

Essential oil production, chemical composition, bioactive compounds, and antioxidant activity of *Thymus vulgaris* as affected by harvesting season and drying conditions

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Abstract

In this study, the effects of seven distinct drying methods (sun, shade, freeze-drying [FD], oven drying at 40°C [OV40] and 70°C [OV70], and microwave 360 W [MW360] and 720 W [MW720]) were assessed in two harvesting seasons (spring and autumn) on the essential oil (EO) content, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and volatile compounds of thyme (*Thymus vulgaris* L.). The analysis of essential oils facilitated the identification of numerous compounds, 18 of which accounted for approximately 67.84–64.94% of the total thyme EO compounds. Six significant compounds were investigated, including thymol, *p*-cymene, γ -terpinene, carvacrol, linalool, and borneol. The results demonstrated that the thyme samples harvested in spring possessed higher amounts of γ -terpinene, thymol, carvacrol, EO content, and TFC, while samples harvested in autumn exhibited the highest amounts of borneol, *p*-cymene, TPC, and higher antioxidant activity. Principal component analysis (PCA) findings revealed that thyme samples harvested in spring, dried in the sun, shade, and FD conditions were rich in EO content, γ -terpinene and samples dried in MW720, OV40, and FD conditions possessed the highest amounts of thymol, carvacrol, and TFC. Drying the samples under MW360, OV40, and FD indicated significantly higher TPC and linalool. Drying the autumn-harvested samples under the sun and shade also exhibited higher linalool and TFC than other methods. Overall, the results conclusively indicated that the harvesting season and drying conditions significantly affected bioactive compounds, antioxidant activity, and EO production and quality.

Introduction

Thyme (*Thymus vulgaris* L.), a member of the Lamiaceae family, is native to the Mediterranean but grows abundantly in large areas of Asia, Europe, North America, and Oceania [1]. Fresh or dried thyme leaves are typically utilized as a spice or seasoning. Thyme is considered an important medicinal plant with unique medicinal properties. In addition to being used as a seasoning and medicinal herb, thyme extract is essential and has a variety of applications in the pharmaceutical industry. Thyme's volatile phenolic oil, classified as one of the top 10 essential oils, possesses antibacterial, antifungal, and antioxidant properties [1, 2]. On the other hand, the global demand for high-quality dried food products is continuously rising.

The primary objective of drying is to extend the product's shelf life, minimize packaging needs, and reduce transportation weight [3]. Various post-harvest processing methods in medicinal and aromatic plants are among the most significant factors influencing the quality and composition of their active compounds [4, 5]. Fresh plants, especially those of the Lamiaceae family, typically comprise 75–80% water, which should be reduced to less than 15% for preservation [6]. Drying increases the shelf life of products by inhibiting microorganism growth and preventing specific biochemical reactions that may cause changes in them [3, 5, 6]. The loss of volatile compounds in plants typically depends primarily on drying conditions and the biological characteristics of the plants [7].

Sun drying is a traditional drying method used primarily in semi-tropical and tropical regions to dry agricultural products, where it is still widely used today. This method uses solar energy to reduce the moisture content in various types of plant products. Most plants, such as vegetables, fruits, and medicinal and aromatic plants, are dried under sunlight due to the lower cost [5].

Drying in the shade is preferable to sun drying for preserving light-sensitive compounds and minimizing light-induced chemical reactions, such as oxidation [5]. This method of drying is economical as it requires minimal initial capital investment. Nonetheless, sun and shade drying presents several challenges, such as a slow process and

uncertain weather conditions, which can be overcome by ensuring good weather conditions and continuously monitoring the drying process to improve the final product's quality [5].

Over the past two decades, another popular drying method that has gained popularity is microwave drying. Microwave drying has numerous advantages over conventional hot air drying, including shorter drying times and improved final product quality [8]. In microwave drying, internal heat generation leads to an increase in internal temperature and vapor pressure. Both of these processes increase the flow of liquid toward the surface, which increases the drying rate. However, the most significant issue with microwave drying is food surface moisture accumulation. A key factor in maximizing the quality of the dried product is the suitable adjustment of microwave power to prevent excessive heating of the dried material [5].

Freeze drying is another method that produces high-quality products with very low moisture content, good nutritional qualities, and good rehydration. Freeze drying is performed based on sublimation at freezing temperatures and vacuum conditions without liquid water to preserve the primary tissue characteristics of the product's materials. However, its limited production capacity and high capital and energy costs primarily limit its application to heat-sensitive foods and pharmaceuticals [8].

The chemical composition of essential oils varies depending on the type of plant, harvest season, post-harvest methods, plant age, and storage conditions. Aromatic volatile compounds are the most sensitive components in drying food materials [7]. Numerous studies on the effect of drying on the volatile compounds of aromatic plants and herbs have revealed that changes in the concentration of volatile compounds during drying depend on several factors, including the drying method and the characteristics of the product being dried [9]. Although many drying methods have been introduced in various studies on medicinal and aromatic plants, the quality of dried plants is still lower than that of fresh ones [5], warranting further investigation.

The essential oil production and quality of herbal plants are greatly influenced by environmental conditions such as the growing season and harvesting time [10, 11]. Thymes are harvested from a wide range of periods in a growing season. Furthermore, thyme samples harvested from the spring and autumn showed morphologically-different characteristics. No study has evaluated the influence of the drying conditions on *T. vulgaris* harvested from two different seasons. Thus, due to the possible interaction of harvesting season with different methods of post-harvest processing, we aimed to evaluate their impact on the quality responses of *T. vulgaris*.

Materials and Methods

Drying methods and equipments

The aerial parts of thyme plants were harvested during autumn and spring from the Shahid Bahonar University research farm, Kerman (30.1440 °N, 57.0715 °E, and 1774 m altitude), Iran. The plant leaves, and stems were selected as the study sample and divided into eight parts. One part was used as a control and analyzed as a fresh sample. The remaining parts were dried in three replicates using one of the following drying methods: (a) shade drying, (b) sun drying, (c) oven drying at 40°C, (d) oven drying at 70°C, (e) microwave drying at 360 W, (f) microwave drying at 720 W, and (g) freeze drying.

The shade-drying process was performed under natural airflow and at room temperature. For sun drying, the leaves were dried in a tray under direct sunlight in July (for spring-harvested samples) and in November (for autumn-harvested samples) in the Kerman region. For oven drying at 40 and 70°C, the plants were placed in the oven

(Labcon, USA) for 72 and 24 h, respectively. The microwave drying process was performed using a microwave oven (LG, WAVEDOM South Korea) for 18 and 8 min, at 360 and 720 W power levels, respectively. Freeze drying was performed using a freeze-dryer (SUBLIMATOR-VACO5, Zirbus, Germany) at -35°C and a vacuum system of < 1 mbar.

Essential oil extraction

A Clevenger apparatus was utilized to hydro-distill 50 grams of dried sample in 500 mL of distilled water for 2 h. The collected essential oils (EOs) were maintained in glass vials. The percentage of essential oil was calculated using the following formula:

$$\text{EO (\%)} = \frac{\text{Weight of the collected essential oil (g)}}{\text{Total weight of the sample (g)}} \times 100$$

Gas chromatography-mass spectrometry (GC–MS) analysis

To determine the EO compositions, 0.2 µL of the extracted oil from the aerial parts of the thyme plant were injected into a GC-MS instrument (7890A, Agilent, CA, USA) equipped with a mass selective detector (5975C VL MSD), a triple-axis detector (RTX5 MS), and a capillary column (length: 30 meters, I.D.: 0.250 mm, film thickness: 25 micrometers). The injection port temperature was set to 250°C, with an initial temperature of 40°C, followed by an increase in temperature up to 270°C with a ramp-up of 3°C/min, based on the predefined thermal program of the column. In addition, helium gas was used as a carrier gas at a volumetric flow rate of 1 mL/min. Furthermore, an ionization energy of 70 electron volts was utilized to create mass spectral fragmentation.

Extract preparation and bioactive components assay

To prepare the methanolic extract, 10 mL of 80% methanol (v/v, methanol:distilled water) was added to 250 mg of the powdered thyme sample and gently shaken (110 rpm) using a shaker for 8 h under dark conditions. The resulting solution was filtered through filter paper and stored at 4°C for further evaluation. The extract's total phenolic content (TPC) was determined using the Folin-Ciocalteu and spectrophotometry methods and the aluminum chloride colorimetric method was used to measure the total flavonoid content (TFC) [12].

The antioxidant activity of each sample extract was determined based on its ability to scavenge free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Ghanbari et al. [13]. After calculating the inhibition percentage, the IC₅₀ value, which represents the sample concentration required to neutralize 50% of DPPH radicals, was determined by linear regression analysis of decolorization versus sample concentration [13].

Statistical analyses

Data analyses were conducted using SAS statistical software (v. 9.1, SAS, NC, USA). Significant differences were evaluated by two-way analysis of variance (ANOVA) and the least significant difference (LSD, $P \leq 0.05$). The results were expressed as mean ± standard error (SE). In addition, principal component analysis (PCA) was performed using XLSTAT (v. 2016, Addinsoft, NY, USA) to identify the interrelationships among the drying conditions in two harvesting seasons and the main examined characteristics.

Results and Discussion

Total phenolic content

The results indicated that TPC in thyme was significantly influenced by different drying methods, harvesting seasons, and their interactions (Table 1). Samples harvested from the autumn season exhibited higher TPC than in spring (Table 1). Samples dried through different methods increased the concentration of TPC relative to fresh thyme. The highest TPC among drying methods in the autumn season was obtained from the 360 W microwave drying treatment (32.34 mg GAE/g DW), and the lowest was obtained through the 70 W oven drying treatment (13.84 mg GAE/g DW). In contrast, in the spring, the highest and lowest phenolic content was observed in the oven-drying treatment at 40°C (31.96 mg GAE/g DW) and sun-drying treatment (8.20 mg GAE/g DW), respectively (Table 1).

Drying techniques substantially impact plant materials' properties, both positively and negatively, and alter the amount of phytochemicals, such as phenolic compounds, which are essential for enzyme inhibition and antioxidant capacity. Identifying phytochemicals plays a crucial role in detecting bioactive compounds in plants and determining their antioxidant and bioactive capacity. Phenolic compounds are useful for describing and interpreting the protective metabolites of plants and their effect on certain non-transmissible chronic diseases [14].

Microwave drying is desirable for preserving polyphenols due to its short drying time, which reduces the oxidation and degradation of polyphenols [15]. Jimenez-Garcia et al. [14] reported the highest levels of phenolic content in *Melissa officinalis* and peppermint plants through microwave drying. High temperatures and excessively long drying times typically lead to increased oxidation and degradation of polyphenols [16]. Rapid heating inactivates degradative enzymes but may also degrade heat-sensitive phenolic compounds [17]. Loss of phenolic compounds during traditional drying methods (shade and sun) may be induced by enzymatic reactions. These drying methods cannot inactivate degradative enzymes such as polyphenol oxidases so that they can break down phenolic compounds during prolonged drying stages [17]. Tan et al. [18] and Bernard et al. [19] reported the highest TPC from *Momordica charantia* and *Cinnamomum zeylanicum*, respectively, when dried at 50°C oven temperature.

Table 1
Effect of different drying methods and harvesting season interaction effect on total phenolic content (TPC), and total flavonoid content (TFC).

Drying methods	TPC (mg GAE/g dry weight)		TFC (mg QE/ g dry weight)	
	Autumn	Spring	Autumn	Spring
Fresh	4.86 ± 0.67	7.20 ± 1.53	2.93 ± 0.52	32.80 ± 2.31
FD	23.70 ± 1.35 ^b	13.85 ± 1.80 ^{bc}	16.80 ± 4.00 ^b	99.47 ± 30.0 ^a
MV 360	32.34 ± 6.73 ^a	15.92 ± 2.10 ^{bc}	46.13 ± 5.81 ^{ab}	40.80 ± 4.62 ^b
MV 720	14.85 ± 1.20 ^c	16.62 ± 3.10 ^b	22.13 ± 11.6 ^b	64.80 ± 12.9 ^{ab}
OV40	17.69 ± 0.63 ^{bc}	31.96 ± 6.72 ^a	7.47 ± 1.33 ^b	96.80 ± 12.2 ^a
OV70	13.84 ± 1.01 ^c	9.83 ± 4.03 ^{bc}	36.8 ± 22.3 ^{ab}	76.80 ± 10.6 ^{ab}
Shade	22.04 ± 0.18 ^{bc}	9.03 ± 2.00 ^{bc}	50.13 ± 9.61 ^{ab}	40.80 ± 4.62 ^b
Sun	24.37 ± 0.74 ^{ab}	8.20 ± 0.84 ^c	71.47 ± 24.9 ^a	44.80 ± 4.62 ^b
Pvalue	0.0002		0.03	

For each season, means $\pm$ standard errors ($n = 3$) with the same letter are not significantly different according to LSD test ($P < 0.05$). **FD**: Freeze drying; **MW360** and **MW720**: microwave 360 W and microwave 720 W; **Ov40**: oven drying at 40 °C.

Total flavonoid content

The results showed that TFC in thyme was significantly affected by different seasons and their interaction with various drying methods (Table 1). The level of TFC was higher in the samples harvested from the spring season than in the autumn. Among the drying methods studied, the highest TFC in the spring was obtained from the freeze-drying treatment (99.47 mg QE/ g DW), while the lowest level was obtained through the shade and microwave treatments (40.80 mg QE/ g DW). In autumn, the highest and lowest TFC were observed in the sun drying (71.47 mg QE/ g DW) and oven drying at 40°C (7.47 mg QE/ g DW) methods, respectively.

Jimenez-Garcia et al. [14] reported microwave drying and freeze-drying as superior methods for flavonoid extraction. The study also concluded that these two drying techniques significantly increased the flavonoid content of mint by 88%. Nguyen et al. [20][20] observed the highest TFC in guava plants through the freeze-drying method compared to oven drying at 50°C, microwave at 450 W, vacuum drying, and sun drying. This finding demonstrated that freeze-drying at a high hydration level increased the concentration of bioactive compounds after bound water removal, which was limited in the guava leaf extract. In addition, due to its low drying temperature, freeze-drying has been regarded as an effective method for preserving maximum nutrients and bioactive compounds [21].

Antioxidant activity

As shown in Table 2, the IC_{50} values of methanolic extracts were affected by different drying methods and seasons, but their interaction was insignificant. Generally, the samples harvested from autumn showed lower IC_{50} values (higher antioxidant power) than the spring (Table 2). Freeze drying (at the lowest IC_{50} ; 50.9 g/mL) exhibited the highest antioxidant activity and did not significantly differ from MW720, MW360, and OV40. However, the highest IC_{50} value (lower antioxidant activity) was obtained from samples dried in an oven at 70°C (92.7 μ g/mL), which did not show a significant difference with sun and shade drying methods (Table 2).

In the samples collected in autumn, a higher content of phenolics was observed compared to the samples collected in spring. Accordingly, a higher antioxidant capacity was observed in autumn samples (Tables 1 and 2). Therefore, it can be concluded that the content of polyphenols is largely responsible for antioxidant activity [22, 23].

Orphanides et al. [24] stated that freeze-drying spearmint yields the highest antioxidant activity. In addition, other studies have demonstrated the superiority of freeze-drying compared to conventional drying methods for various plant materials [25]. The advantage of freeze drying can be attributed to the fact that this technique minimizes the destruction of heat-sensitive compounds by drying plant materials at low temperatures [5, 21].

Table 2
Essential oil content and IC₅₀ of thyme plants obtained from
harvesting seasons and dried by different methods

Drying methods	Essential oil (%)	IC ₅₀ (µg/mL)
Fresh	0.99 ± 0.09	267.11 ± 53.92
FD	0.64 ± 0.13 ^{ab}	50.9 ± 16.23 ^c
MV 360	0.135 ± 0.06 ^c	67.6 ± 22.30 ^{bc}
MV 720	0.149 ± 0.02 ^c	54.1 ± 14.76 ^{bc}
OV40	0.582 ± 0.07 ^b	69.4 ± 9.51 ^{abc}
OV70	0.016 ± 0.00 ^c	92.7 ± 16.75 ^a
Shade	0.784 ± 0.08 ^a	73.3 ± 18.84 ^{abc}
Sun	0.729 ± 0.08 ^{ab}	75.7 ± 19.22 ^{ab}
<i>P</i> value	< .0001	0.022
Harvesting seasons		
Autumn	0.33 ± 0.05 ^b	35.78 ± 4.24 ^b
Spring	0.53 ± 0.09 ^a	102.40 ± 6.01 ^a
<i>P</i> value	< .0001	< .0001

For each studied factor, means ± standard errors (n = 3) with the same letter are not significantly different according to LSD test (*P* < 0.05). **FD**: Freeze drying; **MW360** and **MW720**: microwave 360 W and microwave 720 W; **Ov40**: oven drying at 40 °C.

Previous research has demonstrated that drying methods similarly affect polyphenol content and antioxidant capacity [22, 23]. Drying processes at high temperatures (i.e., 60 and 70°C) and prolonged drying exposure may destroy some phenolic compounds, resulting in a loss of antioxidant activity [26]. Furthermore, drying methods that utilize high temperatures for drying spearmint have been shown to lead to a severe loss of phenolic compounds and antioxidant activity [24]. As shown in the results of the present study (Tables 1 and 2), drying at 70°C also yielded similar results.

In addition, it has been observed that plants subjected to microwave drying preserve bioactive compounds better and exhibit greater antioxidant activity than samples dried by conventional air drying [27]. Jimenez-Garcia et al. [14] reported that microwave-dried samples of thyme, *Melissa officinalis*, and peppermint had the highest antioxidant capacity. During the drying of *Morus alba* leaves, a decrease in antioxidant activity was observed between sun-dried and oven-dried samples at 50°C [18].

Essential oil percentage

The results showed that the EO content of thyme was influenced by different drying methods and harvesting seasons, but their interaction did not indicate a significant effect. Generally, the EO content of thyme samples

harvested in spring was higher compared to autumn. Furthermore, fresh plants had a higher EO content compared to dried ones. Among drying methods, the highest EO content (0.78%) was obtained from shade-dried plants, which was not significantly different from freeze-dried and sun-dried plants. The lowest percentage was observed in OV70 (0.016%), which did not show a significant difference with MW360 and 720 W (Table 2).

Drying time and temperature were regarded as two crucial parameters for interpreting variations in essential oil yield through various techniques [3]. Our results demonstrated that a rise in drying temperature, from 40 to 70°C, led to a significant decrease in EO content. The high temperature is likely to damage the gland that produces essential oil, resulting in the greatest loss of essential oil [7]. *Origanum vulgare* [9] and *Laurus nobilis* [28] exhibited similar trends of decreasing essential oil content in response to high temperatures. Sellami et al. ⁽²⁰¹¹⁾ reported the highest and lowest essential oil yield in *Laurus nobilis*, respectively, by drying in ambient air (shade) and at 65°C. Ghasemi Pirbalouti et al. [29] found that drying in the shade produced the highest essential oil yield in two basil landraces, while oven drying at 60°C for green landrace and microwave treatment for purple landrace produced the lowest yield. Hassanpouraghdam and Hassani [30] also reported that the drying method significantly affects the amount of essential oil in *Mentha pulegium*. Among the various drying methods, such as shade, sunlight, and oven at 40 and 60°C, drying in the shade had the highest EO content, while drying at 60°C had the lowest EO content. It may be possible to prevent the loss of heat-sensitive compounds in plants when they are dried in sunlight and shade at a lower temperature [5]. In most cases, increasing the drying temperature by 20°C may cause a loss in EO yield of up to 92.3% [3].

Essential oil compositions

GC-MS analysis was performed to quantify the EO compositions in the samples harvested from different seasons and dried under different drying conditions, where the results are presented in Table 3. Due to the low EO content of 70°C oven-dried plants, GC-MS analysis was not performed on them. The analysis of EOs extracted from aerial parts of thyme facilitated the identification of numerous compounds, of which 18 compounds comprised approximately 67.84–64.94% of the total thyme oil compounds (Table 3).

In the present study, only the changes in the levels of six important compounds present in thyme EO, such as thymol, *p*-cymene, γ -terpinene, carvacrol, linalool, and borneol, were statistically compared. Depending on the drying method, the amount of these compounds varied from 69.36–77.41% of the total EO. Generally, the most important compound in thyme essential oil is a phenolic compound called thymol (37.2–44.4%), and other compounds include *p*-cymene (7.66–29.94%), γ -terpinene (3.22–9.68%), carvacrol (3.23–8.14%), linalool (2.45–4.25%), and borneol (2.1–3.22%). According to Safaei-Ghomi et al. [31][31], carvacrol, thymol, *p*-cymene, γ -terpinene, and borneol were the most abundant compounds in *Thymus caramanicus*, comprising 95.56% of the plant EO. Several factors, including plant species, drying method, plant age, and cultivation conditions, influence changes in the oil composition [32]. Drying may activate hydrolytic enzymes, resulting in distinct variations in the ratio of volatile compounds released [33].

Table 3

Volatile compounds (%) of thyme essential oils obtained from harvesting seasons and dried by different methods.

Identified compound	RT	Drying methods							Harvesting seasons	
		FD	Fresh	MV 360	MV 720	OV40	Shade	Sun	Autumn	Spring
α -Thujene	8.9	1.08	1.79	0.74	0.51	1.09	1.14	1.07	0.80	1.32
α -Pinene	9.13	0.91	1.07	0.66	0.49	1.12	1.12	1.10	0.85	1.00
1-Octen-3-ol	11.24	1.47	1.30	1.10	0.87	1.43	1.44	1.35	1.33	1.23
β -Myrcene	11.68	1.38	1.87	0.81	0.61	1.45	1.55	1.54	1.14	1.49
α -Terpinen	12.72	1.17	1.49	0.73	0.55	1.26	1.26	1.22	0.70	1.49
p-Cymene	13.39	16.7 $\pm$ 1.81 b	22.9 $\pm$ 3.69 a	11.2 $\pm$ 1.46 c	9.6 $\pm$ 1.52 c	19.2 $\pm$ 1.42 ab	20.1 $\pm$ 1.30 ab	21.0 $\pm$ 1.40 a	18.9 $\pm$ 1.89 ^a	15.6 $\pm$ 0.53 b
1,8-Cineole	13.51	1.27	1.42	1.49	1.50	1.35	1.05	1.08	1.48	1.14
γ-Terpinene	14.8	6.95 $\pm$ 1.52 b	9.68 $\pm$ 1.41 a	4.02 $\pm$ 1.07 c	3.22 $\pm$ 0.85 c	6.72 $\pm$ 1.09 b	7.57 $\pm$ 1.16 b	6.87 $\pm$ 1.35 b	3.93 $\pm$ 0.42 ^b	8.94 $\pm$ 0.60 a
Cis-Sabinenehydrate	15.12	0.80	1.25	1.08	1.14	0.77	0.74	0.74	1.05	0.82
Linalool	16.79	3.73 $\pm$ 0.37 ab	2.51 $\pm$ 0.27 d	3.36 $\pm$ 0.21 bc	3.16 $\pm$ 0.18 bc	4.02 $\pm$ 0.27 a	3.07 $\pm$ 0.22 cd	3.17 $\pm$ 0.30 bc	3.44 $\pm$ 0.19 ^a	3.13 $\pm$ 0.13 a
Borneol	19.66	2.47 $\pm$ 0.38 bc	2.10 $\pm$ 0.22 c	3.22 $\pm$ 0.14 a	2.27 $\pm$ 0.42 bc	2.79 $\pm$ 0.32 ab	2.48 $\pm$ 0.35 bc	2.14 $\pm$ 0.25 c	3.02 $\pm$ 0.12 ^a	1.97 $\pm$ 0.14 b
Terpinene-4-ol	20.19	1.79	1.48	2.20	1.39	1.54	1.73	1.62	1.36	2.00
Thymol methyl ether	22.9	1.55	1.45	1.57	1.41	1.31	1.49	1.65	1.67	1.31
Carvacrol methyl ether	23.3	0.84	1.06	0.88	0.78	0.82	0.93	1.11	1.02	0.81
Thymol	26.08	37.7 $\pm$ 1.26 b	36.8 $\pm$ 0.96 b	42.0 $\pm$ 1.12 a	44.4 $\pm$ 1.36 a	37.2 $\pm$ 1.09 b	38.4 $\pm$ 1.36 b	37.9 $\pm$ 1.50 b	38.2 $\pm$ 0.93 ^b	40.3 $\pm$ 0.72 a
Carvacrol	26.3	6.52 $\pm$ 1.15 a	3.48 $\pm$ 0.28 d	5.71 $\pm$ 0.38 ab	6.70 $\pm$ 0.40 a	4.66 $\pm$ 0.20 bcd	4.74 $\pm$ 0.11 bc	4.40 $\pm$ 0.06 cd	4.96 $\pm$ 0.24 ^a	5.38 $\pm$ 0.43 ^a

Identified compound	RT	Drying methods							Harvesting seasons	
		FD	Fresh	MV 360	MV 720	OV40	Shade	Sun	Autumn	Spring
β-Caryophyllene	30.84	1.22	1.81	2.18	2.64	1.22	1.20	1.07	1.54	1.70
Caryophyllene oxide	37.35	1.50	1.26	2.90	3.40	1.59	1.52	1.56	2.56	1.37

For each compound, means $\pm$ standard errors ($n = 3$) with the same letter are not significantly different according to LSD test ($P < 0.05$). **FD**: Freeze drying; **MW360** and **MW720**: microwave 360 W and microwave 720 W; **Ov40**: oven drying at 40 °C.

Thymol

As shown in Table 3, the thymol content was affected by different drying methods and seasons. Based on GC-MS analysis, all drying methods increased the thymol content compared to the fresh plant. However, this increase was only significantly different from the other methods when MW360 W and MW720 W were used. Among the different drying methods, the highest (44.44%) and lowest (37.23%) levels of thymol were obtained from MW720 W and OV40, respectively (Table 3). Generally, thymol was identified as the main component of the EO in both harvesting seasons, and higher levels of thymol were observed in the spring compared to the autumn (Table 3).

Rahimmalek and Goli [4] observed that microwave and oven drying at 50°C produced the highest and lowest levels of thymol in *Thymus daenensis* compared to the sun, shade, microwave, freeze-drying, OV40, and OV70, respectively. Sárosi et al. [2] reported that longer drying time leads to a much lower ratio of thymol in EO, and the lowest thymol ratio was observed at a low drying temperature (30°C) in *Thymus vulgaris*. Asekun et al. [32] observed the highest loss of major compounds in *Mentha longifolia* L. in plants dried at OV40. Notably, prolonged drying time often causes heat damage and ultimately has a negative effect on the essential oil.

γ-Terpinene

γ-Terpinene was affected by different drying methods and seasons, with higher amounts observed in the samples harvested in the spring compared to autumn (Table 3). After fresh plants with the highest γ-terpinene content, shade drying (7.57%), freeze drying, sun drying, and oven drying at 40°C were ranked in order of γ-terpinene content, respectively. Drying with MW720 W (3.22%) caused a significant decrease in the amount of γ-terpinene, with no significant difference, observed compared to MW360 W (Table 3).

In the study conducted by Safaei-Ghomi et al. [31] various compounds were identified in *Thymus caramanicus* with thymol being the main compound, followed by *p*-cymene and γ-terpinene. The highest amount of γ-terpinene in *Thymus linearis* was observed by Punetha et al. [34] using the shade drying method, corroborating the results of the current study. Rahimmalek and Goli [4] reported the highest amount of γ-terpinene in fresh plants of *Thymus daenensis* and the lowest amount in plants dried by microwave. Sadowska et al. [35] observed the highest amount of γ-terpinene in *Thymus vulgaris* using the shade drying method compared to freeze drying and oven drying at 35°C and 40°C. Similarly, Sárosi et al. [2] reported the highest and lowest amount of γ-terpinene in *Thymus vulgaris* using shade drying and oven drying at 50°C, respectively, among the different drying methods including freeze drying, shade drying, oven drying at 30°C, 40°C, and 50°C.

Borneol

The amount of borneol was influenced by different drying methods and harvesting seasons. Generally, higher values were observed in the autumn compared to spring. Microwave drying at 360 W (3.22%) and OV40 exhibited the highest borneol content, while sun drying (2.14%) demonstrated the lowest amount (Table 3). Consistent with the results of this study, Punetha et al. [34] reported the lowest amount of borneol in *Thymus linearis* from sun drying compared to drying methods in the shade, oven drying at 40°C, and microwave drying at 50°C. Calín-Sánchez et al. [36] found that *Origanum majorana* L. borneol concentrations were highest after freeze-drying and lowest after oven-drying at 60°C, while Rahimmalek and Goli [4] found similar results for *Thymys daenensis* borneol concentrations after oven drying at 50°C and freeze-drying.

p -Cymene

The amount of *p*-cymene was affected by various drying techniques and seasons and their interaction. In contrast to the observed trend in thymol, the *p*-cymene content was higher in autumn compared to spring (Table 3). After fresh samples collected in autumn, the highest percentage of *p*-cymene was found in plants dried in the sun, with 24.14% in autumn and 17.7% in spring, which did not differ significantly from plants dried in the shade or at OV40. The lowest percentage was also observed in plants dried with MW720 W, with 7.66% and 11.49% in autumn and spring, respectively (Table 4).

Punetha et al. [34] reported the highest amount of *p*-cymene in *Thymus linearis* dried in the sun compared to drying methods in the shade, OV40, and microwave drying at 50°C, which is consistent with the results of both seasons of the current study (Table 4). Rahimmalek and Goli [4] reported the highest and lowest amount of *p*-cymene in *Thymus daenensis* from shade drying and microwave methods, respectively, corroborating the results obtained in the current investigation (Table 4). Asekun et al. [32] reported the highest amount of pulegone (the second major compound of *Mentha longifolia* L.), and Kumar et al. [37] reported the highest amount of β -Asarone (one of the important constituents of *Acorus calamus* L.) from the sun drying method. Calín-Sánchez et al. [36] observed the lowest amount of *p*-cymene in *Origanum majorana* L. when dried using MW360 W. The highest amount of *p*-cymene in *Satureja hortensis* was reported by Sefidkon et al. ⁽²⁰⁰⁶⁾ using the sun-drying method compared to shade and oven drying at 40°C. Based on the results detailed in Tables 3 and 4, *p*-cymene decreased in microwave drying methods, while the levels of thymol and carvacrol increased. One possible reason for this may be due to enzymatic reactions resulting from higher temperatures, which lead to the conversion of *p*-cymene to thymol. In comparison, the enzyme responsible for converting γ -terpinene to *p*-cymene, has less activity at higher temperatures [39].

Table 4

Effect of different drying methods and harvesting season interaction effect on *p*-cymene, linalool, and carvacrol.

Drying methods	<i>p</i> -Cymene (%)		Linalool (%)		Carvacrol (%)	
	Autumn	Spring	Autumn	Spring	Autumn	Spring
FD	16.49 ± 3.77 _{ef}	16.94 ± 1.42 _e	4.25 ± 0.61 _a	3.20 ± 0.15 _{bcd}	4.91 ± 0.27 _{cde}	8.14 ± 1.99 _a
Fresh	29.94 ± 4.24 _a	15.80 ± 0.23 _{ef}	2.45 ± 0.59 _d	2.56 ± 0.06 _{cd}	3.23 ± 0.55 _e	3.73 ± 0.11 _e
MV 360	8.80 ± 2.05 _{gh}	13.65 ± 0.72 _{efg}	2.95 ± 0.14 _{bcd}	3.78 ± 0.16 _{ab}	6.47 ± 0.23 _{abc}	4.94 ± 0.32 _{cde}
MV 720	7.66 ± 2.58 _h	11.49 ± 1.14 _{fgh}	2.94 ± 0.26 _{bcd}	3.37 ± 0.21 _{abc}	6.22 ± 0.25 _{bcd}	7.19 ± 0.70 _{ab}
OV40	22.33 ± 0.31 _{bcd}	16.06 ± 0.42 _{ef}	4.21 ± 0.43 _a	3.83 ± 0.39 _{ab}	4.49 ± 0.17 _{de}	4.83 ± 0.36 _{cde}
Shade	22.98 ± 0.56 _{bc}	17.26 ± 0.12 _{de}	3.53 ± 0.12 _{ab}	2.61 ± 0.14 _{cd}	4.9 ± 0.13 _{cde}	4.57 ± 0.11 _{de}
Sun	24.14 ± 0.46 _b	17.94 ± 0.16 _{cde}	3.77 ± 0.26 _{ab}	2.57 ± 0.14 _{cd}	4.53 ± 0.05 _{de}	4.27 ± 0.03 _e
<i>P</i> value	0.0002		0.017		0.025	

For each season, means ± standard errors (n = 3) with the same letter are not significantly different according to LSD test ($P < 0.05$).

FD: Freeze drying; MW360 and MW720: microwave 360 W and microwave 720 W; Ov40: oven drying at 40 °C.

Carvacrol

The results showed that the carvacrol content was significantly affected by different drying methods and their interactions in different seasons (Table 4). All drying methods increased the amount of carvacrol compared to the fresh plant. The highest amount of carvacrol was obtained from MW360 W in the autumn (6.47%), which did not indicate a significant difference with MW720 W, while the lowest amount was observed in the OV40 (4.49%). In spring-harvested samples, freeze-drying yielded the highest concentration of carvacrol (8.14%), failing to show a significant difference compared to MW720 W dried samples. The lowest amount of carvacrol was obtained from the sun-drying method (4.27%), similar to the shade-drying, OV40, and MW360 W methods (Table 3).

The results of this study were consistent with the findings of other researchers. For example, Rahimmalek and Goli [4] study on the *Thymus daenensis* plant indicated that the levels of thymol, carvacrol, and caryophyllene increased at higher temperatures, and drying methods in microwave and oven at 70°C resulted in higher levels of thymol, carvacrol, and beta-caryophyllene compared to other treatments. Furthermore, they reported the highest carvacrol content from drying with an oven at 70°C, which did not differ significantly from drying with a microwave, while drying in the shade resulted in the lowest carvacrol content. Sadowska et al. [35] found that freeze-drying the *Thymus vulgaris* plant yielded the most carvacrol compared to shade drying and OV 35 and 40°C. Calín-Sánchez et

al. [36] also reported the highest amount of carvacrol from microwave-vacuum drying (240 W) for the *Origanum majorana* L. plant. Moreover, Sárosi et al. [2] reported the highest and lowest amounts of carvacrol from freeze-drying and shade-drying, respectively, for the *Thymus vulgaris* plant and stated that the final product, lyophilized, caused a 32% increase in carvacrol. The ratio of carvacrol in *Satureja hortensis* EO also decreased during shade drying [38], which is consistent with the results of the current study.

Linalool

While all drying methods increased linalool content compared to the fresh sample, the highest linalool content was obtained from freeze-drying (4.25%) and OV40 in the autumn, while the lowest level was obtained from MW720 W (2.94%). In contrast, in the spring, OV40 (3.83%) and MW360 W exhibited the highest levels, while sun-drying yielded the lowest level (2.57%) (Table 3).

Rahimmalek and Goli [4] reported the highest and lowest levels of linalool in *Thymus daenensis* from shade-drying and microwave-drying, respectively. In addition, Sefidkon et al. [38] reported that sun drying reduced the linalool content of *Satureja hortensis*. Venskutonis [7] observed the highest level of linalool in *Thymus vulgaris* from freeze drying, consistent with the results obtained during the autumn. Kumar et al. [37] observed the highest and lowest levels of linalool in *Acorus calamus* due to OV40 and sun drying, respectively.

Principal component analysis (PCA)

The PCA results showed that the first two PCs explained most of the variation (65.85%). The first PC accounted for 43.32% of the total variance, which established positive correlations with EO content (0.85), IC₅₀ (0.81), and γ -terpinene (0.73). The second PC described 22.53% of the total variation and established positive correlations with borneol (0.76) and *p*-cymene (0.60). The PCA results also demonstrated that the thyme samples harvested in spring (green circles; Fig. 1) possessed higher amounts of γ -terpinene (127.5%), thymol (5.5%), carvacrol (8.5%), EO content (56.1%), and TFC (95.8%) while samples harvested in the autumn (purple circles; Fig. 1) exhibited the highest amounts of borneol (53.29%) and *p*-cymene (21.15%), TPC (36.5%), and antioxidant activity (26.7%). Also, spring-harvested thyme samples, dried in sun and FD were rich in EO content and γ -terpinene and that thyme samples dried in MW720, OV40, and FD conditions contained the highest amounts of thymol, carvacrol, and TFC (Fig. 1). The drying conditions that had the most significant effect on TPC and linalool were the MW360, OV40 drying, and freeze-dried methods. Samples dried through OV40 and MW360 possessed the highest borneol amounts. Drying the autumn-harvested samples under the sun and shade also recorded higher linalool and TFC than other methods (Fig. 1).

Conclusion

In conclusion, several factors, including harvest time, plant species, energy requirements, cost, quantity, and quality of essential oil, should be considered when determining the optimal drying method for medicinal plants. This study shows that all drying methods under investigation increased the phytochemical compounds compared to the fresh plant. The results also revealed that the samples of thyme harvested in spring possessed higher amounts of γ -terpinene, thymol, carvacrol, EO content, and TFC, while samples harvested in the autumn exhibited the highest amounts of borneol, *p*-cymene, TPC, and antioxidant activity. In addition, the PCA plot demonstrated that thyme samples harvested in spring, dried in the sun, shade, and FD conditions were rich in EO content and γ -terpinene and dried in MW720, OV40, and FD conditions possessed the highest amounts of thymol, carvacrol, and TFC. Furthermore, the drying conditions that exerted the greatest effect on TPC and linalool were MW360, OV40, and FD methods. The findings also revealed the significant effect of OV40 and MW360 on borneol content. Drying the

autumn-harvested samples under sun and shade also recorded higher linalool and TFC than other methods. Overall, the results obtained demonstrate conclusively that the production and quality of EO were significantly impacted by harvest season and drying conditions.

Declarations

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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Consent to Participate Not applicable.

Consent for Publication Not applicable.

Author Contribution Zahra Tohidi-Nejad: Investigation, Data curation, Methodology, Writing – original draft. Gholamreza Khajoei-Nejad: Supervision, Project administration, Funding acquisition, Review & editing. Enayatollah Tohidi-Nejad: Supervision, Funding acquisition, Review & editing. Jalal Ghanbari: Conceptualization, Methodology, Software, Supervision, Writing – review & editing.

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Figures

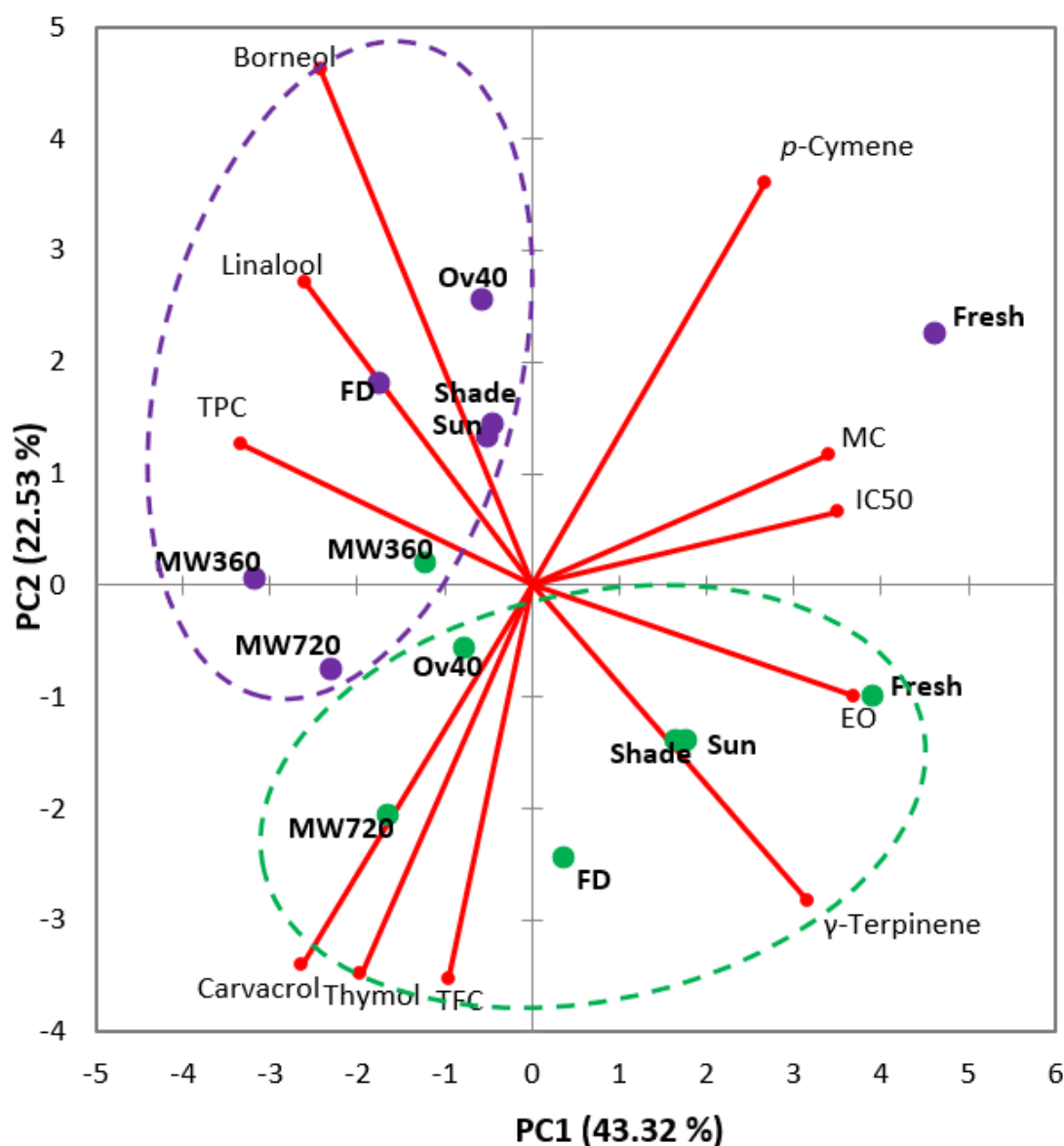


Figure 1

PCA analysis to identify the interrelationships among the drying conditions in two harvesting seasons (spring: the green circles; and autumn: purple circles) and the main examined characteristics. **MW360** and **MW720**: microwave 360 W and microwave 720 W; **Ov40**: oven drying at 40 °C; **FD**: Freeze drying; **MC**: moisture content; **EO**: essential oil content; **TFC**: Total flavonoid content; **TPC**: Total phenolic content.