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Chemical Variability in the Composition of *Zhumeria majdae* (Rech. F. & Wendelbo) Essential Oil According to Storage Time and Temperature

Akbar Karami [1], Fatemeh Tashani [1], Aminallah Tahmasebi [2] and Filippo Maggi [3,*]

[1] Department of Horticultural Science, School of Agriculture, Shiraz University, 71441-65186 Shiraz, Iran; akarami2004@gmail.com (A.K.); ftashani2014@gmail.com (F.T.)
[2] Minab Higher Education Center, Department of Agriculture, University of Hormozgan, 79161-93145 Bandar Abbas, Iran; Tahmasebi.info@yahoo.com
[3] School of Pharmacy, University of Camerino, 62032 Camerino, Italy
* Correspondence: filippo.maggi@unicam.it

Abstract: *Zhumeria majdae (*Rech. F. & Wendelbo*)* is an aromatic herb belonging to the Lamiaceae family, traditionally employed in the Persian medicine for the treatment of a wide number of diseases. In the present study, the chemical composition of *Z. majdae* essential oil obtained from the plant's aerial features, and stored at various temperatures (refrigerator temperature 4 °C, freezer temperature −20 °C, and room temperature 20 ± 3 °C) and times (0, 3, 6, and 9 months) was studied. The essential oil was isolated through hydrodistillation, and its composition was evaluated by gas chromatography/mass spectrometry (GC/MS). The results showed that the composition of essential oils changed as a function of the various storage temperatures and times. Linalool (34.85–48.45%), camphor (27.09–39.17%), limonene (1.97–4.88%), and camphene (1.6–4.84%) made up the main volatile compounds which showed differences in their concentrations according to the various storage conditions. Notably, when compared to a non-stored treatment sample (analyzed immediately after essential oil collection), the amount of linalool and camphor increased in all samples stored in all conditions of temperature and time, with the exception of the samples stored for nine months at room temperature. On the other hand, limonene and camphene contents decreased during the storage treatments, showing that the highest content of these compounds occurred in the non-stored treatment. Essential oil storage at the freezer temperature and for three months storage time resulted in the highest average value of the major constituents, highlighting these as the best conditions for obtaining the highest content of the major compounds.

Keywords: chemical constituents; essential oil; storage temperature; storage time; *Zhumeria majdae*

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1. Introduction

Essential oils produced by aromatic plants are made up of various phytochemical combinations, including hydrocarbons, monoterpenes, sesquiterpenes, and phenylpropanoids [1]. *Zhumeria majdae* (Rech. F. & Wendelbo) (Lamiaceae family) is an aromatic herb that grows wild in Hormozgan Province, southern Iran [2,3]. Its aerial parts are used in Iranian traditional medicine to cure dysmenorrhea, stomachache, acid reflux, cold, headache, diarrhea, wounds, and indigestion [4–7]. A few pharmacological properties have been evidenced for the *Z. majdae* extracts, namely anti-nociceptive and anti-inflammatory properties [2]. It has been previously shown that linalool, camphor, and limonene constitute the major *Z. majdae* essential oil components [8–11]. This essential oil has been proven to exhibit insecticidal, antioxidant, antifungal, and antibacterial properties [9,11,12–16]. A recent study showed that *Z. majdae* has also an effective on morphine withdrawal syndrome in mice [8].

Essential oils are recognized as valuable products worthy of industrial exploitation, since they show a wide range of biological properties including antimicrobial, antidiabetic, repellent, anticancer, antioxidant, and anti-inflammatory [17–21]. Thus, they are extensively used in the food, pharmaceutical, cosmetics, and perfume industries [1,22–26]. Notably, there is a growing interest in the use of plant essential oils as natural substitutes for synthetic compounds in human health [27].

The storage process of essential oil is considered a pivotal step in conserving the product quality [28]. On the other hand, the chemical composition of essential oils can be affected by a number of factors, including environmental conditions, collection time, phenological stages, and extraction methods [29–33]. Moreover, it has been shown that some environmental factors, including light, temperature, and oxygen availability, change the composition of essential oils during the storage process [34]. Instability and degradation of essential oils are caused by environmental factors during storage conditions [28]. Temperature is one of the most important factors affecting the quality of essential oils [35–38]. Little is known in terms of the effect of storage conditions on the chemical composition of *Z. majdae* essential oil. Therefore, the present study was conducted to evaluate the effect of various storage factors, including temperature and time durations, on *Z. majdae* essential oil composition during 9 months of storage at various temperatures, which may represent the best storage conditions to obtain high quality essential oil.

2. Materials and Methods

2.1. Plant Material and Essential Oil Isolation Procedure

The aerial features of *Z. majdae* were collected at the flowering stage from the Geno mountains, Hormozgan Province, southern Iran (27°23′10″ N latitude, 56°11′55″ E longitude), in March 2018. For plant collection, the aerial parts of five plants in three replications were harvested from the natural habitat; the distance between each replication was approximately 50 m. The aerial parts were air-dried at ambient temperature. The *Z. majdae* essential oil was obtained through hydrodistillation for 3 h in three replications, using a Clevenger apparatus. The essential oil was separated, dried with anhydrous sodium sulfate, and kept in closed dark vials.

2.2. Essential Oil Storage Conditions

Z. majdae essential oil composition was investigated using various storage temperatures and times through the methods as described previously [35,36,38]. In this regard, the effect of three storage temperatures, namely room temperature (20 ± 3 °C), refrigerator (4 °C), and freezer temperature (−20 °C) and four storage time levels (non-stored: this sample was analyzed immediately after its collection from the Clevenger apparatus; three months; six months; and nine months) on the chemical composition was evaluated. The essential oil samples were stored in closed dark vials, and the essential oil composition of each treatment was assessed by gas chromatography/mass spectrometry (GC/MS).

2.3. Essential Oil Analysis

The essential oil compounds were identified using a gas chromatograph (Model 7890A, Agilent Technologies, Wilmington, DE, USA) containing a flame ionization detector (FID) and an HP−5 column (30 m l. × 0.25 mm i.d., 0.25 μm f.t.). The temperature program of the column began at 60 °C, increased to 210 °C at 3 °C /min, and then reached 240 °C at 20 °C/min; the program continued for 8.5 min at 240 °C. The temperature of the injector was 280 °C. Nitrogen was the carrier gas, with a flow rate of 1 mL/min. GC/MS analysis was conducted by the GC connected to a mass-spectrometer (Model MS-5975C, Agilent Technologies, Wilmington, DE, USA). The stationary phase was a HP-5MS (30 m l. × 0.32 mm i.d., 0.25 μm f.t.). The temperature of the MS injector and detector was 280 °C. The carrier gas was applied at a 1 mL/min flow rate. The ionization energy used here was

equal to 70 eV. The gas chromatography/flame ionization detection (GC-FID) semi-quantitative determination of each essential oil component was based on the peak area normalization without using response factors.

2.4. Correlation, Principal Component, Cluster and Statistical Analyses

Pearson correlation analysis was performed in order to evaluate the relationship between the major essential oil compounds (linalool, camphor, limonene, and camphene) using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). Moreover, principal component analysis of the major essential oil compounds during the various storage conditions and cluster analysis of the storage times (0, 3, 6, and 9 months) were conducted using Minitab 16 statistical software. Data were analyzed as a completely randomized design with three replications. Data were expressed as means ± standard deviation (SD). The statistical significance of differences between treatments were determined by analysis of variance (ANOVA), and testing for differences between means were measured by the least significant difference (LSD) test at $p \leq 0.05$.

3. Results and Discussion

In this study, the essential oil composition of *Z. majdae* was assessed at different temperatures and storage times. The harvesting and storage are important factors affecting the essential oil quality and chemical profile [39,40]. It has also been shown that the increase of temperature during storage decreases product quality [41–43]. Therefore, in this study, we evaluated the effect of various storage conditions, including temperature and storage times, on the composition of *Z. majdae* essential oil. A number of previous studies highlighted the influence of different storage conditions on the overall essential oil profile [35,36,38,44,45]. The results showed that the quali-quantitative composition of *Z. majdae* essential oil changed as a function of the various temperatures and storage times (Table 1). GC-FID and GC/MS analyses revealed four main volatile constituents, namely linalool (34.85–48.45%), camphor (27.09–39.17%), limonene (1.97–4.88%), and camphene (1.6–4.84%), with fluctuations in composition percentages according to the storage conditions applied (Table 1). Compared to the non-stored sample, linalool and camphor increased in all of the storage treatments with the exception of the sample analyzed after nine months at room temperature. Linalool showed the highest increment at three- and nine-months storage at 4 °C (i.e., 22.89, and 22.94%, respectively) (Figure 1). In addition, camphor exhibited the highest increment (26.60%) at three months storage at 4 °C, when compared to the non-stored sample (Figure 2). The highest concentrations of linalool and camphor were obtained at three months storage. On the other hand, the greatest decrease for linalool and camphor was detected after 9 months at room temperature (11.57 and 12.44%, respectively). With regard to limonene and camphene, they decreased during all storage treatments, compared to non-stored sample (Figures 3 and 4). Notably, limonene showed the greatest reduction at three- and nine-months storage at 4 °C (59.63% decreased rate) (Figure 3), whereas camphene revealed the highest decline rate during three- and nine-months storage at 4 °C (66.94 and 65.29%, respectively) (Figure 4). It has been shown that some environmental factors, including temperature, light, and oxygen availability, change essential oil composition during the storage periods [35,36,38]. Temperature is one of the most important factors affecting the quality of essential oils [35–38]. In this study, the major components of *Z. majdae* were affected by the temperature of the storage conditions. In the previous study, the major constituents of balm mint (*Melissa officinalis* L.) essential oil, including citronellal, neral, and geranial, were decreased in storage conditions, and the highest and lowest reductions occurred at room and freezer temperatures, respectively [36]. The molecular weights of linalool (154.25) and camphor (152.23) are higher than those of limonene (136.23) and camphene (136.23). The findings of our results indicated that the content of compounds with a lower molecular weight decreased by prolonging the storage time, especially at room temperature. Previous research shows that the evaporation,

oxidation, and other unwanted changes in essential oil components during the storage period could be related to molecular weights of compounds [45].

Other compounds including α-pinene, 3-octanone, myrcene, γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, geranial, isophorone, *cis*-jasmone, (*E*)-caryophyllene, and neral, revealed a higher content in the non-stored sample (Table 1). Notably, some constituents were specifically identified in samples analyzed with particular storage treatments. In this regard, β-pinene, *n*-octanal, (*E*)-β-ocimene, and thymol were found only in the non-stored sample. Moreover, *cis*-linalool oxide, *trans*-linalool oxide and β-eudesmol were found in the non-stored and six-month storage samples (Table 1). *p*-Cymene and α-humulene were detected in the six-month storage sample (Table 1). However, a variety of compounds, including borneol, nerol, geraniol, and caryophyllene oxide, were not found in the non-stored sample (Table 1). Moreover, α-terpineol, 2,6-octadiene, and neral were not found in the nine-month storage sample (Table 1).

The findings also revealed that, when compared to refrigerator and room temperatures, essential oil storage at the freezer temperature resulted in the most average value of the major constituents, which may be due to the protection of antioxidant compounds of the essential oil at this temperature. In addition, storage treatments decreased the number of compounds present. Indeed, a total of 25 constituents were found in the non-stored essential oil sample in the highest amount (Table 1). Compared to three- and nine-month storage samples, the number of compounds increased in the six-month storage sample. The changes in the content and number of the essential oil compounds could be attributed to a number of factors, including evaporation and oxidation during the storage period [36]. Moreover, essential oils might be transformed to other phytochemical constituents through a number of chemical reactions, including isomerization, dehydrogenation, and cyclization [36,46]. Therefore, less stable compounds quickly degrade due to their chemical interactions with other constituents [47], occurring as a function of storage times and temperatures.

Table 1. Chemical composition of the essential oil *Zhumeria majdae* at various storage times and temperatures.

NO	Compound	RIa	RIb	No Storage	Compounds (%)								
					After 3 Months			After 6 Months			After 9 Months		
					RT	R	F	RT	R	F	RT	R	F
1	α-Pinene	938	932	2.38*	1.36	0.53	1.34	1.26	0.73	1.34	1.77	0.61	1.33
2	Camphene	935	946	4.84	3.32	1.60	3.11	2.92	2.01	3.08	2.37	1.68	3.03
3	β-Pinene	977	974	0.21	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	3-Octanone	981	979	1.86	1.11	0.76	0.94	0.95	0.87	0.93	0.77	0.74	0.91
5	Myrcene	988	988	1.03	ND	ND	0.38	0.37	0.27	0.37	0.33	ND	ND
6	*n*-Octanal	989	989	0.10	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	*p*-Cymene	1022	1020	ND	ND	ND	ND	0.21	0.14	0.10	ND	ND	ND
8	Limonene	1032	1024	4.88	3.05	1.97	2.64	2.66	2.21	2.60	2.11	1.97	2.59
9	(*E*)-β-Ocimene	1045	1044	0.24	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	γ-Terpinene	1060	1054	0.55	ND	ND	0.32	ND	0.31	0.30	ND	ND	ND
11	*cis*-Linalool oxide	1067	1067	0.44	ND	ND	ND	0.21	0.21	0.20	ND	ND	ND
12	*trans*-Linalool oxide	1069	1084	0.26	ND	ND	ND	0.19	ND	ND	ND	ND	ND
13	Terpinolene	1090	1086	1	ND	ND	0.49	0.33	0.40	0.47	ND	ND	0.43
14	Linalool	1100	1095	39.41	48.20	48.43	47.07	45.60	46.24	46	34.85	48.45	48.23
15	Camphor	1140	1141	30.94	37.30	39.17	36.86	36.49	36.08	36	27.09	37.21	35.98
16	Borneol	1164	1165	ND	2.03	2.10	1.89	1.95	2.19	1.99	1.42	2.03	1.81
17	Terpinen-4-ol	1174	1174	0.63	ND	0.40	0.27	0.19	0.44	0.25	ND	0.49	ND

18	α-Terpineol	1185	1186	3.67	ND	ND	0.35	0.42	0.22	0.32	ND	ND	ND
19	Nerol	1231	1227	ND	ND	ND	0.77	ND	ND	0.81	ND	1.05	1.09
20	Neral	1240	1235	0.77	0.1	0.37	0.22	0.2	0.4	0.2	0.12	0.2	0.2
21	(*Z*)-Anethole	1249	1249	0.44	ND	0.50	ND	0.08	0.54	ND	ND	0.50	ND
22	Geraniol	1252	1252	0.2	1.90	0.74	1.37	2.06	2.14	2.18	1.99	2.11	2.08
23	Geranial	1268	1264	1.67	0.2	0.77	0.48	0.29	0.78	0.39	0.28	0.45	0.37
24	Thymol	1291	1289	0.09	ND	ND	ND	ND	ND	ND	ND	ND	ND
25	(*Z*)-jasmone	1400	1392	0.29	ND	0.26	0.26	0.23	0.27	0.26	0.24	0.26	0.23
26	(*E*)-Caryo-phyllene	1417	1417	2.17	1.75	1.35	1.10	1.69	1.53	1.17	1.93	1.47	1.22
27	α-Humulene	1453	1452	ND	ND	ND	ND	0.17	0.21	0.15	ND	ND	ND
28	Caryophyllene oxide	1589	1582	ND	ND	0.38	0.25	0.30	0.78	0.48	0.38	0.80	0.51
29	β-Eudesmol	1646	1649	3.23	ND	ND	ND	ND	ND	0.20	ND	ND	ND
	Total			99.99	99.99	100	99.99	99.67	99.95	99.89	99.67	99.95	99.89

RIa: Retention indices analyses on HP-5MS column; RIb: Retention index value taken from ADAMS library; ND: not detected; RT: Room Temperature; R: Refrigerator Temperature; F: Freezer Temperature. *Data of this table is the mean of three replicated analyzed samples.

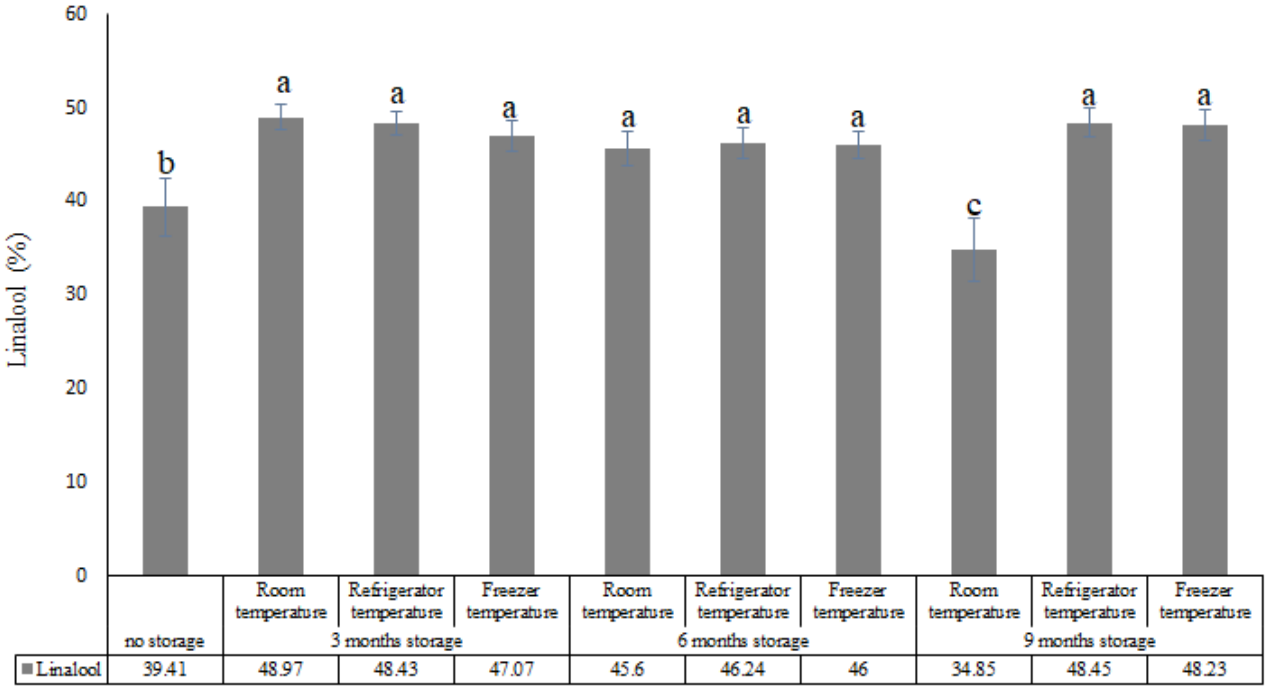

Figure 1. The linalool content of *Zhumeria majdae* essential oil at various storage times and temperatures; the different letters denote a statistically significant difference at $p \leq 0.05$, as determined by LSD tests.

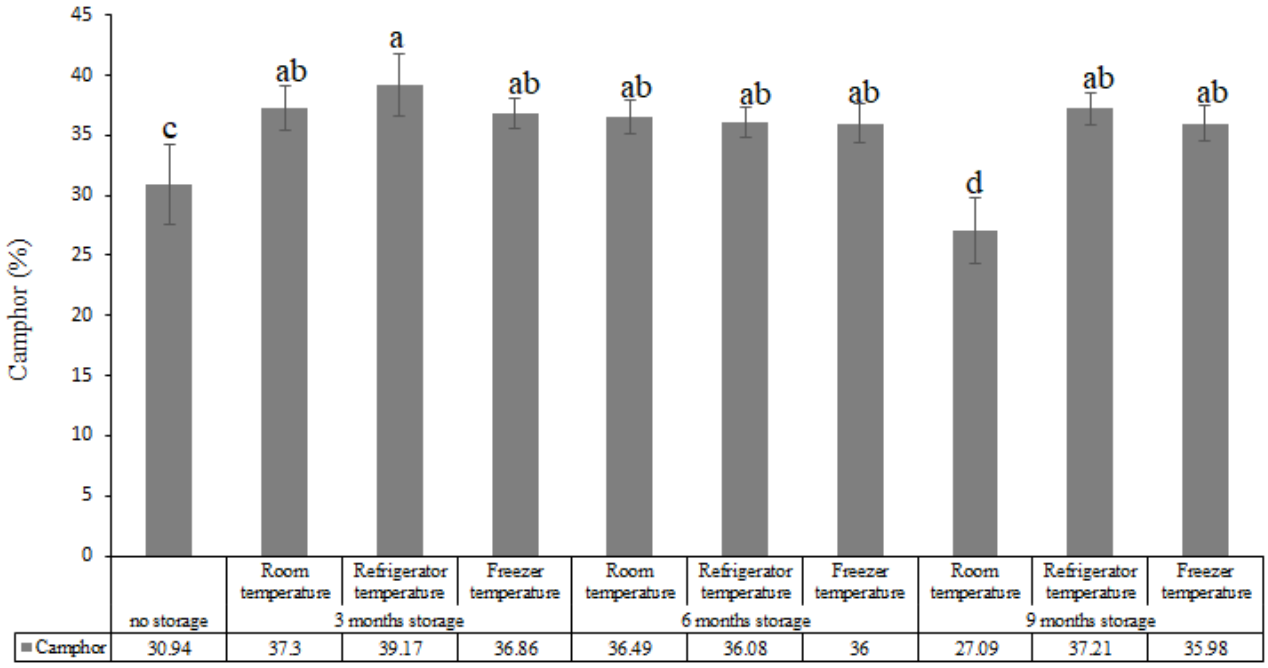

Figure 2. The camphor content of *Zhumeria majdae* essential oil at various storage times and temperatures; the different letters denote a statistically significant difference at $p \leq 0.05$, as determined by LSD tests.

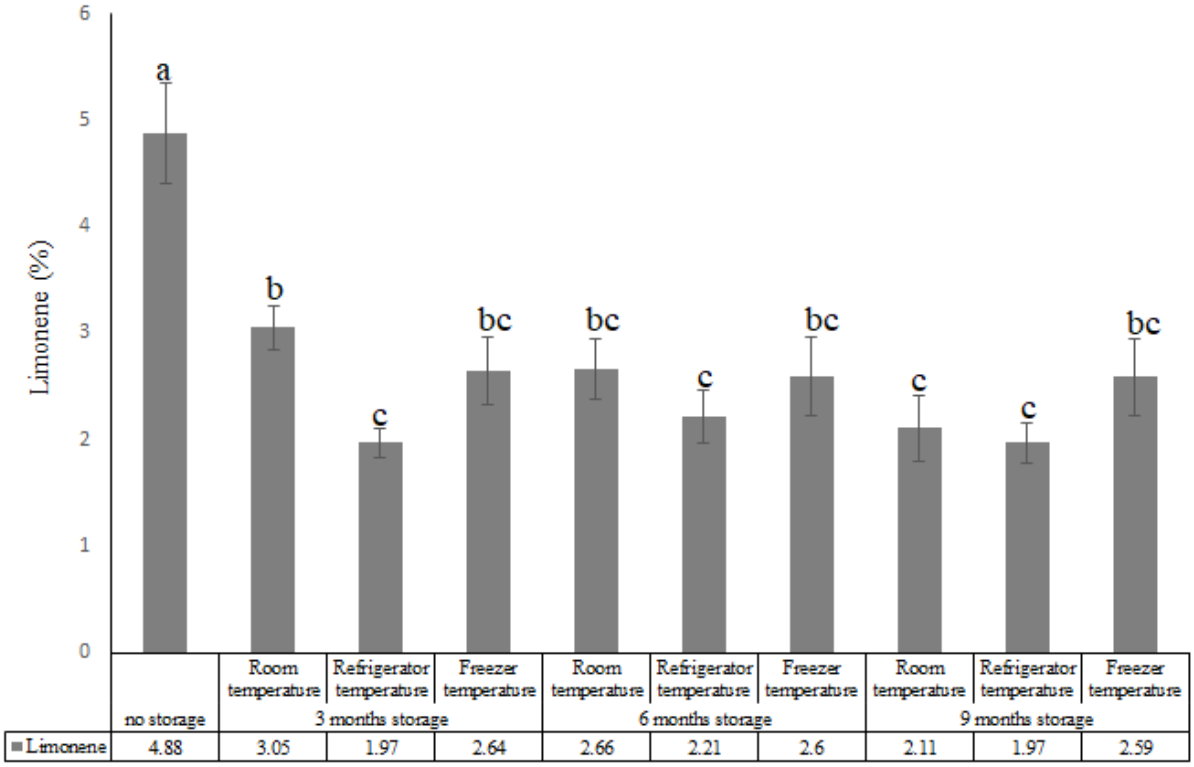

Figure 3. The limonene content of *Zhumeria majdae* essential oil at various storage times and temperatures; the different letters denote a statistically significant difference at $p \leq 0.05$, as determined by LSD tests.

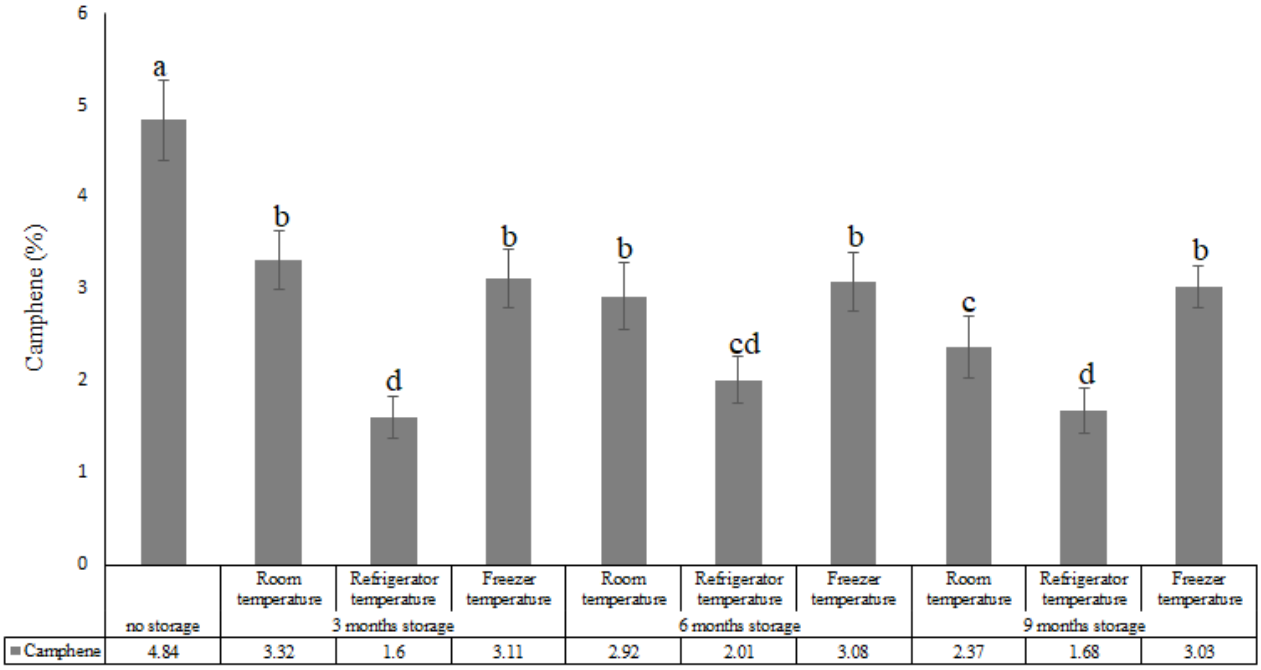

Figure 4. The camphene content of *Zhumeria majdae* essential oil at various storage times and temperatures; the different letters denote a statistically significant difference at $p \leq 0.05$, as determined by LSD tests.

Pearson correlation analysis exhibited a significant positive correlation between linalool and camphor (r = 0.986, significant at the 0.05 level) (Table 2). In addition, camphene showed a significant positive correlation with limonene content (r = 1, significant at the 0.01 level) (Table 2). On the other hand, limonene had a non-significant negative correlation with linalool and camphor (r = −0.824 and −0.731, respectively) (Table 2). Camphene revealed a non-significant negative correlation with linalool and camphor (r = −0.820 and −0.725, respectively) (Table 2). Therefore, the correlation findings highlighted that increases in linalool and camphor were associated with a decrease in camphene and limonene contents. Principal component analysis was performed based on the major *Z. majdae* essential oil compounds (i.e., linalool, camphor, limonene, and camphene) under the various storage conditions (Figure 5). The findings showed that essential oils stored under various storage conditions were placed into three different groups: three-, six-, and nine-months storage, and non-stored samples (Figure 5). In addition, the essential oil sample which underwent no storage treatment was placed close to limonene and camphene, showing the highest content of these compounds in the non-stored group. Meanwhile, the essential oil kept for three months was placed close to linalool and camphor, highlighting the highest concentration of linalool and camphor at three months storage (Figure 5). Furthermore, the hierarchical cluster analysis of the *Z. majdae* essential oils during the different storage times showed various degrees of similarity among the storage times (Figure 6). In this respect, the cluster analysis revealed that the similarity of essential oil storage times ranged from 24.29 to 74.76% (Figure 6). The non-stored sample demonstrated the lowest similarity (24.29%) in the major essential oil compounds, compared with other storage times. Moreover, three- and six-month storage times showed the highest similarity (74.76%) in the major essential oil compounds. The treatments, namely three-, six-, and nine-months storage times, represented a 36.91% similarity level (Figure 6).

Table 2. The Pearson correlation coefficient between the major essential oil components of *Zhumeria majdae* at various storage times and temperatures.

	Linalool	Camphor	Limonene	Camphene
Linalool	1			
Camphor	0.986 *	1		
Limonene	−0.824 ns	−0.731 ns	1	
Camphene	−0.820 ns	−0.725 ns	1 **	1

Ns, * and ** represent no significant and significant correlation at the 0.05 and 0.01 levels (two-tailed), respectively.

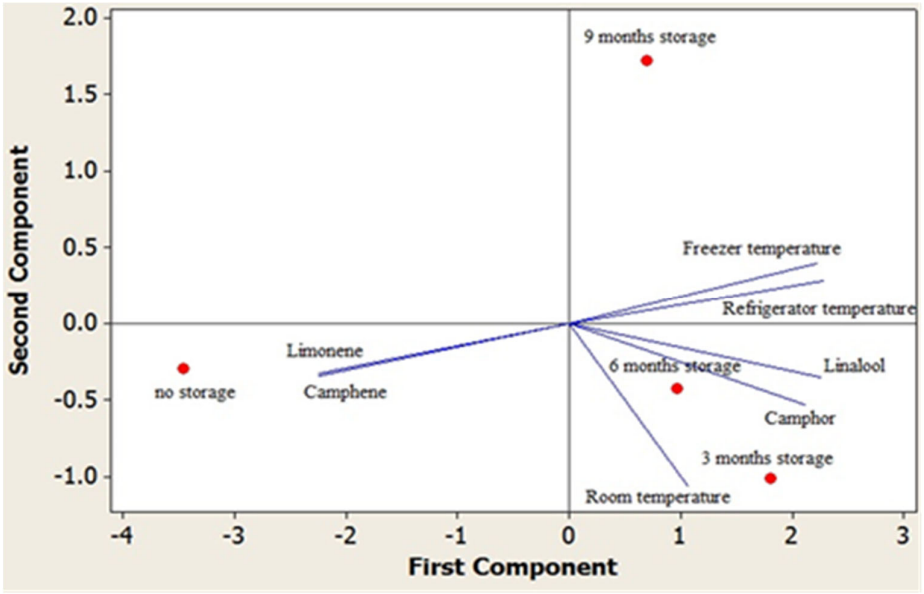

Figure 5. Biplot of the first two principal components for the major *Zhumeria majdae* essential compounds (linalool, camphor, limonene, and camphene) at various temperatures and storage times.

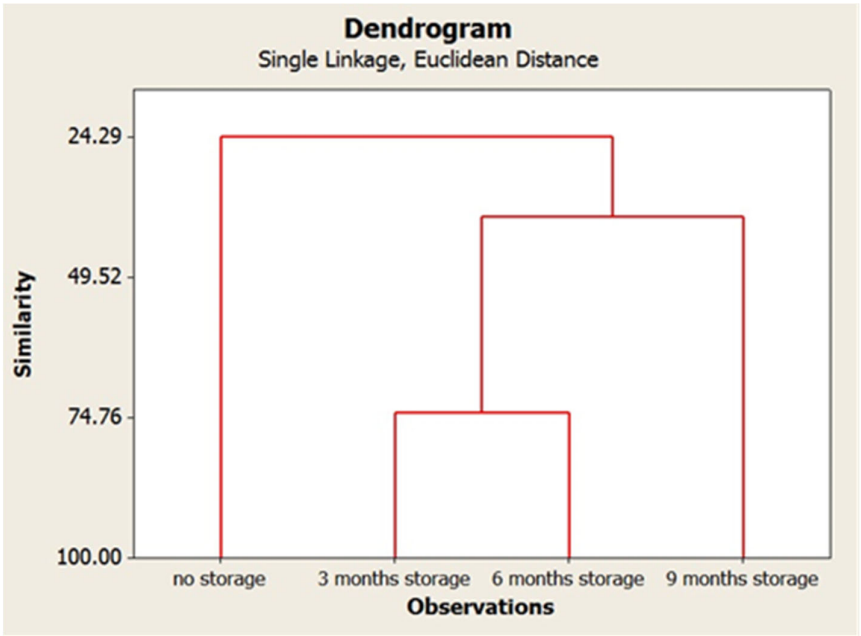

Figure 6. Hierarchical cluster analysis of the major *Zhumeria majdae* essential compounds (linalool, camphor, limonene, and camphene) at various temperatures and storage times.

4. Conclusions

In the present work, we studied the variation in the major constituents of *Z. majdae* essential oil as affected by storage conditions. The results showed that, compared to the non-stored sample, linalool and camphor increased under all the storage treatments, with the exception of the sample analyzed after nine months at room temperature. With regard to limonene and camphene, they decreased during all storage treatments, compared to the non-stored sample; however, this reduction was very low under freezer storage conditions, as compared to refrigerator temperature. Thus, the essential oil stored in the freezer contained higher amounts of limonene and camphene, which may have prevented their contents decreasing after nine months. Therefore, the information generated here may aid the food, cosmetic, and pharmaceutical industries to be aware of the effect of storage conditions on *Z. majdae* essential oil quality, and its storage at the most appropriate conditions.

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