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Research article

Post-harvest processing effects on essential oil composition and antioxidant activity of *Mentha piperita* L.

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Abstract: Peppermint (*Mentha piperita*) is a widely used medicinal and aromatic plant whose preservation of bioactive compounds and essential oil (EO) yield is crucial for maintaining its quality. In this study, we evaluated the effects of five post-harvest treatments applied to peppermint leaves: control (fresh leaves–T1), oven drying at 40 °C with forced-air ventilation (T2), oven drying at 40 °C without forced-air ventilation (T3), room-temperature storage (23−27 °C) (T4), and freezing at −20 °C for 30 days (T5). For each treatment, antioxidant activity (FRAP and DPPH), total phenolic content, EO yield and composition, and EO density and refractive index, were evaluated. Treatments T2 and T4 showed the highest EO yields among the evaluated treatments. Carvone (ranging from 75.79% in T1 to 33.08% in T5) and limonene (ranging from 25.83% in T4 to 7.77% in T3) were the major tentative compounds identified, with their relative abundances varying according to the post-harvest treatment applied. Freezing was associated with lower EO yields and reduced antioxidant activity compared with the other treatments. Overall, room-temperature storage (T4) and oven drying with forced-air ventilation (T2) showed the most favorable results for EO yield and antioxidant activity of *M. piperita*. Although both treatments showed favorable overall performance, differences in EO chemical composition indicate that treatment selection should consider the desired chemical profile

and intended application.

Keywords: carvone; drying methods; freezing; limonene; phenolic compounds

Abbreviations: AD: absolute density; EO: essential oil; FM: fresh mass; GC/MS: gas chromatography/mass spectrometry; nD: refractive index; PCA: principal component analysis; TPC: total phenolic compounds.

1. Introduction

Mentha piperita L. (peppermint) has a long history of traditional and industrial use, largely attributed to its medicinal properties and pleasant aroma. These properties have attracted considerable scientific interest, and numerous researchers have investigated the potential biological and therapeutic applications of peppermint essential oils (EOs) and extracts, including anticancer, radioprotective, and gastrointestinal effects [1,2].

Species of the genus *Mentha* are widely incorporated into pharmaceutical formulations, mouthwashes, toothpastes, and cosmetic products, reflecting their broad applicability and economic relevance. This genus has attracted substantial global industrial interest, particularly in the United States and China, and is associated with a high number of patents, mainly due to the biological and commercial value of key bioactive compounds [3]. In *M. piperita*, menthol and menthone are generally recognized as the major constituents of the EO and are of considerable economic importance [4]. However, variations in chemical composition have been reported, and some researchers have identified carvone as a major constituent, highlighting the relevance of menthol, menthone, pulegone, and carvone to the chemical and commercial value of *M. piperita* EO [1,5].

Medicinal plants are rich sources of secondary metabolites, whose stability and concentration can be markedly influenced by post-harvest handling practices. Changes in drying, storage, and preservation conditions may affect the recovery of bioactive compounds and, consequently, the yield and chemical profile of EOs [6]. For *M. piperita*, several researchers have investigated specific aspects of post-harvest processing, providing evidence that the response of the plant material depends strongly on the processing conditions applied.

For example, Beigi et al. [7] compared oven drying at 50 °C with microwave drying and demonstrated that the drying method affects the EO yield and composition of *M. piperita*. Hubert et al. [6], in turn, evaluated whether the stage at which leaves were separated from the plant during drying influenced EO characteristics, showing that the organization of the plant material during drying can also affect the resulting EO profile. Moreover, Xylia et al. [8] compared sun, shade, and oven drying at 42 °C and evaluated not only EO yield and composition but also biological properties. These studies collectively demonstrate that post-harvest processing can influence the chemical characteristics of peppermint EOs and their associated biological properties while highlighting that the effects depend on the processing conditions evaluated.

Nevertheless, the available literature has predominantly addressed individual drying strategies or specific processing variables. Comparisons involving different types of post-harvest treatment within the same experimental framework remain less explored, particularly when contrasting drying conditions, room-temperature storage, and freezing are evaluated simultaneously. In addition, studies

integrating EO yield and chemical composition with antioxidant-related parameters of peppermint extracts under these contrasting post-harvest conditions are limited. Thus, a comparative approach encompassing different preservation conditions may provide a broader understanding of how post-harvest handling affects the quality and bioactive characteristics of *M. piperita* plant material.

Accordingly, we aimed to comparatively evaluate the effects of different post-harvest treatments on *M. piperita* leaves by integrating EO yield, physicochemical characteristics, chemical composition, and extract-based antioxidant parameters.

2. Materials and methods

2.1. Cultivation of M. piperita

The plants used in this study were cultivated on a private property in the municipality of Pérola, Paraná, Brazil (−23.8559, −53.7508). Planting was carried out in October 2022, and plant material was harvested in December 2022. The region has a subtropical climate, with a mean spring temperature of 22.24 °C [9], and the average precipitation during the cultivation period was 87.76 mm [10].

Prior to planting, soil analyses were performed in September 2022 using samples collected at depths of 0−50 cm for physical analysis and 0−20 cm for chemical analysis [11,12]. The soil composition consisted of 8% clay, 1.45% silt, and 90.55% sand. Base saturation was corrected to 70% by liming, with the application of 1.41 t ha^{-1} of limestone (PRNT 85%). After 30 days, six beds (3.5 × 3 m) were prepared and fertilized with cured bovine manure (15 kg bed^{-1}).

M. piperita seedlings were taxonomically identified by the Herbarium of the Medicinal Garden of UNIPAR (voucher specimen no. 2398). After acquisition, seedlings were transferred to plastic bags and maintained for 30 days for rooting. Subsequently, plants were propagated by cuttings in the beds, using a spacing of 0.6 × 0.35 m. After planting, seedlings were irrigated once daily and cultivated for 90 days, after which the plants were harvested and subjected to the post-harvest treatments described below.

2.2. Processing-harvest

2.2.1. Post-harvest treatments

After the cultivation period, *M. piperita* leaves were harvested and subjected to different post-harvest conservation conditions. Fresh plant material was analyzed immediately after collection and used as the control treatment (T1), as described by Duarte et al. [13]. For the drying treatments, leaves were dried at 40 °C with forced-air circulation until constant weight using an oven manufactured by TECNAL (Piracicaba, São Paulo, Brazil), model TE-394/3 (T2), according to Costa et al. [14], or without forced ventilation under the same temperature conditions using an oven manufactured by Olidef (Ribeirao Preto, São Paulo, Brazil) model CZ (T3), following Cordeiro et al. [15]. The drying process was monitored to ensure uniform dehydration.

Additionally, leaves were stored in brown paper at room temperature (23−27 °C) without controlled humidity for 30 days prior to analysis (T4), as described by Barbosa et al. [16]. A limitation of this treatment was that constant weight was not monitored during the storage period; therefore, complete drying of the leaves could not be confirmed. For the freezing treatment (T5), plant material

was packaged in plastic bags and wrapped in aluminum foil, then stored at −20 °C for 30 days prior to analysis, according to Maus et al. [17].

2.2.2. Essential oil extraction

EO extraction was performed by hydrodistillation using a modified Clevenger-type apparatus, as described by Duarte et al. [13], for 3 h. The plant material was crushed in 3.5 L of distilled water, with plant material masses of 20 g (T1), 18 g (T2), 16 g (T3), 22 g (T4), and 16 g (T5), expressed as mean values obtained from triplicate experiments, ensuring complete immersion of the samples during the extraction process.

Hexane was used to recover the EO adhering to the walls and surfaces of the apparatus. After complete removal of hexane, the extraction yield was calculated based on the mass ratio between the plant material and EO (% w/w). The EO was then stored in an amber glass vial wrapped in aluminum foil, sealed, and refrigerated at approximately −5 °C.

2.2.3. Extract preparation

After each post-harvest treatment (T1, T2, T3, T4, and T5), the leaves were macerated in liquid nitrogen and stored in Falcon tubes. For the preparation of extracts for the DPPH, total phenolic content, and FRAP assays, 0.1 g of frozen, macerated leaf material was extracted with 4 mL of 50% methanol. The samples were allowed to stand for 1 h at room temperature and then centrifuged at 12,000 g. The supernatant was collected, and 4 mL of 70% acetone was subsequently added to the remaining residue. After an additional 1 h at room temperature, the samples were centrifuged under the same conditions, and the second supernatant was collected. The two supernatants were combined, and the final volume was adjusted to 10 mL with distilled water, as described by Waterhouse [18]. All analyses were performed in triplicate.

2.3. Characterization of M. piperita essential oils

2.3.1. Chemical composition

The chemical composition of *M. piperita* EOs was analyzed using a gas chromatograph (Agilent 7890B) coupled to a mass spectrometer (Agilent 5977A), equipped with an Agilent HP-5MS UI capillary column (30 m × 0.250 mm × 0.25 μm). A volume of 2.0 μL of each sample diluted in dichloromethane was injected. The injector temperature was set at 260 °C and operated in split mode (20:1). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}, and the transfer line temperature was maintained at 280 °C.

The oven temperature program started at 50 °C (held for 2 min), increased at 5 °C min^{-1} to 60 °C (held for 1 min), followed by a ramp of 4 °C min^{-1} to 280 °C (held for 5 min), and a final increase of 40 °C min^{-1} to 300 °C (held for 1 min). Mass spectrometric detection was performed in scan mode using electron impact ionization at 70 eV, with a mass range of *m/z* 40−550. Ion source and quadrupole temperatures were set at 230 and 150 °C, respectively. Compound identification was achieved by comparison of mass spectra with the NIST 11.0 library and by calculation of retention indices (RI) relative to a homologous series of n-alkanes (C7−C28), according to Adams [19]. Only compounds

showing agreement between mass spectral data and retention indices were considered tentatively identified.

2.3.2. Determination of density and refractive index

The refractive index (nD) of the EOs was determined using an ABBE refractometer (model RL3, PZO Warszawa) at 20 °C. Prior to analysis, instrument calibration and temperature control were verified using distilled water, with reference nD values of 1.3330 at 20 °C and 1.3325 at 25 °C [20]. EO samples were placed in the refractometer prism, and nD values were read directly from the instrument scale.

Absolute density was determined gravimetrically using a graduated capillary with 1 µL graduations. Density was calculated as the ratio between sample mass and EO volume (4 µL) at 20 °C [20]. Sample mass was determined using an analytical balance with a precision of 0.001 g. Three technical replicates were performed for each treatment.

2.4. Characterization of M. piperita extracts

2.4.1. Determination of total phenolic compounds (TPC)

An aliquot of 500 µL of each extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 4% sodium carbonate in test tubes, according to Larrauri et al. [21]. Absorbance was measured using a spectrophotometer at 750 nm. Results were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} of dry matter. For samples T1 and T5, values originally expressed on a fresh mass basis were converted to a dry matter basis using an estimated moisture content of 67.6% (unpublished data), based on a calibration curve prepared with gallic acid (0−50 µg mL^{-1}), following the equation:

$$y = 0.0076x - 0.02 \ (R^2 = 0.9959) \quad (1)$$

2.4.2. Antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

Antioxidant activity was determined based on the scavenging of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, 60 µM), following the methodology described by Rufino et al. [22], and performed in triplicate. A control solution was prepared by mixing 40 mL of 50% methanol, 40 mL of 70% acetone, and 20 mL of Milli-Q water, resulting in a final volume of 100 mL. For the control, 100 µL of the control solution was added to 3.9 mL of DPPH radical solution. For each sample, 100 µL of the corresponding extract was added to 3.9 mL of the DPPH radical solution. The mixtures were homogenized using a tube shaker, and the tubes were wrapped in aluminum foil to protect them from light.

Absorbance was monitored at 515 nm at 30 min intervals until stabilization. Results were expressed as percentage of free radical scavenging (%FRS), calculated according to the equation:

$$\%FRS = [(Ac - As)/Ac] \times 100 \quad (2)$$

where *Ac* is the absorbance of the control and *As* is the absorbance of the sample. Antioxidant activity was expressed as a function of extract concentration by plotting the percentage inhibition of the DPPH radical.

2.4.3. Antioxidant activity by the ferric reducing antioxidant power (FRAP) assay

Under light-protected conditions, a 90 µL aliquot of each extract dilution was mixed with 270 µL of distilled water and 2.7 mL of FRAP reagent, consisting of sodium acetate buffer (0.3 M, pH 3.6), ferric chloride (20 mM), and 2,4,6-tripyridyl-s-triazine (TPTZ, 10 mM). The mixture was homogenized and incubated in a water bath at 37 °C [23]. Absorbance was measured at 595 nm after 30 min, using the FRAP reagent as blank for spectrophotometer calibration. Results were expressed as mg of Trolox equivalents 100 g^{-1}, based on a calibration curve (0−1600 µmol L^{-1}) following the equation:

$$y = 0.4136x - 0.2515\ (R^2 = 0.99) \quad (3)$$

2.4.4. Determination of flavonoids and anthocyanins

Anthocyanin and flavonoid contents were determined according to the method described by Francis [24]. Leaf samples (0.1 g) from each treatment were macerated in liquid nitrogen, and compounds were extracted using 5 mL of ethanol−HCl (1.5 M). Samples were protected from light, centrifuged at 7,000 g for 10 min, and the supernatant was collected. Absorbance readings were performed at 535 nm for anthocyanins and 374 nm for flavonoids. Results were expressed as mg per 100 g of fresh mass (FM), using a dilution factor of 5000, and calculated using the following equations:

$$Anthocyanins = (Abs_{535} \times dilution\ factor) / 98.2 \quad (4)$$

$$Flavonoids = (Abs_{374} \times dilution\ factor) / 76.6 \quad (5)$$

2.5. Statistical analysis

The experiments were conducted using a completely randomized design (CRD). Biological replication consisted of independent plant material batches subjected to each treatment, with three biological replicates (n = 3) for each treatment. All analytical determinations were performed in triplicate for each biological sample (n = 3 technical replicates). For EO extraction and yield determination, three independent distillation runs were performed for each treatment (n = 3). After confirming normality and homogeneity of variances, the data were subjected to one-way analysis of variance (ANOVA) using a general linear model, followed by Tukey's post hoc test ($p \leq 0.05$) for multiple comparisons. Results were expressed as mean ± standard error. Statistical analyses were performed using IBM SPSS Statistics® software, version 22.0.

For multivariate analysis, treatment means were used as observations, with each treatment represented by its mean value for the evaluated variables. The variables included EO yield, the three most abundant EO constituents (carvone, limonene, and germacrene D), antioxidant activity (DPPH and FRAP), TPC, flavonoids, and anthocyanins. The resulting data matrix was used to generate the heat map with hierarchical clustering and PCA using SRplot [25]. Because only five treatments were included, these multivariate analyses were considered exploratory and were used primarily to visualize patterns and relationships among treatments.

3. Results and discussion

Quality control analyses of the EOs revealed variations in the yield, absolute density, and refractive index among the evaluated treatments (Table 1).

T1, T2, and T3 showed density values ranging from 0.89 to 0.92 g mL^{-1} near the range reported by the Brazilian Pharmacopoeia [20] (0.9−0.916), whereas higher values were observed for T4 (1.02 g mL^{-1}) and especially T5 (1.13 g mL^{-1}), exceeding the recommended limits (Table 1).

Table 1. Yield, refractive index (nD), and absolute density (AD) of *M. piperita* essential oil.

Conservation conditions	Yield (%)	nD	AD (g mL^{-1})
T1	0.20 ± 0.02b	1.493 ± 0.00a	0.89 ± 0.03b
T2	1.52 ± 0.06a	1.491 ± 0.00b	0.92 ± 0.01b
T3	0.26 ± 0.02b	1.492 ± 0.00ab	0.91 ± 0.04b
T4	2.06 ± 0.27a	1.491 ± 0.00b	1.02 ± 0.02ab
T5	0.10 ± 0.02b	1.492 ± 0.00ab	1.13 ± 0.06a

Means ± standard error ($n = 3$) followed by the same letter within a column do not differ significantly according to Tukey's test ($p < 0.05$). T1: control group (fresh leaves); T2: leaves dried in a forced-air oven; T3: leaves dried in an oven without forced ventilation; T4: leaves stored at room temperature; T5: frozen leaves.

The nD of the analyzed samples ranged from 1.491 to 1.493, exceeding the values reported by the Brazilian Pharmacopoeia [20] (1.457−1.467) but remaining consistent with those described by Singh et al. [26], who reported a value of 1.482 for *M. piperita* EO. Because nD is temperature-dependent, all measurements were performed under controlled temperature conditions (20 °C).

EO yield is an important parameter for evaluating the effectiveness of post-harvest treatments. Among the evaluated post-harvest treatments, drying in a forced-air oven at 40 °C (T2) and room-temperature storage (23−27 °C) for 30 days (T4) resulted in higher extraction yields compared to the other treatments when expressed on a FM basis. However, this difference should be interpreted with caution, as treatments involving fresh (T1) and frozen (T5) plant material are inherently associated with higher moisture content, which may result in lower apparent EO yields when expressed on an as-is mass basis.

When an estimated dry-matter-based approach was applied using moisture values obtained under similar cultivation conditions (unpublished data), the yields for T1 and T5 increased to 0.62% and 0.31%, respectively, reducing the apparent magnitude of differences among treatments. Nevertheless, treatment T4 maintained the highest yield values, indicating a consistent trend of superior performance even after considering the effect of water content. Similar trends have been reported by Beigi et al. [7] and Chen et al. [27], who observed higher apparent yields in dried plant material compared to fresh samples. However, these corrected values should be interpreted with caution, as the moisture data were not directly determined from the samples analyzed in this study and were derived from unpublished data obtained under similar, but not identical, experimental conditions.

Our results suggest that drying conditions can influence the preservation of antioxidant activity in peppermint. Research has reported that air-drying, a commonly used method to extend the shelf life of peppermint, may preserve or result in slightly higher antioxidant activity compared with other processing conditions, indicating that this drying method does not necessarily promote the degradation of compounds associated with antioxidant activity [28]. During the drying process, changes in the plant

matrix may also facilitate the release of antioxidant substances, particularly phenolic compounds, that are initially bound to cellular components [29].

In contrast, drying at high temperatures may contribute to the reduction of antioxidant activity, as evidenced by lower values obtained using DPPH and FRAP assays [30,31]. These findings highlight the importance of selecting appropriate drying conditions, as temperature and processing methods may affect the stability and preservation of antioxidant activity in plant materials. From an applied perspective, the choice of post-harvest treatment should also consider energy consumption, equipment availability, and processing time [32], which represents an important consideration for producers, particularly regarding the financial investment required for equipment acquisition.

The influence of post-harvest treatments on antioxidant activity is an important factor when selecting appropriate processing conditions. Compared with the other treatments, the fresh (T1) and frozen (T5) samples showed lower antioxidant activity, as evidenced by lower DPPH and FRAP values, even after correction to the estimated dry matter basis. However, these dry matter-adjusted values should be interpreted with caution because the moisture content was estimated rather than directly determined in the analyzed samples. In contrast, the TPC contents were higher in T1 (fresh leaves) and T5 (freezing) after correction to the estimated dry matter basis (Table 2).

The highest antioxidant capacities determined by DPPH and FRAP were observed in T2 and T4, while T3 showed the highest TPC in the extract solution, followed by T5, T4, and T1 (Table 2). Studies by Djamila et al. [33] and Xylia et al. [8] reported higher DPPH radical-scavenging activity in dried *Mentha* species than in fresh plant material, suggesting that appropriate drying conditions may help maintain antioxidant activity during post-harvest processing. Consistent with these reports, the lack of direct correspondence between TPC and antioxidant activity in study suggests that the antioxidant response was not determined solely by TPC and that other compounds or interactions among constituents may also contribute to the observed activity.

Treatment T1 had the lowest number of detected compounds (18) in the EO, whereas T3 had the highest (32) (Table 3). This difference indicates that post-harvest treatment affected the qualitative profile of the compounds detected by GC–MS. Most EO samples were characterized by a predominance of oxygenated monoterpenes, in agreement with other reports [8], except for T5, which showed a higher relative abundance of sesquiterpene hydrocarbons.

Species of the genus *Mentha* exhibit considerable chemical and genetic variability, and different EO chemotypes may occur within the same species. Among these, chemotypes characterized predominantly by carvone and D-limonene have been reported [34]. The EOs obtained from all treatments showed a carvone-dominant chemical profile, with carvone identified as the major compound. This profile is consistent with a carvone chemotype reported for *M. piperita* [34,35]. Its relative abundance varied according to the post-harvest processing conditions, ranging from 75.79% (T1) to 33.08% (T5). The predominance of carvone at comparable relative abundances was also reported by Hubert et al. [6] for two *Mentha* varieties.

Limonene was identified as the second major compound, with relative abundances ranging from 25.83% (T4) to 7.77% (T3) (Table 3). Similar results were reported by Alsaraf et al. [36], who identified carvone as the major component in peppermint EO and Rezende et al. [37], who identified carvone and limonene as the main constituents of *M. piperita* EO. However, a high content of carvone and limonene was also reported by Barros et al. [38], around 58.79%.

Table 2. Characterization of *M. piperita* extracts.

	Parameter	Conservation				
		T1	T2	T3	T4	T5
Extract solution	TPC (mg_{GAE} 100 g^{-1})	293.88 ± 38.73ab*	253.03 ± 58.56b	470.88 ± 61.03a	349.12 ± 55.64ab	356.75 ± 26.53ab
	DPPH (%)	3.94 ± 0.14c	67.79 ± 1.25a	44.75 ± 2.66b	68.58 ± 2.35a	3.53 ± 0.59c
	FRAP (mg_{TROLOX} 100 g^{-1})	25.08 ± 9.11c	298,609.00 ± 4,029.27a	117.16 ± 10.89c	265,835.96 ± 4,007.84b	19.02 ± 4.27c
	Flavonoids (mg 100 g^{-1})	193.79 ± 37.20b	324.45 ± 24.22a	369.08 ± 28.41a	339.07 ± 16.00a	112.76 ± 2.85b
	Anthocyanins (mg 100 g^{-1})	19.08 ± 1.13a	19.05 ± 1.03a	17.40 ± 0.76a	19.11 ± 0.93a	9.05 ± 0.22b
Estimated dry-matter-adjusted values	TPC (mg_{GAE} 100g^{-1})	904.86 ± 119.24a	253.03 ± 58.56b	470.88 ± 61.03b	349.12 ± 55.64b	1,098.42 ± 81.70a
	DPPH (%)	12.14 ± 0.45c	67.79 ± 1.25a	44.75 ± 2.66b	68.58 ± 2.35a	10.88 ± 1.83c
	FRAP (mg_{TROLOX} 100 g^{-1})	77.21 ± 28.05c	298,609.05 ± 4,029.27a	117.16 ± 10.89c	265,835.96 ± 4,007.84b	58.55 ± 13.15 c
	Flavonoids (mg 100 g^{-1})	596.67 ± 114.53a	324.45 ± 24.22b	369.08 ± 28.41b	339.07 ± 16.00b	347.17 ± 8.79b
	Anthocyanins (mg 100 g^{-1})	58.76 ± 3.49a	19.05 ± 1.03c	17.40 ± 0.76c	19.11 ± 0.93c	27.87 ± 0.67b

*Means ± standard error (n = 3) followed by the same letter within a row do not differ significantly according to Tukey's test ($p \leq 0.05$). T1: control group (fresh leaves); T2: leaves dried in a forced-air oven; T3: leaves dried in an oven without forced ventilation; T4: leaves stored at room temperature; T5: frozen leaves; TPC: total phenolic compounds. For T1 and T5, values originally expressed on a fresh-mass basis were converted to a dry-matter basis using an estimated moisture content of 67.6%. Because moisture content was not directly determined in the samples analyzed, the resulting dry-matter-adjusted values should be considered exploratory.

The relative abundance and chemical composition of the EOs were also influenced by the applied treatments (Table 3).

Table 3. Chemical composition of *M. piperita* essential oils.

Peak	RT	Compound	T1	T2	T3	T4	T5	RI lit.	RI calc.	Reference	Identification criteria
			Relative area %								
1	5.416	α-pinene	0.24	0.28	0.18	0.35	-	932	938	[19]	a, b, c
2	6.459	Sabinene	0.18	0.14	-	0.48	-	969	977	[19]	a, b, c
3	6.556	β-pinene	0.38	0.43	0.22	0.82	0.28	974	980	[19]	a, b, c
4	6.954	Myrcene	0.59	0.61	0.34	1.19	0.80	988	993	[19]	a, b, c
5	8.175	Limonene	15.01	15.04	7.77	25.83	19.72	1024	1032	[19]	a, b, c
6	8.253	Eucalyptol	-	-	-	-	1.15	1026	1034	[19]	a, b, c
7	8.478	*Trans*-β-ocimene	-	0.29	0.17	0.17	-	1039	1041	[39]	a, b, c
8	8.480	*Cis*-ocimene	0.30	-	-	0.53	0.58	1040	1041	[40]	a, b, c
9	13.293	L- borneol	0.37	0.38	0.32	0.34	-	1165	1166	[19]	a, b, c
10	14.588	*Cis*-dihydrocarvone	-	0.36	0.38	0.70	-	1191	1195	[19]	a, b, c
11	15.602	Carveol	0.52	-	0.48	-	-	1246	1248	[41]	a, b, c
12	16.667	Carvone	75.79	68.25	64.99	60.72	33.08	1239	1247	[19]	a, b, c
13	20.580	Piperitenone	-	0.36	0.36	-	-	1340	1339	[19]	a, b, c
14	21.615	*Cis*-carvyl acetate	-	-	0.36	0.32	-	1365	1364	[19]	a, b, c
15	22.480	β-bourbonene	1.23	2.58	3.56	1.99	6.24	1387	1384	[19]	a, b, c
16	22.814	β-elemene	-	-	0.29	-	0.46	1389	1391	[19]	a, b, c
17	22.817	α-copaene	-	0.27	0.36	-	0.72	1394	1392	[39]	a, b, c
18	23.500	α-gurjunene	-	-	0.24	-	0.4	1409	1408	[19]	a, b, c
19	23.871	β-caryophyllene	1.15	2.02	3.15	1.49	5.76	1417	1417	[19]	a, b, c
20	24.274	β-copaene	-	0.25	0.37	-	0.72	1428	1428	[42]	a, b, c
21	24.887	γ-Muurolene	-	0.24	0.36	-	0.76	1449	1444	[43]	a, b, c
22	25.234	Humulene	-	0.32	0.52	0.22	0.97	1447	1446	[44]	a, b, c
23	25.512	Trans-β-farnesene	0.23	0.62	1.01	0.31	1.96	1454	1459	[19]	a, b, c

Continued on the next page

Peak	RT	Compound	T1	T2	T3	T4	T5	RI lit.	RI calc.	Reference	Identification criteria
			Relative area %								
24	25.627	Bicyclosesquiphellandrene	0.45	1.04	1.61	0.61	2.98	1462	1465	[19]	a, b, c
25	25.761	n.i.	-	0.27	0.40	-	0.72	-	1465	-	a, b, c
26	26.357	Germacrene-D	2.04	3.77	6.21	2.44	11.49	1484	1479	[19]	a, b, c
27	26.969	Bicyclogermacrene	0.33	0.46	0.79	0.27	1.64	1500	1494	[19]	a, b, c
28	27.669	γ-Cadinene	-	-	0.25	-	0.42	1511	1512	[45]	a, b, c
29	27.981	(1S)-*cis*-Calamenene	0.46	0.71	1.30	0.45	1.81	1521	1520	[40]	a, b, c
30	28.062	δ-Cadinene	-	-	-	-	0.53	1524	1523	[42]	a, b, c
31	28.580	α-Muurolene	-	-	0.31	-	0.56	1537	1536	[46]	a, b, c
32	30.012	(-)-Spatulenol	-	-	0.36	-	0.69	1572	1573	[47]	a, b, c
33	31.494	Cubenol	0.34	0.46	0.87	0.30	1.58	1607	1612	[48]	a, b, c
34	32.447	T-cadinol	-	-	0.39	-	0.81	1638	1638	[45]	a, b, c
35	32.945	α-Cadinol	0.37	0.45	0.97	0.27	1.42	1653	1652	[42]	a, b, c
36	34.074	Aromadendrene oxide-(2)	-	-	0.34	-	0.47	1678	1682	[49]	a, b, c
37	34.207	6-epi-shyobunol	-	0.30	0.56	-	0.90	1881	1886	[50]	a, b, c
Total (%)			99.80	99.90	99.79	99.81	99.62				
Hydrocarbon Monoterpenes			16.54	16.79	8.68	29.37	21.38				
Oxygenated Monoterpenes			76.68	69.35	66.53	61.76	34.23				
Hydrocarbon Sesquiterpenes			5.89	12.55	20.73	7.78	38.14				
Oxygenated Sesquiterpenes			0.71	1.21	3.49	0.58	5.87				
Other Compounds			-	-	0.36	0.32	-				

a: Compounds listed according to their elution order on the HP-5MS column; b: Retention index (RI) calculated using a homologous series of *n*-alkanes on a capillary column (HP-5MS); c: Identification based on comparison of mass spectra with the NIST 11.0 spectral library; Area (%): percentage of the chromatographic area corresponding to each compound; (–): compound not detected in the sample; RT: retention time; RI calc: calculated RI; RI lit: literature RI; n.i.: not identified. T1: control group (fresh leaves); T2: leaves dried in a forced-air oven; T3: leaves dried in an oven without forced ventilation; T4: leaves stored at room temperature; T5: frozen leaves.

The predominance of carvone and limonene observed in this study differs from the composition reported in some other investigations of *M. piperita* EO [7]. Such differences may be associated with the geographic origin of the plant material and the environmental conditions during cultivation, particularly temperature and rainfall patterns, which can influence the biosynthesis of monoterpenes and other volatile constituents [5].

Moreover, variations in harvest conditions and post-harvest storage may affect the relative proportions of EO constituents due to their different volatilities and susceptibilities to degradation or evaporation [51]. Other compounds detected at relatively high relative abundances included germacrene D, β-bourbonene, and β-caryophyllene, whose proportions also varied according to the post-harvest conservation methods. Moreover, the adopted conservation strategies influenced the total amount of substances extracted into the EO, resulting in distinct chemical profiles, particularly regarding the relative abundances of major constituents such as carvone and limonene.

The different post-harvest treatments showed three main clustering patterns in the heat map combined with hierarchical clustering (Figure 1).

B

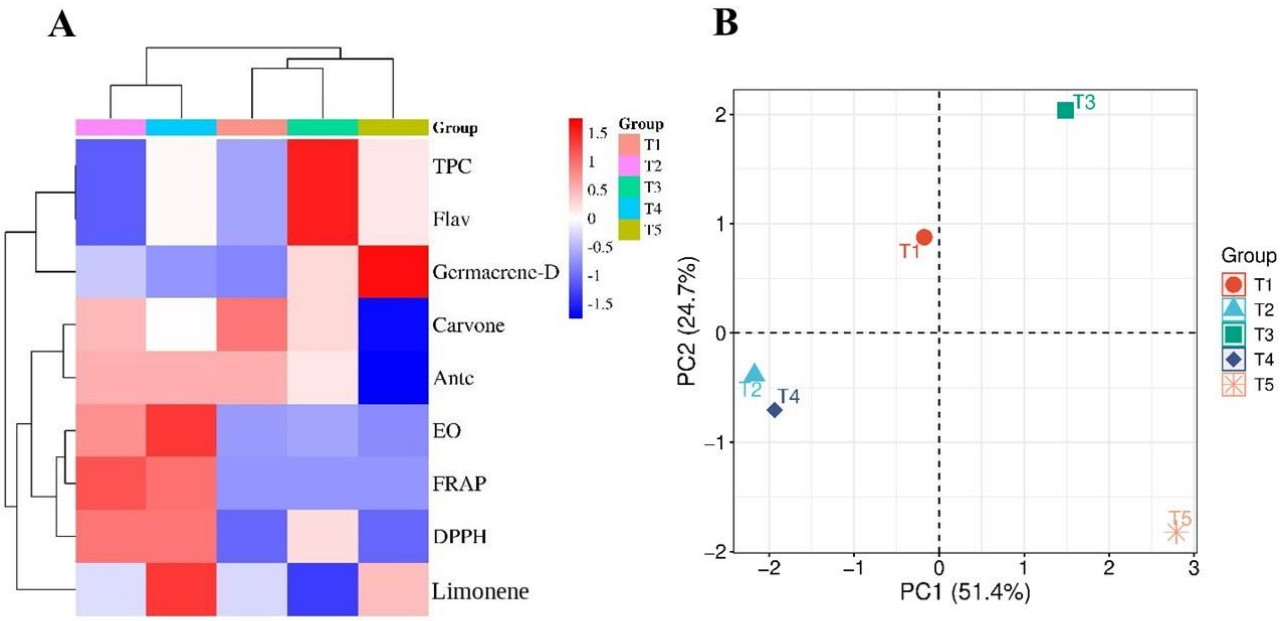


Figure 1. Heat map combined with hierarchical clustering (A) and Principal Component Analysis (PCA) (B) revealed three main patterns among treatments based on essential oil (EO) yield and the three most abundant EO constituents (carvone, limonene, and germacrene D), as well as antioxidant activity, total phenolic content (TPC), flavonoids (Flav), and anthocyanins (Antc) determined in the *Mentha piperita* extract solution. T1: control group (fresh leaves); T2: leaves dried in an oven with forced-air ventilation; T3: leaves dried in an oven without forced-air ventilation; T4: leaves stored at room temperature; T5: frozen leaves.

The freezing treatment (T5), fresh leaves and oven drying without forced-air ventilation (T1 and

T3, respectively), and room-temperature storage and forced-air oven drying (T4 and T2) formed separate clusters, indicating differentiated responses in terms of EO yield, phenolic compounds, flavonoids, major EO constituents, and antioxidant activity (Figure 1).

Treatments T2 (forced-air oven drying) and T4 (room-temperature storage) clustered closely, indicating similarities in their chemical and antioxidant profiles. These similarities indicate that T2 and T4 showed comparable patterns across the variables included in the analysis (Figure 1).

Variable clustering analysis revealed that total phenolic compounds, flavonoids, and germacrene-D formed one distinct group, whereas carvone and anthocyanins formed another. Additionally, EO yield, FRAP, and DPPH clustered separately, suggesting similar patterns of variation among these variables.

The exploratory PCA revealed that T2 and T4 occupied relatively similar positions, whereas T3 and, particularly, T5 showed distinct positioning relative to the other treatments. Overall, the PCA and hierarchical clustering provided complementary exploratory visualizations of patterns among the treatments (Figure 1).

4. Conclusions

The results indicate that post-harvest treatments influenced EO yield, antioxidant activity, and chemical composition. The treatments that showed the most favorable results for antioxidant activity and EO yield were room-temperature storage and oven drying with forced-air ventilation.

Variations were observed in the EO chemical profile, indicating that the choice of treatment should consider the intended application. In addition, factors such as equipment availability, investment requirements, and processing time may be relevant criteria when selecting a post-harvest treatment.

Use of AI tools declaration

Artificial Intelligence (ChatGPT-OpenAI) was used solely as an assistive tool for translation and language refinement of text originally written by the authors.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: GOC, HMM, OA; Methodology: GOC, HMM, OA; Data curation: GOC, OA, JH; Formal analysis: GOC, ZCG, JEG; Funding acquisition: HMM, OA; Supervision: HMM, OA, JH; Validation: OA, JH; Writing–original draft: GOC, HMM, ZCG, JEG, JH; Writing review and editing: GOC, OA, JH. All authors read and approved the final manuscript.

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