosmarinic acid, carnosol, carnosic acid and caffeic acid [6-8]. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity [9]. The essential oil of rosemary has been reported to be a tonic stimulant and is used as a pulmonary antiseptic, a choleric, a colagogic, and also shows stomachic, antidiarrhoeal and antirheumatic properties [10].

Several studies on the chemical composition of the essential oils of *R. officinalis* L. from different geographic origins have been performed. A survey of the literature reveals that there are mainly three chemotypes: a 1,8-cineole chemotype from France, Greece, Italy and Tunisia; a camphor-borneol chemotype from Spain; and a α -pinene and verbenone chemotype from Corsica and Algeria [10-13]. The Indian rosemary oil is characterised by relative high amounts of 1,8- cineole, camphor and α -pinene [14]. The chemical composition of a plant essential oil generally depends on a number of factors such as heredity, part and age of plant, isolation method, environmental condition, collecting season, dehydration procedure and storage condition under which the collected plant is kept until the essential oil is extracted [11,15-20].

A literature survey revealed that the essential oil composition of rosemary has been investigated in India at few occasions [14, 21-23], but there were no reports on postharvest storage from any part of the country. The aim of this work is therefore to observe the changes that occur in the yield and composition of the essential oil of *R. officinalis* (cv. CIM-Hariyali) leaves and leaf powder during storage.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *R. officinalis* cv. CIM-Hariyali [24] for study was collected from the experimental farm of Central Institute of Medicinal and Aromatic Plants Research Centre, Purara, Uttarakhand, India in the month of May, 2008. The site is located between the coordinates 28° 60' to 31° 29' and 77° 49' to 80° 60' E at an altitude of 1250m in Kattiyur valley. Climatologically, the site falls in the temperate zone of western Himalaya, with the monsoon usually breaking in June and continuing up to September. A total 60 random samples, weighing 100 g each (fresh weight basis) were divided in to 20 batches of three samples/batch. The first batch was distilled on the day of harvesting. Ten batches were kept in shade and the rest were kept under the sun. Observations, viz. moisture loss, oil yield, oil composition, etc., were taken for up to 30 days for under-sun (open) samples and up to one year for under-shade samples. In addition to this, shade-dried leaves were powdered with an electric grinder and stored under shade at room temperature and observations pertaining to oil yield and composition were taken at regular intervals for up to 270 days.

Isolation of Essential Oil

The essential oil was isolated by hydro-distillation for 3 hours using a Clevenger type apparatus. The oil content (% v/w) of leaves and leaf powder was estimated on the fresh weight basis and dry weight basis respectively. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analysis.

GC and GC-MS

The gas chromatographic analysis of the oil samples was carried out on a Nucon gas chromatograph model 5765 equipped with FID and BP-20 (30m×0.25mm; film thickness 0.25 µm) fused silica capillary column, and on a Perkin-Elmer Auto XL GC equipped with FID and PE-5 (60m×0.32mm; film thickness 0.25 µm) fused silica capillary column. Hydrogen was the carrier gas at 1.0 mL/min. Temperature programming was 70-230°C at 4°C/min with initial and final hold time of 2 min (for BP-20) and 70-250°C at 3°C/min (for PE-5). Split ratio was 1:30. The injector and detector temperatures were 200°C and 230°C on BP-20 column, and 220°C and 300°C on PE-5 column respectively. GC-MS was performed on a Perkin Elmer Auto System XL GC and Turbo mass spectrometer fitted with a PE-5 fused silica capillary column (50 m x 0.32 mm; film thickness 0.25 µm). The column temperature programme was 100-280°C at 3°C/min, using helium as carrier gas at a constant pressure of 10 psi. MS conditions were: EI mode 70 eV, ion source temperature 250°C.

Identification of Compounds

Compounds identification was done on the basis of retention time, retention indices, MS library search (NIST & WILEY), *n*-alkane (C₉-C₂₂) hydrocarbons pattern (Nile, Italy) and by comparing mass spectra with the MS literature data [25-26]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Statistical Analysis

The data of oil content were subjected to statistical analysis following analysis of variance (ANOVA) technique as applicable to randomised block design [27]. The significance of treatments variance was tested with variance (F) ratio at 5.0% probability level.

RESULTS AND DISCUSSION

Essential Oil Content

The changes in moisture level, essential oil content and chemical composition during post harvest storage of rosemary leaves and leaves powder are given in Tables 1-4. The essential oil content of the unpowdered leaves was not affected significantly by postharvest storage. It was found to vary from 1.2% to 1.05% and 1.12% to 1.1% in leaves stored under shade and sun respectively, while the essential oil content in fresh leaves was 1.3 %. Maximum oil loss was noticed on the second day (48 hours) under shade (15.4% loss) and on the first day (24 hours) under sun (15.4 % loss). Thereafter the oil content (1.1%) was virtually unchanged under both conditions. The leaves stored for up to 1 year under shade recorded 19.2% oil loss. On the other hand, the leaves stored in powdered form showed a substantial oil loss (about 59% after 270 days).

Table 1. Changes in essential oil content of rosemary (*Rosmarinus officinalis*) during storage

Storage period (no. of days)	Oil content (A), oil loss (B) and moisture loss (C) (%)								
	Leaves stored under shade			Leaves stored under sun			Leaves stored in powdered form		
	A	B	C	A	B	C	A	B	
0	1.3	-	-	-	-	-	2.70	-	
1	1.2	7.7	47.3	1.12	13.84	51.30	-	-	
2	1.1	15.4	60.1	1.1	15.38	63.76	-	-	
3	1.1	15.4	65.4	1.1	15.38	65.54	-	-	
4	1.1	15.4	65.6	1.1	15.38	65.58	-	-	
5	1.1	15.4	65.7	1.1	15.38	65.66	-	-	
6	1.1	15.4	65.7	1.1	15.38	65.72	-	-	
7	1.1	15.4	65.8	1.1	15.38	65.78	-	-	
15	1.1	15.4	65.8	1.1	15.38	65.80	-	-	
30	1.1	15.4	65.8	1.1	15.38	66.00	1.90	29.63	
90	1.1	15.4	65.8	-	-	-	1.85	31.48	
150	1.1	15.4	65.8	-	-	-	1.50	44.44	
210	1.1	15.4	65.8	-	-	-	1.33	50.74	
270	1.1	15.4	65.8	-	-	-	1.10	59.26	
360	1.05	19.2	65.8	-	-	-	-	-	
CD=0.05	NS	-	-	NS	-	-	0.14	-	

Note: CD = Critical difference; NS = Non-significant

Essential Oil Composition

Of a total of 31 components identified (Table 2), the major components in the essential oil from fresh leaves were camphor (23.91%), 1,8-cineole (22.36%), α -pinene (11.45%), camphene (5.82%), verbenone (5.81%), α -terpineol + borneol (4.26%), β -pinene (4.05%), bornyl acetate (2.67%), myrcene (2.37%), limonene (2.16%) and linalool (1.11%). Most of the components were found to be affected by postharvest storage/drying. Amounts of α -pinene, camphene and β -pinene were reduced substantially after 1 day of storage. However, concentrations of α -pinene and camphene were observed to increase after 90 days while that of β -pinene, after 4 days of storage under shade. Under sun, the concentrations of α -pinene and camphene were maximal after 30 days of storage while that of β -pinene, after 2 days of storage (Table 3). The concentrations of camphor and 1,8-cineole were observed to be generally higher in stored leaves than in fresh ones under both the conditions. The concentration of verbenone was highest after 1 day of storage in the shade and 3 days of storage in the sun (Tables 2-3).

The oil composition of the powdered leaves (Table 4) was quite different from the unpowdered ones. Overall, the former contained a higher percentage of oxygenated monoterpenoids (71.7-83.7%) than those in the latter (63.3-70.5% and 65.7-67.4% for shade-dried and sun-dried leaves respectively), probably due to a more favourable formation of oxidation products during postharvest storage. The amount of monoterpene hydrocarbons, however, was found to be highest in shade-dried leaves (19.7-31.1%) and lowest in powdered leaves (14.3-18.2%).

In general, most of the changes in essential oil composition of aromatic plants occur during the early hours of storage (initial 12-24 hours). These changes may be due to some physiological processes that continue even after the harvesting of the plant material and/or due to loss of some molecular constituents as the oil glands start to deteriorate on storage. Once the tissues become dried, the changes

Table 2. Changes in essential oil composition of rosemary (*Rosmarinus officinalis*) leaves stored under shade

Compound (%) *	Number of days of storage															
	0	1	2	3	4	5	6	7	15	30	90	150	210	270	360	
<i>α</i> -Pinene	11.4	6.72	11.1	11.4	11.6	12.1	10.6	11.7	11.3	10.6	15.6	13.7	13.5	15.5	11.1 9	
Camphene	5.82	3.20	4.91	5.12	5.29	5.13	4.53	4.95	4.70	4.45	6.3	5.9	5.6	6.1	6.05	
<i>β</i> -Pinene	4.05	3.10	4.12	3.97	4.32	4.06	3.77	4.15	3.26	2.85	2.2	1.1	0.4	0.2	3.33	
Sabinene	t	-	-	-	-	-	-	-	-	-	t	t	t	-	-	
Myrcene	2.37	2.28	2.39	2.35	2.84	2.58	2.75	3.20	2.45	2.71	2.4	1.5	1.4	1.7	1.53	
<i>α</i> -Terpinene	0.31	0.29	0.22	0.29	0.61	0.22	0.55	t	0.32	0.71	0.4	0.2	0.4	0.6	0.24	
Limonene	2.16	2.90	2.92	2.81	2.78	2.68	2.63	2.85	2.62	3.03	2.7	2.4	2.4	2.8	2.48	
1,8-Cineole	22.3	21.4	25.3	24.5	24.6	24.5	24.5	26.3	24.0	24.9	26.6	27.8	28.4	27.0	24.4 4	
<i>β</i> -Phellandrene	t	-	t	-	0.27	t	0.23	t	t	0.37	-	t	t	-	-	
(<i>Z</i>)- <i>β</i> -Ocimene	0.67	0.78	0.72	0.77	0.98	0.85	0.83	0.77	0.91	0.98	0.8	0.4	0.4	0.4	0.57	
<i>γ</i> -Terpinene	0.18	0.13	0.11	0.10	0.09	0.11	t	0.11	0.11	t	t	t	t	t	0.13	
<i>p</i> -Cymene	0.32	0.31	0.36	0.37	0.40	0.45	0.41	0.33	0.51	0.54	0.7	0.8	1.0	1.4	0.90	
1-Octen-3-ol	0.33	0.11	0.24	0.24	0.23	0.28	0.20	0.49	0.24	0.21	t	t	0.1	0.1	0.31	
(<i>E</i>)-Sabinene hydrate	0.46	0.55	0.68	0.83	0.69	0.72	0.66	0.77	0.54	0.39	t	t	0.1	0.1	0.40	
Camphor	23.9	26.5	24.9	24.3	24.1	24.4	24.4	24.8	24.8	25.0	25.1	28.7	28.4	26.5	28.5 7	
Chrysanthenone	0.26	0.32	0.27	0.26	0.25	0.25	0.27	0.24	0.25	0.24	0.1	-	-	-	0.28	
Linalool	1.11	1.60	1.17	1.18	1.10	1.20	1.14	1.26	1.08	1.03	0.8	0.5	0.6	0.5	0.76	
Linalyl acetate	0.39	0.50	0.47	0.46	0.40	0.42	0.45	0.41	0.41	0.37	0.1	-	t	-	0.30	
Bornyl acetate	2.67	3.53	2.36	2.53	2.42	2.46	2.55	2.18	2.37	2.30	2.0	1.9	1.9	1.6	0.81	
<i>β</i> -Caryophyllene	0.75	1.41	0.86	1.24	1.00	1.18	1.21	1.00	1.38	1.09	1.2	0.3	0.8	0.9	0.30	
Terpinen-4-ol	0.82	0.82	0.76	0.96	0.66	0.74	0.77	1.46	0.68	0.81	0.6	0.5	0.4	0.4	0.97	
<i>α</i> -Humulene	0.45	0.82	0.68	0.58	0.71	0.82	0.85	0.71	0.80	0.62	0.8	0.1	0.4	0.5	0.63	
<i>α</i> -Terpineol+borneol	4.26	5.50	4.35	4.09	4.28	4.39	4.44	3.78	4.40	4.05	3.8	6.4	5.9	6.0	6.83	
Verbenone	5.81	7.26	4.80	5.19	3.96	5.25	5.44	3.23	5.71	4.58	6.3	4.2	4.5	4.1	4.79	
Citronellol	t	0.32	0.19	0.16	0.14	0.18	0.14	0.12	0.16	t	t	t	t	t	0.11	
Myrtenol	0.14	0.19	0.13	0.53	0.21	0.12	0.13	0.31	0.16	0.11	t	t	t	t	0.17	
Geraniol	0.25	0.64	0.17	t	t	0.10	t	t	0.60	0.17	-	t	t	0.2	0.22	
Caryophyllene oxide	t	0.45	0.31	t	0.17	0.26	t	t	0.20	0.90	-	0.1	0.1	0.1	0.10	
Methyl eugenol	-	t	t	t	t	t	t	t	t	-	-	0.1	0.1	t	t	
Eugenol	t	0.24	0.84	0.18	0.23	0.28	0.21	0.21	0.40	0.86	0.2	0.1	0.1	0.1	0.39	
Class composition																
Monoterpene hydrocarbons	27.3	19.7	26.9	27.2	29.2	28.2	26.3	28.1	26.2	26.2	31.1	26.0	25.1	28.7	26.4 2	
Oxygenated monoterpenes	62.7	69.5	66.4	65.4	63.3	65.3	65.4	66.2	65.9	65.1	65.5	70.2	70.5	66.6	69.3 5	
Sesquiterpene hydrocarbons	1.2	2.23	1.54	1.82	1.71	2	2.06	1.71	2.18	1.71	t	0.1	0.1	0.1	0.93	
Oxygenated sesquiterpenes	t	0.45	0.31	t	0.17	0.26	t	t	0.20	0.90	2.0	0.4	1.2	1.4	0.10	
Total identified (%)	91.3 0	91.9 7	95.1 7	94.5 1	94.4 1	95.8 9	93.9 0	96.0 9	94.5 1	93.9 9	98.6 0	96.7 0	96.9 0	96.8 0	96.8 0	

* Mode of identification: RI (retention index based on homologous series of *n*-alkanes: C₈-C₂₄), co-injection with standards compounds, and MS (GC-MS)

Note: t = trace (<0.10%)

Table 3. Changes in essential oil composition of rosemary (*Rosmarinus officinalis*) leaves stored in open (sun) condition

Compound (%)	Number of days of storage									
	1	2	3	4	5	6	7	15	30	
α -Pinene	11.02	11.49	10.59	10.80	10.53	11.35	11.43	11.30	12.11	
Camphene	4.82	5.08	4.72	4.70	4.52	4.89	4.82	4.77	5.17	
β -Pinene	3.92	4.15	3.63	3.89	3.88	3.78	4.00	3.48	3.44	
Myrcene	2.44	2.32	2.29	2.40	2.36	2.26	2.37	2.29	2.44	
α -Terpinene	0.51	0.17	0.25	0.25	0.51	0.17	0.21	0.21	0.31	
Limonene	2.89	2.93	2.89	2.81	2.67	2.71	2.93	2.67	3.01	
1,8-Cineole	23.68	24.70	22.87	23.60	24.92	23.61	27.07	24.32	26.03	
β -Phellandrene	-	t	-	t	0.28	t	t	t	t	
(Z)- β -Ocimene	0.72	0.71	0.70	0.77	0.75	0.82	0.76	0.88	0.95	
γ -Terpinene	t	t	0.18	0.11	0.10	0.11	0.11	0.10	0.12	
<i>p</i> -Cymene	0.33	0.41	0.36	0.41	0.41	0.37	0.41	0.46	0.61	
1-Octen-3-ol	0.24	0.24	0.42	0.91	1.05	0.29	0.25	0.88	0.25	
(E)-Sabinene hydrate	0.70	0.72	1.08	0.74	0.75	0.67	0.73	0.55	0.17	
Camphor	25.16	25.67	23.09	25.00	24.68	24.49	26.08	25.87	25.14	
Chrysanthenone	0.25	0.27	0.28	0.24	0.25	0.26	0.26	0.25	0.21	
Linalool	1.22	1.19	1.11	1.20	1.18	1.04	1.21	1.10	1.00	
Linalyl acetate	0.34	0.33	0.37	0.47	0.40	0.35	0.30	0.41	0.27	
Bornyl acetate	2.60	2.54	2.43	2.50	2.38	2.40	2.01	2.24	2.05	
β -Caryophyllene	1.10	0.85	1.10	1.09	1.32	1.22	0.83	0.98	0.87	
Terpinen-4-ol	0.78	0.80	0.77	0.76	0.78	0.65	0.68	0.79	0.78	
α -Humulene	0.59	0.72	0.42	0.82	0.81	0.78	0.58	0.71	0.64	
α -Terpineol+borneol	4.51	4.56	3.77	4.39	4.20	4.85	4.03	4.90	4.13	
Verbenone	5.87	4.91	7.19	5.92	5.49	6.34	3.61	5.26	4.86	
Citronellol	0.13	0.13	t	0.12	0.13	0.15	t	0.13	0.12	
Myrtenol	0.20	0.22	0.32	0.31	0.41	0.27	t	0.48	0.31	
Geraniol	0.26	t	0.11	t	t	t	t	t	t	
Caryophyllene oxide	0.55	0.50	t	0.22	0.44	0.44	0.38	0.40	0.13	
Methyl eugenol	t	t	-	t	t	t	t	t	t	
Eugenol	0.20	0.78	1.96	0.18	0.25	0.70	0.62	0.29	0.54	
Class composition										
Monoterpene hydrocarbons	26.65	27.26	25.61	26.14	26.01	26.46	27.04	26.16	28.16	
Oxygenated monoterpenes	66.14	67.06	65.77	66.34	66.87	66.07	66.85	67.47	65.86	
Sesquiterpenes hydrocarbons	1.69	1.57	1.52	1.91	2.13	2	1.41	1.69	1.51	
Oxygenated sesquiterpenes	0.55	0.50	0	0.22	0.44	0.44	0.38	0.40	0.13	
Total identified (%)	95.03	96.39	92.9	94.61	95.45	94.97	95.68	95.72	95.66	

Note: t = trace (<0.10%)

Table 4. Changes in the essential oil composition of rosemary (*Rosmarinus officinalis*) leaves stored in powdered form

Compound (% peak area)	RI	Number of days of storage				
		30	90	150	210	270
α -Pinene	1026	9.1	9.8	9.6	8.5	7.4
Camphene	1065	4.0	4.3	4.1	3.6	3.5
β -Pinene	1105	1.4	1.3	0.3	0.1	0.3
Sabinene	1119	t	0.1	-	-	0.4
Myrcene	1158	0.9	1.1	0.2	0.3	0.9
α -Terpinene	1177	0.2	0.5	t	0.1	0.6
Limonene	1194	1.1	t	0.2	0.9	1.3
1,8-Cineole	1204	29.5	27.9	31.6	27.8	24.7
β -Phellandrene	1206	-	t	-	t	0.1
(Z)- β -Ocimene	1234	0.6	0.7	0.1	0.2	0.6
γ -Terpinene	1240	-	-	t	-	0.2
p-Cymene	1271	0.3	0.4	0.4	0.6	1.1
1-Octen-3-ol	1411	t	t	t	t	0.6
(E)-Sabinene hydrate	1463	t	t	t	t	0.7
Camphor	1507	31.2	29.0	35.8	35.0	30.1
Chrysanthenone	1512	0.1	0.2	-	-	-
Linalool	1550	1.0	0.9	0.4	0.4	0.5
Linalyl acetate	1561	0.1	0.2	t	t	t
Bornyl acetate	1585	2.6	2.5	2.2	2.2	2.4
β -Caryophyllene	1594	1.3	1.8	0.3	0.7	0.9
Terpinen-4-ol	1606	0.9	0.9	0.7	0.4	1.1
α -Humulene	1670	0.5	1.3	0.1	0.4	0.6
α -Terpineol+borneol	1682	5.4	5.8	7.3	9.1	6.8
Verbenone	1702	7.8	7.9	5.6	5.1	4.6
Citronellol	1778	t	0.1	-	0.2	0.1
Myrtenol	1792	t	0.1	-	0.1	t
Geraniol	1848	-	t	-	0.2	0.1
Caryophyllene oxide	1995	-	t	t	t	t
Methyl eugenol	2132	t	0.1	t	t	-
Eugenol	2192	0.2	0.3	0.1	0.2	t
Class composition						
Monoterpene hydrocarbons		17.6	18.2	14.9	14.3	16.4
Oxygenated monoterpenes		78.8	75.9	83.7	80.7	71.7
Sesquiterpenes hydrocarbons		1.8	3.1	0.4	1.1	1.5
Oxygenated sesquiterpenes		0	t	t	t	t
Total identified (%)		98.2	97.2	99	96.1	89.6

Note: t = trace (<0.10%)

in the essential oil composition are mainly due to loss of molecules from stored biomass. So, if we look at the content of Table 2 again, the major changes in composition are noticed in leaves stored for 1 day (24 hours). Oxygenated monoterpenes (69.5%) dramatically increased when compared to those in the oil of fresh leaves (62.7%). Oxygenated monoterpenes which increased after 1 day were sabinene hydrate, camphor, bornyl acetate, linalool, linalyl acetate, verbenone, α -terpineol + borneol, citronellol, geraniol, myrtenol and caryophyllene oxide. Thus due to increase (or decrease) in some components the relative percentages of other constituents automatically become less (or more). However, the changes notices on long storage are due to loss of oil constituents. Similar variations in essential oil

content and composition have also been noticed in other plant materials [17, 19, 20, 28]. Therefore, the results of the present study reinforce the fact that there are quantitative and qualitative differences in the essential oil of fresh and dried plant materials.

CONCLUSIONS

The essential oil content of shade-dried and sun-dried rosemary leaves were not significantly different and oil loss was less than 20% after 1 year and 1 month respectively. In contrast, oil loss in powdered leaves was quite substantial (nearly 60% after 9 months). The oil composition of all stored leaves seemed to change continuously during storage. The monoterpene hydrocarbons content was lowest whereas the oxygenated monoterpenes content was highest in the powdered leaves compared with unpowdered leaves kept in the shade and sun.

. During the process of shade drying the green colour of the leaves remained unchanged while the sun-dried leaves turned to brown. Rosemary leaves should therefore be dried and stored as such under shade condition. Storage in powdered form should also be avoided in order to prevent excessive oil loss.

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