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Effect of Storage Duration and Condition on Essential Oil Components of *Mentha aquatica* L.

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ABSTRACT

Mentha aquatica is an important traditional herb in north Iranian cuisine and folklore medicine, with high potential for storage as dried leaves. This study examines how different storage conditions and times affect the essential oil percentage and components of dried *M. aquatica*. Samples were collected from *M. aquatica* flowering branches at a natural habitat. The experiment had two storage treatments, i.e., refrigeration at 4 ± 1 °C and storage at 25 ± 2 °C with adequate airflow. There were three storage durations, i.e., 60, 120, and 365 d after drying, and three drying methods, i.e., shade-drying at 25 ± 2 °C, oven-drying at 40 °C, and microwave-drying at 200 W. The essential oil components decreased through storage duration, which was more noticeable in samples stored at 25 ± 2 °C compared to those stored at 4 ± 1 °C. Additionally, the type and amount of essential oil components differed under various storage conditions. The main compound in *M. aquatica* essential oil was 1,8-cineole, with its highest content (26.55%) in oven-dried samples. The second-most prominent essential oil compound was *trans*-caryophyllene, with a high content in shade-dried plants stored at 25 ± 2 °C for 120 d and a lower content in samples dried with a 200 W microwave, followed by storage at 2 ± 4 °C for 365 d. The optimal treatment for maintaining more of the essential oil components was oven-drying at 40 °C and storage at 25 ± 2 °C. In sum, the current research showed that *M. aquatica* optimally retains its EO components when the foliage is oven-dried and subsequently stored at 2 ± 4 °C. This finding can benefit the EO industry in harvesting *M. aquatica* on a sustainable basis through drying and storage.

Abbreviation: Essential oil (EO), gas chromatography (GC), gas chromatography-mass spectroscopy (GC/MS), statistical analysis of data using (SAS), *Mentha aquatica* (*M. aquatica*), and flame ionization detection (FID)

Introduction

M. aquatica is a species of the Lamiaceae family. Its morphology may include or be without trichomes and blue flowers on an erect stem with flipped trichomes (Safaiee et al., 2019). It grows naturally in humid regions and is locally known as "Oji" in northern Iran. The plant's chemical composition comprises phenols and flavonoids in its EO (Alara et al., 2021). *M. aquatica* is highly effective in dealing with respiratory complications such as colds, allergies, asthma, bronchitis, itches, and abdominal pain (Sajjadi et al., 2013; Salmanian et al., 2012). Furthermore, its extract is an efficient antioxidant and acts as an alternative to synthetic chemicals in controlling microbial growth and extending food shelf life

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(Salehi et al., 2018; Salmanian et al., 2010).

EO composition depends on various factors, including plant type, harvest season, post-harvest period, plant age, and storage conditions (Dokhani et al., 2005). Drying is one of the most effective ways of reducing product bulk and enabling easy transportation. Correct drying, packing, and storage procedures also help to reduce waste volume and improve the quality of the final product (Ahmadi et al., 2008). The humidity level of medicinal plants should be maintained at 10-14% at the end of the drying process to ensure extended storage (Poós and Varju, 2017). Plant EO compounds are affected by drying and storage parameters, such as duration and packaging methods, which can lead to changes in biochemical properties; for example, the anthocyanin, vitamin C, acidity, and Brix values of seedless barberry were found to be significantly influenced by the different drying methods and storage periods (Talebzadeh et al., 2013).

Extended storage duration can also affect EO components, as seen in studies on *Echinophora tenuifolia*, where significant changes were detected after 365 d of storage at 4 ± 1 °C (Chalchat et al., 2007). An increase in storage duration caused a significant decrease in the amount of EO in lemon verbena (Ebadi et al., 2017). Drying of *Mentha haplocalyx* plant led to lower amounts of certain EO components, such as alpha-pinene, except for beta-caryophyllene and thymol, which increased in their concentrations. However, prolonged storage of the dried plants led to significant changes in some EO components, including alpha-thujene, myrcene, and caryophyllene oxide, while other compounds were unaffected (Zhu et al., 2015). A study on tea leaves found reduced amounts of tannin and caffeine with prolonged storage, though the quantities of 1-octen-3-ol, ocimene, cis-farnesene, and cedrol increased (Mei et al., 2022).

Due to limited studies on *M. aquatica*, the bioactive components of this medicinal plant remain unknown in Iran. This research aimed to measure the chemical components of EO obtained from the aerial parts of *M. aquatica* and to draw comparisons between components after storage in various conditions. *M. aquatica* was selected as the primary material for this research because of novelty in the current approach to the processing and storage of this plant, since there is a lack of available literature on the methods of processing and drying this plant. The research question prompted efforts to explore the optimum drying method and storage conditions for the sustainable use of this plant species throughout the year, with a particular focus on EO components and their ability to remain stable while in storage.

Material and Methods

Plant collection and identification

To evaluate the effects of time and storage conditions, undamaged dried samples of *M. aquatica* were selected. The samples were dried either in an oven at temperatures of 40, 45, 50 °C, in a microwave 200, 400 and 600 W, sun-dried, or air-dried under the shade. In September 2020, the plants were transferred from Balamarznak, Iran, to Marzun Abad, Babol, Mazandaran, where blooming began. The location was characterized by an altitude of 250 m above sea level and an annual rainfall of 671 mm. Voucher specimens were deposited in the Kevin Herbarium.

Drying methods

The drying methods were 1) shade-drying at 25 ± 2 °C indoors (light intensity 200 lm m-2) 2) drying in an oven at 40, 45, 50 °C, and 3) drying in a microwave operating at 200, 400, 600 W. By calculating the moisture percentage of the final dried sample, the weight of the sample was measured before and after drying. Weight change was calculated by subtracting the final weight from the initial weight. Finally, using the following formula, the final moisture percentage was determined according to Eq. 1:

$$\text{Moisture percentage (\%)} = \frac{\text{Change in weight (g)}}{\text{Initial weight (g)}} \times 100 \quad (1)$$

Essential oil content

One-hundred g of dried plant sample was powdered and added to 1200 mL of distilled water. The solution was placed in a Clevenger-type apparatus and extracted for 4 h. The essential oil content was stored in an Eppendorf tube, surrounded by aluminum foil, and placed in a refrigerator until further use for GC-MS analysis of essential oil quality.

Storage location, time, and drying methods

The samples were placed either in the refrigerator (1 ± 4 °C) or in a shaded environment at 25 ± 2 °C. Three storage durations were considered, i.e., 60, 120, and 365 d after drying. The samples were dried either at 25 ± 2 °C, in an oven at 40 °C, or with a microwave at 200 W.

Measurement of EO percentage and components

The EO content was measured by distillation and

with an EO extraction device (Clevenger-type apparatus) (McLafferty and Stauffer, 1989). GC and GC/MS determined the essential oil components and their amounts. The gas chromatography device (Younglin Acme 6000) was equipped with a capillary column (30 m and 0.25 mm diameter). It had a 0.25 µm stable phase layer thickness of Bp5. The essential oil sample (1 µL) was diluted with n-hexane and injected into the GC device to categorize essential oil components. The column temperature was set at an initial oven temperature of 50 °C, which remained for 2 min, and increased up to 130 °C at a rate of 3 °C min^{-1}. The temperature was maintained at 130 °C for 2 min and then the temperature increased to 270 °C at a rate of 5 °C min^{-1}. The temperature was maintained at 270 °C for 3 min. The injector temperature was 280 °C and helium gas was applied as carrier gas, at a flow rate of 1 mm min^{-1}. The FID detector temperature was 300 °C (McLafferty and Stauffer, 1989).

Characterization of GC/MS

The chromatographic device (Agilent 7890A) was equipped with a mass spectrometer (Agilent 5975). A Bpx5-type capillary column (30 m in length and 0.25 µm internal diameter) was used to identify the essential oil components. After diluting the essential oil sample with n-hexane, 0.01% was mixed with 0.04% of n-hexane, and 1 µL of this solution was injected into the GC/MS device. The temperature of the capillary column began from an initial oven temperature of 50 °C, remaining at this temperature for 2 min, and then increased to 130 °C at a rate of 3 °C min^{-1}. It was maintained at 130 °C for 2 min. Then, the temperature increased to 5 °C min^{-1} and reached 270 °C, where it remained for 3 min.

The injector temperature was set at 280 °C, and helium gas was used as the carrier gas at a 1 mL min-1 flow rate. The Agilent 5975 mass spectrometer was used in association with 70 electron volts as the ionization voltage. In the ionization method, EI was set as the temperature of the ionization source equal to 220 °C, and the scan range was specified from 40 to 465. The software for this purpose was Chemstation, and the spectra were identified by considering their inhibition index and comparing it to the indexes available in reference books and articles. Using mass spectra, comparisons of standard components were made, and available information from libraries was used.

Statistical analysis

Statistical analysis of data was done by SAS software (ver. 9.4) (SAS Institute, Cary, NC, USA) and the comparison of means was done using Duncan's multiple range test at the 5% probability level. Microsoft Excel (2016) was used for illustrating the graphs.

Results

The analysis of variance showed that the interaction effects of storage temperature (a), storage duration (b), and drying method (c) caused significant differences in the EO content and components ($P \leq 0.01$) (Table 1). Mean comparisons showed that the highest EO content (1.2%) was measured in shade-dried samples stored at 4 ± 1 °C for 60 d. A longer storage period was associated with a decrease in the EO content of *M. aquatica*. The lowest EO content occurred in the microwave drying method (200 W), followed by storage at 25 ± 2 °C for 365 d, which reduced one-third of the EO percentage compared to the optimal treatment (Table 1).

EO components

According to the analysis of variance, the EO components were α-thujene, α-pinene, β-pinene, β-myrcene, cymene, *trans*-beta-ocimene, γ-terpinene, 4-(terpineol), borneol, ISO menthol, menthol, endo borneol, lavandulyl acetate, α-copaene, β-elmene, β-farnesene, α-humulene, delta-cadinene, aromadendrene, t-muurolol, octen3ol(3-octanol), benzene, methyl dihydroedulan, and eugenol. The highest components of the essential oil were 1,8-cineole (30.56%), mentafuran (17.68%), germacrene-D (10.76%), transcaryophyllene (17.31%), and viridifloral (4.29%). The essential oil had significant amounts of hydrocarbon monoterpenes, oxygenated monoterpenes, sesquiterpenes, monoterpenes: sesquiterpenes ratio, 1,8-cineole: EO ratio, alphapinene, camphene, sabinene, betapinene, *trans*-alpha ocimene, gammaterpinene, 1,8-cineole, menthafuran, 4-terpineol, menthol, endobrunol, lavendol acetate, alphacopene, deltacadinen, gammamorulene, caryophyllene oxide, vidrifloral, 3-octanol, one-methyl benzene, dihydrodolane, and mintfuran ($P \leq 0.05$) (Table 1). Significant differences were observed in α-cardinal by the simple effect of the storage temperature treatment (a), the effects of storage temperature (a) × storage duration (b), as well as the storage temperature (a) × drying method (c) ($P \leq 0.05$). Also, significant differences were observed by the simple effects of storage duration (b) and drying method (c), the effects of these two and the interaction effects of treatments ($P \leq 0.01$) (Table 2).

Table 1. Mentha aquatica under the influence of different drying methods shade-drying (200 mL m^{-2}), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 ℃ and 4 ± 1 ℃) affecting EO components.

S.O.V	D.f	Mean of squares								
		Essential oil percent	*α*-thujene	*α*-pinene	*β*-pinene	*β*-myrcene	cymene	*trans*-beta-ocimene	*γ*-terpinene	4-(Terpineol)
A	1	0.025 **	0.017 **	0.127 **	44.65 **	0.0044 **	0.0012 **	0.112 **	0.240 **	0.053 **
B	2	1.006 **	0.169 **	30.52 **	8.65 **	4.24 **	2.08 **	0.806 **	1.66 **	0.680 **
C	2	0.177 **	0.082 **	10.04 **	26.95 **	0.040 **	0.531 **	0.474 **	1.58 **	0.196 **
AB	2	0.004 ns	0.017 **	3.07 **	11.96 **	0.0044 **	0.0012 **	0.108 **	0.404 **	0.043 **
AC	2	0.0063 ns	0.022 **	0.145 **	0.736 **	0.0023 **	0.0025 **	0.109 **	0.110 **	0.60 **
BC	4	0.017 **	0.066 **	1.34 **	2.64 **	0.0401 **	0.531 **	0.344 **	0.735 **	0.687 **
ABC	4	0.0062 ns	0.022 **	1.59 **	1.47 **	0.0023 **	0.0025 **	0.109 **	0.564 **	0.066 **
Error	36	0.0028	0.0001	0.0097	0.075	0.0005	0.0001	0.00008	0.0016	0.0003
Coeff var		7.17	13.15	4.60	7.37	8.14	5.36	4.94	5.87	8.00

S.O.V	D.f	Mean of squares								
		borneol	ISO menthol	menthol	endo-borneol	lavandulyl acetate	*α*-copaene	*β*-elmene	*β*-farnesene	*α*-humulene
A	1	0.657 **	0.416 **	0.453 **	8.88 **	0.429 **	0.034 **	0.932 **	0.072 **	1.12 **
B	2	27.99 **	1.07 **	3.09 **	45.41 **	1.89 **	0.189 **	10.36 **	2.80 **	7.32 **
C	2	1.41 **	1.07 **	0.561 **	7.002 **	3.86 **	0.2005 **	31.49 **	0.042 **	13.19 **
AB	2	0.657 **	0.448 **	1.29 **	5.42 **	0.71 **	0.196 **	1.28 **	0.069 **	0.542 **
AC	2	0.593 **	0.448 **	0.733 **	13.46 **	1.40 **	0.028 **	0.498 **	0.072 **	1.08 **
BC	4	1.41 **	2.26 **	0.210 **	7.77 **	0.675 **	0.192 **	6.08 **	0.127 **	14.14 **
ABC	4	0.593 **	0.432 **	0.282 **	18.21 **	1.19 **	0.207 **	0.932 **	0.069 **	0.397 **
Error	36	0.0011	0.00022	0.00022	0.012	0.0079	0.00015	0.030	0.00052	0.013
Coeff var		4.60	5.91	5.05	6.18	11.08	10.82	11.10	8.16	5.19

S.O.V	D.f	Mean of squares							
		delta-cadinene	aromadendrene	t-muurolol	octen3ol(3-octanol)	benzene 1methyl	dihydroedulan	min sulfide	eugenol
A	1	1.12 **	0.876 **	3.03 **	6.13 **	0.458 **	0.037 **	0.058 **	0.029 **
B	2	0.631 **	2.96 **	16.53 **	16.23 **	6.58 **	0.621 **	0.097 **	1.08 **
C	2	7.23 **	0.891 **	12.33 **	5.96 **	0.986 **	1.61 **	0.091 **	0.331 **
AB	2	0.834 **	0.235 **	0.403 **	2.55 **	0.373 **	0.123 **	0.057 **	0.029 **
AC	2	1.85 **	2.40 **	0.751 **	0.189 **	0.085 **	0.033 **	0.146 **	0.028 **
BC	4	2.15 **	1.47 **	0.597 **	2.08 **	0.335 **	0.589 **	0.121 **	0.33 **
ABC	4	0.757 **	3.23 **	0.342 **	1.87 **	0.122 **	0.037 **	0.049 **	0.0287 **
Error	36	0.0044	0.00073	0.026	0.0047	0.0036	0.00067	0.00007	0.00052
Coeff var		6.61	7.74	14.38	6.82	13.39	12.83	10.66	16.12

**: significant at 1% level, *: significant at 5% level, ns: not significant. A: storage temperature conditions, B: storage duration and C: drying method.

Table 2. *Mentha aquatica* under the influence of different drying methods shade-drying (200 mL m^{-2}), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 °C and 4 ± 1 °C) affecting EO components.

S.O.V	D.f	Mean of squares						
		camphene	sabinenee	1, 8-cineol	limonene	menthone	menthofuran	(-)-bornyl acetate
A	1	0.0056 ^{ns}	0.907 **	246.30 **	0.0020 ^{ns}	0.0009 *	1.03 ^{ns}	0.010 *
B	2	1.64 **	12.41 **	185.74 **	17.35 **	2.87 **	264.23 **	0.870 **
C	2	3.03 **	2.96 **	148.05 **	0.412 **	0.056 **	131.47 **	0.103 **
AB	2	1.99 **	0.324 **	11.12 **	0.0020 ^{ns}	0.00097 *	10.14 **	0.258 **
AC	2	0.076 **	0.0088 ^{ns}	2.22 ^{ns}	0.0049 *	0.00094 *	6.75 **	0.219 **
BC	4	0.510 **	2.10 **	40.30 **	0.412 **	0.056 **	46.57 **	1.51 **
ABC	4	2.48 **	0.191 **	8.23 **	0.004 **	0.0009 **	7.56 **	0.093 **
Error	36	0.0056	0.0038	0.733	0.00087	0.00022	0.325	0.0022
Coeff var		11.39	4.64	5.77	4.94	6.94	5.70	13.50

S.O.V	D.f	Mean of squares						
		trans caryophyllene	germacrene D	*y*-cadinene	*α*-amorphen	caryophyllen oxid	viridiflorol	α cadinol
A	1	0.892 ^{ns}	16.23 **	0.0031 ^{ns}	0.00097 **	0.045 ^{ns}	15.06 **	0.99 *
B	2	44.31 **	342.54 **	3.22 **	0.2352 **	0.595 **	95.00 **	0.211 **
C	2	33.57 **	104.35 **	0.201 **	0.235 **	1.60 **	51.41 **	0.2113 **
AB	2	7.13 **	1.95 ^{ns}	0.0031 ^{ns}	0.0002 ^{ns}	0.022 ^{ns}	0.022 ^{ns}	0.06 *
AC	2	2.12 *	24.68 **	0.0018 ^{ns}	0.00025 ^{ns}	0.675 **	8.42 **	0.066 *
BC	4	23.51 **	8.53 **	0.201 **	0.413 **	0.620 **	4.08 **	0.430 **
ABC	4	31.86 **	4.64 **	0.0018 ^{ns}	0.00061 **	1.17 **	1.53 *	0.083 **
Error	36	0.622	0.844	0.0031	0.00	0.014	0.460	0.013
Coeff var		6.61	10.40	23.16	0.00	16.84	13.36	10.08

**: significant at 1% level, *: significant at 5% level, ns: not significant. A: storage temperature conditions, B: storage duration and C: drying method.

Based on the comparison of mean values, the highest amount of α-thujene resulted from drying at 40°C in the oven, followed by storage at 25 ± 2 °C for 30 d, leading to a numerical value of 0.51%, which was 3.64-fold higher than the shade treatment group stored for 60 d at 25 ± 2 °C and at 4 ± 1 °C. The highest amount of α-pinene occurred by drying in the microwave (200 W), stored at 25 ± 2 °C for 120 d. The highest amount of β-pinene was observed in the shade-dried samples stored at 4 ± 1 °C for 120 d, and the highest amount of β-myrcene occurred in plant samples dried in the oven and then stored at 25 ± 2 °C temperature for 60 d. The highest amount of cymene (0.97%) occurred in shade-dried plant samples that were stored at 4 ± 1 °C for 60 d, which was 30.1-fold higher than the amount observed in samples dried in the oven (40 °C) and in samples stored for 60 d at 4 ± 1 °C. The highest amount of *trans*-beta-ocimene (0.81%) resulted from the microwave drying method (200 W), stored at 25 ± 2 °C for 120 d, which was 55.77% higher than the value caused by the same drying method followed by 60-d storage at 4 ± 1 °C. The highest amount of γ-terpinene resulted from the microwave treatment stored at 4 ± 1 °C for 365 d. The highest borneol content occurred in samples dried at 40°C and stored at 25 ± 2 °C for 60 d. The highest ISO menthol (1.62%) occurred by drying with microwave (200 W), which was 12.5% higher compared to the value obtained by oven drying (40 °C) followed by 60-d storage at 25 ± 2 °C temperature. The highest amount of menthol occurred by drying with microwave and storage at 25 ± 2 °C for 120 d. The highest amount of endo-borneol was obtained by drying in the oven

and storage at 25 ± 2 °C for 365 d. The highest amount of lavandulyl acetate was obtained by drying in the shade and storage at 25 ± 2 °C for 365 d. The highest amount of α-copaene was obtained by drying in microwave and storage at 25 ± 2 °C for 365 d. The highest amount of β-elmene occurred in conditions of storage at 4 ± 1 °C and 25 ± 2 °C for 60 d. The highest amount of β-farnesene was observed in samples dried at 40 °C, stored for 60 d at 4 ± 1 °C and 25 ± 2 °C. The highest amount of α-humulene occurred in samples dried in the microwave and stored at 25 ± 2 °C for 365 d. The microwave method, followed by storage at 25 ± 2 °C for 365 d, resulted in the highest amount of delta-cadinene. The highest amount of aromadendrene was recorded in samples dried in the oven and stored at 4 ± 1 °C for 365 d.

The highest amount of t-muurolol occurred in samples stored at 25 ± 2 °C temperature for 60 d, and the highest amount of 3-octanol was recorded in samples dried in the shade and stored for 60 d at 25 ± 2 °C. The highest amount of dihydroedulan component was obtained by microwave drying and storage at 4 ± 1 °C for 365 d. The highest amount of eugenol (1.02%) was observed in plant samples dried in the microwave (200 W), followed by storage at 25 ± 2 °C for 120 d, which was 2.21-fold higher than the value observed in the shade treatment with 120 d of storage at 4 ± 1 °C and 25 ± 2 °C (Table 3).

According to the analysis of variance, the amount of camphene under the influence of storage temperature treatment did not show any significant difference, whereas the simple effects of other treatments and the interaction effect of the treatments caused a significant difference in camphene content ($P \leq 0.01$) (Table 2). The comparison of mean values showed that the microwave treatment increased the amount of camphene in plant samples, compared to the other methods of drying, and the maximum amount of this substance was measured in storage conditions of 4 ± 1 °C for 365 d and at 25 ± 2 °C for 120 d. Its lowest amount was measured under the conditions of storage at 25 ± 2 °C after oven drying with a storage period of 365 d and shade drying with a storage period of 365 d (Table 4).

According to the analysis of variance, the interaction effects of storage temperature (a), storage duration (b), and drying method (c) ($P \leq 0.01$) were significant on the amount of sabinene, whereas the interaction effect of the storage temperature (a) × drying method (c) had no significant effect on sabinene (Table 2). The comparison of mean values indicated that oven drying increased the amount of sabinene in plant samples. During storage for 365 d, both at 25 ± 2 °C and 4 ± 1 °C, the amount of this component was more than in the other treatment groups. However, the amount of sabinene showed a significant decrease in microwave-assisted drying (Table 4).

According to the analysis of variance, significant differences were observed in 1, 8-cineol under the simple effects of storage temperature conditions (a), storage duration (b), and drying method (c) and their interaction effect ($P \leq 0.01$), despite the fact that the interaction effect of storage temperature × drying method on the amount of the mentioned substance was not significant (Table 2). Based on the results, the highest amount of 1,8-cineol component was obtained by oven drying and storage at 4 ± 1 °C for 120 d. In contrast, the lowest amount occurred in plant samples dried in the microwave and then stored at 25 ± 2 °C temperature for 365 d (Table 4).

The analysis of variance indicated that the amount of limonene was significantly affected by the interaction effects of storage duration (b) and drying method (c) ($P \leq 0.01$). The interaction effect of storage temperature (a) × drying method (c) caused a significant difference in limonene content ($P \leq 0.05$). The simple effect of storage temperature conditions (a) and the interaction effect of storage temperature (a) × storage duration (b) did not make a significant difference in the limonene component (Table 2). According to the results, plant samples that were dried in the oven and then stored at 25 ± 2 °C temperature for 60 d contained the highest amount of limonene (2.26%), which was 72.52% higher than the lowest limonene content (1.31%) obtained from samples dried in the shade and stored at 4 ± 1 °C for 60 d (Table 4).

According to the analysis of variance, the amount of menthone under the effect of storage temperature treatment (a) and the interaction effect of storage temperature conditions (a) × storage duration (b) and storage temperature conditions (a) × drying method (c) showed significant differences ($P \leq 0.05$). Also, significant differences were observed under the simple effects of storage duration (b) and drying method (c) and the interaction effect of storage duration (b) × drying method (c) ($P \leq 0.01$) on the menthone component (Table 2).

Oven drying yielded higher amounts of menthone from plant samples. The highest amount of this substance was measured in the conditions of storage for 60 d at 4 ± 1 °C and 25 ± 2 °C temperatures. Its lowest amount was measured under the conditions of storage at 25 ± 2 °C temperature for 60 d after microwave drying (Table 4).

Table 3. *Mentha aquatica* under the influence of different drying methods shade-drying (200 mL m-2), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 °C and 4 ± 1 °C) affecting EO components.

	at 4 ± 1 °C									at 25 ± 2 °C									KI
	60 d			120 d			365 d			60 d			120 d			365 d			
	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	
α-thujene	0.14 ± 0.0066^{e}	-	-	0.24 ± 0.003^{b}	-	0.16 ± 0.003^{d}	-	-	-	0.14 ± 0.014^{e}	-	-	0.21 ± 0.011^{c}	-	0.51 ± 0.038^{a}	-	-	-	938
α-pinene	3.52 ± 0.078^{b}	3.01 ± 0.023^{c}	2.56 ± 0.101d^{e}	2.71 ± 0.126^{d}	2.42 ± 0.071^{ef}	2.32 ± 0.121^{f}	2.30 ± 0.068^{f}	-	-	2.56 ± 0.201^{de}	2.71 ± 0.126^{d}	2.32 ± 0.021^{f}	5.33 ± 0.183^{a}	2.73 ± 0.016^{d}	2.51 ± 0.126^{e}	1.56 ± 0.101^{g}	-	-	945
β-pinene	4.43 ± 0.243^{cd}	3.29 ± 0.026^{c}	3.25 ± 0.146^{c}	6.68 ± 0.631^{a}	5.42 ± 0.261^{b}	4.70 ± 0.378^{c}	6.50 ± 0.278^{a}	4.89 ± 0.473^{c}	2.53 ± 0.293^{f}	4.43 ± 0.243^{cd}	3.29 ± 0.126^{c}	3.25 ± 0.346^{c}	4.80 ± 0.071^{c}	4.18 ± 0.118^{d}	0.90 ± 0.062^{g}	2.41 ± 0.233^{f}	2.06 ± 0.058^{f}	-	987
β-myrcene	0.92 ± 0.024^{ab}	0.90 ± 0.034^{b}	0.61 ± 0.047^{d}	-	-	-	-	-	-	0.91 ± 0.026^{ab}	0.94 ± 0.028^{a}	0.74 ± 0.061^{c}	-	-	-	-	-	-	1003
cymene	0.97 ± 0.018^{a}	0.75 ± 0.016^{d}	-	-	-	-	-	-	-	0.94 ± 0.022^{b}	0.86 ± 0.029^{c}	-	-	-	-	-	-	-	1032
trans-beta-ocimene	-	0.72 ± 0.011^{b}	0.52 ± 0.029^{c}	-	-	-	-	-	-	-	0.726 ± 0.10^{b}	0.523 ± 0.012^{c}	-	-	0.81 ± 0.016^{a}	-	-	-	1049
γ-terpinene	0.87 ± 0.012^{d}	0.55 ± 0.022^{g}	0.74 ± 0.077^{c}	1.06 ± 0.082^{c}	0.87 ± 0.0128^{d}	0.92 ± 0.0121^{d}	-	-	1.86 ± 0.082^{a}	0.87 ± 0.012^{d}	0.55 ± 0.027^{g}	0.74 ± 0.017^{c}	1.03 ± 0.067^{c}	0.63 ± 0.32^{f}	1.42 ± 0.0378^{b}	-	-	0.43 ± 0.0371^{h}	1066
4-(terpineol)	0.293 ± 0.028^{d}	0.84 ± 0.0014^{a}	-	0.53 ± 0.013^{c}	-	0.59 ± 0.016^{b}	-	-	-	0.256 ± 0.019	0.84 ± 0.058^{a}	-	0.59 ± 0.020^{b}	-	-	-	-	-	1172
borneol	1.70 ± 0.057^{d}	2.17 ± 0.005^{b}	1.61 ± 0.066^{c}	-	-	-	-	-	-	1.77 ± 0.095^{c}	4.09 ± 0.035^{a}	1.61 ± 0.041^{c}	-	-	-	-	-	-	1166
ISO menthol	-	1.48 ± 0.013^{b}	-	-	-	-	-	-	-	-	1.44 ± 0.031^{c}	-	-	-	1.62 ± 0.053^{a}	-	-	-	1181
menthol	-	-	-	1.07 ± 0.002^{c}	0.81 ± 0.280^{d}	1.61 ± 0.028^{a}	-	-	-	-	-	-	1.13 ± 0.032^{b}	-	-	0.71 ± 0.038^{e}	-	-	1167
endo-borneol	-	-	-	2.65 ± 0.060^{e}	2.20 ± 0.058^{f}	3.24 ± 0.022^{d}	-	-	4.69 ± 0.103^{b}	-	-	-	2.73 ± 0.077^{e}	2.13 ± 0.058^{f}	4.35 ± 0.133^{c}	1.31 ± 0.013^{g}	8.13 ± 0.423^{a}	1.43 ± 0.073^{g}	1181
lavandulyl acetate	1.93 ± 0.10^{b}	1.03 ± 0.001^{c}	0.614 ± 0.016d^{e}	0.94 ± 0.043^{c}	0.39 ± 0.018^{f}	0.61 ± 0.011^{de}	-	-	0.91 ± 0.058^{c}	1.83 ± 0.101^{b}	1.03 ± 0.0017^{c}	0.63 ± 0.021^{d}	0.97 ± 0.028^{c}	0.75 ± 0.058^{d}	-	2.36 ± 0.333^{a}	-	0.46 ± 0.016^{f}	1287
α-copaene	-	-	-	0.38 ± 0.024^{c}	-	0.413 ± 0.0246^{b}	-	-	-	-	-	-	0.406 ± 0.015^{b}	-	-	-	-	0.840 ± 0.036^{a}	1379
β-elmene	0.235 ± 0.008^{h}	2.58 ± 0.269^{d}	4.94 ± 0.55^{a}	1.27 ± 0.114^{f}	0.77 ± 0.035^{g}	1.32 ± 0.060^{f}	-	-	1.96 ± 0.040^{e}	0.180 ± 0.019^{h}	2.58 ± 0.269^{d}	3.94 ± 0.050^{b}	1.32 ± 0.039^{f}	1.23 ± 0.105^{f}	2.65 ± 0.104^{d}	-	-	3.54 ± 0.250^{c}	1392
β-farnesene	0.65 ± 0.029^{bc}	0.86 ± 0.070^{a}	0.69 ± 0.014^{b}	0.65 ± 0.034^{bc}	-	-	-	-	-	0.64 ± 0.039^{c}	0.87 ± 0.015^{a}	0.67 ± 0.019^{bc}	-	-	-	-	-	-	1460
α-humulene	1.63 ± 0.109^{e}	3.58 ± 0034^{b}	2.16 ± 0.075^{d}	2.65 ± 0.030^{c}	2.07 ± 0.120^{d}	2.64 ± 0.035^{c}	0.669 ± 0.032^{f}	-	3.64 ± 0.201^{b}	1.60 ± 0.055^{e}	3.54 ± 0.218^{b}	2.16 ± 0.075^{d}	2.84 ± 0.064^{c}	2.80 ± 0.044^{c}	3.71 ± 0.20^{b}	-	-	4.99 ± 0.26^{a}	1464
delta-cadinene	1.22 ± 0.037^{c}	1.06 ± 0.117^{c}	1.27 ± 0.012^{c}	1.08 ± 0.107^{cd}	0.72 ± 0.055^{f}	1.19 ± 0.052^{cd}	-	-	1.26 ± 0.017^{c}	1.22 ± 0.037^{c}	1.06 ± 0.117^{c}	1.27 ± 0.012^{c}	-	0.99 ± 0.152^{e}	2.25 ± 0.077^{b}	0.39 ± 0.043^{g}	-	3.22 ± 0.037^{a}	1521
aromadendrene	-	-	-	0.57 ± 0.045^{d}	-	0.67 ± 0.032^{c}	-	3.05 ± 0.095^{a}	-	-	-	-	-	-	0.27 ± 0.012^{e}	-	-	1.72 ± 0.030^{b}	1439
t- muurolol	2.87 ± 0.200^{b}	1.72 ± 0.074^{c}	1.02 ± 0.551^{e}	1.46 ± 0.104^{cd}	-	-	1.04 ± 0.114^{e}	-	-	3.63 ± 0.258^{a}	2.87 ± 0.100^{b}	1.24 ± 0.114^{de}	1.66 ± 0.104^{c}	1.52 ± 0.074^{cd}	-	1.45 ± 0.040^{cd}	-	-	1642
octen3ol(3-octanol)	2.62 ± 0.106^{b}	2.12 ± 0.056^{c}	1.56 ± 0.046^{c}	1.90 ± 0.053^{d}	1.53 ± 0.138^{e}	1.15 ± 0.128^{f}	1.19 ± 0079^{f}	-	-	3.89 ± 0.141^{a}	2.12 ± 0.056^{c}	-	-	-	-	-	-	-	992
benzene 1methyl	1.51 ± 0.042^{a}	1.44 ± 0.107^{a}	1.24 ± 0.067^{b}	0.70 ± 0.037^{d}	-	-	-	-	-	1.44 ± 0.107^{a}	0.96 ± 0.032^{c}	0.24 ± 0.183^{f}	0.59 ± 0.017^{c}	-	-	-	-	-	988
dihydroedulan	-	-	-	-	-	0.47 ± 0.046^{d}	-	-	1.18 ± 0.029^{a}	-	-	-	0.456 ± 0.032^{d}	-	0.73 ± 0.016^{c}	-	-	0.94 ± 0.089^{b}	1305
min sulfide	-	-	-	0.43 ± 0.013^{b}	-	-	-	-	-	-	-	-	0.21 ± 0.022^{c}	-	0.22 ± 0.012^{c}	-	-	0.59 ± 0.023^{a}	1719
eugenol	-	-	-	0.46 ± 0.020^{c}	-	0.60 ± 0.0208^{b}	-	-	-	-	-	-	0.463 ± 0.020^{c}	-	1.02 ± 0.090^{a}	-	-	-	1340

Numbers with common letters in each column do not have a significant difference ($P \leq 0.05$).

Table 4. *Mentha aquatica* under the influence of different drying methods shade-drying (200 mL m^{-2}), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 °C and 4 ± 1 °C) affecting EO components.

	at 4±1 °C									at 25±2 °C								
	60 d			120 d			365 d			60 d			120 d			365 d		
	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200 W	Shade	Oven 40 °C	Microwave 200	Shade	Oven 40	Microwave 200
camphene	0.63 ± 0.0096^{cd}	0.32 ± 0.035^{e}	0.62 ± 0.054^{cd}	0.80 ± 0.024^{b}	0.66 ± 0.044^{cd}	0.60 ± 0.02^{d}	-	-	2.39 ± 0.22^{a}	0.63 ± 0.020^{cd}	0.30 ± 0.017^{e}	0.62 ± 0.039^{cd}	0.75 ± 0.049^{bc}	0.73 ± 0.059^{bcd}	2.49 ± 0.17^{a}	0.30±0.021e	-	-
sabinene	1.65 ± 0.034^{e}	2.78 ± 0.071^{a}	1.56 ± 0.039^{ef}	2.03 ± 0.155^{c}	2.22 ± 0.066^{b}	1.38 ± 0.104^{g}	1.58 ± 0.069^{ef}	-	-	1.65 ± 0.034^{e}	2.78 ± 0.071^{a}	1.56 ± 0.079^{ef}	1.83 ± 0.055^{d}	1.48 ± 0.029^{fg}	0.72 ± 0.0012^{i}	0.85±0.026h	-	-
1,8-cineol	16.57 ± 0.999^{c}	19.44 ± 0.305^{b}	15.85 ± 0.185^{cde}	19.20 ± 0.772^{b}	26.55 ± 1.16^{a}	15.65 ± 0.285^{cde}	14.88 ± 0.670^{de}	14.39 ± 0.915^{e}	10.17 ± 1.02^{fg}	10.59 ± 0.743^{fg}	16.44 ± 1.60^{cd}	15.85 ± 0.185^{cde}	14.62 ± 0.80^{e}	18.76±1.26b	9.94 ± 0.140^{g}	11.03±0.595fg	11.56±1.33f	5.46±0.380h
limonene	1.31 ± 0.033^{f}	2.16 ± 0.048^{b}	1.57 ± 0.044^{c}	-	-	-	-	-	-	1.39 ± 0.029^{e}	2.26 ± 0.042^{a}	1.50 ± 0.078^{d}	-	-	-	-	-	-
menthone	0.76 ± 0.018^{b}	0.866 ± 0.036^{a}	0.58 ± 0.013^{d}	-	-	-	-	-	-	0.63 ± 0.024^{c}	0.89 ± 0.033^{a}	0.52 ± 0.023^{e}	-	-	-	-	-	-
menthofuran	14.94 ± 0.00^{a}	10.43 ± 0.00^{ef}	11.87 ± 0.00^{cd}	13.98 ± 0.00^{b}	12.28 ± 0.00^{c}	7.12 ± 0.00^{h}	9.78 ± 0.00^{f}	8.34 ± 0.00^{g}	-	13.46 ± 0.00^{b}	13.90 ± 0.00^{b}	15.86 ± 0.00^{a}	11.89 ± 0.00^{cd}	11.13 ± 0.00^{de}	7.98 ± 0.00^{gh}	10.99±0.00de	6.02±0.00i	-
(-)-bornyl acetate	-	0.51 ± 0.038^{d}	1.01 ± 0.056^{a}	0.91 ± 0.061^{b}	-	-	-	-	0.59 ± 0.028^{d}	-	0.51 ± 0.061^{d}	1.01 ± 0.111^{a}	1.02 ± 0.116^{a}	0.73 ± 0.028^{c}	-	-	-	-
trans caryophyllene	13.66 ± 0.378^{bcd}	11.95 ± 1.09^{efg}	14.66 ± 0.771^{b}	12.21 ± 0.848^{defg}	12.96 ± 0.078^{cdef}	12.87 ± 0.123^{cdef}	13.55 ± 0.216^{bcd}	8.63 ± 0.243^{i}	5.72 ± 0.698^{j}	12.18 ± 1.096^{defg}	11.61 ± 1.18^{fgh}	14.32 ± 0.396^{bc}	17.48 ± 0.818^{a}	13.16 ± 0.021^{cde}	6.82 ± 0.944^{j}	11.63±0.743fgh	10.82±114gh	10.49±1.31h
germacrene D	11.41 ± 0.809^{d}	8.75 ± 0.74^{e}	10.76 ± 0.435^{d}	13.75 ± 1.38^{b}	14.34 ± 1.22^{b}	12.13 ± 1.12^{cd}	7.24 ± 0.675^{ef}	3.38 ± 0.245^{g}	2.62 ± 0.245^{g}	13.36 ± 0.824^{bc}	7.28 ± 1.13^{ef}	8.46 ± 0.714^{ef}	16.51 ± 1.31^{a}	10.76 ± 0.435^{d}	7.41 ± 0.605^{ef}	7.02±0.565f	3.29±0.198g	0.439±0.036 h
y-cadinene	0.616 ± 0032^{b}	1.09 ± 0.150^{a}	0.56 ± 0.031^{b}	-	-	-	-	-	-	0.58 ± 0.021^{b}	1.09 ± 0.174^{a}	0.45 ± 0.045^{c}	-	-	-	-	-	-
α-amorphen	-	-	-	0.24 ± 0.011^{d}	-	-	-	-	0.66 ± 0.020^{b}	-	-	-	0.28 ± 0.022^{c}	-	-	-	-	0.69±0.033a
caryophyllen oxid	-	2.09 ± 0.20^{a}	-	1.06 ± 0.115^{d}	0.52 ± 0.383^{c}	1.14 ± 0.045^{d}	0.64 ± 0.025^{e}	-	1.14 ± 0.024^{d}	-	2.09 ± 0.10^{a}	-	-	1.58 ± 0.145^{b}	1.03 ± 0.130^{d}	1.37±0.040c	-	-
viridiflorol	4.36 ± 0.559^{b}	5.71 ± 0.446^{de}	6.79 ± 0.119^{bcd}	5.98 ± 0.475^{cde}	4.29 ± 0.130^{f}	4.37 ± 0.248^{f}	4.10 ± 0.225^{f}	2.30 ± 0.125^{g}	-	10.73 ± 0.989^{a}	7.10 ± 0.825^{bc}	5.36 ± 0.595^{ef}	7.62 ± 0.534b	5.99 ± 0.277^{cde}	4.29 ± 0.130^{f}	5.23±0.660ef	4.10±0.225f	-
α cadinol	-	-	0.70 ± 0.021^{a}	-	0.67 ± 0.49^{a}	-	-	-	-	-	-	0.60 ± 0.012^{a}	-	-	-	-	-	-

Numbers with common letters in each column do not have a significant difference ($P \leq 0.05$).

According to the variance analysis, the simple effect of storage temperature (a) was not significant on the amount of menthofuran. However, the simple effects of other treatments, their interaction effect, were important in the amount of menthofuran ($P \leq 0.01$) (Table 2). The comparison of mean values indicated that the storage period of 60 d increased the amount of menthofuran in plant samples. After drying in the shade with storage at 4 ± 1 °C and, separately, drying with microwave and storage at 25 ± 2 °C temperature, the amount of menthofuran was more than the other treatments. However, the amount showed a noticeable decrease after drying with microwave and storage for 365 d at 25 ± 2 °C temperature (Table 4).

According to the analysis of variance, (-)-bornyl acetate was significantly affected under the simple effect of storage temperature (a) ($P \leq 0.05$) and under the simple effect of storage duration treatments (b) and drying method (c). The interaction effect caused significant differences as well ($P \leq 0.01$) (Table 2). According to the comparison of mean values, the highest amount of (-)-bornyl acetate (1.02%) was obtained in samples dried in the microwave and then stored at 25 ± 2 °C temperature, or at 4 ± 1 °C, for 60 d, as well as plant samples dried in the shade at 25 ± 2 °C temperature for 120 d. In contrast, samples dried in the oven and stored at 4 ± 1 °C and 25 ± 2 °C temperature for 60 d had the lowest amount (0.51%) of the mentioned component (Table 4).

The analysis of variance showed that the amount of trans-caryophyllene under the simple effect of storage temperature treatment (a) was not significantly different by the different treatments, whereas the simple effects of storage duration (b) and drying method (c) and the interaction effect caused significant differences ($P \leq 0.01$). Nonetheless, the interaction effect of storage temperature conditions (a) × drying method (c) caused a significant difference ($P \leq 0.05$) in trans-caryophyllene (Table 2).

The comparison of mean values indicated that drying in the shade increased the amount of trans-caryophyllene in the plant samples. In samples stored at 25 ± 2 °C for 120 d, the amount of trans-caryophyllene was more than in other treatment groups. In microwave drying and storage for 365 d at 4 ± 1 °C, the amount of trans-caryophyllene showed a substantial decrease (Table 4). Based on the analysis of variance, the shade drying method caused the highest amount of germacrene-D in plant samples stored at 25 ± 2 °C for 120 d (Table 4).

Hydrocarbon monoterpenes

According to the analysis of variance, the simple effect of treatments of storage temperature (a), storage duration (b), and drying method (c) and the interaction effect of the studied treatments was significant ($P \leq 0.01$) on the amount of hydrocarbon monoterpenes (Table 5). The comparison of mean values showed that the highest amount of hydrocarbon monoterpenes (13.95%) was observed in plant samples dried in the shade and after 120 d of storage at 25 ± 2 °C. However, the same statistical significance was observed for samples obtained from the treatment of drying in the shade and storage at 4 ± 1 °C for 60 d. Also, when the samples were dried in the shade and stored at 4 ± 1 °C for 120 d, they were not statistically different from the said treatment and had no significant difference compared to each other. The lowest amount of hydrocarbon monoterpenes (0.43%) was obtained in microwave drying and storage at 25 ± 2 °C for 365 d (Table 3).

Oxygenated monoterpenes

The analysis of variance showed significant changes in the amount of oxygenated monoterpenes ($P \leq 0.01$) under the simple effect of storage temperature conditions (a), storage duration (b), and drying method (c) and their interaction effect (Table 5).

The comparison of mean values showed that the highest amount of oxygenated monoterpenes (42.23%) occurred in samples dried in the oven and stored at 4 ± 1 °C for 120 d. Also, a high amount of oxygenated monoterpenes was observed in plant samples dried in the oven and stored at 25 ± 2 °C for 60 d. Nonetheless, these two were placed in the same statistical category and did not differ significantly. The lowest amount of oxygenated monoterpene (7.35%) was observed in samples of microwave drying, followed by storage at 25 ± 2 °C for 365 d (Table 2). The highest value of oxygenated monoterpenes was 5.75 times higher than the lowest value. Storing the dried *M. aquatica* samples at 25 ± 2 °C reduced the amount of oxygenated monoterpene components in the EO, thereby indicating the decomposition of these components.

Hydrocarbon sesquiterpenes

The analysis of variance showed no significant changes caused by the simple effects of storage temperature (a) on the amount of hydrocarbon sesquiterpenes. However, the simple effects of storage duration (b) and drying method (c), as well as their interaction effect led to significant

differences ($P \leq 0.01$) (Table 5).
The comparison of mean values indicated that extending the storage duration was associated with a reduction in the amount of sesquiterpene hydrocarbons in the samples. The interaction effect of drying in the shade and storage at 25 ± 2 °C for 120 d led to higher amounts of hydrocarbon sesquiterpenes (38.84%) in the EO. In contrast, the oven treatment at 40 °C and 365 d of storage at 25 ±2 °C caused the hydrocarbon sesquiterpenes to decrease by 2.75-fold (Table 4). It was apparent that storing the dried *M. aquatica* samples at 4 ± 1 °C caused the conversion of sesquiterpene components into other components because of their decomposition and the reactions between components.

Table 5. Analysis variance of *Mentha aquatica* under the influence of different drying methods shade-drying (200 mL m-2), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 °C and 4 ± 1 °C) affecting EO components.

S.O.V	D.f	Mean of squares				
		hydrocarbon monoterpenes	oxygenated monoterpenes	hydrocarbon sesquiterpenes	oxygenated sesquiterpenes	non-terpene components
A	1	54.38 **	101.06 **	1.107 ns	25.95 **	6.42 **
B	2	267.88 **	1278.72 **	903.62 **	205.61 **	31.59 **
C	2	70.51 **	449.15 **	81.98 **	101.80 **	9.59 **
Ab	2	26.58 **	46.57 **	21.60 **	0.734 ns	0.940 **
Ac	2	0.622 **	9.96 **	9.58 **	17.44 **	0.067 **
Bc	4	5.53 **	90.96 **	68.02 **	3.99 **	8.59 **
Abc	4	3.30 **	60.102 **	70.84 **	20.63 **	3.64 **
Error	36	0.092	0.915	1.139	0.518	0.0125
Coeff var		3.23	3.17	3.98	10.24	5.83

**: significant at 1% level, *: significant at 5% level, ns: not significant. A: storage temperature conditions, B: storage duration and C: drying method.

Oxygenated sesquiterpenes

The analysis of variance revealed that the amount and types of oxygenated sesquiterpenes changed significantly as a result of the simple effects of storage temperature (a), storage duration (b), and drying method (c), as well as their interaction effect ($P \leq 0.01$), although the interaction effect of storage temperature conditions (a) × storage duration (b) were not significant on oxygenated sesquiterpenes (Table 5).
The comparison of mean values showed higher amounts of oxygenated sesquiterpene in the shade drying method. With an increase in storage duration, the amount of these components in the plant samples decreased. Generally, the highest amount of oxygenated sesquiterpenes (14.36%) occurred in the shade-drying treatment and by storage at 25 ± 2 °C for 60 d. In the microwave-assisted method and storage at 25 ± 2 °C for 365 d, sesquiterpenes decreased significantly in the plant samples (Table 5).
Storing the dried *M. aquatica* samples at 4 ± 1 °C reduced these components, which suggests the decomposition of oxygenated sesquiterpenes and their conversion into other elements.
Regarding sesquiterpenes, such as caryophyllene, spatholinol, caryophyllene oxide, bisabolene, and aromadendrene, the samples stored at 4 ± 1 °C had more sesquiterpenes than the samples stored at room temperature.

Non-terpene components

The analysis of variance indicated significant differences ($P \leq 0.01$) in non-terpene components caused by the simple effects of storage temperature (a), storage duration (b), and drying method (c) and the interaction effect of the treatments (Table 5).
The comparison of mean values showed that the amount of non-terpene components (5.65%) in the EO of shade-dried plant samples, stored at 25 ± 2 °C for 60 d, was more than in other plant samples. With an increase in storage duration, a significant decrease occurred in the mentioned components via the oven-drying method at 40 °C, so that the amount of non-terpene components neared zero (Fig .1).

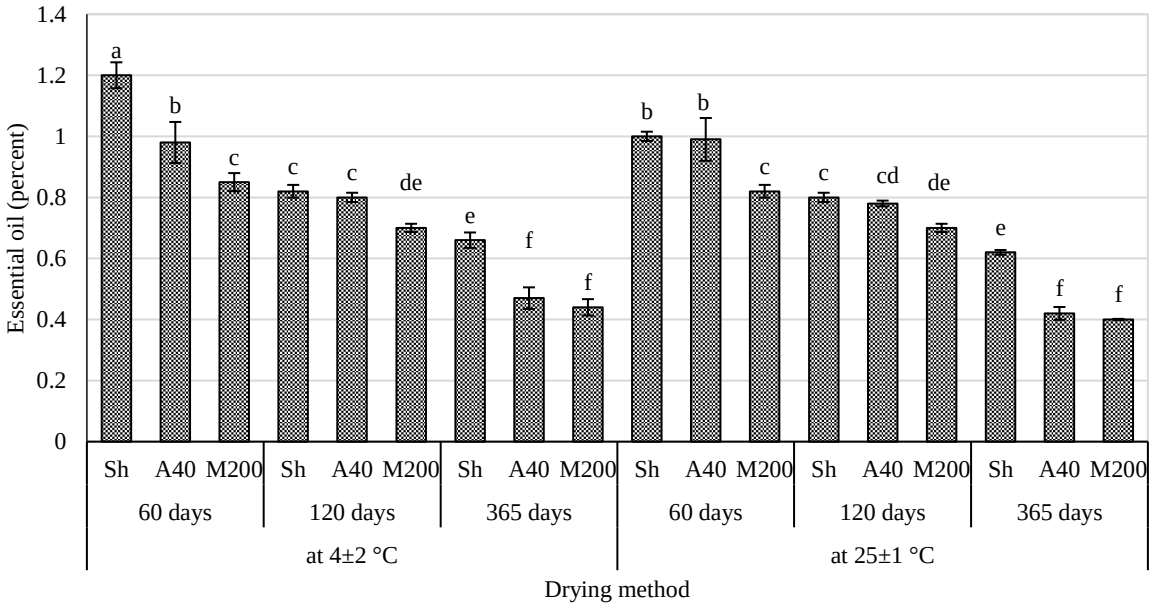

Fig. 1. *Mentha aquatica* under the influence of different drying methods shade-drying (200 mL m^{-2}), oven-drying (40 °C), microwave-assisted drying (200 W), storage duration (60, 120, and 365 d), and storage temperatures (25 ± 2 °C and 4 ± 1 °C) affecting EO components.
Sh: Shade-drying at 200 mL m^{-2}, A40: drying in the oven at 40 °C, M200: drying in the microwave with a power of 200 W.

Discussion

The results of this study show that different storage conditions can lead to different changes in essential oil compounds. For example, lower temperatures can preserve essential oil components optimally. In addition, some enzymatic activities and microorganisms can play a role in the process of changing essential compounds. To arrive at ideal outcomes in farm conditions, it is important to determine the proper storage conditions for each plant species. In sum, the findings indicated that during storage, the EO components of *M. aquatica* decreased in amount, due to the conversion of components into each other or their decomposition. As a result of storage at 4 ± 1 °C, more EO components were preserved, compared to storage at 25 ± 2 °C. This indicates the positive role of low temperatures in maintaining the phytochemical components of *M. aquatica* EO. The 1,8-cineole component was known as the dominant component in *M. aquatica* EO. This component had a higher concentration when the samples were oven-dried and stored at 4 ± 1 °C. However, increasing the storage duration to 120 d caused an increase in the 1,8-cineole component, followed by a decrease after 365 d of storage, which suggests an optimal condition for preserving this component. Indeed, 1,8-cineole was reportedly a prominent component of mint essential oil and a strong antibacterial agent in previous research (Bokić et al., 2020).

Research on folklore medicine indicated that *M. aquatica* is a traditional herb which can be used as a mouthwash, a remedy for headaches and digestive disorders, and its leaves can be used as a natural antioxidant in various food industries (Thi et al., 2020). Essential oils obtained from mint species act as a good expectorant and are used as a treatment for respiratory diseases such as bronchitis, sinusitis, tuberculosis and colds. Exploitation of mint species in pharmaceutical formulations requires more research in the field of antibacterial, antifungal, antiviral and anticancer activities. Indeed, mint species, and especially essential oils, are used to reduce microbial load. (Tafrihi et al., 2021). It has been suggested that *M. aquatica* is an economically important plant used as a medicinal, traditional and culinary herb.

According to relevant applications in the food and pharmaceutical industries, this plant can have high potential for commercialization as a native plant of the northern regions of Iran. Its EO contains a variety of compounds including beta caryophyllene, viridifloral, and 1,8 cineole (Gupta et al., 2023). The current research provides information for the localization and conservation of essential oil compounds in storage, which confirms previous indications in similar research on mint storage for 60, 120, and 365 d (Gupta et al., 2023).

According to the previous research, the amount and composition of EO in chrysanthemum plants were significantly affected by storage duration (Kumar et al., 2013). After 1.5 d of storage, the EO of *Rosa damascena* decreased by 40% (Baydar and Baydar, 2005). In a relevant study, the highest EO content in *Rosa damascena* was observed when the petals were stored at -20 °C, so the petals showed insignificant changes in EO content for three weeks (Mohamadi et al., 2011). Similar to the findings of the current research, storage duration and temperature (Kazaz et al., 2009) affected the EO content and quality in *Rosa damascena*.

In a previous study, storage at 25 ± 2 °C without light for 30-150 d caused significant changes in EO components of *Tilia platyphyllos*, possibly due to changes in EO composition during storage, which may cause oxidation or other chemical changes (Selvi, 2020). In a relevant case of research, the highest amount of EO was observed after 60 d of storage, where shade drying of Danai thyme without pre-drying and with storage at 4 °C resulted in the maximum amount of EO (Dehghani Meshkani et al., 2018). Storage duration can significantly affect the physicochemical properties and essential oil content (Mieso et al., 2022). Oxidases, peroxidases, hydrolases and isomerases are the most important enzymes that cause changes in essential oil components during storage. A decrease in the amount of alpha togen, beta pinene, alpha pinene and myrcene, which have a low boiling point, has been reported in savory plants (Mohtashami et al., 2018). Research by Azizi et al. (2008) showed the conservative effects of different drying treatments (40, 50, and 60 °C in oven, shade, normal air flow) on essential oil components of chamomile flowers (cv). Bodegold). High oven temperature and near-fermentation conditions caused significant reduction due to bisabolol oxidation. Therefore, it was reported that the bisabolol component in chamomile, in addition to genetic and environmental factors, depends significantly on the conditions of drying and storage of plant materials after harvest. Previous research showed that the essential components and color of tarragon leaves dried at 40 °C changed less than samples dried at 90 °C during 120 d of storage, indicating that tarragon storage duration changed the composition of the essential oil and its color. Also, the results showed that the composition of methyl eugenol had higher stability compared to other essential oil components (ocimene and estragole) and was less affected by the storage period (Arabhosseini et al., 2007). Normally, the concentrations of components with lower molecular weight decrease in response to prolonged storage duration at 25 ± 2°C. This phenomenon can be due to evaporation, oxidation and other changes in essential oil components during storage (Gasemi Pirbaluti et al., 2012; Mokote et al., 2005).

Conclusion

The drying method, storage duration, and storage condition were effective on the chemical components of *M. aquatica* EO. The optimal use of *M. aquatica* as a traditional medicine and natural antioxidant can have good scope for storage and drying if used under proper circumstances. In general, a comprehensive investigation of the effects of drying and storage on the moisture of medicinal plants can help the better development of this industry. The limitations of this research were an absence of genetic studies, whereas genetic diversity can play an important role in determining the physiological and chemical characteristics of plants. Future research can focus on the biochemical profile of different accessions of *M. aquatica* and their response to specific drying and storage conditions.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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