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Enhanced extraction of wild mint (*Mentha longifolia* L.) leaf essential oil by ultrasound pre-treatment prior to hydrodistillation

Areeg S. Shoukair[1]. Morsi M.K.S. [1]. Nashwa F.S. Morsy[1]*. Karima S.M. Hammad[1].

[1]*Food Science Department, Faculty of Agriculture, Cairo University, Egypt*

Abstract

Ultrasound pre-treatment (U) followed by conventional hydrodistillation (HD) was used to extract wild mint leaf essential oil in a short time with high quality compared with the HD. The effects of ultrasound power, ultrasound time, extraction time and essential oil yield were studied. The volatile oil constituents of the essential oil samples were analyzed by GC-MS. Extraction of essential oil with U+HD decreased total extraction time (< 1 h vs. 4 h in HD) with the same essential oil yield (5.4%). Scanning electron microscopy of mint leaves indicated the efficiency of U+HD in the eruption and destruction of oil glands. The oxygenated monoterpenes/monoterpene hydrocarbons (O/H) ratio, as a quality index for the distinct aroma of wild mint essential oil, and sensory analysis indicated the superior quality of the samples obtained by U at 60 W power for 10 min followed by HD for 33 min. These results suggest that ultrasound assisted extraction is an appropriate pretreatment technique prior to HD for the extraction of high-quality wild mint essential oil in minutes. The effect of direct incorporation of the investigated essential oil into olive oil on its sensory attributes and oxidative stability was studied.

Keywords: Wild mint ; hydrodistillation ; ultrasound pretreatment ; essential oil ; short extraction time.

1. Introduction

Wild mint (*Mentha longifolia* L., horsemint, a member of the Lamiaceae family), is a medicinal and aromatic herb that is used in many countries as food flavoring [1,2]. The Egyptians are using the wild mint leaf in pharmaceutical industries [3]. There are several chemotypes of *M. longifolia* essential oil. They are classified according to their main components [4]. Characteristic natural flavor of an essential oil depends mainly on its components and their concentrations [5]. Pulegone, an oxygenated monoterpene, was the major compound of the fresh leaves of *M. longifolia* essential oil [6].

There are many techniques for the extraction of essential oils that influence the quality and chemical composition of EO, such as HD, solvent extraction, ultrasound-assisted extraction (U) and supercritical extraction using CO_2 [7]. Application of U as a new technique has attracted lots of attention due to the short time required and the high extraction efficiency [8]. Shortening extraction time can control the drawbacks of HD [9].

Conventional extraction methods are less efficient and consume more energy compared with the U method, for low molecular weight compounds from plant sources [10]. The ultrasound waves enhance the breaking of plant cells and the release of their contents into the extraction medium [11]. Ultrasound power and time are the main factors that affect the efficiency of the ultrasound process [12].

Virgin olive oil is highly consumed throughout the world. It has potential protective effects against several pathologies [13]. Flavoring olive oil with essential oils improved its sensory attributes and consumer acceptance [14]. In terms of innovation flavored olive oils have just entered the market [15]. The innovative consumption patterns of the past few decades have shown that consumers are more concerned with their health and are looking for new sensory experiences.

To our knowledge, there are no available data on the utilization of ultrasound assisted extraction of wild mint essential oil as a pretreatment prior to hydrodistillation. Therefore, the present study

*Corresponding author e-mail: nanafsm@agr.cu.edu.eg

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provides novelty by using ultrasound as a pretreatment to extract wild mint essential oil. The influence of ultrasound assisted extraction pretreatment parameters on the oil yield, the quality index for the unique distinct aroma of wild mint volatile oil, and sensory analysis were studied. In addition, the sensory attributes and oxidative stability of wild mint-flavored olive oil were evaluated.

2. Materials and methods

Materials

The dried leaves of wild mint (*M. longifolia* L.) were purchased from Harraz Company for Medicinal Plants, Cairo, Egypt. Extra-virgin olive oil (one kilogram) was obtained from Agricultural Research Center, Giza, Egypt. Butylated hydroxytoluene (BHT), and n-alkanes (C8–C20) were obtained from Sigma-Aldrich, USA.

Essential oil isolation

a. Hydrodistllation (HD)

Dried leaves of wild mint (50 g) were ground into fine powder and sieved. Hydrodistillation was carried out for 4 h using a Clevenger–type apparatus according to European pharmacopoeia [16], until no more essential oil was obtained. The essential oils were dried with anhydrous Na_2SO_4 and kept in amber vials at 4 °C prior to analysis and use.

b. Ultrasound assisted extraction (U) followed by HD

Fifty grams of the dried leaves powder were immersed in 600 mL distilled water. The mixture was sonicated using Fisher Sonic Dismemberator (Model 300, 50 Hz, USA) as reported by Morsy [17]. The U process was carried out at 20% and 30% of the maximal output power (60 W and 90 W, respectively) at room temperature. This extraction process was performed at 60 W for 10 min (technique A), 20 min (technique B) and 30 min (technique C). Meanwhile, the same process was conducted at 90 W for 10 min (technique D), 20 min (technique E) and 30 min (technique F). After each U treatment, the resulting mixture + 950 mL of distilled water were subjected to HD as reported previously. Each extraction process was carried out three times and yield was recorded as mean ± SD.

Identification of volatile compounds by Gas Chromatography-Mass Spectrometry (GC/MS)

Chemical composition of wild mint oil samples was analyzed by a TRACE Ultra GC–MS as described by Morsy and Hammad [18]. (THERMO Scientific Corp., USA) equipped with a TR-5MS capillary column (0.25 mm × 30 m, 0.25 μm). Thermo mass detector (ISQ Single Quadrupole Mass Spectrometer) was used. The temperatures of injection port and detector were set at 240 °C. One μL of the diluted sample (1:10 hexane, *v/v*) was injected into the GC with a split ratio of 1:10. Helium flow rate was 1.3 mL/min. The temperature was first held at 60 °C for 1 min, then increased to 240 °C by 3 °C/min and held for 1 min. The analysis was performed using electron ionization at 70 eV, and a spectral range of *m/z* 40-450. Identification was carried out by Kovats indices using n-alkanes (C_8–C_{20}), mass spectra of authentic standards, Wiley spectral library collection and NIST library. Retention indices of mint oil constituents were compared with those reported by Adams [19].

Sensory evaluation of wild mint essential oil

The aroma profile of essential oil samples was evaluated with quantitative descriptive analyses. Samples were placed in dark glass bottles in randomly coded samples. Ten selected panelists were asked to score the intensity of five descriptive adjectives: freshness, minty cool-minty, herbal minty, floral, camphoraceous and burnt note. Sensory tests were performed in triplicate at room temperature. Intensity was evaluated on a scale of 0–10 (0 = not perceptible, and 10 = high intensity). The results of the sensory profile analysis were averaged for each parameter and then plotted in a radar graph.

Fourier-transform infrared (FTIR) of wild mint essential oil

FTIR spectrometer (Thermo Scientifc Nicolet 380 FTIR) was used to analyze the functional groups of the essential oil sample obtained by the new technique (U pretreatment followed by HD) with the highest quality and that produced with the conventional technique (HD). Samples were mixed with KBr and pressed into pellets using a hydraulic press. The FTIR spectra were recorded in the wavenumber range 4000-500 cm^{-1}. For each spectrum, 16 scans were accumulated (resolution 4 cm^{-1}).

Analysis of extra virgin olive oil

The acidity (oleic acid %), peroxide value (meq O_2/kg), conjugated diene (K_{232}) and conjugated triene (K_{268}) were determined in olive oil according to European Union Commission Regulations EEC/2568/91 [20].

Oxidative stability of olive oil

The oxidative stability was measured using Rancimat 679 apparatus (Metrohm, Herisau, Switzerland). To 5 g of olive oil heated at 110 ± 1.6°C air was incorporated at a rate of 20 L/h. The resulting volatile oxidation products were collected in water. The conductivity of water was continuously measured. The time (h) required to reach the conductivity inflection (induction period) was recorded [21]. BHT was used at 200 ppm for comparison. Relative stability was calculated as the ratio of induction period of olive oil in presence of the tested essential oil to induction period of olive oil without any additive.

Preparation of flavored olive oil

Flavored olive oil was prepared by direct addition (200 and 400 ppm) of the selected essential oil (with the highest quality index) to olive oil. Flavored olive oil samples were placed in closed amber glass bottles, stored at 5°C. Flavoring was carried out in triplicate for each concentration. Olive oil sample without essential oil was used as a control.

Sensory evaluation of flavored olive oil

Sensory evaluation of flavored olive oil samples was carried out according to Moldão-Martins et al. [22], and Asensio et al. [14] by ten panelists, who were olive oils consumers, and aged between 30 and 45. The intensity of odor, taste (fruity, bitterness and pungency) and acceptability was evaluated on a 9-point scale with 1 (dislike extremely) to 9 (like extremely).

Scanning electron microscope

The solid residue obtained after the extraction techniques was observed under an electronic scanning microscope (SEM) (model JSM 5200LV; JEOL Ltd, Tokyo, Japan). The samples were coated by a sputtering process using Jeol JFC-1200 auto fine sputter coater, before they were placed for microscopy and was examined at 25 kV (x500-x750 magnification) [23].

Statistical analysis

Data of essential oils recovery were expressed as mean ± standard deviation. One-way ANOVA was used to calculate significant difference and Duncan's test was used to compare the significance of differences at $p < 0.05$.

3. Results and Discussion

Effect of the ultrasound time, power, and total extraction time on essential oil yield

Ultrasound technique as a pretreatment prior to hydrodistillation method (U+HD) accelerated extraction without significant difference in the yield of essential oil (5.4±0.02%, *v/w*), regardless ultrasonic power (60 and 90W) used (Table 1). Jaimand and Rezaee [24] found that essential oil content of wild mint leaves at flowering stage was 3.5%. The extraction yield of tarragon (*Artemisia dracunculus* L.) leaf essential oil obtained by ultrasound power at 250, 350 and 500 W for 20, 30 and 40 min as a pretreatment was significantly similar [9]. Pretreatment with ultrasound at 160 W for 15 min prior to HD of *Prangos ferulacea* Lindl. and *Satureja macrosiphonia* Bornm leaves did not cause significant difference in the essential oil yield [25].

Extraction efficiency is dependent on sonication time [26]. The extraction time of HD of wild mint leaf powder without ultrasonic pretreatment was carried out at 240 min (four hours) according to the European Pharmacopoeia [16].

Total extraction time ranged from 43 to 56 min when ultrasonic was set at 60 W during ultrasonic pretreatment. Meanwhile, time ranged from 35 to 56 min when ultrasonic pretreatment was performed at 90 W. Ultrasound is suitable for the extraction of thermally labile components [27]. Incorporating ultrasound with HD reduces energy demand and improves extraction rate [28] and [10].

Ultrasonic pretreatment causes the glandular walls to rupture more rapidly than in hydrodistillation [29]. Ultrasound pretreatment accelerates the mass transfer of the compounds from the cells [30]. This effect reduces the extraction time [23]. Increasing the duration of ultrasonic pretreatment from 10 to 20 min decreased the HD time required to reach the maximum yield of essential oil (5.4%) regardless of the ultrasonic power used. Extending ultrasonic time from 20 to 30 min did not reduce HD time. The ideal sonication time to attain the highest yield was up to 25 min [31].

Chemical profile of wild mint essential oil

Thirty-six aromatic constituents were identified by GC-MS in the investigated samples of wild mint essential oil representing > 96% as shown in Table 1 and Figure 1 and Figure 2.

The volatile constituents of the wild mint essential oil extracted by different techniques (HD and U+HD) are almost similar with some differences in their percentage as reported by Pingret et al. [32].

Oxygenated monoterpenes (88.73-94.13%) were predominant in M. longifolia essential oil, followed by monoterpene hydrocarbons (4.29-6.08%), oxygenated sesquiterpenes (0.23-0.57%) and sesquiterpene hydrocarbons (0.84-1.72%).

Monoterpene hydrocarbons comprised β-pinene, α-pinene and sabinene as the major compounds in this group. On the other hand, pulegone, 1,8-cineole, L-menthone, isomenthone, trans-isopulegone and α-terpineol were the main constituents that comprised the oxygenated monoterpenes, which distinguish the aroma of wild mint oil. This result is in agreement with Soilhi et al. [33].

Table 1: Chemical composition of volatile components relative percentage (%)[1] of *Mentha Longifolia* oil obtained by different techniques

Compounds	RI[2]	RI[3] LIT	HD[4]	A[5]	B[5]	C[5]	D[5]	E[5]	F[5]
Monoterpene Hydrocarbons									
α-Pinene	920.32	932	1.02	0.90	0.95	0.97	1.05	1.21	0.94
Camphene	935.86	946	0.03	0.03	0.03	0.03	0.03	0.04	0.03
Sabinene	956.57	969	1.19	1.01	1.03	1.10	1.18	1.58	1.09
β-Pinene	961.75	974	2.46	2.19	2.31	2.34	2.45	2.71	2.35
β-Myrcene	976.10	988	0.20	0.16	0.18	0.17	0.15	0.54	0.20
γ-Terpinene	1045.92	1059	0.02	-	0.01	-	0.01	-	0.03
Oxygenated Monoterpenes									
α-Thujene	914.34	920	-	0.01	0.01	0.01	0.01	-	0.01
1,8-Cineole	1017.82	1026	18.09	19.57	19.65	19.34	20.72	17.85	18.68
trans Sabinene hydrate	1058.61	1065	0.06	0.04	0.05	0.05	0.05	0.06	0.02
cis-Verbenol	1075.23	1080	0.02	-	-	0.02	0.02	0.04	0.02
cis-Sabinol	1129.80	1142	0.34	0.23	0.23	0.26	0.25	0.39	0.34
trans-Verbenol	1135.61	1144	0.23	0.17	0.17	0.19	0.18	0.26	0.07
ρ-Menth-2-en-1-ol	1140.40	1145	0.01	-	-	0.01	-	0.06	0.05
L-Menthone	1146.97	1148	14.39	13.00	13.06	14.01	12.99	14.19	14.30
Isomenthone	1155.30	1167	8.99	7.97	7.99	8.52	7.91	8.79	8.85
ρ-Menthan-3-one	1159.85	1169	0.03	0.01	0.01	0.02	0.01	0.04	0.03
α-Terpinyl acetate	1162.12	1170	0.80	0.62	0.62	0.69	0.65	0.85	0.85
cis-Isopulegone	1166.92	1175	0.39	-	-	-	-	0.56	0.43
trans-Isopulegone	1168.94	1177	1.46	1.31	1.30	1.54	1.41	1.79	1.59
α-Terpineol	1188.13	1197	1.60	1.04	1.06	1.22	1.18	1.72	1.57

Compounds	**RI[2]**	**RI[3] LIT**	**HD[4]**	**A[5]**	**B[5]**	**C[5]**	**D[5]**	**E[5]**	**F[5]**
Ascaridole epoxide	1210.21	1215	0.07	0.03	0.05	0.06	0.04	0.05	0.05
5-Caranol	1223.99	1230	0.01	-	-	-	-	-	0.01
Pulegone	1233.02	1233	45.90	50.13	49.59	47.84	48.42	42.08	47.12
cis-Piperitone oxide	1253.92	1250	0.03	-	0.02	0.02	0.01	-	-
D-Verbenone	1357.86	1360	0.04	-	-	-	-	-	-
Chrysanthenone	1359.29	1364	0.02	-	-	-	-	-	-
Sesquiterpene Hydrocarbons									
trans-β-Caryophyllene	1396.90	1417	1.25	0.92	0.90	0.82	0.64	0.73	0.69
α-Humulene	1434.31	1440	0.08	0.06	0.06	0.05	0.04	0.05	0.04
Germacrene D	1460.78	1484	0.27	0.17	0.18	0.16	0.10	0.16	0.13
ç-Muurolene	1494.61	1499	0.12	0.09	0.08	0.07	0.06	0.07	0.05
Oxygenated Sesquiterpenes									
Caryophyllene oxide	1563.59	1582	0.11	0.07	0.07	0.07	0.08	0.09	0.07
Viridiflorol	1576.92	1585	0.09	-	-	-	-	-	-
Alloaromadendrene oxide	1584.62	1591	0.02	0.02	0.02	0.02	0.01	-	0.01
α-Humulene epoxide	1592.82	1608	0.01	-	-	-	-	-	-
Cubenol	1598.46	1612	0.04	0.02	0.02	0.02	0.02	0.03	0.02
τ-Cadinol	1628.42	1644	0.30	0.19	0.21	0.20	0.16	0.19	0.13
Monoterpene Hydrocarbons			4.92	4.29	4.51	4.61	4.87	6.08	4.64
Oxygenated Monoterpenes			92.48	94.13	93.81	93.80	93.85	88.73	93.99
Sesquiterpene Hydrocarbons			1.72	1.24	1.22	1.10	0.84	1.01	0.91
Oxygenated Sesquiterpenes			0.57	0.30	0.32	0.31	0.27	0.31	0.23
O/H*			18.80	21.94	20.80	20.35	19.27	14.59	20.26
Total oxygenated compounds (%)			93.05	94.43	94.13	94.11	94.12	89.04	94.22
Total non-oxygenated compounds (%)			6.64	5.53	5.73	5.71	5.71	7.09	5.55
Total identified components			99.69	99.96	99.86	99.82	99.83	96.13	99.77
Total unidentified components			0.31	0.04	0.14	0.18	0.17	3.87	0.23
% Yield (v/w)			5.4	5.4	5.4	5.4	5.4	5.4	5.4
Ultrasonic Extraction time (min)			-	10	20	30	10	20	30
HD Extraction time (min)			240	33	35	26	25	24	26
Total Extraction time (min)			240	43	55	56	35	44	56

1 Relative area percent (peak area relative to total peak area)
2 Retention indices using a RTX–5 column
3 RILIT: alphabetical listing of compounds with their retention time and arithmetic retention index on DB-5 [19].
4 Hydrodistillation (HD) for 240 min;

5 Ultrasonic assisted extraction (U) at 60 W for 10 min + HD for 33 min (Technique A); U at 60 W for 20 min + HD for 35 min (Technique B); U at 60 W for 30 min + HD for 26 min (Technique C); U at 90 W for 10 min + HD for 25 min (Technique D); U at 90 W for 20 min + HD for 24 min (Technique E) and U at 90 W for 30 min + HD for 26 min (Technique F)
*O/H oxygenated monoterpene/monoterpene hydrocarbon ratio

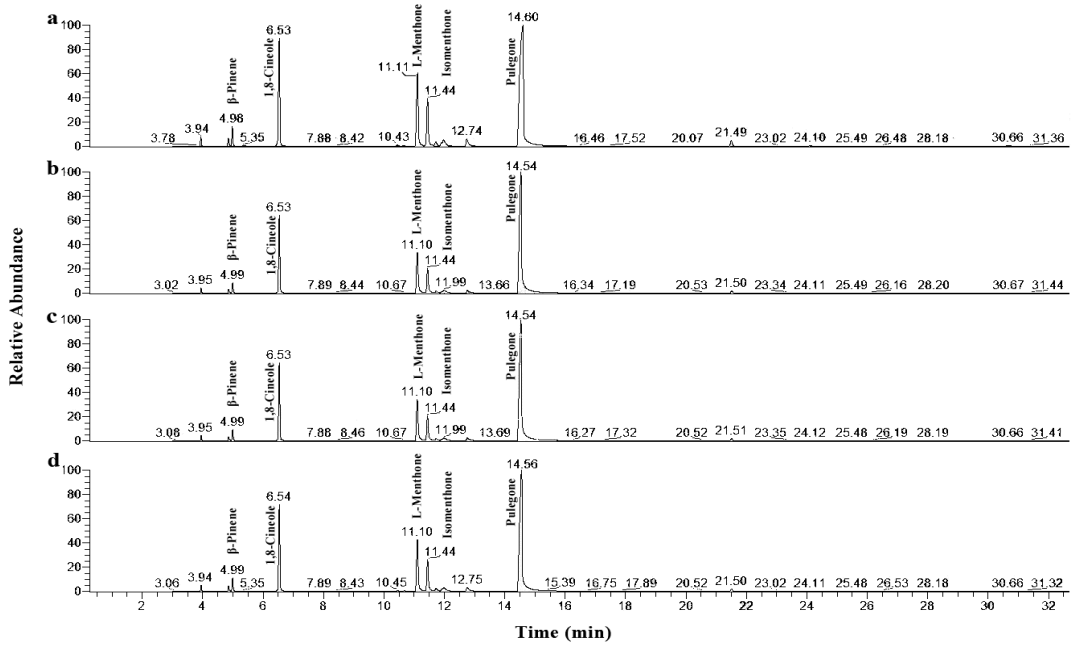

Fig. 1. GC-MS chromatograms of wild mint (*Mentha longifolia* L.) leaves volatile compounds obtained by a: Hydrodistillation (HD) for 240 min; b: U (60 W) for 10 min + Hydrodistillation (HD) for 33 min (Technique A); b: U (60 W) for 20 min + HD for 35 min (Technique B); c: U (60 W) for 30 min + HD for 26 min (Technique C)

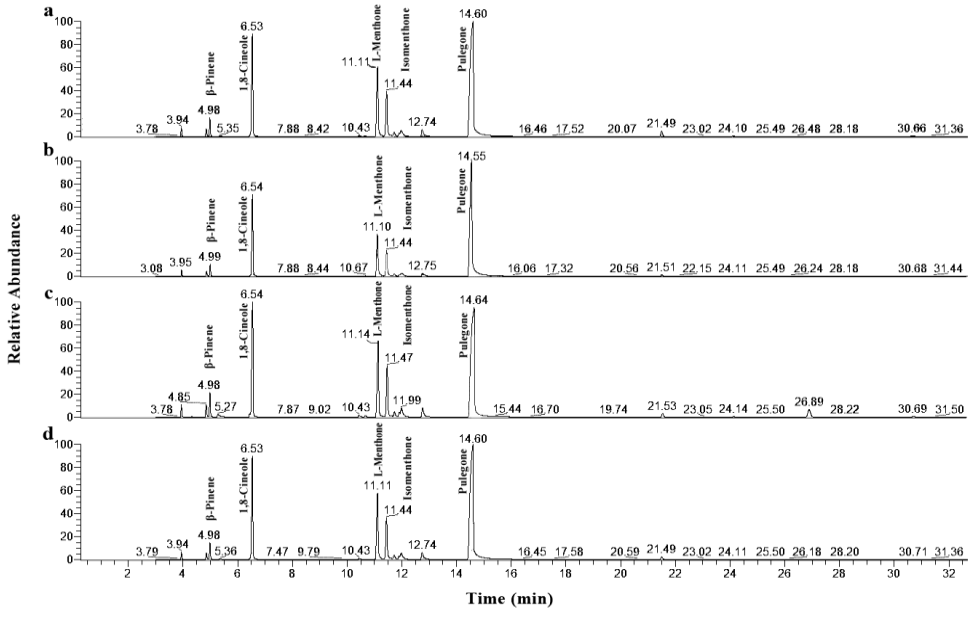

Fig. 2. GC-MS chromatograms of wild mint (*Mentha longifolia* L.) leaves volatile compounds obtained by a: Hydrodistillation (HD) for 240 min; b: U (90 W) for 10 min + HD for 25 min (Technique D); c: U (90 W) for 20 min + HD for 24 min (Technique E) and d: U (90 W) for 30 min + HD for 26 min (Technique F)

Sesquiterpene hydrocarbons contained trans-β-caryophyllene, Germacrene D, α-humulene and ç-muurolene. Oxygenated sesquiterpenes comprised caryophyllene oxide, viridiflorol, alloaromadendrene oxide, α-humulene epoxide, cubenol and τ-cadinol. D-Verbenone, Chrysanthenone, Viridiflorol and α-humulene epoxide were identified in the hydrodistilled M. longifolia essential oil but were not detected in all the ultrasonic hydrodistilled essential oil samples. These compounds resulted from thermal degradation during the long extraction time at 100 °C as reported by Damyeh et al. [25]. Ultrasound did not affect the composition of oil due to short extraction time [34].

The total oxygenated compounds (%) comprised >89.00% while the total non-oxygenated compounds (%) represented <7.1%. The aromatic attributes of wild mint essential oils are related to the oxygenated compounds and mainly to the oxygenated monoterpene odor [35].

Oxygenated monoterpene / monoterpene hydrocarbons (O/H) ratio can be used as an important characteristic for the distinct aroma of wild mint essential oil. Results in Table 1 indicated that O/H ratio in the essential oil samples obtained by technique A (U pretreatment at 60 W for 10 min followed by HD for 33 min) was ~ 22 instead of ~ 19 in oil samples that obtained by the conventional technique (HD) only.

Sensory evaluation of the wild mint essential oils

The sensory evaluation of the wild mint oils extracted by HD or U+HD is illustrated in the following graph (Figure 3).

A burnt note was observed only in the HD sample. This could be attributed to the long heating time (240 min) during extraction, as reported by Binello et al. [36]. Technique A was the most appreciated in terms of freshness, minty, herbal minty, and camphoraceous odor. Schmidt et al. [37] reported that mint oil rich in pulegone, 1,8-cineole, L-menthone, isomenthone, trans-isopulegone and α-terpineol possessed herbal minty, camphoraceous, eucalyptus-like, fresh, minty and floral notes. The same oil sample obtained by technique A (U (60 W) for 10 min + HD for 33 min) was characterized with the lowest score of burnt note followed by floral then cool minty odor. Meanwhile, the wild mint oil samples extracted by techniques A, B, C and D were characterized by their high scores of camphoraceous odor. The camphor odor of this oil is related to the presence of a very large amount of 1.8-cineole and pulegone [37].

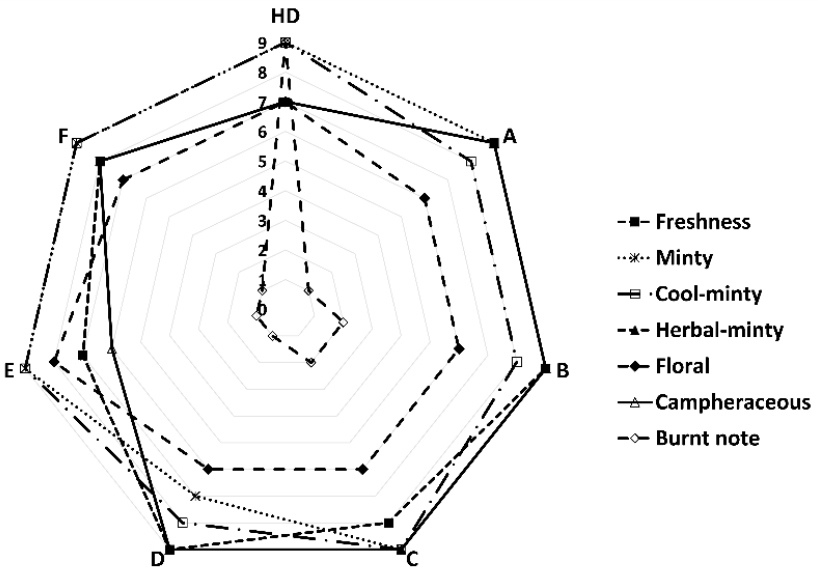

Fig. 3. Aromatic profiles of the essential oils of wild mint obtained by a: U (60 W) for 10 min + HD for 34 min (Technique A); b: U (60 W) for 20 min + HD for 25 min (Technique B); c: U (60 W) for 30 min + HD for 25 min (Technique C); d: U (90 W) for 10 min + HD for 25 min (Technique D); e: U (90 W) for 20 min + HD for 14 min (Technique E) and f: U (90 W) for 30 min + HD for 15 min (Technique F)

On the other hand, the lowest camphoraceous and freshness odor scores were recorded for the oil sample obtained by the E technique. According to results, the highest quality oil was recorded for that obtained by technique A.

FTIR spectrum of the essential oil

The FTIR analysis spectra for the essential oil samples either extracted directly through hydro-distillation (Figure 4a) or the highest quality ultrasound pretreated sample (Figure 4b) showed similar results in terms of the different functional groups present in the samples. These results indicated that the ultrasound pre-treatment did not affect the composition of the essential oil obtained from the dried leaves of wild mint [38]. The FTIR spectra of the investigated essential oil samples show broad O-H stretch (~3400 cm^{-1}), methyl C-H stretch (~2954 cm^{-1}), C−H asymmetric stretch (~ 2924 cm^{-1}), −CH2− sym stretching (~2872 cm^{-1}), C=O stretch (~1680 cm^{-1}), and C-O stretch (~1100 cm^{-1}) of terpenoid components (Figure 4a,b) [39]. The vibrational modes of monoterpenes are noticed at 877, 1451, and 1614 cm^{-1} as reported by Agatonovic-Kustrin et al. [40].

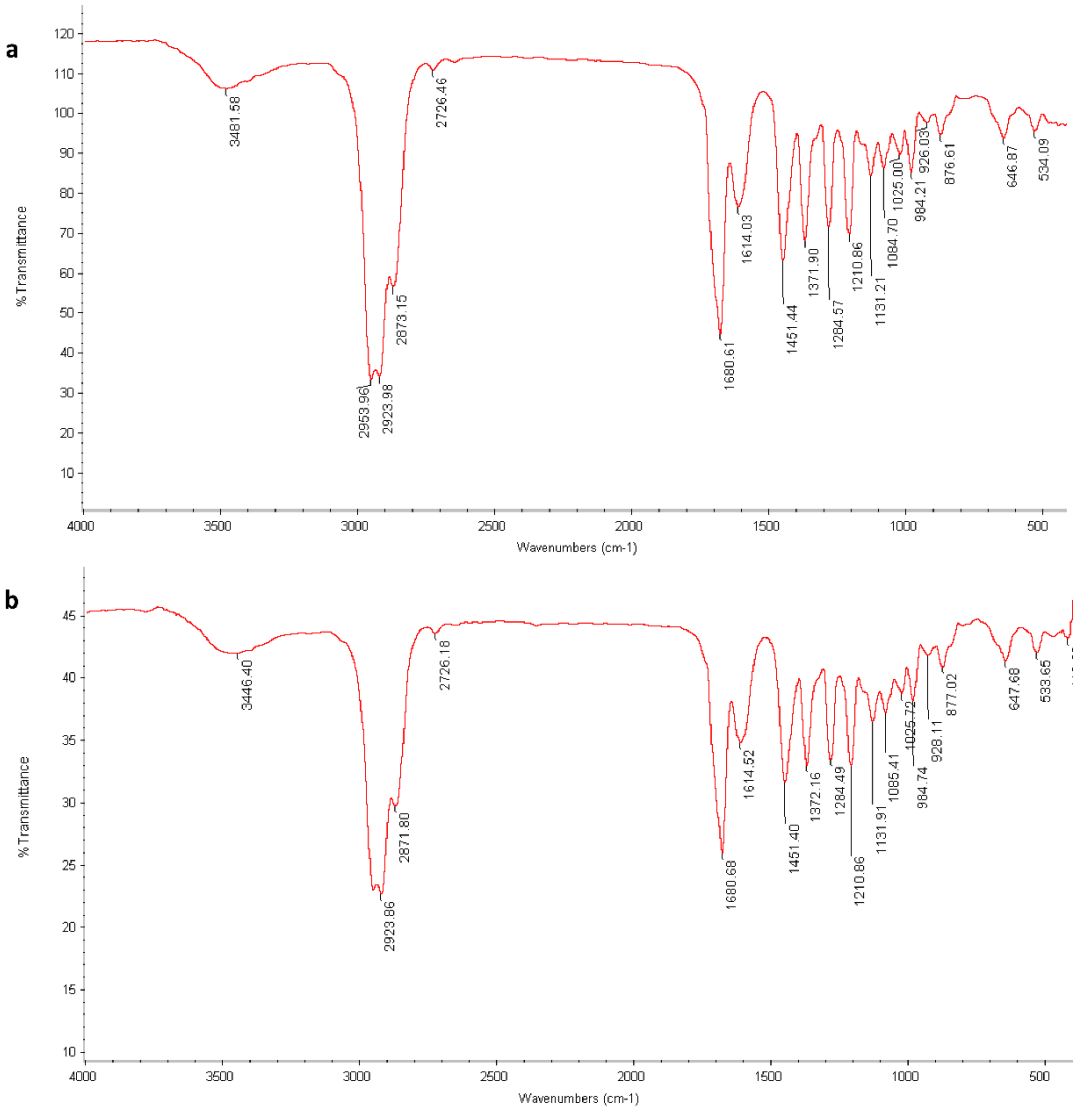

Fig. 4. FTIR spectra of wild mint essential oil a: hydrodistilled; b: U pretreated + HD (Technique A)

SEM analysis

Changes in the oil glands of dried wild mint leaves, before and after the selected extraction technique (Technique A), are illustrated in scanning electron microscopy (SEM) images (Figure 5).

The microscopic imaging of dried mint leaves before extraction shows that there was no rupture or destruction in the oil glands of the control sample (Figure 5a). Hydrodistillation of wild mint leaf powder left oil glands intact (Figure 5b). On the other hand, sonication of wild mint leaf powder resulted in widespread opening and an explosion effect of oil glands during extraction (Figure 5c).

This action facilitated the secretion of volatile oil from oil glands as reported by Damyeh et al. [25].

This finding confirms the strong mechanical effect of ultrasound as reported by Veillet et al. [41] and Chemat and Esveld [42]. Severe eruption and rupture of the oil glands were observed when ultrasound followed by HD (U+HD) (Figure 5d). This micrograph may explain why ultrasound assisted extraction is faster than conventional extraction [43].

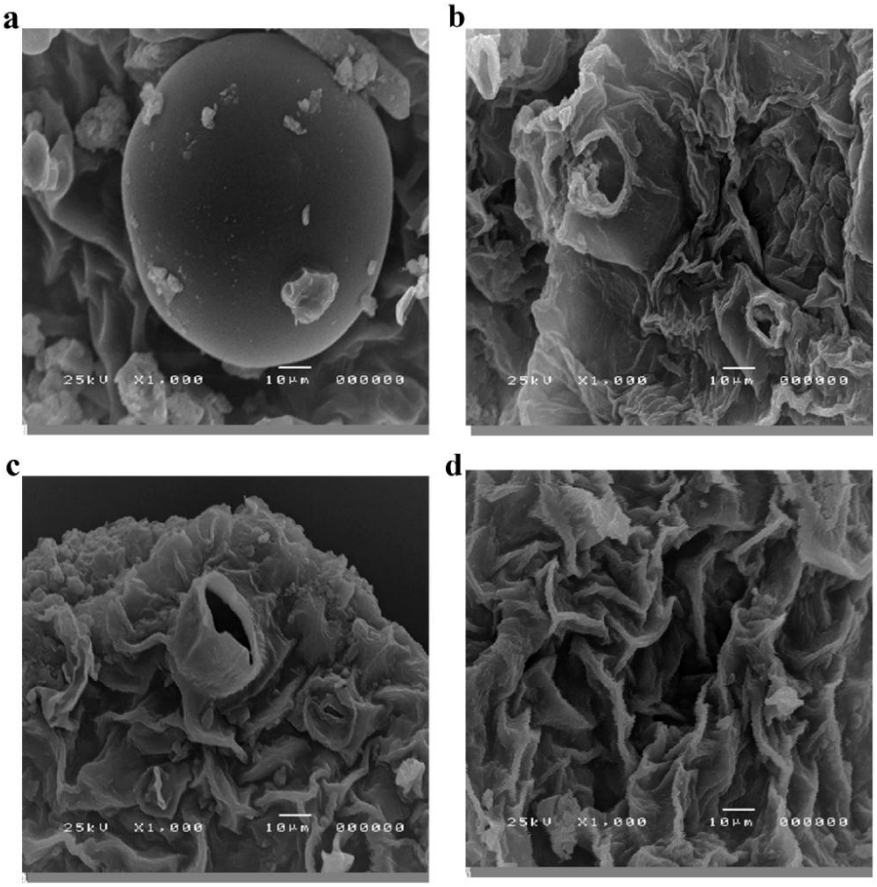

Fig. 5. SEM image of the wild mint leaves: a: dried leaves (control sample), b: after extraction with HD, c: after extraction with U (60 W) for 10 min and d: after extraction with U (60 W) for 10 min + HD for 33 min (Technique A) (x500 – x750 magnification, 25 kV)

Quality indices of olive oil

The investigated olive oil showed the following chemical characteristics: acidity 0.534 ± 0.04%; PV 5.563 ± 0.41; K_{232} 0.147 ± 0.01; and K_{270} 0.006 ± 0.00. These values are within the limits for extra virgin olive oil according to European Union Commission Regulations EEC/2568/91 [20].

Oxidative stability

The oxidative stability of vegetable oils verifies their resistance to oxidation [15]. The induction period of the olive oil (control) measured by the Rancimat apparatus at 110°C was 16.2 ± 0.1 h (Table 2). This indicates the high quality and oxidative status of the oil [44]. The induction periods of olive oil enriched with wild mint essential oil (Technique A) at 200 and 400 ppm were 15.17 ± 0.21 and 14.17 ± 0.21 h, respectively. Oxidative stability of olive oil decreased significantly with the increase of the incorporation level of wild mint essential oil. This result is consistent with Mulagić et al. [45]. According to Cherif et al. [46] some flavoring agents support the pro-oxidant effect, due to their specific composition (e.g., presence of reducing compounds). The induction period of olive oil treated with BHT at 200 ppm was significantly (P<0.05) higher than the control and all samples of flavored olive oil.

Table 2: Oxidative stability of olive oil enriched with different levels of wild mint essential oil using the Rancimat method

Olive oil samples	Induction period (h)	Relative stability*

Control (unflavored olive oil)	16.2 [b] ± 0.10	1.00
Olive oil flavored with wild mint EO at 200 mg/Kg	15.17 [c] ± 0.21	0.94
Olive oil flavored with wild mint EO at 400 mg/Kg	14.17 [d] ± 0.21	0.87
Olive oil enriched with BHT at 200 mg/Kg	19.6 [a] ± 0.20	1.21

Data are expressed as mean ± SD (n=3). Means followed by different letters are significantly different ($P < 0.05$).
*Relative stability is the ratio of induction period (h) of the olive oil containing the investigated material and the induction period (h) of the fresh olive oil without any additive.

Sensory evaluation of flavored olive oil

Enriched olive oil samples with wild mint essential oil at 200 ppm showed significantly (P<0.05) the same intensity of odor, taste (fruity, bitterness and pungency) and overall acceptability as control (Table 3). However, this flavored oil showed marginally higher scores than control for fruity taste and pungency while it showed slightly lower scores than control for bitterness. Increasing the flavoring level of mint oil to 400 ppm showed significantly (P<0.05) lower scores in odor and overall acceptability than the control sample. It could be concluded that enrichment with wild mint essential oil at 200 ppm preserved the sensory attributes of olive oil. However, enrichment with a higher level of wild mint essential oil did not preserve these attributes but imparted undesirable sensory characteristics (pungency and bitterness). Moldão-Martins et al. [22] reported that the *Mentha piperita* essential oil affects significantly the pungency, aromatic plant aroma and cooling of the flavored olive oil. The consumer panel didn't prefer the incorporation of *Mentha piperita* essential oil at a high level. Gaeini et al. [47] reported that the pulegone is the original compound of this oil that has a specific mint aroma ranging from an intense to balsamic and pungent aroma. The addition of aromatizers to olive oil influences several characteristics and properties. Their inclusion improves sensorial characteristics of olive oils, but the concentration must be kept at low level in terms of sensorial acceptability by consumers to avoid over-aromatization [48].

Table 3: Sensory evaluation of olive oil enriched with wild mint EO

Olive oil samples	Odor	Taste			Overall acceptability
		Fruity	Bitterness	Pungency	
Unflavored Oil	8.60 [a] ± 0.55	7.80 [a] ± 0.45	3.18 [ab] ± 0.46	3.00 [b] ± 0.35	8.20 [a] ± 0.84
Flavored oil with wild mint essential oil at					
200 mg/Kg	7.80 [ab] ± 0.84	8.80 [a] ± 0.84	3.10 [b] ± 0.81	3.40 [ab] ± 0.54	8.60 [a] ± 0.55
400 mg/Kg	6.60 [b] ± 0.55	8.20 [a] ± 0.84	4.20 [a] ± 0.95	4.00 [a] ± 0.70	7.20 [b] ± 0.84

Means within columns followed by different letters are significantly different ($P < 0.05$)

4. Conclusion

The use of ultrasound prior to HD accelerated the extraction process of wild mint essential oil without

significant effect on the yield. It decreased the total extraction time to less than 1 h instead of 4 h using conventional extraction. In this work, the essential oil obtained with U+HD technique was of high quality and almost free of burnt note. The FTIR analysis indicated that the chemical composition of both products was similar. Flavoring olive oil with wild mint essential oil at 200 ppm did not affect its quality.

5. Conflict of interest

The authors declare that there is no conflict of interest.

6. Formatting of funding sources

No competing financial interests exist.

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