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Research Article

A Compositional Perspective of Sour Orange (*Citrus aurantium* L.) Flowers Essential Oil under Different Storage Conditions

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Abstract: Sour orange (*Citrus aurantium* L.) is a tree with medicinal properties. This study assessed the impact of various storage conditions and times on the Essential Oil (EO) content of sour orange blossoms. The changes in the EO composition were recorded at the beginning of extraction and during three, six, and nine months of storage at the temperature of 4 °C (refrigerator), -20 °C (freezer), and 25 °C (room temperature) under conditions of light and darkness. According to the Gas Chromatography (GC, FID detector) and Gas Chromatography-Mass Spectrometry (GC-MS, FID detector) results, 35 compounds were identified. Linalool acetate (14.56-31.4%), linalool (18.9-23.1%), limonene (7.51-15.3%), farnesol (2.1-9%), nerolidol (6.1-8.9%), β-pinene (5.7-8.7%), trans-β-ocimene (0-7.9%), and geranyl acetate (4.2-5.7%) constituted the major EO components in different storage treatments. After nine months, linalool acetate climbed by more than twice as much. Overall, the principal EO compositions are significantly impacted by the storage circumstances. Such knowledge is essential for the food and pharmaceutical industries to use EO effectively in their intended products.

***Keywords*:** storage periods, temperature, limonene, linalool, sour orange, food industry

1. Introduction

Essential Oils (EOs) are natural substances that give them their distinctive aroma and lower molecular weight than water. EOs are extracted from different parts of plants such as flowers, barks, leaves, fruits, and woods [1]. They are used in the pharmaceutical, perfumery, sanitary, and food industries. EOs were initially used in the pharmaceutical industry, but have been increasingly used as enhancers of aroma and flavor since the 19th century [2]. The antibacterial, insecticidal, antifungal, and antioxidant activities of these plant secondary metabolites are among their numerous advantageous traits [3].

Citrus aurantium L., Rutaceae, commonly named bitter or sour orange [4], is a Citrus species native to the Asian equatorial region, countries such as India, China, and Iran. Its blossom is recognized with the common name "neroli" [5] and is one of the most intoxicating aromas being used in aromatherapy, perfumery industries, and medicinal purposes. The fragrance of neroli is complex, slightly sweet, and floral. The color of the EO ranges from pale to golden yellow [6]. Numerous bioactive substances, including phenolics, alkaloids, flavonoids, vitamins, and EOs with various therapeutic

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properties, have been extracted from the sour orange [7, 8]. Additionally, EOs are expensively priced in the global liqueur and aromatherapy markets. They can also be employed in the food and cosmetics sectors as flavoring agents [9, 10]. Due to its antioxidant, antibacterial, antifungal, anti-parasitic, anti-inflammatory, and anti-aging characteristics, multiple research has identified its various medical benefits [11]. Neroli EO can reduce anxiety, cure bacterial infections and eczema, relieve emotional stress and exhaustion, and reduce high blood pressure [12]. In addition to having anti-depressant properties, it is a treatment for insomnia, irritable bowel syndrome, neuralgia, and soothes panic attacks [13]. Neroli has a significant impact on skin cell regeneration and increased skin elasticity. Also, it protects the skin during X-Ray treatments [14]. The bitter orange-flower water is used to flavor sweets, and the blooms are utilized in teas. It is frequently used in perfumery for sweetness and freshness and has a nice, distinguishing aroma [15].

Studies demonstrate differences among the EOs extracted from different parts of sour orange. Hydrocarbon monoterpenes such as limonene and myrcene are compounds that are present abundantly in the peel, while oxygen monoterpenes such as linalool, linalool acetate, geranyl acetate, and α-terpineol are the greatest compounds in the leaves and flowers of sour orange [16]. Researchers discovered that linalool, linalool acetate, and limonene were the primary components of Egyptian sour orange essential oil [17]. Reported stated that the main EO components of sour orange of Darab county in the south of Iran were geraniol (26.6%), α-terpineol (20.7%), linalool (15.4%), benzene acetaldehyde (5.5%), methyl anthranilate (11.8%) and oxidized linalool (6.1%) [18]. Researchers reported that the main compositions of sour orange flowers are linalool (44.2%), α-terpinol (18.5%), and geranyl (6.4%) [19].

The majority of scientific studies so far have concentrated on the storage conditions and durability of bioactive components like EO of aromatic plants. There hasn't been much research that looks at how storage conditions affect the quality of basic ingredients in medicinal plants. Also, to guarantee the safety of EOs, their efficacy and quality are important for consumers and others [20]. Most research has documented how storage time affects the secondary metabolites of medicinal and aromatic plants. The effects are primarily found to influence EOs and their qualities. The number of EO compounds was seen to increase as a result of oxidation, hydrolysis, isomerization, color change, and changes in flavor and aroma, although it has been observed that EOs and their primary components generally decrease, notably in the monoterpenes group [21]. The effect of storage conditions on the EO compositions of *Leonurus cardiac* L. showed that exposure to air led to oxidation of β-caryophyllene and α-humulene when storage takes place in the freezer [22].

The effect of storage time and temperature on the quality of EO of *Melissa officinalis* L. was investigated. Changes in EO compositions were detected during storage for four months in the refrigerator (4 °C), freezer (-20 °C), and at room temperature. Regarding damask rose (*Rosa* × *damascena* Mill.), the temperature and duration of storage affected the EOs extracted from flowers. The preservation of flowers under various conditions specifically influenced the content and composition of EO. However, changes in EO compositions were observed during cold storage at 4 ± 0.5 °C and -20 °C for up to one month as compared with the control group. Flowers preserved their primary quality when kept at low temperatures, especially at -20 °C, because EO compositions changed the least. The optimal results in terms of rose oil content and components resulted from rose flowers that were immediately distilled after harvest, as well as from flowers stored at -20 °C for 20 days [23].

The objective of the current study was to assess the EO compositions of sour orange flowers for nine months in the refrigerator (4 °C), freezer (-20 °C), and at room temperature (25 °C), with and without light. To the best of our knowledge, this is the first work that shows how storage conditions change the EO compositions of sour orange flowers.

2. Materials and methods

2.1 *Plant materials*

At the full flowering stage, the flowers of the sour orange tree were collected from Fasa, Fars province, Iran. The plant species were identified and authenticated by A. Khosravi, a plant taxonomist at Shiraz University, Iran. The voucher specimen (No: 625) was deposited in the herbarium.

2.2 *Essential oil extraction*

The EO of fresh sour orange flowers was extracted by the hydrodistillation method using an industrial method (distillation flask). Together with 400 liters of water, 150 kg of the flowers were used for this. The water was heated with vapor (direct and indirect) for three hours (Temperature kept constant during the procedure.). After boiling, the vapors of water and EO were imported to a condenser where they were converted into liquid (Liquescence). The liquefied product was collected in an EO separator tank. The obtained EO was kept at 4 °C before GC and GC-MS (FID detector) and storage experiments.

2.3 *Essential oil storage conditions*

The extracted EO was stored under six different storage conditions, i.e. the refrigerator (4 °C), freezer (-20 °C), and at room temperature (25 °C), with and without light for nine months. The EO samples were analyzed every three months using GC and GC-MS devices (FID detector). Moreover, an EO sample without treatment operation was analyzed at the beginning of the experiment (control) at the extraction time, which was considered the control sample. The evaluations were performed every three months.

2.4 *The essential oil analysis procedure*

The components of volatile oil from the flowers were identified using GC and GC-MS analyses (FID detector). The GC analysis (FID detector) was performed using an Agilent gas chromatograph series 7890-A equipped with a Flame Ionization Detector (FID). The analysis was carried out on a fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; with a film thickness of 0.25 µm). The sample volume injected into the GC was 0.2 µL of pure EO. The temperatures of the injector and detector were set at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1ml per min; the oven temperature program was set to increase from 60 to 210 °C at the rate of 4 °C per min, which was then programmed to reach 240 °C at the rate of 20 °C per min and, finally, was held isothermally for 8.5 min. The split ratio was 1:50. The GC-MS analysis (FID detector) was carried out by the use of an Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30m × 0.25mm i.d.; film thickness 0.25µm) coupled with a 5975-C mass spectrometer. The sample volume injected into the capillary column was 0.1 µL of pure EO in the split mode (1:50). Helium was used as carrier gas with an ionization voltage of 70 eV. The temperatures of the ion source and interface were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. The oven temperature program was the same as for the GC. The retention indices for all components were determined according to the method using n-alkanes as standard.

2.5 *Identification of essential oil components*

The compounds were identified by comparing their Retention Indices (RI, HP-5) with those reported in the literature and by comparing their mass spectra with the Wiley GC-MS Library, Adams Library, and Mass Finder 2.1 Library data, as well as the published mass spectra data [24, 25].

3. Results and discussion

The EOs of sour orange flowers contained a total of 35 compounds, according to the findings of this investigation. The major EO compounds were linalool acetate (4.6-31.4%), linalool (18.9-23.2%), limonene (10.9-15.4%), farnesol (2*Z*, 6*E*) (2.1-9.0%), nerolidol-*E* (6.1-8.9%), β- pinene (5.8-8.7%), trans-β-ocimene (3.7-7.9%) and geranyl acetate (4.2-5.7%) (Table 1). Because EOs are volatile and may be exposed to varied modifications by storage conditions, there are limited studies about the storage of plant secondary metabolites [26]. Some modifications were found in the composition of EOs from clary (*Salvia sclarea* L.) [27], savory (*Satureja hortensis* L.) [28], and cloves (*Caryophyllus aromaticus* L.) [29].

Table 1. Compositions of sour orange flower EO during 9 months as influenced by different storage treatments

Number	Compounds	Class	RI_C *	RI_r **	Control (%)	3 months				6 months			
						Refrigerator temp. (%)	Freezer temp. (%)	Room temperature		Refrigerator temp. (%)	Freezer temp. (%)	Room temperature	
								Without light	With light			Without light	With light
1	Alpha Pinene	MT***	932	939	0.99	0.65	0.64	0.58	0.56	0.76	0.7	1.74	0.61
2	Sabinene	MT	974	975	1.06	1.64	1.53	1.55	1.51	1.14	1.21	-	1.5
3	Beta- Pinene	MT	979	979	7.37	7.76	7.62	8.06	7.69	6.61	6.8	5.76	7.36
4	Beta Myrcene	MT	990	990	4.08	1.93	2.01	1.73	1.65	2.01	2.04	0.36	1.36
5	Limonene	MT	1,029	1,029	7.51	12.86	12.88	13.07	12.74	12.44	12.52	12.20	12.3
6	Gamma-Terpinene	MT	-	-	0.35	-	-	-	-	-	-	0.76	-
7	Cis-Ocimene	MT	1,036	1,037	1.72	0.83	0.89	0.76	0.71	0.83	0.88	-	0.72
8	Trans-beta-Ocimene	MT	1,048	1,050	7.31	5.85	6.31	5.83	5.25	5.23	5.84	-	5.1
9	Cis-Linalool oxide	MT	1,073	1,072	-	0.4	-	-	-	-	-	1.8	-
10	Trans-Linalool oxide	MT	1,089	1,086	0.6	-	0.43	0.39	0.37	0.44	0.43	1.66	0.46
11	Linalool	MT	1,103	1,096	19.07	19.34	19.12	19.19	19.66	19.15	18.95	22.07	19.24
12	Trans-Pinocarveol	MT	1,141	1,139	-	-	-	-	-	-	-	-	-
13	Alpha-Terpineol	MT	1,194	1,188	1.85	0.92	0.9	0.82	0.88	1.1	1.02	0.9	0.97
14	Trans-Carveol	MT	1,220	1,216	-	-	-	-	-	-	-	-	-
15	Nerol	MT	1,229	1,229	0.59	0.37	0.37	0.32	0.34	0.45	0.43	-	0.39
16	Carvone	MT	1,246	1,246	0.03	-	-	-	-	-	-	-	-
17	Linalool acetate	MT	1,263	1,257	14.56	25.73	25.52	26.48	26.67	25.42	25.43	31.42	26.45
18	Geranial	MT	1,274	1,267	0.05	-	-	-	-	-	-	-	-
19	Indole	ST****	1,296	1,291	0.09	-	0.4	0.34	0.33	0.43	0.45	-	0.31
20	Methyl anthranilate	-*****	1,344	1,337	0.14	-	-	-	-	-	-	-	-
21	α-terpinyl acetate	MT	1,352	1,349	0.24	-	-	-	-	-	-	-	-
22	Neryl acetate	MT	1,365	1,361	3.85	2.42	2.38	2.26	2.37	2.73	2.64	2.99	2.58
23	Geranyl acetate	MT	1,384	1,381	7.08	4.44	4.37	4.22	4.38	4.85	4.72	5.57	4.69
24	Trans-Caryophyllene	ST	1,425	1,419	1.24	0.51	0.5	0.47	0.48	0.59	0.57	-	0.52
25	Trans β-Farnesene	ST	1,458	1,456	1.71	-	-	-	-	-	-	-	-
26	Geramacrene-D	ST	1,486	1,485	0.11	-	-	-	-	-	-	-	-
27	Bicyclogermacrene	ST	1,502	1,500	0.28	-	-	-	-	-	-	-	-
28	Nerolidol-E	ST	1,565	1,563	7.93	6.81	6.62	6.61	6.88	7.34	7.14	6.95	7.17
29	(-)-Caryophyllene oxide	ST	1,589	1,583	-	-	-	--	-	-	-	-	-
30	Heptadecane	-	1,699	1,700	0.17	-	-	-	-	-	-	-	-
31	Farnesol (2*Z*, 6*E*)	ST	1,723	1,723	9.09	6.95	6.85	6.78	6.94	7.48	7.29`	2.72	7.17
32	Farnesal (2*Z*, 6*E*)	ST	1,746	1,741	0.22	-	-	-	-	-	-	-	-
33	Farnesyl acetate (2*Z*, 6*E*)	ST	1,843	1,846	0.43	0.28	0.29	0.27	0.28	0.38	0.36	1.19	0.39
34	Palmitic acid	-	1,961	1,960	0.19	-	-	-	-	-	-	-	-
35	Tricosane	-	2,298	2,300	0.33	0.31	0.3	0.28	0.31	0.38	0.31	0.42	0.36
36	Unknown compounds	-	-	-	-	-	-	-	0.56	0.24	0.27	0.66	0.35
37	Total identification				100	100	100	100	99.44	99.76	99.73	99.34	99.65
38	Monoterpenes				78.29	85.14	85.04	85.25	84.22	83.16	83.61	87.23	83.73
39	Sesquiterpenes				20.51	14.55	14.66	14.47	14.91	16.22	15.81	11.69	15.56
40	Other compounds	-	-	-	1.2	0.31	0.3	0.28	0.31	0.38	0.31	0.42	0.36

9 months					
Refrigerator temp. (%)	Freezer temp. (%)	Room temperature			
		Without light	With light		
0.69	0.68	0.44	0.65		
1.62	1.64	0.51	1.19		
7.64	7.67	6.34	7.71		
1.8	2.06		1.53		
12.82	12.86	10.95	12.62		
-	-	-	-		
0.6	0.8	-	0.55		
3.76	5.2	-	3.69		
-	-	1.64	-		
0.29	0.34	1.53	0.36		
19.72	19.31	21.33	19.63		
-	-	0.73	-		
1.03	0.97	0.73	1.01		
-	-	0.38	-		
0.41	0.4	-	0.38		
-	-	0.36	-		
26.75	25.91	30.9	27.07		
-	-	-	-		
0.34	0.41	-	-		
-	-	-	-		
-	-	-	-		
2.66	2.54	2.77	2.65		
4.8	4.71	4.98	4.83		
0.51	0.52		0.49		
-	-	-	-		
-	-	-	-		
-	-	-	-		
-	-	-	-		
7.10	6.89	6.11	7.45		
-	-	0.45	-		
-	-	-	-		
6.80	6.79	2.15	7.05		
-	-	0.4	0.3		
0.34	0.3	1.37	0.44		
-	-	-	-		
0.32	-	0.39	0.38		
-	-	5.54	-		
100	100	94.46	100		
84.59	85.09	83.59	83.89		
14.75	14.5	10.48	15.73		
0.66	0.41	0.39	0.38		

IRc*: Retention ratio calculated by Kovats' equation, RI**: literature retention rate, MT***:Monoterpenes, ST****: Sesquiterpenes, -*****: Unknown category/ Not detected. Treatments at refrigerator and freezer temperatures were without light. Control treatment stands for immediately after distillation

Understanding the metabolic basis of EOs may assist in monitoring structural alterations to EO constituents. Constituents in EOs generally originate from several major biosynthetic pathways [9]. While the aromatic phenylpropanoids are formed via the shikimic acid pathway leading to phenylalanine [30], terpenoids are derived from the C_5- the building blocks isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) [31]. In plants, the biosynthesis of terpenes occurs through the methylerythritol phosphate (MEP) pathway, which is the predominant pathway for monoterpenes and diterpenes, or through the mevalonate pathway (MVA), which is the primary pathway for sesquiterpenes [32].

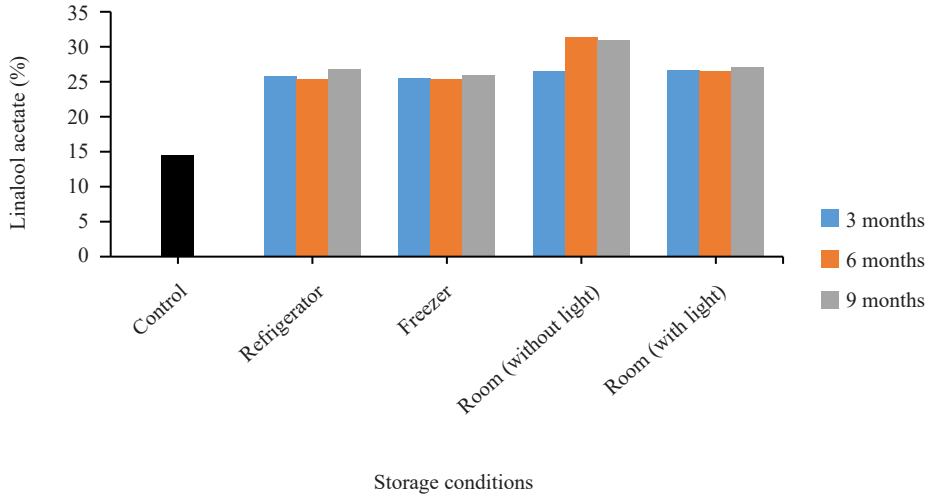

Figure 1. Changes of linalool acetate content during different storage conditions. Treatments at refrigerator and freezer temperatures were without light. Control treatment stands for immediately after distillation

Generally, some external factors such as temperature, light, oxygen, heat, and moisture may cause the destruction (transformation, oxidation, etc.) and change of EO compositions. This makes the storage of the EOs appear an important and vital practice [33]. Based on the various times and storage techniques used in the current investigation, each chemical in the EO changed uniquely. One of the main ingredients in neroli essential oil, linalool acetate, changed significantly during various storage times and storage circumstances. The lowest content of this compound was observed

at the control treatment (14.56%). The highest content (31.4%) was observed at room temperature (without light) after six months. Its content was also high at room temperature (without light) after nine months of storage (30.9%). The light impact at room temperature, could decrease linalool acetate content in comparison with darkness after six and nine months of storage (18.8 and 14.1% reduction, respectively) (Table 1 and Figure 1). Linalool acetate is responsible for the floral scent of neroli EO [34]. This component may fluctuate to varying degrees depending on the temperature and length of storage. Additionally, less stable substances may change as a result of chemical reactions with other components [35].

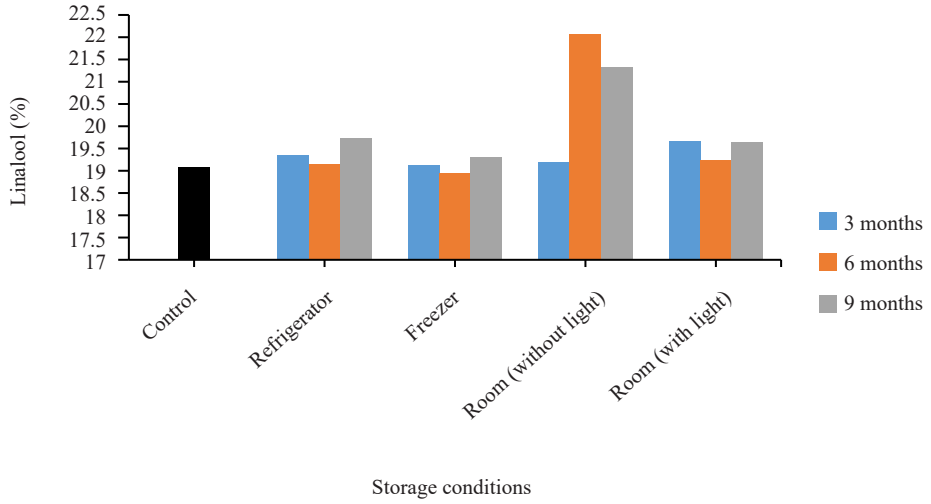

Figure 2. Changes of linalool content during different storage conditions. Treatments at refrigerator and freezer temperatures were without light. Control treatment stands for immediately after distillation

Linalool as the second major component of neroli EO showed significant changes during different storage times and conditions. The highest content of linalool was detected in the control (19.07%). Keeping in the freezer after six months caused the lowest content of linalool (18.9%) which showed a 22.4% reduction. Generally, in all treatments, linalool showed a declining trend from 5% (at room temperature without light after six months) to 22.4% (at freezer temperature after six months) in comparison with the control. According to the light impact at room temperature, after three months, linalool was higher in light conditions (2.4% more) than in darkness but light decreased linalool content in comparison with darkness after six and nine months of storage (14.7 and 8.7% reduction, respectively) (Table 1 and Figure 2).

It appears that the soonest after EO extraction is the ideal period to obtain greater linalool from neroli EO. Linalool is responsible for the floral aromatic note [34]. Linalool reduction is accompanied by the appearance of oxidation products (isomeric linalool oxides). Moreover, linalool is easily isomerized into geraniol and nerol, and its oxidation leads to the formation of neral and geranial. Dihydroxylation and degradation of linalool produce compounds including β-myrcene, cis-ocimene, and trans-ocimene [36]. Cyclization of these compounds is followed by the formation of isomers, para- and meta cymenes. Other changes also occurred when the EO was stored in the light. It is possible to delay fermentation by freezing [37]. The capacity of linalool to change into different molecules explains why there is a decrease in linalool during storage. In this study, linalool oxidation led primarily to a considerable increase (by 28-fold) in the contents of two isomeric linalool oxides in the oil stored for nine months (Table 1). Additionally, it is known that linalool readily isomerizes into geraniol and nerol, its oxidation produces neral and geranial, and these compounds may then combine to create rings that produce isomeric para- and meta-cimenes. Such a kind of transformation may be responsible for the continuously increasing levels of these benzene derivatives in stored oil [38]. Among the major components, by their higher content, linalool and limonene were considered to contribute principally to the aroma of neroli EO. EO components are known to readily transform into one another by oxidation, isomerization, cyclization, or dehydrogenation processes that are initiated either enzymatically or chemically because of their structural link within

the same chemical group [39].

Limonene is a major monoterpene in neroli EO and showed the highest content in the control treatment (7.5%). All examined storage treatments caused a reduction in this component concentration. Its minimum quantity was observed at room temperature without light after nine months (10.9%) which showed a 40.5% decline in comparison with the control (Table 1 and Figure 3).

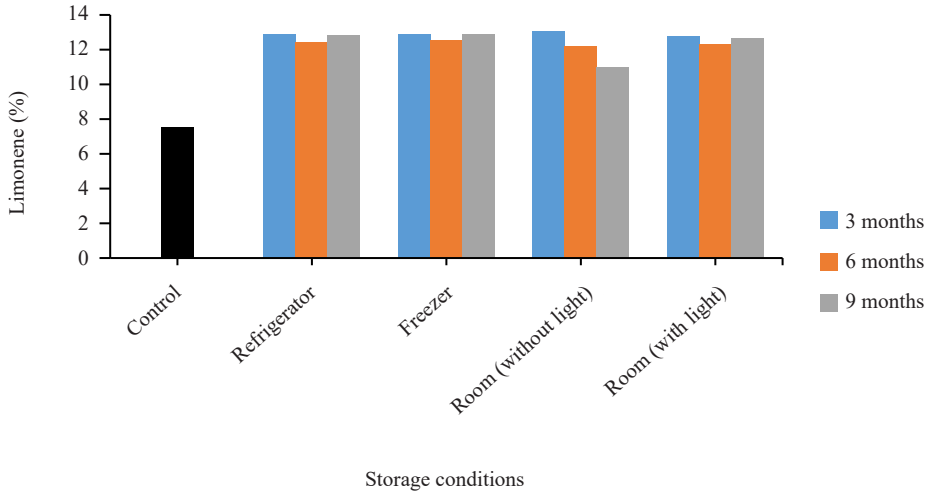

Figure 3. Changes of limonene content during different storage conditions. Treatments at refrigerator and freezer temperatures were without light. Control treatment stands for immediately after distillation

Limonene causes citrus aromatic notes in the EOs [34]. The concentration of limonene which is a monoterpene decreased after different storage periods. It could be suggested that the synthesis of limonene, starts with α-pinene as a preliminary monoterpene substrate and proceed via the α-terpinyl cation as an intermediate [40]. In the presence of air, limonene can thermally decompose and produce a variety of oxidative degradation products. In a report, limonene content of lemon EO decreased from 67.1 to 30.7% after 12 months when the EO was stored at 25 °C with the cap removed for 3 min every day. However, storage at 5 °C, with the cap removed for three min only once a month, resulted in minimal degradation [16]. To maintain quality over time, EOs should be kept in tightly closed, darkened glass containers in a cool location. Researchers reported that the concentrations of hydrocarbon in the EO of rose flowers, stored for various durations under storage conditions at 4 °C, displayed higher scores than the petals distilled immediately [41]. Additionally, rose blossom EO that had been held under room conditions for a variety of lengths of time performed better than petals that had been instantly distilled [26]. When a model system containing either limonene or linalool was stored, both compounds turned into α-terpineol, while the conversion rate of linalool far exceeded that of limonene. Limonene and linalool may be α-terpineol precursors [42]. In *Lippia citriodora*, parallel to the increase in the storage duration, citral content decreased, whereas the amounts of limonene and 1,8-cineole were increased. With increasing storage duration up to four months, the amount of citral (geranial + neral) initially increased and then gradually decreased until the eighth month [21]. A study on *Citrus tamurana* Hort. showed that the contents of myrcene, γ-terpinene, and terpinolene decreased with increasing storage duration and temperature. The reverse was true for p-cymene. It has been suggested that the increase of p-cymene during storage could occur by rearrangement, hydrogenation, or dehydrogenation of α- and γ-terpinenes and limonene [43].

Farnesol (2*Z*, 6*E*) is the first major sesquiterpene in sour orange flower EO. It showed changes during different storage durations and conditions. The highest content of farnesol was obtained in the control treatment (9.09%). The maximum reduction was observed at room temperature with darkness after nine months (322.8% decline towards control). According to light's impact on its content, darkness could reduce farnesol content much more than being in light (Table 1). Farnesol has been reported to impinge on at least three central regulatory pathways that are directly or indirectly related to oxidative stress resistance [44].

Nerolidol-*E* is a sesquiterpene in the EO of the sour orange flower. It showed changes during different storage durations and conditions in this research. The highest content of nerolidol-*E* was obtained in the control treatment (8.9%). The maximum reduction was observed at room temperature with darkness after nine months, which was 6.1% (that had declined by 45.8%). Other treatments did not change their concentration significantly (Table 1). In a report on cucumber and lima bean, a sesquiterpene synthase was observed catalyzing the formation of (3S)-(E)-nerolidol from farnesyl diphosphate. Nerolidol is the sesquiterpene analog of the monoterpenoid linalool [45, 46].

A study showed that inhaling neroli vapor may induce anti-anxiety effects and that these characteristics originate from different compounds such as nerolidol [47, 48].

β-pinene is another major monoterpene in sour orange flower EO, which showed the highest content in the control treatment (8.7%). After six months, the minimal quantity was noticed in complete darkness and at room temperature (5.8%). In general, all treatments showed reductions in β-pinene content in the control treatment. All storage durations and conditions reduced the β-pinene content after six months, but the content increased again after nine months. The impact of light on the EO content was noticeable during three months of storage at room temperature, while a lower quantity was obtained in the presence of light than darkness (4.8% lesser), but higher quantities were observed at six and nine months in the presence of light, compared to darkness (27.8 and 21.6% more, respectively) (Table 1). β-pinene is responsible for the green odor in aromatic note assessments of citrus EOs [49]. Both optical antipodes of α-pinene are natural products, and they may co-occur with either isomer predominant. By contrast, β-pinene usually occurs as the optically pure isomer. Cyclization of the neryl cation can occur to make a monocyclic α-terpinyl intermediate. The acyclic trienes cis-ocimene and myrcene were suggested as precursors of α and β-pinene, respectively. Finally, the favorable isomerization of the exocyclic to the endocyclic isomer raises the possibility of a single cyclization of an acyclic precursor to β-pinene, followed by enzymic (or nonenzymic) conversions to α-pinene [50]. Meanwhile, β-pinene is known to be isomerized into α-pinene under a variety of conditions [51].

Trans-β-ocimene as a monoterpene in neroli EO showed the highest content at the control treatment (7.9%). Other treatments showed reduction after six and nine months. The minimum quantity was observed at room temperature in light after nine months (3.7%) which showed a 115.4% reduction in comparison with the control. A significant point observed in the pattern of changes occurring in the content of trans-β-ocimene was the omission of this component from the EO profile after six and nine months at room temperature under dark conditions. Over time, its content decreased after nine months in the presence of light at room temperature in comparison with the same storage conditions after three or six months (about a 38.2% decrease) (Table 1). This monoterpene occurs in many EOs that are responsible for the citrus aromatic note in the EO [52]. The structure of trans-β-ocimene contains isoprene units. The reduction in its content may be because of the migration of volatile compounds into the packaging material, which can cause changes in the content. This migration has already been reported regarding citral [53]. Two problems that need to be solved for its synthesis are the formation of the acid-sensitive terminal conjugated double bonds and the trans configuration in the C_3-C_4 double bonds [54].

Geranyl acetate is a monoterpene in sour orange flower EO. Here, it showed the highest content in the control treatment (5.7%). The minimum quantity (4.2%) was observed at room temperature and darkness after three months which showed a 36.2% decline. In general, the examined storage conditions did not cause significant changes in geranyl acetate content (Table 1).

Geranyl acetate is responsible for the metal aromatic quality in the EO of bitter orange [55]. EO constituents are especially prone to oxidative damage, chemical transformations, or polymerization reactions. Representative structures are depicted in Figure 4. The trends of changes in different compounds of EOs are various during storage treatments. Moreover, the changes regarding the chemical groups such as monoterpenes or sesquiterpenes that constituted the EO composition at various storage conditions are different. In this study, monoterpenes were the major group of EOs, and they showed significant changes during different storage durations and conditions. Linalool, limonene, β-pinene and trans-β-ocimene were major monoterpenes in the neroli EO. The highest content of monoterpenes was obtained at room temperature and darkness after six months (87.2%). However, control treatments had the lowest content of monoterpenes (78.3%) which showed an 11.4% reduction. This phenomenon can be due to evaporation, oxidation, and other unwanted changes in EOs components during the storage period. It is clear that over time, lower boiling compounds markedly decreased at room temperature [56, 57]. The α-Terpinyl cation is the main precursor for all cyclic monoterpenes such as limonene or α-terpineol. Also, it can be applied as the raw material for the synthesis of

α-terpinyl acetate. α-pinene might be also transformed to limonene by dehydration in the refrigerator and freezer and/or by α-terpineol at room temperature which may happen under storage conditions. Oxidation, reduction, rearrangement (via hydride shifts and additional ring closures), conjugation, double bond isomerization, and hydration result in a high diversity of monoterpene structures [58, 59]. The monoterpene accumulation correlated with an increase in the transcript abundance of early terpenoid pathway enzymes [60].

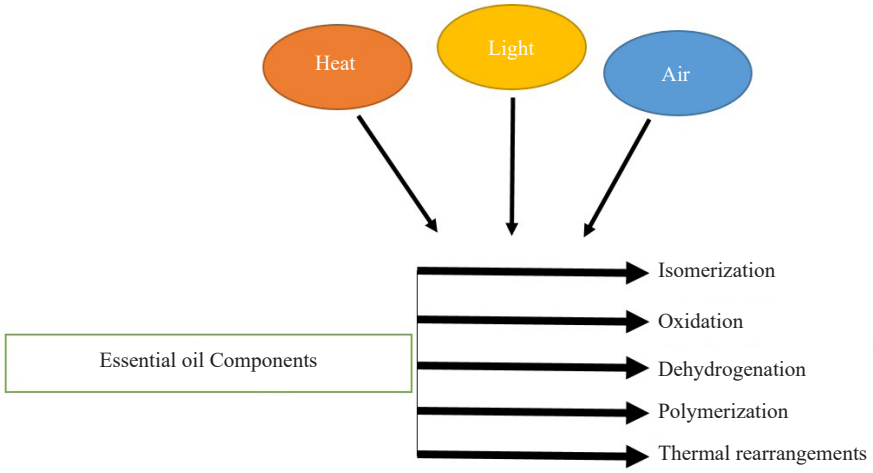

Figure 4. Possible conversion reactions in essential oils [31]

A study on EO of *Rosa damascena* Mill showed that with the increase in storage duration, citronellol and nerol content increased at 4 °C, 18 ± 1 °C, and 25 ± 1 °C, whereas the geraniol content decreased as the storage duration increased. Methyl eugenol content increased contrary to the content of geraniol when the duration of storage increased from 4 to 24 h, while the rate of geraniol and neryl acetate decreased. Another report explained that the percentage of monoterpene alcohols increased from 39.92 to 49.23%, whereas linalool and geraniol decreased after five days of storage at -20 °C [61]. Changes in the *Citrus tamurana* Hort. EO composition was analyzed after storage for one, three, six, and nine weeks at -21, 5, 20, and 30 °C. The total amount of oxides and monoterpene alcohols and ketones increased. The contents of myrcene, γ-terpinene, and terpinolene decreased, while the amount of p-cymene increased considerably [16]. Changes in the compositions of EOs from the aerial parts of Thymus daenensis were determined at different temperatures and storage times including three months in the refrigerator (4 °C), freezer (-20 °C), and at room temperature. The results indicated that at room temperature, the proportions of compounds with lower boiling temperatures such as α-pinene (4.3-0.5%), α-terpinene (1.8-0.5%), and myrcene (1-0.4%) along with γ-terpinene (10.1-4.7%) and p-cymene (8.3-4.7%) as thymol and carvacrol precursors decreased. However, the amounts of thymol and carvacrol considerably increased by 26.6 and 23% after three months, respectively.

Oxidative and polymerization processes may result in a loss of quality and pharmacological properties. Focusing on individual EOs, the various paths of degradation upon exposure to extrinsic parameters are outlined [62]. An example of the structural pattern as a function of harvest time was given by [63], who found a decreasing concentration of a hydrocarbon monoterpene in the EO throughout the year while the amount of an oxidized immediate metabolic successor rose at the same time. Consequently, plant volatiles is subject to natural fluctuations in their composition [64] that need to be considered upon quality evaluation. The tendency of terpenoids to be volatile and thermolabile means that, depending on the structure, they may be easily oxidized or hydrolyzed. Accordingly, it is established that the chemical composition of EOs is largely dependent on the conditions during the processing and storage of the plant material, upon distillation as well as in the course of subsequent handling of the oil itself [65, 66]. Studies reported when storage was at room temperature, the organoleptic properties (correlated with EO components) of dill in 12 months of storage were better preserved [67]. In general, the change in the EO composition during storage depends on the type of compound and the storage conditions [68]. Researchers mentioned that terpene biotransformation can be a reason

for these changes. He clarified that EO components or trace pollutants may catalyze or start these reactions in which terpenes can bind or release a water molecule, isomerize, or rearrange. It has been observed that the composition of EOs can be altered as a result of processing and storage, whereby factors such as temperature, light, and oxygen availability would have a crucial impact on alteration processes [69-71].

In the present study, sesquiterpenes were another major group of EO components that include many major and effective compounds. They showed changes during different storage durations and conditions. Farnesol (2*Z*, 6*E*) and nerolidol-*E* were major sesquiterpenes in the sour orange flower EO. The highest content of sesquiterpenes was obtained in the control treatment (20.5%). After nine months of storage at room temperature under darkness, the lowest content of sesquiterpenes (10.5%) was observed (95.2% reduction towards the maximum content). It seems that a long duration of storage causes significant reductions in sesquiterpenes of the bitter oranges' EO. Changes detected in the composition of EO after storage in the daylight could result from chemical modifications of terpenes including oxidative processes initiated by light. Cyclic monoterpene hydrocarbons contain two double bonds per cycle and can be easily oxidized when they lose two hydrogen atoms. Terpene dienes can bind or release a water molecule and isomerize or rearrange it. Then, EO components or trace contaminants may catalyze or initiate these reactions. Based on the investigations of the stability and reactivity of specific terpenes and model systems, including data on terpene biotransformation, the variations in the EO composition can only find a partial explanation at this time [72, 73]. It is known that some microorganisms can change mono- and sesquiterpene hydrocarbons by bioconversion. Monoterpenes may also undergo such a transformation under the influence of factors other than microorganisms [74, 75].

When EOs are evaluated for stability, the chemical composition of the initial raw material utilized may change due to factors such as plant health, growth stage, habitat, climate, edaphic conditions, and harvest time [76, 77]. A report on coriander EO expressed that γ-terpinene is known to be oxidized by air oxygen giving dihydrocoumarol, which in turn releases a water molecule to yield p-cimene. Also, γ-terpinene can bind a water molecule to give carvacrol, in stored EOs [78]. Ultraviolet (UV) and visible lights tend to increase the rate of self-oxidation via hydrogen stimulation, and this process leads to the release of free radicals [79]. Acetates, estrols, and certain monoterpenes are primarily reduced when EOs are stored in the presence of light. A study on Lime EO revealed that the exposure of EO to light leads to the reduction in the amounts of geraniol, terpinolene, α-terpinene, and especially monoterpenes. Additionally, the stability and speed of chemical processes are impacted by temperature. While low temperatures can result in oxygen being dissolved in the liquid medium, which could have a negative impact on the stability of the EO, high temperatures promote self-oxidation and the catalysis of hydro-peroxides [80-82].

The concentration of the majority of the low-molecular-weight components was found to decrease over the course of storage, and this drop was more pronounced at ambient temperature than in the refrigerator and freezer. This event may be due to evaporation, oxidation, and the occurrence of undesirable changes during the time of storage [83, 84]. Our results are in agreement with similar findings on Lemon balm (*Melissa officinalis* L.) [26], *Hyptis pectinata* [85], and *Thymus daenensis* [86]. It might be found that the EO keeps its original quality when stored at freezer temperature.

According to the present results, the amount of β-pinene, limonene, linalool, trans-β-ocimene, nerolidol-*E*, and farnesol showed trends that declined through the storage time from three to nine months. However, some of the mentioned components such as geranyl acetate, nerolidol, and farnesol showed a moderate rise through the duration from three to six months and then decreased from the sixth to the ninth month. Linalool acetate which was a minor component for the EO extraction time (at the beginning of the experiment) increased dramatically (at least more than 80%) during all storage stages. It is suggested that, even at low concentrations, components that generate authentic flavors such as γ-terpinene, terpinolene, geraniol, β-pinene, and myrcene have high antioxidant activities. Consequently, even minor components that are derived from oxidation or degradation reactions may have a strong impact on the flavor and aroma [84]. Studies evaluated the effect of storage conditions on the EO of *Melissa officinalis* L. for four months and reported that the ratio of some compositions such as citronellal, neral, and geranial decreased at room temperature [26]. On the other hand, experiments assessed changes in the compositions of sweet basil EO during storage at -20, 4, and 25 °C. They discovered that the primary EO constituents are not significantly affected by storage temperatures over the course of a year, although they did notice significant variations in the levels of linalool and geraniol [87]. Researchers evaluated the changes in compositions of *Hyptis pectinata* EO during an experimental time of one year. They claimed that storage duration and temperature had an impact on how the EO compositions behaved. Furthermore, the storage at room temperature increased the concentration of β-almin, α-kupein, and caryophyline oxide, but lowered

the amount of α-hyomaline and β-caryophyllene [33]. In another work, four common EOs were subjected to different storage conditions to reveal the impact of light and temperature on the physicochemical properties as well as on the chemical composition of the respective oil. For this purpose, aliquots of lavender, pine, rosemary, and thyme oil were stored for up to 72 weeks in the presence of atmospheric oxygen at 23 °C in the dark as well as at 23 °C and 38 °C under cool white light, respectively. Changes in the characteristics occurred for each EO, revealing individual impacts of extrinsic parameters on particular samples. The greatest occurrence of degradation in monoterpenes was observed in rosemary oil, while α-terpinene was reduced to less than 10% within three weeks of storage at 38 °C under daylight [31].

An increase in the oil quality index could be denoted by the increase in the amount of thymol and carvacrol when stored at room temperature. Furthermore, the oil compositions showed the least changes and kept the primary quality when stored at low temperatures, particularly at -20 °C [86].

Researchers evaluated changes in the compositions of EO of *Thymus daenensis* for three months in the refrigerator (4 °C), freezer (-20 °C), and at room temperature. Reductions were reported in the amounts of compositions at room temperature with low boiling points such as α-pinene, α-terpinene, myrcene as well as γ-terpinene and p-cymene (as pre-formative for thymol and carvacrol). However, after three months of storage at room temperature, the concentrations of thymol and carvacrol considerably rose. The increase in the amounts of thymol and carvacrol during storage at room temperature indicates an increase in the qualitative index of EO. Additionally, they stated that EO quality remained steady when kept at freezer temperature [86]. Studies assessed the effects of different storage temperatures and durations (7, 14, 21, and 28 days) on the compositions of Damask rose EO. They discovered that whereas storage period had a considerable impact on the amount of EO components, storage temperature had no discernible impact. The amounts of hexadian, citranol, and methyl eugenol increased, whereas the amount of neral and geranial decreased. The highest amount of citronellol was observed during storage at 3 and 7 °C after 28 days [88]. The increase in the content of p-cymene is probably associated with the oxidation of α- and γ-terpinenes. Previous studies showed that, during the storage of the EO, the oxidation of α- and γ-terpinenes results in the formation of p-cymene. Storage of the EO in the dark was followed by insignificant changes in the content of substances, depending on their structure and reactivity [89]. The proportions of compounds with lower boiling temperatures decreased at room temperature. These were compounds such as citronellal (25.8-12.6%), neral (18.9-4.0%), and geranial (27.0-4.6%). It was found that the primary quality of EO extracted from *M. officinalis* could be maintained when stored at low temperatures, particularly at -20 °C [90].

In the present study, some minor compounds such as indole, nerol, and carvone disappeared after nine months of storage. The increase in the α-terpineol concentration was probably related to chemical transformations of limonene and linalool, which may transform into this compound. It should be emphasized that linalool is converted into α-terpineol more rapidly than limonene [91, 92]. According to the findings of the current study, storage decreased several EO compositions, while storage at low temperatures prevented both EO composition decreases and increases. This led to the preservation of the primary quality of the EO with the lowest changes. The antibacterial, anti-cancer, and antioxidant properties of the EOs and extracts from medicinal plants are evident. Some agents can eradicate free radicals with a high potential to be utilized as new natural preservatives for the protection of raw and processed foods [93, 94]. These could be categorized as immunological chemicals as well. Therefore, it's crucial to preserve the essential characteristics of EOs and extracts [95]. Some microbial secondary transformations may also happen in EOs [96] as they need more research.

4. Conclusion

The acquired data showed that, compared to low temperatures, room temperature caused a decrease in the amounts of compounds with low boiling points, including linalool, limonene, and farnesol, which may be due to evaporation. Lower boiling chemicals dramatically decreased with increased storage duration in the refrigerator, especially at room temperature. However, these changes were very slight in freezing temperature. Our data suggest that limonene and linalool are two major compounds in neroli EO. They are responsible for the aroma of the EO. Its therapeutic effects are most effective just after EO extraction, however after six months of storage, the amount of linalool acetate dramatically increased. Therefore, it can be advised for the food and pharmaceutical industries to store the EO at room temperature and in complete darkness. Our data suggest that the use of natural EOs in various fields such as medicine, cosmetics,

and the food industry, requires consideration of storage conditions and their effects that change the composition and quality of the EOs. Therefore, producers and consumers can take advantage of this method. Consequently, the storage of secondary metabolites extracted from plants, such as EOs, can be investigated more than other metabolites.

Author contributions

Supervision: M. J. S.; data curation: A. M.; funding acquisition: M. J. S.; writing-review and editing: M. J. S., A. M., and T. M. H.; investigation, methodology: A. M.; validation, writing-original draft preparation, M. J. S., A. M., and T. M. H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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