Essential Oil Composition and Menthol Production of Mint Types Grown in Different Regions in Turkey

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Summary Menthol is one of the key components of mint oil and it is a chemical component that is widely used in many sectors such as cosmetics, medicine, and food. Menthol can be made by synthesis or by various extraction and distillation methods. In this study, first, mint oil was obtained by microwave extraction and hydrodistillation from the mint that is an agricultural and industrial product cultivated in a wide geography in Turkey. Later, qualitative and quantitative analysis of this oil was executed and the differences in the chemical contents of the oils manufactured by different methods and the amount of menthol contained were compared. Lastly purification of the menthol from the oils was done. The dissimilarities in the chemical contents of the oils were compared with GC-MS analysis. The amount of menthol % obtained as a result of the studies was found as 31.65 by titration method and 29.58 by gradual crystallization. The melting point range of menthol separated from mint oil by gradual crystallization was found as 42.4 - 43.1°C as stated in the literature.

Key words: Hydrodistillation, Microwave-assisted extraction, Mint oil, Menthol, GC-MS, TLC.

Introduction

Mints (genus *Mentha*) are from the *Lamiaceae* family [1]. They have 25-30 species and they have significant importance as aromatic and medicinal plants. They are mostly widespread throughout the northern hemisphere with a distribution across Asia, Europe, Australia, Africa and North America as they are fast growing perennial plants and they are able to tolerate a wide range of climatic conditions [2]. In Turkey, there are fifteen *mentha* taxa with six hybrids. The region mentioned in this study has eleven taxa including four hybrids [3]. However, studies are mostly done on the three most significant species of the mint, namely *Mentha piperita, Mentha arvensis, Mentha spicata* [4]. Mint is called "nane (nah`-neh) in Turkish.

Some of the traditional methods to extract mint oil from mint are distillation, pressing and enfleurage. These traditional extraction methods however are reported to have an adverse effect on the final product quality as some of the volatiles are lost or some unsaturated ingredients are degraded due to the heat or the solvents used [5]. Other methods used to extract mint oil from mint are microwave extraction [5], hydro-distillation [3, 6-9], supercritical liquid extraction [8, 10] and headspace / solid-phase micro-extraction (HS/SPME) [9].

Although menthol, which is the main component of the crude essential oil obtained from mint species, usually makes up 30-40% of the oil [11], the rate can reach up to 80-85% depending on the climatic conditions in which the plant is grown and the cultivated plant [1, 12]. Menthol as an ingredient is mainly used in the pharmaceutical industry where it is used in tablets, cough syrups, pain balms, ointments and inhalers. Other than its pharmaceutical use, menthol is very commonly used in industries like foods and beverages including alcoholic beverages, mouthwash, dental preparations, toothpaste and several cosmetics [11].

The fact that the mint oil market is growing recently is mainly due to the escalating demand for the organic and natural personal care products that use mint or menthol as an ingredient. This has increased mainly due to the increasing awareness about the benefits of preferring organic certified products and also the increasing awareness of the possible adverse reactions from the chemical based products; as natural ingredients like mint oil is used in organic certified and

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natural products as a flavoring agent, fragrance or a denaturant [13]. The available supply is not enough to cover the annual demand of 25,000-30,000 metric tons as menthol is widely used in new product launches [14].

Menthol, also known as mint camphor, is a cyclic monoterpene alcohol and it is the main component of the essential oils of *Mentha piperita* L. (peppermint) and *Mentha canadensis* L. (cornmint) [15]. The most important natural isolate of menthol is the L- or (-) menthol, hence this isolate is referred to when talking about menthol commercially. Annually, 30,000-32,000 metric tons of menthol is estimated to be used. It is one of the majorly important flavouring substances, following citrus and vanilla [11].

Menthol is also known as p-menthan-3-ol, 2-isopropyl-5-methylcyclohexanol or 5-methyl-2-(1-methylethyl)-cyclohexanol and its molecular formula is $C_{10}H_{20}O$ with a molecular weight of 156.27. It has three asymmetric carbon atoms, hence, it has four pairs of optical isomers which are (+)- and (-)-neoisomenthol, (+)- and (-)-neomenthol, (+)- and (-)-menthol and (+)- and (-)-isomenthol. Besides the cosmetic and other uses, menthol is also known for its anti plasmid activity that can reach to 100% that indicate that it has an important role in the anti plasmid activity of mint oil (Fig. 1) [11, 16, 17].

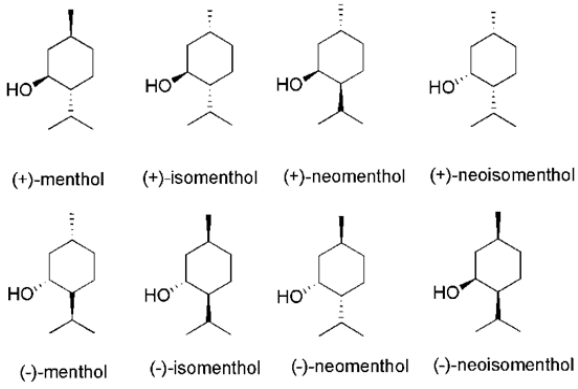

Fig. 1: The various stereoisomers of menthol.

In a local study in Turkey, 90 samples were collected from the 11 mint species from the Marmara region in northwest Turkey. Samples were then hydro-distilled for their essential oils. These mint oils were fed to gas chromatographic (GC) and GCMS analysis. 25% of these study samples contained 3-oxo compounds which are isopulegol, piperitenone, piperitone, menthofuran, pulegone, isomenthone and menthone [3].

In this study, commercially available and widely grown local mint plants from different regions are collected. These mints are then fed to microwave extraction and Clevenger type hydrodistillation to get their essential oils. Yields for both methods are calculated and presented. These essential oils are tested qualitatively and quantitatively. The chemical contents of these essential oils and their menthol contents are compared. Lastly, menthol was derived from these essential oils and its qualitative and quantitative values are presented.

Since there is no similar study conducted in Turkey on this subject, our study will constitute the first step of our efforts to draw attention to the development of local mint crop harvesting, mint oil and menthol production in Turkey. Because our research has shown that menthol is the most abundant chemical in mint oil after *D*-Carvone.

Experimental

Plant material

In the experiments, mint species from the Marmara region in northwest Turkey and from the Gaziantep region in southeast of Turkey were used. Samples were collected during the months of April and July 2018 from these regions. Those collected in the Marmara Region are *Mentha spicata* and those collected from Gaziantep region are *Mentha piperita*. In the experiments, *Mentha spicata* was used in its fresh and dried form and *Mentha piperita* in its dried form only. The fresh plant material was dried to constant weight at room temperature without direct sunlight. The samples were labelled and stored in sealed bags until analyzed.

Chemicals used

To prepare the solutions for the analyses, 8 mΩ deionised water from a Millipore Milli-Q water purification system was used. In Menthol recovery from mint oil used in crystallization analysis; Merck brand Na_2SO_4, Sigma Aldrich brand boric acid, Merck brand NaOH were used. In TLC analysis; Sigma Aldrich brand pure menthol, Merck brand methanol, Merck brand ethyl acetate, Merck brand toluene were used. In HPLC analysis; Sigma Aldrich brand pure menthol, Merck brand methanol, Sigma Aldrich brand di-sodium hydrogen phosphate were used. In titration analysis; Sigma Aldrich brand acetic anhydride, Merck brand sodium acetate anhydrous, Fischer brand ethanol, Merck brand KOH, Merck brand HCI were used. For GC-MS analysis; Merck brand acetone was used.

Isolation of essential oil

Two different methods were used to obtain mint oil from mint. These methods are hydrodistillation with Clevenger-type apparatus and microwave extraction.

Hydrodistillation with Clevenger apparatus

250, 400 or 500 ml of deionized water is added into 21 volumetric flasks. 100, 150, 200 or 250 g mint (fresh or dried) is weighed into the volumetric flask and stirred. The heater is turned on and the cooling water temperature was adjusted to 10 °C in the circulating water bath for rapid cooling of the resulting steam. Distillation is performed for 90 or 120 minutes. The system is wrapped with glass wool and aluminum foil to prevent heat loss. The distilled essential oils were then dried over anhydrous sodium sulphate, filtered and stored in sealed vials at -18 °C.

Microwave-assisted Extraction

Microwave assisted extraction draws attention as a relatively low cost and environmentally friendly method with a short extraction time compared to other methods (eg, HD and supercritical liquid extraction) in essential oil extraction [18-20]

A laboratory microwave-assisted extraction system (NEOS, Milestone, Italy) was used to extract mint oil (Fig. 2). In the experiment, 150 grams of dried mint and 150 ml of distilled water were used. and extraction was performed in 800-1000 Watt and 2-30 minutes.

Fig. 2: NEOS Microwave Extraction System.

Separation of menthol from essential oil

For this purpose, the crystallization method applied in two stages was used [21]. In the first stage, Na_2SO_4 is added to the mint oil sample and it is filtered to remove the water in it. Filtration was done with 0.45 RC filter. The remaining oil amount after filtration is determined by weighing. Later, this sample was then cooled by keeping it at +14 °C, +10 ℃ and -5 ℃, respectively, for 8 hours in each step. Menthol crystals were separated from mint oil. As dementholised mint oil still contained menthol, racemic menthol, isomenthol and menthone; for the complete recovery of menthol crystals, the dementholised mint oil was treated with 8 g boric acid in distillation flask for 3 h to distill off menthone. Distillation residues containing borates of menthol were saponified by steam distillation over 70 g of 15% NaOH solution and thereby separating the menthol crystals from liquid mixture containing racemic and isomenthol. Crystals were dried at 25±1°C and yield was determined.

Qualitative and quantitative analysis of menthol

Qualitative analysis of menthol was performed by thin-layer chromatography (TLC) method and quantitative analysis by titration method and gas chromatography/mass spectrometry (GC-MS) method. The melting points of the obtained menthol crystals were determined and compared with the literature.

Thin Layer Chromatography (TLC)

We have employed thin layer chromatography (TLC) method for preliminary analysis of the essential oil because of its simplicity, speed, reliability and economy [22-23]: The method found for levomenthol and menthol racemic from European pharmacopoeia. As standard, 25 mg of pure menthol was weighed and put in a 5 ml volumetric flask and some methanol was added, mixed and completed to 5 ml with methanol. For the sample, 25 mg of mint oil was weighed and put in a 5 ml volumetric flask and some methanol was added. It was kept in ultrasonic bath for 10 minutes and completed to 5 ml with methanol. The mobile phase is a mixture of 5 ml ethyl acetate and 95 ml toluene. 'TLC Silica Gel Plate' (Merck) was used. Afterwards, the anisaldehyde solution was sprayed and the spots formed in the chromatogram were compared with the layer observed at 254 nm in the UV device [24].

Titration Method

The purpose of this method is to find the total amount of menthol in mint oil. The method consists of two stages. As a first step, 10 g mint oil is weighed into an empty flask. 10 ml of acetic anhydride and 2 g of anhydrous sodium acetate are added. Erlenmeyer flask is boiled with the reflux for 1 hour. After 1 hour, the flask is cooled. After cooling, 30 ml of deionized water is added. Erlenmeyer flask is heated by mixing for 15 minutes in boiled water. The mixture is then taken into the separator funnel. It is kept for at least 1 hour. Phase

formation is observed after holding. The supernatant is oil and this oil is called acetylated oil.

Titration analysis is performed for the acetylated oil obtained from the second stage, the non-acetylated oil obtained from the second stage, the non-acetylated oil sample and the blank sample made without adding any oil. Two Erlenmeyer flasks are taken. The first flask weighs 2 g of acetylated oil, while the other flask weighs 5 g of unacetylated oil. 5 ml of ethanol and 2 drops of phenolphthalein solution are added to them. The flasks are carefully stirred and 25 ml of 0.5 M potassium hydroxide-ethanol solution are added. After stirring the flasks carefully by hand, it is boiled for 1 hour together with reflux. After an hour, 25 ml of distilled water is added to the flasks immediately, mixed and allowed to cool. After cooling, both solutions are titrated with 0.5 M HCI and the amounts spent are noted.

The second step is repeated as blank sample solution without adding any oil and 0.5 M HCI spent for blank sample solution is recorded. After analysis is completed, the calculation is made with Equation 1 and the total amount of menthol in the oil is found.

$$Total\ menthol\ \% = \frac{7.814 \times (c-a) \times \left(1 - \left(\frac{0.021 \times (c-b)}{acetylated\ oil\ (g)}\right)\right)}{non\ acetylated\ oil\ (g) - 0.021 \times (c-a)}$$

a and *b* indicate the amount of 0.5 M HCI solution used to titrate the acetylated and non-acetylated oil samples, respectively. *c* is the 0.5 M HCI used for titration in the blind sample [25].

Gas chromatography/mass spectrometry analysis (GC-MS)

Mint oil samples are diluted with acetone in a ratio of 1/10 (v/v) and injected to GC-MS using a 1.0 µL injector. Capillary column HP-5 MS (30 m×0.25 mm×0.25 µm) were used and injector temperature was set at 25℃. The column temperature was increased to 50 ℃ for the first 3 minutes, then to 150 ℃ in 10 ℃/min. Increments and after holding it for 10 minutes, it was raised to 25 ℃/min. Also injection split ratio was 50/1, carrier gas was helium (1.23 ml/min) [26].

Identification of the components

The identification of components was achieved through retention time and by comparing them with reference spectra in the computer library (Wiley/NIST version 2.0). The retention indices (RI) were calculated by comparation of for all volatile constituents using a n-alkanes homologous serie, Kovats indices and mass spectra with corresponding data in the literature [27].

Melting point determination

To prove the purity of the menthol obtained from mint oil, the melting point for menthol was determined. Büchi brand B-540 Model device was used. A certain amount of menthol obtained at the end of the experiments is put into the capillary tube at a height of 4-6 mm and placed in the device. The first temperature at which the sample begins to melt and the temperature at which it melts completely is recorded through the magnifying window. Menthol exhibits four polymorphs with melting temperatures ranging from 31.5 to 42.5 ºC [28, 29].

Result and Discussion

During the hydrodistillation experiments performed with Clevenger Apparatus, parameters were changed to find the optimum condition. The different parameters examined and the results obtained are given in Table-1 and Fig. 3. As can be seen from Table-1, a total of 49 trials were conducted to examine the effect of different parameters on essential oil yield.

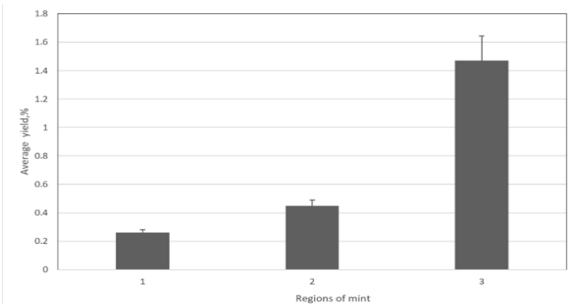

Fig. 3: Average yield values obtained by hydrodistillation for different mint sample. (1. Marmara Region-fresh mint, 2. Marmara Region dried mint, 3. Gaziantep Region dried mint)

The best yield was determined as 1.47% with 250 g dry mint, 400 ml deionised water and 120 mins distillation time. The yield was found to be 0.56% in the microwave extraction performed with the laboratory type microwave Clevenger apparatus (NEOS, Milestone, Italy). GC-MS analysis was performed with the mint oil obtained from the dried mint obtained from Gaziantep Region by the method of distillation method with Clevenger apparatus. While there was 10.22% menthol in the oil content obtained by Clevenger, no menthol was found in the oil obtained by microwave extraction.

Table-1: Distillation efficiency with Clevenger apparatus.

Region	Amount of mint, g	Amount of solvent, ml	Distillation time, min	Experiment Number	Average yield, %
Marmara (*Mentha spicata*)					
Fresh Mint	100	250	120	1-3	0.24±0.010
Fresh Mint	100	400	120	4-6	0.28±0.009
Fresh Mint	100	500	120	7-10	0.26±0.009
Dry Mint	100	250	90	11-13	0.41±0.011
Dry Mint	100	400	120	13-15	0.49±0.009
Dry Mint	200	500	120	16-19	0.45±0.011
Gaziantep (*Mentha piperita*)					
Dry Mint	150	400	120	20-23	1.03±0.053
Dry Mint	200	400	120	24-27	0.95±0.053
Dry Mint	300	400	120	28-31	0.89±0.055
Dry Mint	250	500	120	32-35	1.31±0.028
Dry Mint	250	250	120	36-40	0.89±0.024
Dry Mint	250	400	90	41-44	1.17±0.015
Dry Mint	250	400	120	45-49	1.47±0.171

Table-2: GC-MS analysis result of mint oil (Clevenger apparatus, Gaziantep region, dried mint).

Compound		RI	Area (%)	
			Clevenger	Microwave
1	α- pinene	937	0.15	0.02
2	β-pinene	979	0.21	0.07
3	β-Myrcene	992	0.19	0.06
4	3-Octanol	994	0.71	0.58
5	Limonene	1030	3.15	2.18
6	1,8-Cineole	1033	4.49	4.44
7	Linalool	1098	0.27	0.31
8	cis-β-Terpineol	1140	0.23	0.16
9	L-(-)-Menthol	1171	10.22	-
10	Terpinen-4-ol	1180	-	0.32
11	cis-Dihydrocarvone	1194	2.22	3.26
12	Dihydrocarveol	1195	0.24	0.78
13	D-Verbenone	1206	0.10	0.05
14	iso-Dihydrocarveol	1214	1.09	0.23
15	trans-Carveol	1217	-	0.19
16	Pulegone	1237	0.32	0.25
17	D-Carvone	1245	63.03	77.68
18	cis-Carvone oxide	1263	0.12	0.24
19	trans-Carvone oxide	1276	0.05	0.11
20	Geraniol formate	1298	0.35	0.07
21	Dihydrocarvylacatate	1306	0.19	0.24
22	β-Bourbonene	1387	1.00	0.56
23	β-Cubebene	1387	0.26	0.12
24	β-Elemene	1389	0.62	0.26
25	Cedrene	1399	0.20	0.08
26	isocaryophyllen	1415	5.29	3.46
27	trans-elemene	1436	0.35	0.21
28	(Z)-β-Farnesene	1445	0.44	-
29	α-Caryophyllene	1453	0.24	0.37
30	D-Germacrene	1481	0.67	0.35
31	β-Selinene	1486	0.18	0.19
32	(1*S*)-cis- Calamenene	1529	0.05	0.12
33	Spathulenol	1577	0.24	0.42
34	Caryophyllene oxide	1582	0.50	1.29
35	Cubenol	1645	0.08	0.11
36	α-cadinol	1652	0.05	0.12
37	Corymbolone	1898	0.17	-
		Total	97.67	98.90

The most abundant ingredient in mint oil obtained by both methods was Carvone with 63.03% and 77.68% for Clevenger and microwave extraction, respectively. RT (Retention Time) in GC-MS analysis of the oil obtained with the Clevenger apparatus was similar to RT in the GC-MS analysis of the oil obtained by microwave extraction. The GC-MS analysis results of the oils obtained by Clevenger and microwave-assisted extraction are comparatively given in Table-2 and Fig. 4 and 5. In addition, the amounts and retention times of the most abundant compounds in the oil content are given in Table 6.

Methods such as TLC, GC-MS analysis and titration method were used to detect the presence of menthol in the essential oil obtained. Qualitative analysis of menthol was performed by TLC method and quantitative analysis by titration method and GC-MS analysis. The melting points of the obtained menthol crystals were determined and compared with the literature [29].

TLC method, which is found in European Pharmacopoeia, was used [24].

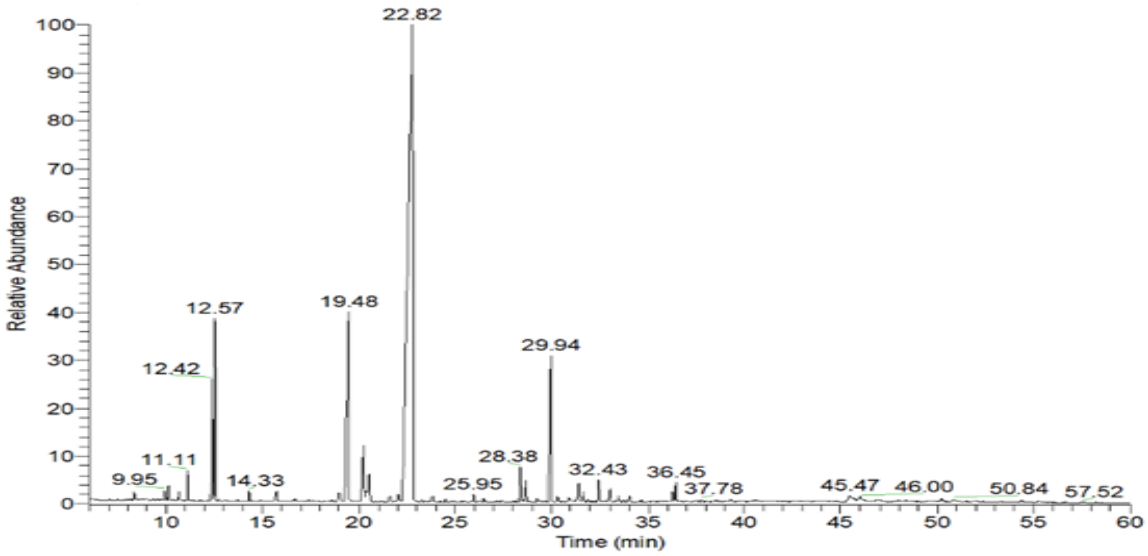

Fig. 4: GC-MS chromatogram of mint essential oil (obtained by Clevenger)

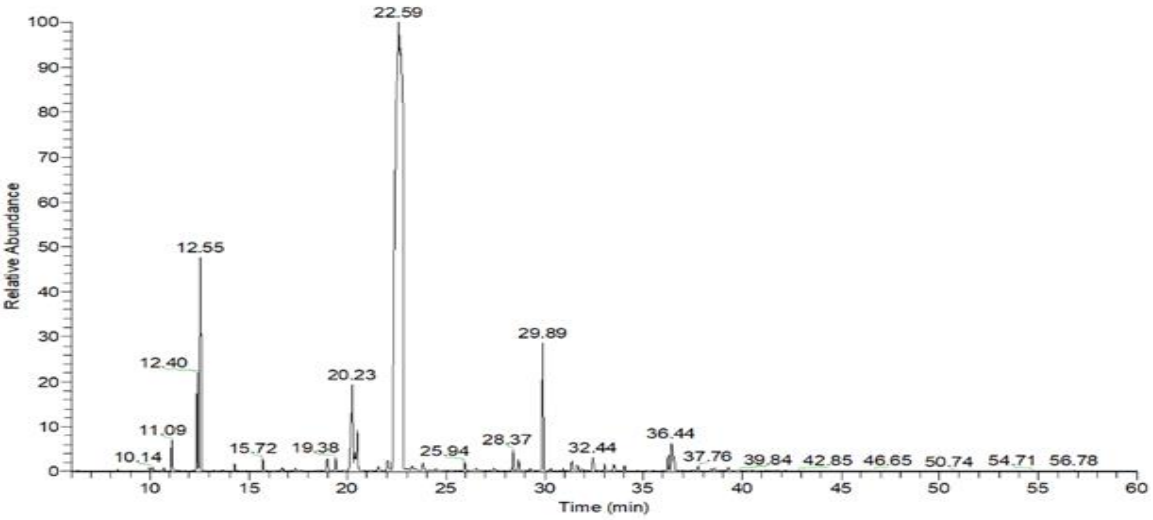

Fig. 5: GC-MS chromatogram of mint essential oil (obtained by Microwave-assisted Extraction).

Quantitative results were obtained with the titration method in the Japanese Pharmacopoeia [25]:

Amount of non-acetylated oil: 4.0120 g
Amount of acetylated oil: 2.7124 g
a value: 4.0 ml (0.5 M HCl required for acetylated oil)
b-value: 49.1 ml (0.5 M HCl required for non-acetylated oil)
c-value: 29.0 ml (blank)

As a result, the amount of menthol was found to be 25.00%. The other two analysis results were calculated as 36.25% and 33.70%. The highest yield was found in mint oil obtained from dried mint grown in Gaziantep region. The average menthol content in the oil was found as 31.65%. According to the test yield, there is 1.13 g menthol in 3.58 g oil obtained from 250 g dry mint.

Experimental data for separation of menthol from essential oil by gradual crystallization method are given in Table-3. The melting point of the menthol samples obtained was determined by Büchi brand B-540 Model device, and it was proved that the substance separated from mint oil by crystallization method was menthol. According to the melting test results; the beginning of melting was determined as 42.4 ^{o}C and the end of melting as 43.1 ^{o}C. These results are compatible with the literature data given in Table-4.

Table-3: Menthol production by gradual crystallization method.

Samples[a]	Experiment Number	Amount of oil used, g	Obtained amount, g Menthol,[b] g	Isomenthol, racemic menthol[c], g	Menthol (total), g	Total yield, %
1	32	2.062	0.524	0.095	0.619	30.02
2	37	1.982	0.511	0.063	0.574	28.96
3	21,24,28	8.127	1.869	0.329	2.198	27.05
4	22-32,46	8.828	2.384	0.446	2.830	32.06
	Total	20.999	5.288	0.933	6.221	29.58±2.09[d]

[a] Samples prepared to carry out the experiment with 4 repetitions. Experiment numbers were chosen randomly to ensure the accuracy of the results. [b] The amount of menthol found in the first step in the determination of menthol with the Titration Method. [c] The amount of isomenthol and racemic menthol found in the second step in the determination of menthol with the Titration Method. [d]Average yield ± standard deviation

Table-4: Melting point of the various L-menthol polymorphs [29].

Polymorph	α (stable form)	β	γ	Δ
Melting point,°C	42.5	35.5	33.5	31.5

Mint is a well-known and easy to grow plant in many countries including Turkey. Mint (*Mentha*) is a commercially valuable and important plant used widely for medicinal purposes.

Mint essential oils are highly variable mixtures of terpenoids and aromatics compounds [11]. The literature reports that essential oils production methods can affect the final product quality, due to losses of some volatile compounds during the procedure, low extraction efficiency, and degradation of unsaturated compounds due to thermal effects or solvent used in the extract [5].

The main component of mint oil is menthol which forms nearly 30% - 40% of crude essential oil derived from mint species. The most widespread use of menthol is in the pharmaceutical industry [11]. The percentage of menthol in mint oil of *Mentha arvensis* species increases up to 80% [1]. Clarke (2008) reported that the menthol content of *Mentha piperita* species can reach up to 50.98% [30]. The researchers reported that with the enzymatic pretreatment applied before hydrodistillation, the cell walls of the leaves of *M. arvensis* were allowed to break and an increase of 186.63% was achieved in menthol yield [31].

In this study, mint oil and menthol production methods from mint species grown in different regions of Turkey, have been studied. The first step to obtain menthol from mint is mint oil production. Mint oil was obtained by hydrodistillation and microwave extraction methods. The results have shown that both the mint type, the region where mint is grown, and the method of obtaining mint oil affect both the yield and the mint oil composition. The highest average oil yield was determined as 2.8 g/kg dry matter and 4.8 g/kg dry matter for *Mentha spicata* (Marmara region, fresh and dried leaves, respectively). For *Mentha piperita* (Gaziantep region), the highest average oil yield was 14.7 g/kg dry matter. In Table-5, mint oil yields calculated in this study are given by comparing them with the results of other researchers in the literature [8, 9, 32-34]. When Table-5 is examined, the results obtained from the literature together with this study can be summarized as follows:

- Dried mint should be preferred instead of fresh mint to obtain mint oil, because its oil yield is higher.
- In addition to traditional shade drying of the crops, different methods can be used, such as oven, microwave or freeze drying [6, 33].
- The higher the drying temperature, the lower the mint oil yield [6, 33].
- Freeze drying method did not increase the oil yield [33].
- Ref [26] and Ref [27] have shown that drying processes can significantly change the chemical profiles of essential oils from mint leaves where some essential oil compounds are lost and/or increased due to oxidation, glycoside hydrolysis, esterification, and the formation of new components by other means.
- Methods of obtaining mint oil also have important effects on mint oil yield and chemical content. The yield and oil content of mint oils obtained by two different methods in this study also supports this information in the literature [8, 9].
- Many parameters such as the type of mint, the climate of the place where and how it is grown, the drying method and the oil production method have an effect on the oil yield [8].

Higher mint oil yield was obtained from steam distillation method with Clevenger device. The menthol content in the average oil was found to be 31.65%. Menthol was isolated from mint oil by the crystallization method applied in two stages was used [17]. Qualitative analysis of menthol was performed by thin-layer chromatography (TLC) method and quantitative analysis by titration method and GC-MS method. The melting points of the obtained menthol crystals were determined and compared with the literature. The melting point of the isolated menthol was determined as 42.4 °C - 43.1 °C (Table-4). In Table 6, the most common components, amounts and retention time in the obtained oil content are given in order to compare:

Table-5: Average mint oil yields reported by some researchers for fresh or dried mint leaves.

Mint spices (region)	Fresh or dry leaf	Method of obtaining essential oil	Average oil yield, g/ kg dry matter	Ref. No
M. spicata (Marmara region)	fresh	Hydodistillation by Clevenger	2.8	This study
	Shade-dried leaves		4.8	
M. piperita (Gaziantep region)	Shade-dried leaves	Hydodistillation by Clevenger	14.3	
		Microwave extraction	5.6	
M. piperita	fresh	Hydodistillation by Clevenger	16.15	[6]
	Dried at 50 °C		22.24	
	Dried at 60 °C		19.55	
	Dried at 70 °C		15.26	
	Dried at 200 W by microwave		2.23	
	Dried at 400 W by microwave		1.43	
	Dried at 800 W by microwave		1.33	
	Shade-dried leaves		18.89	
M.spicata	Oven-dried	Supercritical CO_2 extraction	4.13-10.91	[33]
	Freeze-dried		5.47-5.51	
M. spicata var. *Crispa*	Dried	Hydodistillation	15.7	[34]
M. longifolia			10.1	
Mint procured from local markets	Dried	Supercritical CO_2 extraction	0.587-2.732	[8]
M.piperita L.		HS/SPME[a]	4.2	[9]
		Hydodistillation	-	
Mentha × *piperita*	Fresh	Hydodistillation by Clevenger	16.15	[32]
	Dried by hot air,		22.24	
	Dried by microwave power		2.23	
	Dried shade		18.89	

[a] headspace/solid-phase micro-extraction

Table-6: Comparison of the most common components in-with some literature values [34-37].

Component	This study (Area%)				References (Area%)			
	RT[a]	Clevenger	RT[a]	Microwave	[35]	[36]	[34]	[37]
D-Carvone	22.82	63.03	22.59	77.68	76.65	0.2	0.03-43.65	-
L (-) Menthol	19.48	10.22	-	-	-	40.7	0.38-56.27	46.8
Isocaryophyllen	29.94	5.29	29.89	3.46	1.93	1.7	0.53-6.57	-
1,8-Cineole	12.57	4.49	12.55	4.44	-	5.3	0.57-8.38	2.4
Limonene	12.42	3.15	12.40	2.18	9.57	2.6	0.34-0.74	0.6
cis-Dihydrocarvone	20.26	2.22	20.23	3.26	2.04	-	-	-

[a]Retention time

While menthol was determined qualitatively and quantitatively in mint oil obtained by Clevenger method, no menthol was found in mint oil obtained by microwave extraction. As can be seen, both the peppermint oil content and the amount of menthol in the oil are variable.

Conclusion

In this study, mint oil was obtained from different types of mint grown in different regions of Turkey for the first time and the yields were compared. In addition, the separation of menthol from the mint oil obtained was investigated. The amount of menthol % obtained as a result of the studies was found as 31.65 by titration method and 29.58 by gradual crystallization. In GC-MS analysis, the % peak area of menthol is 10.22. The results obtained by titration and gradual crystallization methods are compatible with each other, showing total menthol. Although in the Ref [32] they found high concentrations of menthol and menthyl acetate by drying mint leaves with microwave for a relatively short time (4 minutes in microwave 800 W), the same cannot be said for microwave extraction. Unfortunately, both lower yield and menthol content were not found in the oil obtained under the microwave extraction conditions studied.

When Turkey's climatic conditions and arable land are considered in combination with the local cultivation of the high-menthol-containing mint species, to invest and improve industrial-scale production of these mint and menthol will produce positive results in terms of our country's economy. Because of the use of natural organic products that do not contain chemicals are increasing day by day.

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References

1. S. Kokkini, R. Karousou, E. Hanlidou, *HERBS | Herbs of the Labiatae, In: B. Caballero, P. Finglas, F. Toldrás (Editors). Encyclopedia of Food Sciences and Nutrition*, Academic Press, USA, p.3082 (2003).
2. Z. Soilhi, A. Rhimi, S. Heuskin, M.L. Fauconnier, M. Mekki, Essential oil chemical diversity of Tunisian *Mentha spp*. Collection, *Ind. Crops Prod.* **131,** 330, (2019).
3. K. H. C. Başer, M. Kürkçüoğlu, B. Demirci, T. Özek, G. Tarımcılar, Essential oils of *Mentha* species from Marmara region of Turkey, *J. Essent. Oil Res*. **24**, 265, (2012).
4. H. Baydar, *Tıbbi, Aromatik ve Keyf Bitkileri: Bilimi ve Teknolojisi*, Süleyman Demirel Üniversitesi Yayınları, İstanbul, Turkey, **51**, (2005) (in Turkish).
5. S. S. Costa, Y. Gariepy, S. C. S. Rocha, V. Raghavan, Microwave extraction of mint essential oil-temperature calibration for the oven, *J. Food Eng*. **126**, 1, (2014).
6. L. Mihajlov, S. K. Veličkovska, G. N. Letia, P. V. Podea, H. Mirhosseini, Isolation, chemical composition, antioxidant and antimicrobial potential of essential oil from *Mentha Arvensis* L. organically planted from Macedonia. *Riv. Ital. delle Sostanze Grasse*. **96**, 151, (2019).
7. D. Lorenzo, D. Paz, E. Dellacassa, P. Davies, R. Vila, S. Cañigueral, Essential oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay, *Braz. Arch. Biol. Technol*. **45**, 519, (2002).
8. N. M. Shrigod, N. R. S. Hulle, R. V. Prasad, Supercritical fluid extraction of essential oil from mint leaves (*mentha spicata*): Process optimization and its quality evaluation, *J. Food Process Eng*. **40**, 1, (2017).
9. A. A. Taherpour, S. Khaef, A. Yari, S. Nikeafshar, M. Fathi, S. Ghambari, Chemical composition analysis of the essential oil of *Mentha piperita* L. from Kermanshah, Iran by hydrodistillation and HS/SPME methods, *Anal. Sci. Technol*. **8**, 1, (2017).
10. M. Goto, M. Sato, T. Hirose, Extraction of peppermint oil by supercritical Carbon dioxide, *J. Chem. Eng. Japan*. **26**, 401, (1993).
11. M. Ozturk, D. Egamberdieva, M. Pešić, (Eds.) *Biodiversity and Biomedicine: Our Future, Essential oil of mint: current understanding and future prospects*, Academic Press, UK: (2020).
12. J. Fejéra, D. Gruľová, V. De Feo, Biomass production and essential oil in a new bred cultivar of peppermint (*Mentha × piperita* L.), *Industrial Crops & Products* **109**, 812, (2017).
13. F. Sharmeen, M. Mahomoodally, G. Zengin, F. Maggi, Essential Oils as Natural Sources of Fragrance Compounds for Cosmetics and Cosmeceuticals, *Molecules*. **26**, 666, (2021).
14. BASF (2012). The cool freshness of menthol [online]. Website https://www.basf.com/us/en/media/science-around-us/the-cool-freshness-of-menthol.html (accessed 30 September 2021)
15. G. P. P. Kamatou, I. Vermaak, A. M. Viljoen, B. M. Lawrence, Menthol: A simple monoterpene with remarkable biological properties, *Phytochemistry*, **96**, 15, (2013).
16. V. Schelz, J. Molnar, J. Hohmann, Antimicrobial and antiplasmid activities of essential oils, *Fitoterapia*, **77**, 279, (2006).
17. N. Galeotti, L. D. C. Mannelli, G. Mazzanti, A. Bartolini, C. Ghelardini, Menthol: a natural analgesic compound, *Neurosci. Lett*. **322**, 145, (2002).
18. M. Nekoei, M. Mohammadhosseini, Chemical Compositions of the Essential Oils from the Aerial Parts of *Achillea wilhelmsii* Using Traditional Hydrodistillation, Microwave Assisted Hydro-distillation and Solvent-Free Microwave Extraction Methods: Comparison with the Volatile Compounds Obtained by Headspace Solid-Phase Microextraction, *Journal of Essential Oil Bearing Plants*, **19**:1, 59, (2016).
19. M. Nekoei, M. Mohammadhosseini, Screening of Profiles of Essential Oils from the Aerial Parts of *Sclerorhachis platy-rachis* (Boiss.) Podlech ex Rech.f. Using Classical and Microwave-based Methods: Comparison with the Volatiles Using Headspace Solid-Phase Microextraction, *Journal of Essential Oil Bearing Plants*, **21**:5, 1199, (2018).
20. B. Yingngam, A. Brantner, M. Treichler, N. Brugger, A. Navabhatra, P. Nakonrat, Optimization of the eco-friendly solvent-free microwave extraction of *Limnophila aromatica* essential oil, *Industrial Crops & Products* **165**, 113443 (2021).
21. M. N. Alvi, S. Ahmad, K. Rehman, Preparation of Menthol Crystals from Mint (*Mentha Arvensis*), *Int. J. Agric. Biol*. **3**, 527, (2001).
22. J. A. Llorens-Molina, V. Castell, S. Vacas, M. Verdeguer, TLC-GC/MS Method for identifying and selecting valuable essential Oil Chemotypes from Wild Populations of *Mentha longifolia* L., *Nat. Volatiles & Essent. Oils*, **4**(4), 49, (2017).

23. M. Najdoska, J. Bogdanov, Z. Zdravkovski, TLC and GC-MS Analyses of Essential Oil Isolated from Macedonian *Foeniculi fructus Macedonian pharmaceutical bulletin*, **56** (1,2) 29, (2010).
24. European Pharmacopoeia, 9.0, pp. 2894-2895, 2998-2999.
25. Japanese Pharmacopoeia, 11th Edition.
26. Y. Arslan, D. Katar, I. Subaşı, Determination of Ontogenetic Variability Of Essential oil Content and Components in Menthol Mint (*Mentha arvensis* L.) in Ankara Ecological Conditions, *GOU, Ziraat Fak. Derg*. **27**, 39, (2010). (in Turkish with an abstract in English).
27. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th Edition, Allured publishing Co. Carol Stream, Illinois, (2017).
28. F. E. Wright, Crystallization of Menthol, *J. Am. Chem. Soc*. **39**, 1515, (1917).
29. I. de Albuquerque, M. Mazzotti, Influence of Liquid-Liquid Phase Separation on the Crystallization of L-Menthol from Water, *Chem. Eng. Technol*. **40**, 1339, (2017).
30. S. Clarke, *Essential Chemistry for Aromathreapy*, Second Edition, Churchill Livingstone: Elsevier, (2008).
31. N. S. Reis, A. R. Brito, C. S. V. Pacheco, L. C. B Costa, E. Gross, et al. Improvement in menthol extraction of fresh leaves of *Mentha arvensis* by the application of multi-enzymatic extract of *Aspergillus niger*, *Chem. Eng. Commun*. **206**, 387, (2019).
32. M. Beigi, M. Torki-Harchegani, A.G. Pirbalouti, Quantity and chemical composition of essential oil of peppermint (*Mentha×piperita* L.) leaves under different drying methods. *Int. J. Food Prop*. **21**, 267, (2018).
33. A. H. Al-Marzouqi, M. V. Rao, B. Jobe, Comparative Evaluation of SFE and Steam Distillation Methods on the Yield and Composition of Essential Oil Extracted from Spearmint (*Mentha spicata*), *J. Liq. Chromatogr. Relat*. **30**, 463, (2007).
34. M. A. Hawrył, K. S. Wozniak, R. Swieboda, M. Niemiec, K. Stepak, et al. GC-MS fingerprints of mint essential oils, *Open Chem*. **13**, 1326, (2015).
35. R. S. Chauhan, M. K. Kaul, A. K. Shahi, A. Kumar, G. Rama, et al. Chemical composition of essential oils in *Mentha spicata* L. accession [IIIM(J)26] from North-West Himalayan region, India, *Ind. Crops Prod*. **29**, 654, (2009).
36. E. Schmidt, S. Bail, G. Buchbauer, I. Stoilova, T. Atanosova, et al. Chemical Composition, Olfactory Evaluation and Antioxidant Effects of Essential Oil from *Mentha x piperita*, *Nat. Prod. Commun*. **4**, 1107, (2009).
37. M. Buleandra, E. Oprea, S. E. Popa, I. G. David, Z. Moldovan, et al. Comparative Chemical Analysis of *Mentha piperita* and *M. spicata* and a Fast Assessment of Commercial Peppermint Teas, *Nat. Prod. Commun*. **11**, 551, (2016).