

Research Article

Impacts of temperature and storage duration on the stability of *Salvia officinalis* and *Satureja hortensis* essential oilsSharareh Najafian^{a*} , Seyedeh Fariba Mohammadian Yasuj^b , Mehdi Hosseinfarabi^b ^a Department of Natural Resources and Environmental Engineering, School of Agriculture, Shiraz University, Shiraz, I. R Iran^b Department of Horticultural Science, Yas. C., Islamic Azad University, Yasuj, I. R. Iran

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ABSTRACT- Environmental storage conditions play a crucial role in determining both the quantity and quality of active compounds in medicinal and aromatic plants. This study aimed to evaluate the impacts of various storage conditions on the stability of essential oil constituents in these plants. A factorial experiment was conducted using a completely randomized design with three replications. The experimental treatments included three storage temperatures: room temperature ($21 \pm 3^\circ\text{C}$), freezer temperature ($-18 \pm 2^\circ\text{C}$), and refrigerator temperature (3°C), combined with four storage durations: T1 (immediately after distillation), T2 (after one month), T3 (after two months), and T4 (after three months). Two Lamiaceae species, *Salvia officinalis* and *Satureja hortensis*, were selected for the experiment. Plant samples were dried in air and shade, and essential oils were extracted via hydrodistillation. The essential oils were then analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The analysis revealed a significant reduction in carvacrol and a notable increase in γ -terpinene in *Satureja hortensis* across all three temperature conditions. In *Salvia officinalis*, key compounds such as cis-thujone, trans-thujone, camphor, 1,8-cineole, α -pinene, and camphene exhibited a generally stable trend. Although some fluctuations in these compounds were observed after three months of storage, the variations across temperature treatments were not statistically significant. In conclusion, room temperature appears to be the most cost-effective storage condition, preserving essential oil stability effectively over a three-month period. This was particularly evident in *Salvia officinalis*, which retained its major constituents under all storage conditions. These findings support the use of these essential oils in the medical, cosmetic, health, and food industries.

INTRODUCTION

In recent years, essential oils have gained increasing validation for their therapeutic applications in medical science, demonstrating effectiveness in treating various human conditions such as migraine (Flores et al., 2021), stroke (Contrada et al., 2021), and depression (Zhang et al., 2021). These oils, extracted from different parts of plants, including leaves, flowers, bark, roots, and fruits, not only contribute to the plant's aroma but also possess distinctive therapeutic properties and energizing effects (Nikolova et al., 2015). The synthesis of aromatic compounds in plants, especially under stress conditions, is a key metabolic response, suggesting that essential oils play a vital role in the plant's defense mechanisms (Jahan et al., 2015). Historically, essential oils have been widely used for their bioactive properties. Their antibacterial, antiviral, antioxidant, and antidiabetic effects are well-documented, along with their potential roles in cancer prevention and chemotherapy support. Additionally, essential oils are extensively utilized in the food industry, particularly for

flavoring and aroma enhancement (Tanu et al., 2016). Summer savory (*Satureja hortensis*), a member of the mint genus, is an annual or perennial herb native to the Eastern Mediterranean and southern Europe. In Iran, Summer savory (Lamiaceae) is one of the most important species of *Satureja*, cultivated across various regions. Its dried aerial parts have long been used in the food industry as a flavoring agent and in Iranian traditional medicine for their carminative, stomachic, antidiarrheal, and diuretic effects (Namayandeh et al., 2017). The plant's main compounds have been employed in the treatment of muscle aches, cramps, nausea, infectious diseases, and diarrhea (Tepe and Cilkiz, 2016). Other reported biological activities of its essential oil include antispasmodic effects, acetylcholinesterase inhibition, lipid peroxidation suppression, free radical scavenging, and macrophage stimulation (Sharifi-Rad et al., 2018). Summer savory essential oil is also used in the canning and beverage industries, largely due to its strong aroma, which results from its volatile and aromatic oil content (Tepe and Cilkiz, 2016). Sage, one of the most widely used plants in the

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Lamiaceae family, is a well-known and potent source of essential oils in traditional medicine (Kulak et al., 2020). Numerous studies have focused on the plant's effects on cognitive function, particularly memory enhancement and the treatment of Alzheimer's disease. These effects are attributed to its ability to inhibit acetylcholinesterase, an enzyme closely associated with the progression of Alzheimer's disease symptoms (Ghorbani and Esmaeilzadeh, 2017).

Researchers have identified a structural relationship among essential oil components, noting that many compounds can be readily transformed into one another through oxidation, isomerization, cyclization, or dehydrogenation reactions (Can Başer and Buchbauer, 2015) (Fig. 1). Masotti et al. (2003) demonstrated that the structural patterns of medicinal plant compounds are influenced by harvest time, attributing the seasonal decline in hydrocarbon monoterpenes in various essential oils to their conversion into other metabolites. Similarly, Schweiggert et al. (2007) highlighted the significant impact of different storage conditions on the stability of essential oils following distillation. Further studies have shown that essential oil constituents are particularly susceptible to oxidative degradation, chemical alterations, and polymerization reactions (Turek and Stintzing, 2013). In investigating essential oil degradation, factors such as compositional structure, the presence of impurities, and environmental influences, including oxygen exposure, light, and temperature, must be carefully considered. According to Turek and Stintzing (2013), the high vulnerability of essential oils to oxidation and polymerization can adversely affect both their quality and pharmacological efficacy. Despite increasing consumer demand for natural products, limited research has specifically addressed the stability of key compounds such as γ -terpinene in summer savory and *cis*-thujone in sage under varying storage conditions. Given the importance of temperature, light, and oxygen availability in maintaining essential oil stability, this study aims to fill that gap by examining the chemical changes in these essential oils over time under different temperature regimes. Ensuring consumer confidence and maintaining product quality are essential for the successful application of plant essential oils in the pharmaceutical and food industries (Fig. 2). Therefore, this study was designed to assess the effects of various storage temperatures (room temperature, refrigeration, and freezing) and durations (from post-distillation to three months) on the chemical composition of essential oils. The ultimate goal is to determine the optimal storage conditions for preserving the quality and integrity of essential oils in these medicinal plants.

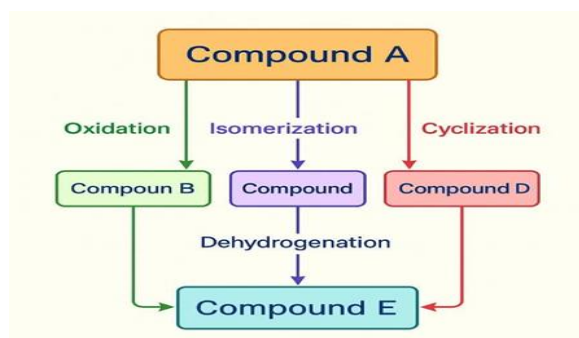


Fig. 1. Possible conversion reactions in essential oils.

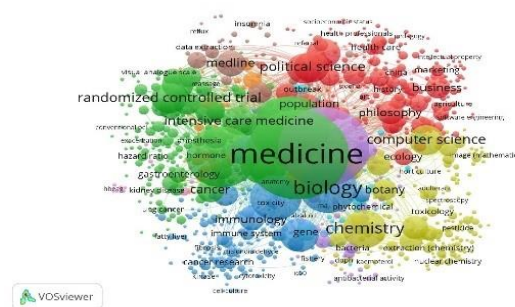


Fig. 2. Co-occurrence analysis of the terms used more than 100 times as author and/or index keywords in horticulture research. The frequency of occurrence is represented by the size of the circle beneath each word. Different colors were employed to depict distinct clusters of highly related keywords, facilitating their categorization. The VOS viewer software was utilized to represent the term cloud, with data collected from the API database.

MATERIALS AND METHODS

Collection of plant samples

Samples of summer savory were collected (2000 g) from Lalehzar in Kerman (29.5245° N, 56.8133° E, nearly 3000 meters above the sea level) and sage were collected (2000 g) from Marvdasht Shiraz (29.52° N, 52.48° E, 1595 m above the sea level). All plants were collected at their peak flowering stage. Identification was performed by Ahmad Hatami, and voucher specimens for summer savory (No. 16453) and sage (No. 14833) were stored in the Herbarium of the Fars Research Center.

Essential oil extraction

The collected plant samples of summer savory and sage were completely dried for 20 days in the shade at room temperature ($21 \pm 3^\circ\text{C}$). To prevent heat buildup and ensure uniform drying, the samples were placed on mesh trays away from direct sunlight, allowing proper air circulation and minimizing thermal degradation of essential oil compounds. The essential oil was extracted by hydrodistillation using a Clevenger type apparatus at a standard time of up to three hours with three repetitions. The essential oils were then collected in special dark jars. The essential oils were dehumidified with dry sodium sulfate (Pharmacopoeia British, 2016).

Different storage conditions of essential oil

A factorial experiment was conducted to investigate the effects of storage conditions and duration on the essential oil composition of summer savory and sage. The study employed a completely randomized design with three replications and included two factors: storage temperature and storage time. Storage temperature was tested at three levels: room temperature ($21 \pm 3^\circ\text{C}$), refrigerator temperature (3°C), and freezer temperature ($-18 \pm 2^\circ\text{C}$). Storage duration included four time points: T1 (immediately after distillation, with no storage, i.e., samples were directly injected into the GC/MS), T2 (after one month of storage), T3 (after two months), and T4 (after three months). Essential oil samples from each plant were stored in dark glass containers under the specified temperature conditions, in a

dark environment, for up to three months. This design allowed for a comprehensive evaluation of the interaction between temperature and storage time on the stability and composition of essential oil compounds.

Essential oil analysis and identification

The essential oils extracted from each treatment were analyzed using two complementary techniques: gas chromatography with flame ionization detection (GC/FID) for quantification, and gas chromatography/mass spectrometry (GC/MS) for compound identification.

Gas Chromatography/Flame Ionization Detector (GC/FID)

Quantitative analysis of essential oil components was carried out using gas chromatography (GC) equipped with a flame ionization detector (FID). An Agilent 7890A gas chromatograph (Agilent Technologies, USA) fitted with an HP-5 capillary column (30 m length $\times$ 0.32 mm internal diameter $\times$ 0.25 μ m film thickness) was used. The oven temperature was programmed to increase from 60 °C to 210 °C at a rate of 3 °C per minute. The injection port temperature was maintained at 280 °C, and detection was performed at 290 °C using nitrogen as the carrier gas at a constant flow rate of 1 mL/min. The relative percentage of each compound was calculated based on the area under its respective peak in the chromatogram.

Gas Chromatography/Mass Spectrometry (GC/MS)

For qualitative identification, essential oils were analyzed using gas chromatography–mass spectrometry (GC/MS). The system consisted of an Agilent 7890A gas chromatograph coupled with a 5975A mass selective detector (Agilent Technologies, USA), equipped with an HP-5MS column (30 m length $\times$ 0.25 mm internal diameter $\times$ 0.25 μ m film thickness). The oven temperature was initially increased from 60 °C to 210 °C at 3 °C/min, followed by a rapid increase to 240 °C at 20 °C/min. The injection port was held at 280 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. Electron ionization (EI) was conducted at 70 eV with an ion source temperature of 230 °C. Compound identification was achieved by comparing retention times, retention indices, and mass spectral fragmentation patterns with those available in the NIST, Wiley, and Adams (2001) spectral libraries.

Statistical analysis

The study employed a factorial experimental design with three replications, arranged in a completely randomized layout. Data analysis was conducted using SAS software (version 9.1.2). Mean comparisons were performed using Duncan's multiple range test at the 5% significance level. Pearson's correlation analysis was used to evaluate relationships among variables, and graphical illustrations were generated using Prism software (version 9).

RESULTS AND DISCUSSION

Given the significance of plant secondary metabolites and their varying behavior under different storage conditions, as well as the species-specific responses of medicinal plants, the storage stability of essential oils has attracted considerable research interest. In this study, the essential oil

profiles of two Lamiaceae species, summer savory and sage, were examined to assess the effects of storage temperature and a three-month storage period.

Satureja hortensis (summer savory)

Analysis of summer savory essential oil identified 40 medicinal compounds (Fig. 3), with the major constituents being carvacrol (50.7%), γ -terpinene (34.4%), p-cymene (3.2%), α -terpinene (3.7%), myrcene (1.9%), α -thujene (1.2%), and α -pinene. These plants were cultivated in the Kerman region of Iran (Fig. 4, Table 1, Table 2, and Table 3). The majority of the compounds were monoterpenoids, comprising 99.3% of the essential oil. Only two sesquiterpenes, i.e., β -bisabolone and viridiflorol, were detected, representing a small fraction of 0.4% of the oil (Table 1, Table 2, and Table 3).

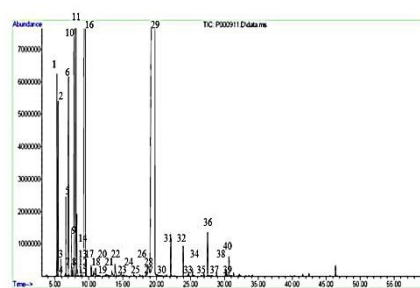


Fig. 3. GC/MS chromatograph of summer savory after hydrodistillation. Peak identification: 1. α -Thujene; 2. α -Pinene; 3. Camphene; 4. Sabinene; 5. β -Pinene; 6. Myrcene; 7. 3-Octanol; 8. α -Phellandrene; 9. p-Mentha-1(7),8-diene; 10. α -Terpinene; 11. p-Cymene; 12. Limonene; 13. 1,8-Cineole; 14. (Z)- β -Ocimene; 15. (E)- β -Ocimene; 16. γ -Terpinene; 17. cis-Sabinene hydrate; 18. Terpinolene; 19. trans-Sabinene hydrate; 20. Unknown; 21. Camphor; 22. Borneol; 23. Terpinen-4-ol; 24. α -Terpineol; 25. trans-Dihydro-Carvone; 26. Carvacrol methyl ether; 27. Unknown; 28. Thymol; 29. Carvacrol; 30. Eugenol; 31. Carvacrol acetate; 32. (E)-Caryophyllene; 33. Aromadendrene; 34. α -Humulene; 35. Viridiflorene; 36. β -Bisabolene; 37. Unknown; 38. Spathulenol; 39. Caryophyllene oxide; 40. Viridiflorol.

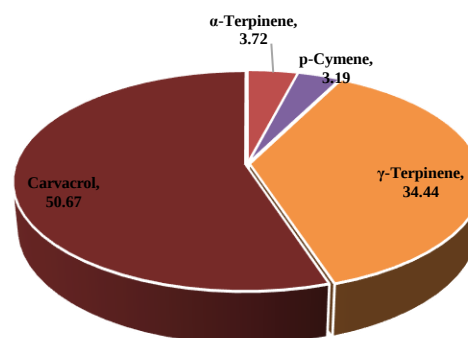


Fig. 4. Percentage of the main compounds of summer savory.

Table 1. Composition of summer savory essential oil during 3 months storage at room temperature

No.	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)	Identification method***
1	α -Thujene	925	8.61	1.16	1.23	1.18	1.26	MS, R
2	α -Pinene	933	8.85	1.07	1.12	1.09	1.16	MS, R
3	Camphene	949	9.33	0.10	0.11	0.10	0.11	MS, R
4	β -Pinene	978	10.22	0.56	0.57	0.56	0.59	MS, R
5	Myrcene	989	10.57	1.88	1.95	1.87	1.98	MS, R, CS
6	α -Phellandrene	1003	11.00	0.32	0.33	0.31	0.32	MS, R
7	p-Mentha-1(7),8-diene	1006	11.10	0.07	0.07	0.07	0.08	MS, R
8	α -Terpinene	1018	11.57	3.72	3.84	3.61	3.72	MS, R, CS
9	p-Cymene	1024	11.80	3.20	3.52	3.69	4.05	MS, R, CS
10	Limonene	1029	12.00	0.52	0.53	0.51	0.54	MS, R
11	1,8-Cineole	1032	12.10	0.06	0.09	0.13	0.06	MS, R
12	(E)-b-Ocimene	1047	12.65	0.10	-	-	-	MS, R
13	γ -terpinene	1060	13.15	34.44	35.79	34.37	35.77	MS, R, CS
14	cis-Sabinene hydrate	1070	13.52	0.05	0.07	0.06	0.06	MS, R
15	Terpinolene	1090	14.28	0.09	0.08	0.08	0.08	MS, R
16	trans-Sabinene hydrate	1099	14.60	0.08	-	-	-	MS, R
17	Borneol	1169	17.50	0.11	-	-	-	MS, R
18	Terpinen-4-ol	1178	17.85	0.21	-	-	-	MS, R
19	α -Terpineol	1190	18.35	0.06	-	-	-	MS, R
20	Thymol	1290	22.50	0.26	0.36	0.25	0.34	MS, R
21	Carvacrol	1299	22.90	50.68	49.43	51.14	49.02	MS, R, CS
22	Carvacrol acetate	1373	25.90	0.17	0.16	0.18	0.16	MS, R
23	(E)-Caryophyllene	1423	27.88	0.23	0.23	0.22	0.21	MS, R
24	α -Humulene	1455	29.10	0.06	-	-	-	MS, R
25	Viridiflorene	1496	30.70	0.05	-	-	-	MS, R
26	β -Bisabolene	1506	31.10	0.31	0.30	0.31	0.29	MS, R
27	Viridiflorol	1598	34.49	0.08	0.13	0.09	0.09	MS, R
Total				99.65%	99.91	99.84%	99.91%	

Grouped components (%)

Monoterpene (Sr. No. 1- 22, 98.92%)

Sesquiterpene (Sr. No. 23-27, 0.73%)

The specific essential oil constituent was identified through the GC/MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley, and Adams; CS, Commercial standard.

Table 2. Composition of summer savory essential oil during 3 months storage at refrigerator temperature

No.	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)	Identification method***
1	α -Thujene	925	8.61	1.16	1.23	1.35	1.26	MS, R
2	α -Pinene	933	8.85	1.07	1.11	1.22	1.15	MS, R
3	Camphene	949	9.33	0.10	0.10	0.12	0.11	MS, R
4	β -Pinene	978	10.22	0.56	0.56	0.60	0.58	MS, R
5	Myrcene	989	10.57	1.88	1.94	2.08	2.01	MS, R, CS
6	α -Phellandrene	1003	11.00	0.32	0.34	0.35	0.33	MS, R
7	p-Mentha-1(7),8-diene	1006	11.10	0.07	0.07	0.08	0.07	MS, R
8	α -Terpinene	1018	11.57	3.72	3.85	3.97	3.83	MS, R, CS
9	p-Cymene	1024	11.80	3.20	3.49	3.76	3.91	MS, R, CS
10	Limonene	1029	12.00	0.52	0.55	0.57	0.55	MS, R
11	1,8-Cineole	1032	12.10	0.06	0.10	0.11	0.07	MS, R
12	(E)-b-Ocimene	1047	12.65	0.10	-	-	-	MS, R
13	γ -terpinene	1060	13.15	34.44	35.63	36.77	36.50	MS, R, CS
14	cis-Sabinene hydrate	1070	13.52	0.05	-	-	-	MS, R
15	Terpinolene	1090	14.28	0.09	0.09	0.09	0.08	MS, R
16	trans-Sabinene hydrate	1099	14.60	0.08	-	-	-	MS, R
17	Borneol	1169	17.50	0.11	-	-	-	MS, R
18	Terpinen-4-ol	1178	17.85	0.21	0.22	0.21	0.21	MS, R
19	α -Terpineol	1190	18.35	0.06	-	-	-	MS, R
20	Thymol	1290	22.50	0.26	0.38	0.22	0.19	MS, R
21	Carvacrol	1299	22.90	50.68	49.20	47.67	48.40	MS, R, CS
22	Carvacrol acetate	1373	25.90	0.17	0.15	0.15	0.15	MS, R
23	(E)-Carvophyllene	1423	27.88	0.23	0.23	0.22	0.21	MS, R
24	α -Humulene	1455	29.10	0.06	-	-	-	MS, R
25	Viridiflorene	1496	30.70	0.05	-	-	-	MS, R
26	β -Bisabolene	1506	31.10	0.31	0.30	0.30	0.29	MS, R
27	Viridiflorol	1598	34.49	0.08	0.04	0.09	0.09	MS, R
Total				99.64%	99.60%	99.93%	99.99%	
Grouped components (%)								
Monoterpene (Sr. No. 1- 22, 98.92%)								
Sesquiterpene (Sr. No. 23-27, 0.73%)								

The specific essential oil constituent was identified through the GC/MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley, and Adams; CS, Commercial standard.

Table 3. Composition of summer savory essential oil during 3 months storage at freezer temperature

No.	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)	Identification method***
1	α -Thujene	925	8.61	1.16	1.28	1.26	1.25	MS, R
2	α -Pinene	933	8.85	1.07	1.16	1.14	1.13	MS, R
3	Camphene	949	9.33	0.10	0.11	0.11	0.11	MS, R
4	β -Pinene	978	10.22	0.56	0.60	0.58	0.58	MS, R
5	Myrcene	989	10.57	1.88	2.00	1.99	1.96	MS, R, CS
6	α -Phellandrene	1003	11.00	0.32	0.33	0.33	0.33	MS, R
7	p-Mentha-1(7),8-diene	1006	11.10	0.07	0.08	0.07	0.07	MS, R
8	α -Terpinene	1018	11.57	3.72	3.99	3.92	3.85	MS, R, CS
9	p-Cymene	1024	11.80	3.20	3.46	3.38	3.36	MS, R, CS
10	Limonene	1029	12.00	0.52	0.58	0.54	0.53	MS, R
11	1,8-Cineole	1032	12.10	0.06	0.07	0.05	0.05	MS, R
12	(E)-b-Ocimene	1047	12.65	0.10	-	-	-	MS, R
13	γ -terpinene	1060	13.15	34.44	37.02	36.17	35.60	MS, R, CS
14	cis-Sabinene hydrate	1070	13.52	0.05	0.06	0.06	0.06	MS, R
15	Terpinolene	1090	14.28	0.09	0.09	0.08	0.08	MS, R
16	trans-Sabinene hydrate	1099	14.60	0.08	-	-	-	MS, R
17	Borneol	1169	17.50	0.11	-	-	-	MS, R
18	Terpinen-4-ol	1178	17.85	0.21	0.22	0.21	0.19	MS, R
19	α -Terpineol	1190	18.35	0.06	-	-	-	MS, R
20	Thymol	1290	22.50	0.26	0.21	0.21	0.31	MS, R
21	Carvacrol	1299	22.90	50.68	47.80	48.92	49.67	MS, R, CS
22	Carvacrol acetate	1373	25.90	0.17	0.15	0.14	0.15	MS, R
23	(E)-Caryophyllene	1423	27.88	0.23	0.22	0.22	0.21	MS, R
24	α -Humulene	1455	29.10	0.06	0.05	0.10	0.05	MS, R
25	Viridiflorene	1496	30.70	0.05	-	-	-	MS, R
26	β -Bisabolene	1506	31.10	0.31	0.29	0.29	0.28	MS, R
27	Viridiflorol	1598	34.49	0.08	0.07	0.07	0.07	MS, R
Total				99.65%	99.87%	99.84%	99.89%	
Grouped components (%)								
Monoterpene (Sr. No. 1- 22, 98.92%)								
Sesquiterpene (Sr. No. 23-27, 0.73%)								

The specific essential oil constituent was identified through the GC/MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley, and Adams; CS, Commercial standar.

Among the detected monoterpenes, α -pinene exhibited a relatively stable profile across all storage conditions. Although slight fluctuations in concentration were noted, the differences were not statistically significant after three months of storage. The content of myrcene showed minimal variation under different temperature and duration settings. Its concentration remained steady, suggesting that myrcene is less susceptible to oxidative or thermal degradation during storage. α -Terpinene demonstrated a consistent trend in all tested storage treatments. The compound's stability indicates a degree of resistance to breakdown under ambient and refrigerated conditions over the study period. No significant changes were observed in the α -Thujene content across varying storage environments. This stability further supports the assumption that this component maintains its integrity over at least a three-month storage duration. After one to three months of storage, carvacrol content decreased by 48.81% to 49.02%, compared to the initial value of 50.67% with no storage. Over time, the highest carvacrol level was observed at room temperature after three months (49.86%), which, in some cases, did not differ significantly from the values recorded under refrigeration (Table 1, Table 2, and Table 3). The next important compound was γ -terpinene, a component of the essential oil that was monitored under different storage conditions over time. An increasing trend was observed across all three temperature conditions after three months, with the most pronounced rise occurring under refrigeration and freezing, followed by a notable increase at room temperature. Overall, γ -terpinene content increased from 34.44% to 36.30%. Similar to γ -terpinene, *p*-cymene exhibited an increasing trend under all three temperature conditions after three months of storage. Compared to the initial value with no storage, the amount of *p*-cymene increased by 3.49%, 3.61%, and 3.77% after one, two, and three months, respectively. The greatest increases were observed under refrigeration and at room temperature, where *p*-cymene rose from 3.19% to 3.75%. The correlation heat map of essential oil compounds in summer savory is presented in Fig. 5.

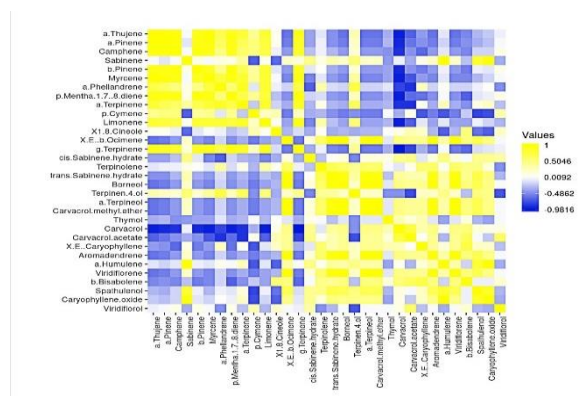


Fig. 5. The correlation heat map of essential oil compounds of summer savory.

Salvia officinalis (sage)

Analysis of sage essential oil revealed 50 medicinal constituents (Fig. 6), with the main compounds in plants grown in the Marvdasht region of Shiraz, Iran, being *cis*-thujone (38.05%), camphor (16.1%), 1,8-cineol (12.0%), *trans*-thujone (5.9%), α -pinene (4.7%), camphene (4.8%), and α -humulene (Fig. 7). Most of the identified compounds

were monoterpenoids, comprising 95.63% of the essential oil. In contrast, only four compounds, caryophyllene oxide, viridiflorol, humulene epoxide, and manool, were sesquiterpene, present in relatively small amounts (3.17%) (Table 4 and Table 5). According to previous studies, the variation in essential oil composition in sage is generally attributed to genetic differences, environmental growing conditions, and the season of harvest (Mot et al., 2022; Rahmani et al., 2019). *Cis*-thujone, the dominant compound in sage essential oil, showed an interesting storage pattern in this study: its content remained stable over three months, with no significant differences observed among the different temperature conditions. Room temperature proving to be the optimal storage condition for these compounds as well. As the composition remained entirely stable at room temperature, a separate table was omitted to avoid unnecessary repetition. This indicates that room temperature is the most economically efficient option for storage. Similarly, other major constituents, including camphor, 1,8-cineol, *trans*-thujone, α -pinene, and camphene, also maintained constant levels after three months, with room temperature proving to be the optimal storage condition for these compounds as well. From an economic standpoint, the ability to store sage essential oil at room temperature for up to three months without energy costs makes it particularly valuable. Furthermore, the stability of its key medicinal compounds supports its potential application in the pharmaceutical, cosmetic, health, and food industries.

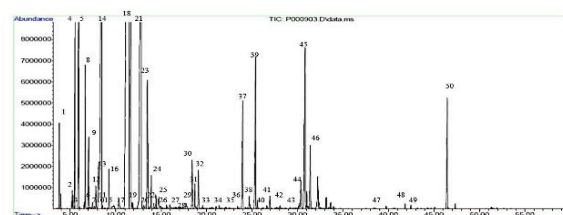


Fig. 6. GC/MS chromatograph of sage after distillation. Peak identification: 1. (Z)-Salvene; 2. Tricyclene; 3. α -Thujene; 4. α -Pinene; 5. Camphene; 6. Thuj-2,4(10)-diene; 7. Sabinene; 8. β -Pinene; 9. Myrcene; 10. α -Phellandrene; 11. α -Terpinene; 12. *p*-Cymene; 13. Limonene; 14. 1,8-Cineole; 15. (Z)- β -Ocimene; 16. *g*-Terpinene; 17. Terpinolene; 18. *cis*-Thujone; 19. *trans*-Thujone; 20. α -Campholenal; 21. Camphor; 22. *trans*-Pinocamphone; 23. Borneol; 24. Terpinen-4-ol; 25. *p*-Cymen-8-ol; 26. α -Terpineol; 27. Myrtenol; 28. *trans*-Carveol; 29. Unknown; 30. Bornyl acetate; 31. Thymol; 32. Carvacrol; 33. *trans*-Carvyl acetate; 34. α -Terpinyl acetate; 35. Eugenol; 36. (Z)-Caryophyllene; 37. (E)-Caryophyllene; 38. Aromadendrene; 39. α -Humulene; 40. allo-Aromadendrene; 41. Viridiflorene; 42. *d*-Cadinene; 43. Spathulenol; 44. Caryophyllene oxide; 45. Viridiflorol; 46. Humulene epoxide II; 47. 6,10,14-trimethyl-2-Pentadecanone; 48. Isopimara-9(11),15-diene; 49. Unknown; 50. Manool.

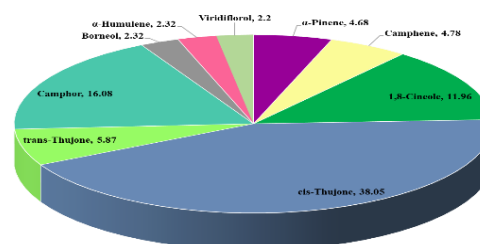


Fig. 7. Percentage of the main compounds of sage.

Table 4. Composition of sage essential oil during 3 months storage at freezer temperature

No.	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)	Identification method***
1	(Z)-Salvene	854	6.89	0.13	0.17	0.07	0.15	MS, R
2	Tricyclene	922	8.53	0.16	0.10	0.13	0.09	MS, R
3	α -Thujene	925	8.61	0.11	0.08	0.11	0.08	MS, R
4	α -Pinene	933	8.87	4.69	4.28	4.01	4.00	MS, R, CS
5	Camphene	949	9.35	4.79	4.41	4.18	4.21	MS, R, CS
6	Sabinene	973	10.08	0.06	1.66	-	-	MS, R
7	β -Pinene	978	10.23	1.79	0.81	1.61	1.64	MS, R, CS
8	Myrcene	989	10.56	0.85	0.06	0.80	0.82	MS, R
9	α -Phellandrene	1006	11.11	0.07	0.26	0.07	0.07	MS, R
10	α -Terpinene	1017	11.54	0.27	0.81	0.26	0.27	MS, R
11	p-Cymene	1025	11.85	0.84	1.66	0.78	0.84	MS, R
12	Limonene	1029	12.00	1.47	1.41	1.38	1.46	MS, R, CS
13	1,8-Cineole	1032	12.10	11.97	11.54	11.38	11.88	MS, R, CS
14	γ -Terpinene	1059	13.10	0.38	0.36	0.36	0.38	MS, R
15	Terpinolene	1090	14.27	0.10	0.12	0.17	0.10	MS, R
16	cis-Thujone	1108	15.00	38.05	38.61	38.52	39.40	MS, R, CS
17	trans-Thujone	1115	15.25	5.88	5.96	5.99	6.19	MS, R, CS
18	Camphor	1148	16.61	16.08	16.52	16.62	16.87	MS, R, CS
19	Borneol	1169	17.50	2.32	2.43	2.45	2.41	MS, R, CS
20	Terpinen-4-ol	1178	17.85	0.33	0.34	0.34	0.34	MS, R
21	α -Terpineol	1190	18.35	0.12	0.05	0.13	0.05	MS, R
22	Myrtenol	1196	18.60	0.16	0.13	0.17	0.13	MS, R
23	Bornyl acetate	1289	22.47	0.45	0.46	0.47	0.45	MS, R
24	Thymol	1290	22.50	0.20	0.21	0.22	0.26	MS, R
25	Carvacrol	1299	22.90	0.25	0.29	0.02	0.29	MS, R
26	(E)-Caryophyllene	1423	27.86	1.39	1.52	1.53	1.47	MS, R, CS
27	Aromadendrene	1440	28.55	0.13	0.17	0.14	0.16	MS, R
28	α -Humulene	1457	29.20	2.32	2.58	2.62	2.48	MS, R, CS
29	allo-Aromadendrene	1460	29.30	0.07	0.11	0.09	0.10	MS, R
30	Viridiflorene	1496	30.70	0.17	0.20	0.19	0.29	MS, R
31	Caryophyllene oxide	1588	34.15	0.22	0.23	0.25	0.14	MS, R
32	Viridiflorol	1595	34.40	2.20	2.55	2.83	2.08	MS, R, CS
33	Humulene epoxide II	1604	34.74	0.11	0.13	0.14	0.11	MS, R
34	Isopimara-9(11),15-diene	1904	45.00	0.04	-	-	-	MS, R
35	Manool	2060	49.40	0.64	0.60	1.05	0.15	MS, R
Total				98.82%	99.17%	99.07%	99.37%	
Monoterpene (%) (Sr. No. 1- 25, 91.51%); Sesquiterpene (%) (Sr. No. 26-35, 7.31 %)								

The specific essential oil constituent was identified through the GC/MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley, and Adams; CS, Commercial standard.

Table 5. Composition of sage essential oil during 3 months storage at refrigerator temperature

No.	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)	Identification method***
1	(Z)-Salvene	854	6.89	0.13	0.12	0.05	0.05	MS, R
2	Tricyclene	922	8.53	0.16	0.10	0.09	0.08	MS, R
3	α -Thuiene	925	8.61	0.11	0.08	0.13	0.07	MS, R
4	α -Pinene	933	8.87	4.69	3.74	3.15	2.24	MS, R, CS
5	Camphene	949	9.35	4.79	3.97	3.46	2.59	MS, R, CS
6	Sabinene	973	10.08	0.06	-	-	-	MS, R
7	β -Pinene	978	10.23	1.79	1.55	1.44	1.17	MS, R, CS
8	Myrcene	989	10.56	0.85	0.78	0.78	0.67	MS, R
9	α -Phellandrene	1006	11.11	0.07	-	-	-	MS, R
10	α -Terpinene	1017	11.54	0.27	0.24	0.24	0.21	MS, R
11	p-Cymene	1025	11.85	0.84	0.81	0.81	0.74	MS, R
12	Limonene	1029	12.00	1.47	1.38	1.35	1.19	MS, R, CS
13	1,8-Cineole	1032	12.10	11.97	11.43	11.33	10.40	MS, R, CS
14	γ -Terpinene	1059	13.10	0.38	0.37	0.37	0.35	MS, R
15	Terpinolene	1090	14.27	0.10	-	-	-	MS, R
16	cis-Thujone	1108	15.00	38.05	39.40	40.45	41.17	MS, R, CS
17	trans-Thujone	1115	15.25	5.88	6.06	6.16	6.35	MS, R, CS
18	Camphor	1148	16.61	16.08	16.81	17.23	18.15	MS, R, CS
19	Borneol	1169	17.50	2.32	2.48	2.55	2.75	MS, R, CS
20	Terpinen-4-ol	1178	17.85	0.33	0.35	0.36	0.38	MS, R
21	α -Terpineol	1190	18.35	0.12	0.13	0.14	0.26	MS, R
22	Myrtenol	1196	18.60	0.16	-	-	-	MS, R
23	Bornyl acetate	1289	22.47	0.45	0.47	0.48	0.53	MS, R
24	Thymol	1290	22.50	0.20	0.21	0.21	0.24	MS, R
25	Carvacrol	1299	22.90	0.25	0.28	0.29	0.35	MS, R
26	(E)-Carvophyllene	1423	27.86	1.39	1.57	1.54	1.70	MS, R, CS
27	Aromadendrene	1440	28.55	0.13	-	-	-	MS, R
28	α -Humulene	1457	29.20	2.32	2.65	2.60	2.90	MS, R, CS
29	allo-Aromadendrene	1460	29.30	0.07	-	-	-	MS, R
30	Viridiflorene	1496	30.70	0.17	0.15	0.14	0.22	MS, R
31	Carvophyllene oxide	1588	34.15	0.22	0.27	0.26	0.28	MS, R
32	Viridiflorol	1595	34.40	2.20	2.72	2.63	3.02	MS, R, CS
33	Humulene epoxide II	1604	34.74	0.11	0.14	0.13	0.16	MS, R
34	Isopimara-9(11),15-diene	1904	45.00	0.04	-	-	-	MS, R
35	Manool	2060	49.40	0.64	0.85	0.76	0.81	MS, R
Total				98.82%	99.11%	99.14%	99.03%	
Grouped components (%)								
Monoterpene								
Sesquiterpene (Sr. No. 26-35, 7.31 %)								

The specific essential oil constituent was identified through the GC/MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley, and Adams; CS, Commercial standard.

The most important findings of the present study revealed that camphor, 1,8-cineol, *trans*-thujone, α -pinene, camphene, and α -humulene exhibited a consistent trend over three months of storage across all three temperature conditions. Another key compound, *cis*-thujone, showed a noteworthy variation pattern. At the time of essential oil extraction, its concentration was 38.05%, which increased to 39.06%, 39.86%, and 39.86% after one, two, and three months, respectively. The most substantial increase was observed under refrigeration. However, no statistically significant differences were found among the three storage temperatures, indicating a high shelf life for *cis*-thujone across all conditions. Fig. 8 presents the correlation heat map of the essential oil constituents in sage. Unlike perishable products such as cream cheese or bread, essential oils do not expire in a predictable manner. When stored properly, they can remain effective for years. However, from the moment a bottle is opened and exposed to air, oxidation begins, a process that gradually alters the chemical composition of the oil. To mitigate this, it is important to store essential oils in dark bottles, away from sunlight, and at room or cool temperatures. Predicting the shelf life of natural products like essential oils is inherently complex due to the numerous influencing factors. Their degradation is slower and less consistent than that of typical perishables, and it largely depends on chemical stability. Any disruption to this stability, especially through exposure to light or oxygen, can initiate gradual degradation. Proper storage, however, significantly enhances the longevity of essential oils. Previous research has shown that oxygen and light are two major environmental factors that influence oxidation during storage (Mohtashami et al., 2018). It is also clear that both temperature and time affect the rate of change in essential oil constituents.

However, compounds that are less stable and interact with other chemical constituents tend to degrade more rapidly (Mohtashami et al., 2018). Among various environmental factors, temperature plays a particularly significant role in preserving the quality and enhancing the storage stability of medicinal compounds (Tan et al., 2006). As storage temperature increases, the quality of essential oil constituents tends to decline (Maalekuu et al., 2006). Determining the precise shelf life of a given essential oil is challenging, as it depends on multiple factors, including the quality and freshness of the plant material, the distillation process, storage conditions, and the level of care in handling. For example, orange essential oil may last only one year if stored improperly, but it can remain stable for over three years when stored correctly and distilled from high-quality plant material (Turek and Stintzing, 2013). Studies conducted in diverse environmental conditions on the essential oils of lavender, pine, rosemary, and thyme have shown that lower temperatures and darker storage environments better preserve monoterpenes. Among the essential oils examined, rosemary showed the highest degree of degradation (Turek and Stintzing, 2012). Research on the storage behavior of essential oils from various plants also highlights the compound-specific nature of their stability. For instance, Kumar et al. (2013) observed that the concentrations of nerol and citronellol in rose

essential oil increased over time. In contrast, the primary constituents of lemon balm, i.e., neral, geranial, and citronellal, declined across all three tested storage conditions (Najafian, 2014). Similarly, in other plant essential oils, compounds such as myrcene, α -pinene, and α -terpinene decreased during storage, while carvacrol and thymol levels increased. Notably, the best preservation of key thyme compounds was observed under freezing conditions (Rowshan et al., 2015).

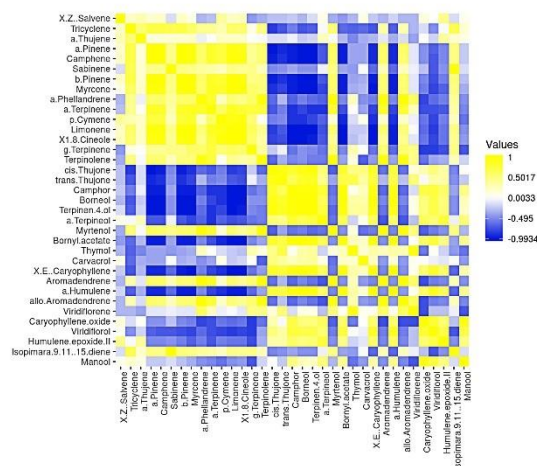


Fig. 8. The correlation heat map of essential oil compounds of sage.

In this study, the behavior of key compounds in In summer savory and sage essential oils showed patterns similar to those reported by other researchers studying different medicinal plants (Mohtashami et al., 2018). Notably, greater changes were observed in the major constituents of savory compared to sage under varying temperature conditions. In savory, several minor compounds, including (E)- β -ocimene, terpinolene, *trans*-sabinene hydrate, borneol, α -terpineol, aromadendrene, viridiflorene, spathulenol, and caryophyllene oxide, as well as the major compound carvacrol, all of which possess relatively low boiling points, decreased by the end of the storage period across all three temperature conditions. In contrast, two important compounds in savory, i.e., *p*-cymene and γ -terpinene, showed a significant increase after three months at freezer, refrigerator, and room temperatures. These findings are consistent with those of previous studies, which demonstrated that essential oil components can readily undergo transformation through cyclic, enzymatic, or chemical dehydrogenation reactions, as well as oxidation and isomerization. Such processes may result in either an increase or a decrease in the concentration of individual compounds (Turek and Stintzing, 2013).

In summer savory, carvacrol and γ -terpinene, i.e., two compounds recognized for contributing most significantly to the plant's biological activity, exhibited a strong negative correlation. A marked decrease in carvacrol accompanied by a significant increase in γ -terpinene was observed consistently across all three storage conditions. This negative correlation has also been reported by other researchers (Mohtashami et al., 2018). Among the tested conditions, the smallest

reduction in carvacrol levels occurred at ambient temperature. Similar observations have been reported in previous studies. For instance, Rowshan et al. (2015) also identified room temperature as the most favorable storage condition for preserving carvacrol, whereas Mohtashami et al. (2018) reported freezer storage as the optimal condition. The discrepancy between these findings has been attributed to the compound's varying responses to different storage environments, as noted by Afshar et al. (2021).

CONCLUSION

Notable findings regarding the stability of key compounds were observed in both savory and sage, which are two members of the Lamiaceae family. In many cases, the main monoterpenes in the essential oils of these plants retained their quality effectively when stored at room temperature. The duration of the experiment was three months; however, the long-term stability of these compounds beyond this period remains uncertain. If storage is extended, the levels of these important compounds may eventually decline. It also remains unclear whether room temperature will continue to be the most suitable condition for prolonged storage. Further research is needed to address these uncertainties. From an economic standpoint, preserving the quality of essential oils at room temperature is highly beneficial, as it significantly reduces electricity costs. Therefore, ongoing research into essential oil storage and the chemical behavior of secondary metabolites in medicinal plants can offer valuable insights for producers and consumers in the pharmaceutical, health, cosmetic, food, and aromatherapy industries.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Sharareh Najafian; Methodology: Seyedeh Fariba Mohammadian Yasuj; Software: Seyedeh Fariba Mohammadian Yasuj; Validation: Mehdi HosseiniFarahi; Formal analysis: Mehdi HosseiniFarahi; Resources: Mehdi HosseiniFarahi; Data curation: Sharareh Najafian; Writing—original: Sharareh Najafian; Writing—review and editing: Mehdi HosseiniFarahi; Visualization: Sharareh Najafian; Supervision: Sharareh Najafian; Project administration: Sharareh Najafian.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author on request.

ETHICAL STATEMENT

The conducted research is not related to either human or animals use. Author is aware of the content of the

manuscript and consented to submit it to Iran Agricultural Research journal.

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