

Comparison of extraction parameters and extracted essential oils from *Mentha piperita* L. using hydrodistillation and steamdistillation

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Abstract

Hydrodistillation (HD) and steamdistillation (SD) are known to be the most prevalent methods of essential oils extraction. Despite many studies on extraction methods, there is no report on comparison of SD and HD and their extracted essential oils. In this study, SD was used for extraction of essential oils from *Mentha piperita* L. (peppermint), which is one of the medicinal plants of high consumption, and the results were compared with those of the HD. The results showed that although extraction with HD started sooner, both methods had the extraction time of about 1 hour. Scanning electron micrographs of mint leaves showed significant changes of glands after extraction in both methods. The required energy for extraction and kinetic parameters as well as quality of extracted essential oils were compared by physical constants and GC-MS analysis. The results of this study revealed that both HD and SD can be used as qualitative reference methods of extraction due to similarity of properties of extracted essential oils.

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Introduction

Peppermint (*Mentha piperita* L.) belongs to mint (*Lamiaceae*) family and is herbaceous and perennial considered as a medical and aromatic plant and were produced extensively for the medicinal and food product industries (The Wealth of India, 1962). This plant was cultivated by the ancient Egyptians and documented in the Icelandic pharmacopoeia of the thirteenth century. It is widely grown in temperate areas of the world, particularly in Europe, North America and North Africa but nowadays cultivated in other regions of the world including Middle East (The Wealth of India, 1962).

The aerial parts of mint bears essential oils containing a large number of aroma chemicals like menthol, menthone, isomenthone and menthofuran which are used in pharmaceutical, food, flavour, cosmetics and beverages industries (The Wealth of India, 1962; Carmines, 2002). Tobacco industry consumes about 40% of the total mint essential oil followed by pharmaceutical and confectionary industries. Usually the major components of peppermint oil include menthol, menthone and menthofuran. Menthol, the main substance that gives the mints their characteristic aromas and flavors, is

used as a raw material in toothpaste, toothpowder, chewing tobacco, confectionary, mouth fresheners, analgesic balms, cough drops, perfumes, chewing gums, candies and tobacco industry (The Wealth of India, 1962). The peppermint oil is reported to have anti-oxidant properties, antibacterial activity and is one of the most important constituents of some over-the-counter remedies in Europe for irritable bowel syndrome (Pittler and Ernst, 1998; Ribeiro *et al.*, 2002; Singh *et al.*, 2011).

There are a number of methods for essential oils isolation, e.g. hydrodistillation (HD), steam distillation (SD) and organic solvent extraction (Presti *et al.*, 2005). Traditional methods for the extraction of essential oils from medicinal plants are known to be SD and HD. It is known that these methods suffer from some disadvantages including losses of volatile compounds, long extraction times and energy intensive. However, these are simplest methods of extraction and their equipments are often more available than novel methods of extraction like microwave-assisted hydrodistillation (MAHD) and ohmic-assisted hydrodistillation (OAHD) which are using microwave and ohmic energy as the heating source, respectively. (Stashenko *et al.*, 2004, Gavahian *et al.*, 2011; Gavahian *et al.*, 2012).

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In addition, some extraction factories already have required facilities for both SD and HD. Furthermore, it is typical in extraction studies to use HD as the reference method for quality of extracted essential oils by other proposed methods (Moyler, 1991; Presti *et al.*, 2005). So it is necessary to delineate the similarities and differences between these methods and possibility of substitution of SD for HD.

Despite many studies reported on the essential oils of *M. piperita*, there is no report on the extraction of essential oils from this medicinal plant using HD and SD to evaluate extraction parameters and extracted oils quality. Therefore, the aim of this work was to use SD technique for the extraction of essential oils from dried peppermint areal parts and to compare the extraction parameters and some physical and chemical properties of the extracted essential oils with those obtained by HD.

Materials and Methods

Plant materials

Fresh aerial parts of peppermint when the plant has enough foliage (before flowering stage) were collected from an indigenous crop in Noor-Abad (Mamasani, Sothern Iran), in July 2013.

The identity of the genus *Mentha* was certified by plant taxonomy experts from Biology Department of the Shiraz University, Shiraz, Iran. The herbs were then dried in a dark room under ambient conditions (30-40 °C) for four days on a large screened tray, packed in high density poly ethylene (HDPE) bags, put in a cardboard box and kept in a dark and cool place for further experiments. The moisture content of the plants was measured in triplicate using a laboratory oven (1H-100 Galenkamp, UK) by drying until constant weight and was about 12.4±0.2%.

Steamdistillation

SD was performed using a laboratory heater (MAG-K; Gerhardt Ltd., Germany; and 500 W) as the heating source. Processing parameters (e.g. processing time and power consumption), were precisely monitored using a software developed and coupled with a Wattmeter to record the input power of apparatus to double check the data given by the software. In SD procedures, 15 g of dried peppermint aerial parts placed in the junction of flask and Clevenger device. The bottom of the junction was perforated to let steam easily travel. 0.5 L distilled water were heated in the apparatus flask for up to 2 h from initial temperature of 27±1°C (similar to initial temperature of material in HD method). The extraction process continued until no more essential

oils were obtained. During the first 1 h, the collected essential oils were decanted from the condensate at 2.5-min intervals followed by decanting of the essential oils every 30-min. To remove water, the extracted essential oils were then dried over anhydrous sodium sulfate and stored in amber vials at 4 °C for further experiments.

Hydrodistillation

HD is an approved method that is used as a reference for the quantification of essential oils (Stahl-Biskup, 2002). HD was carried out in a similar way as SD. The volume and dimensions of the utilized container were exactly similar to that used for the SD. In addition, processing parameters were monitored using the designed software. Alternatively, the input power consumption was monitored using a separate Wattmeter at the entrance of electrical heater power supply. Fifteen grams of dried peppermint aerial parts with 0.5 L distilled water were put into HD with a Cle-venger-type apparatus and essential oils were extracted for 2 h, after which no more essential oils were obtained. During the first 1 h, the collected essential oils were decanted from the condensate at 2.5-min intervals followed by decanting of the essential oils every 30-min. Removal of water from essential oils was performed as described for SD samples. The extracted essential oils were then stored in a cool (4 °C) and dry place for further experiments.

Physical constants

Specific gravity and the refractive index of the essential oils from the mint samples were measured according to Food Chemical Codex (FCC) (FCC, 1996) at 25 and 20°C, respectively. The color parameters of the oils (L: lightness, a: redness-greenness and b: blueness-yellowness) were determined according to the described method by Afshari-Jouybari and Farahnaky (2011). In addition, the color of the oils was determined visually as directed in FCC (FCC, 1996).

Scanning electron microscopy (SEM)

SEM images of dried peppermint leaves were obtained for the untreated samples and also for those extracted by SD (for 60 min) and HD (for 60 min). The leaves were freeze-dried using a freeze drier (Armfield, UK) and fixed on an aluminum sample holder and then sputtered with gold in a sputter coater (Polaron SC7640, UK). All the samples were examined with a scanning electron microscope (Cambridge, UK) under high-vacuum condition and at an accelerating voltage of 20.0 kV and at a working distance of 7.5–9.5 mm (i.e. the distance between the

Table 1. Effect of extraction method on starting time of oil accumulation, extraction duration, total extraction time, mean rate of essential oils accumulation, yield and consumed energy

	HD	SD
Starting time of oil accumulation (min)	34.16 ^b ± 1.93	42.00 ^b ± 1.20
Extraction duration (min)	21.71 ^a ± 1.71	16.75 ^b ± 2.16
Total extraction time (min)	55.88 ^a ± 3.30	58.75 ^a ± 2.85
mean rate of essential oils accumulation (ml/min)	0.024 ^b ± 0.003	0.033 ^a ± 0.005
Yield (%V/W)	2.29 ^a ± 0.16	2.00 ^b ± 0.27
Electric consumption (kWh/ml essential oils)	0.689 ^a ± 0.081	0.835 ^a ± 0.095

The same letters in each row indicate that the means are not significantly different ($p < 0.05$).

surface of the sample and the microscope lens).

Gas chromatography-Mass spectrometry (GC-MS)

GC-MS analysis of the essential oils was performed using an Agilent 7890A chromatograph, coupled with Agilent 5975C mass spectrometer (Agilent Technologies, USA), operating at 70 eV ionization energy, 0.5 s/scan and the mass range: 35-400, equipped with a HP-5MS capillary column (phenyl methyl siloxane, 30×0.25 mm; 0.25 μm film thickness). The oven temperature increased from 60 to 240°C at a rate of 3°C/min, the injector and detector temperatures were 240°C and 250°C, respectively. Helium was used as the carrier gas with a flow rate of 0.9 mL/min and a split ratio of 1:50. Relative percentage data were obtained from electronic integration of peak areas without the use of correction factor. The software adopted to handle mass spectra and chromatograms was ChemStation.

Retention indices were determined by using retention times of normal alkanes that had been injected after the oil under the same chromatographic conditions according to the Van Den Dool and Kratz method (Van Den Dool and Kratz, 1963). The compounds were identified by comparison of their mass spectra with the Wiley library or with the published mass spectra.

Statistical analysis

All extractions with SD and HD were performed in triplicates. An independent samples t-test was performed to determine significant differences between the means using SPSS (version 19.0.0; IBM Institute Inc., USA).

Results and Discussion

Comparison of extraction kinetics, yield and required

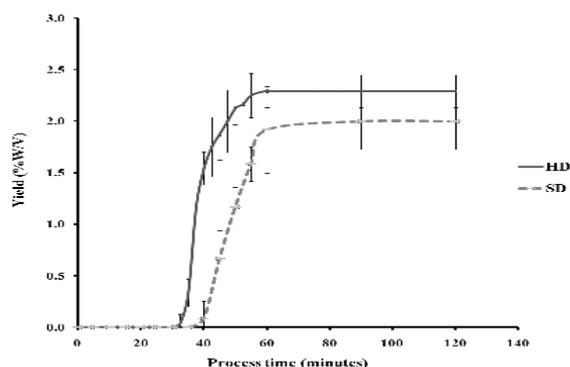


Figure 1. Extraction yield (ml essential oils/100 g dried herb) as a function of time for HD and SD of essential oils from peppermint aerial parts

energy

The extraction kinetics of essential oils from peppermint using SD was compared with that of HD (Figure 1). Extraction with HD started earlier than SD methods (about 34 min for HD and 42 min for SD). This may be due to faster temperature increase in hydro distilled mint areal parts in comparison with steam distilled mint areal parts since mints in HD process are in contact with heat transfer medium (water) while in SD they are in contact with a secondary form of heat transfer medium (steam). Therefore as predicted, in HD mint glands (which contain essential oils) were affected sooner than SD and as a result the extraction process started quicker in HD method. The effect of extraction method on yield, starting time of oil accumulation, extraction duration, total extraction time and the mean rate of essential oils accumulation are presented in Table 1. As the data shows, although HD started extraction of essential oils sooner, both methods (HD and SD) require approximately same time to perform the extraction process. In other words, SD has a shorter extraction duration which compensates its delay in starting of oil accumulation.

The rates of essential oils accumulation of SD and HD (which is also presented in Table 1.) obtained from the slope of linear part of each graph (Figure 1) by dividing the amount of extracted oils (ml) to its corresponding time (min), which is equal to the mean rate of essential oils accumulation (ml/min). The results show that SD extracts essential oils almost 37% faster than the HD method. This data illustrates that the rate of extraction in SD was greater. In addition, SD method has a shorter extraction duration time (i.e. the difference between total extraction time and starting time of oil accumulation). This is due to higher extraction rate in SD. As seen in Figure 1, extraction graphs of SD method are steeper than HD graphs. In HD process, the mint leaves have enough time to soak before process reaching high temperatures

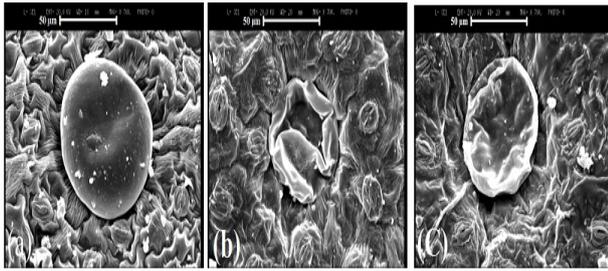


Figure 2. Scanning electron micrographs of the glands from mint leaves: (a) untreated, (b) after HD for 60 min and (c) after SD for 60 min

(i.e. the temperature which can disrupt glands and evaporate essential oils), so the glands (which jailed essential oils) can absorb water and increase their resistance to eruption (since dry plant material are more brittle than soaked rubbery ones). On the other hand, in SD process, dry glands suddenly face hot steam and as a result, some parts of their wall may easily erupt and essential oil can outpour in a shorter time. In addition, higher heat transfer coefficient of steam (condensing water vapor) in comparison to boiling water can also cause more intensive effect on placed mint leaves in SD process in comparison with that placed in HD (Singh and Heldman, 2009).

The essential oil content of medicinal plants can be influenced by harvest time, ecological and climatic conditions (Kastner, 1969; Clark *et al.*, 1980; Cabo *et al.*, 1987; Özgüven and Tansi, 1998; Barauskiene, 2003; Tabatabaie and Nazari, 2007; Khorasaninejad *et al.*, 2010). It was previously reported that the oil content of peppermint can vary by harvest time from 0.72% to more than 3% and oil yield of peppermint depends on growing stage (White *et al.*, 1987). Likewise, Tabatabaie and Nazari (2007) reported that the essential oil content of peppermint can vary from less than 2% to more than 4% by changing farming conditions (soil nutrient solutions and salinity). In this study the resulted yields in both methods were in the range of the previous reports on peppermint. As presented in Table 1, HD yields higher amount of essential oils than SD (2.29 ± 0.16 and 2.00 ± 0.27 %V/W, respectively). The lower yield in SD process can be related to packing of mint leaves in junction of steam distillatory device (i.e. the part of device where mint areal parts were placed). In HD process, plant material can move freely and boiling water (which is heat and mass transfer medium) can access all mint leaves. On the other hand, leaves in SD process are relatively packed and all leaves may not be accessible for steam (i.e. heat and mass transfer medium in this system). In addition, the leaves which are placed in the top of junction of device will face steam of lower temperature and quality in comparison with the leaves which are placed in the bottom of junction.

Table 2. Physical properties of essential oils from mint aerial parts by HD and SD

	HD	SD
Specific gravity	$0.9089^a \pm 0.0071$	$0.9093^a \pm 0.0066$
Refractive index	$1.4377^a \pm 0.0391$	$1.4333^a \pm 0.0200$
Appearance	Pale yellow	Pale yellow
L [#]	$61.0^a \pm 3.6$	$61.0^a \pm 3.6$
a	$-3.3^a \pm 1.5$	$-1.0^a \pm 0.0$
b	$10.7^a \pm 3.5$	$9.7^a \pm 4.0$

The same letters in each row indicate that the means are not significantly different ($p < 0.05$).

[#] L: lightness, a: redness-greenness and b: yellowness-blueness.

This can cause a reduction in the rate of heat and mass transfer for upper leaves. A continuous range of states exists between that of a saturated liquid and that of a saturated vapor, in which the proportions of liquid and vapor vary according to the degree of phase change transition. The extent to which the phase change has progressed is defined as steam quality. Normally, steam quality is expressed as a percentage indicating the heat content of the vapor-liquid mixture and temperature reduction will result in quality reduction of saturated steam (Singh and Heldman, 2009). The energy requirement to perform the extraction, based on the power consumptions of the electromantle for 1 ml of extracted oils, was not significantly different between the SD and HD. This indicates that substitution of HD by SD will not increase energy consumption. This finding can have significant importance for large scale extractors and industries.

Structural changes during the extraction of essential oil

The images of mint glands on the leaves obtained by scanning electron microscopy before and after the extraction process are shown in Figure 2. Figure 2a is a micrograph of the untreated gland (i.e. before the extraction). Figure 2b and c show the SEM images of peppermint glands that had undergone HD (60 min) and SD (60 min), respectively. Both extraction methods resulted in clear physical changes in the glands. The glands that had undergone SD were wrinkled while those that had undergone HD were wrinkled and also erupted to some extent which can be a reason for more extraction yield in HD in comparison to SD. Such eruptions can facilitate the outpour of all jailed essential oils.

Table 3. Physical properties and Chemical compositions of essential oils of mint aerial parts extracted by HD and SD analyzed using GC-MS.

No.	Compound Name	KI*	KI**	Relative peak are [%]***	
				HD	SD
1	(2E)-Hexenal	854	855	t ^a	t ^a
2	α -Thujene	926	930	t ^a	t ^a
3	α -Pinene	936	939	0.7 ^b \pm 0.0	1.0 ^a \pm 0.1
4	Camphene	954	954	t ^a	t ^a
5	Sabinene	971	975	0.5 ^b \pm 0.0	0.7 ^a \pm 0.0
6	β -Pinene	976	979	1.3 ^a \pm 0.1	1.4 ^a \pm 0.1
7	Myrcene	989	990	0.2 ^b \pm 0.0	0.3 ^a \pm 0.1
8	p-Mentha-1(7),8-diene	1004	1008	t ^a	t ^a
9	α -Terpinene	1018	1017	0.1 ^a \pm 0.0	t ^b
10	Limonene	1029	1029	t ^a	t ^a
11	1,8-Cineole	1033	1031	7.5 ^a \pm 0.7	7.3 ^a \pm 0.8
12	(Z)- β -Ocimene	1037	1037	3.1 ^a \pm 0.6	2.2 ^a \pm 0.6
13	(E)- β -Ocimene	1047	1050	0.1 ^a \pm 0.0	0.1 ^a \pm 0.0
14	γ -Terpinene	1059	1059	0.9 ^b \pm 0.0	1.5 ^a \pm 0.0
15	Terpinolene	1087	1088	0.1 ^a \pm 0.0	0.1 ^a \pm 0.0
16	trans-Sabinene hydrate	1099	1098	0.2 ^a \pm 0.0	0.2 ^a \pm 0.0
17	iso-Menthone	1167	1162	26.4 ^a \pm 0.0	23.2 ^b \pm 0.1
18	Menthofuran	1170	1164	15.5 ^b \pm 0.0	16.9 ^a \pm 0.1
19	neois-Menthol	1190	1186	30.3 ^a \pm 0.1	27.7 ^b \pm 0.2
20	Pulegone	1245	1237	5.4 ^a \pm 0.0	5.2 ^b \pm 0.1
21	Piperitone	1259	1252	0.2 ^b \pm 0.0	0.3 ^a \pm 0.0
22	neo-Menthyl acetate	1274	1273	0.4 ^b \pm 0.0	0.5 ^a \pm 0.0
23	Menthyl acetate	1293	1295	2.9 ^b \pm 0.0	3.7 ^a \pm 0.1
24	iso-Menthyl acetate	1306	1305	0.2 ^a \pm 0.0	0.1 ^b \pm 0.0
25	β -Bourbonene	1380	1388	0.2 ^a \pm 0.0	0.2 ^a \pm 0.0
26	β -Elemene	1389	1390	t ^a	t ^a
27	(E)-Caryophyllene	1415	1419	1.2 ^b \pm 0.0	2.6 ^a \pm 0.1
28	β -Copaene	1427	1432	t ^a	t ^a
29	(E)- β -Farnesene	1451	1456	0.1 ^b \pm 0.0	0.4 ^a \pm 0.1
30	Germacrene D	1478	1485	0.8 ^b \pm 0.0	2.5 ^a \pm 0.2
31	Bicyclogermacrene	1498	1500	0.1 ^b \pm 0.0	0.2 ^a \pm 0.0
32	Germacrene A	1504	1509	t ^b	0.1 ^a \pm 0.0
33	δ -Amorphene	1515	1512	t ^a	t ^a
34	Caryophyllene oxide	1580	1583	0.1 ^b \pm 0.0	0.4 ^a \pm 0.0
Total		-	-	98.7	99.0

* Experimentally determined Kovats retention index (KI) relative to C19-C18 n-alkanes on the DB-5MS column

(Kovats, 1965), ** Literature Kovats retention index (Adams, 2007)

t=trace (<0.05), *** Mean \pm SD (n=3), The same letters in each row indicate that the means are not significantly different (p < 0.05).

Physical constants evaluation

The physical properties (specific gravity, refractive index and color) of mint essential oils extracted by HD and SD are shown in Table 2. There is no significant difference between HD and SD for the specific gravity and refractive indices. Every essential oil has a typical range of refractive indexes and densities at specified temperatures. Generally, the densities of essential oils range from 0.780 to 0.970 g.cm⁻³ (Bowles, 2003). Similarly, sensory color perceptions of both samples were similar and within the range indicated by FCC. From the physical tests of the extracted essential oils, it can be concluded that SD, as a possible substitution technique for HD, did not introduce any considerable changes to the studied physical properties of the extracted essential oils from aerial parts of mint.

GC-MS

The identified components in the extracted essential oils of peppermint aerial parts by HD and SD are given in Table 3. The 34 components presented in Table 3 comprise more than 98.7% of the total GC peak areas. The compositions of the essential oils obtained by HD and SD were almost similar and as a result, components extracted by HD were also found in SD. Also, there were significant differences between the quantities of some components extracted by HD and those extracted by SD (for example compound

No. 3, α -pinene). Similar results were obtained for the extraction of essential oils by MAHD (Stashenko *et al.*, 2004) and OAHD (Gavahian *et al.*, 2011, 2012). In the current study, the main compounds of all essential oils were neoiso-Menthol (compound No. 19), iso-menthone (compound No. 17) and menthofuran (compound No. 18). Overall, according to presented data in Table 3 and similarity of major components of extracted essential oils, it can be concluded that extracted essential oils by SD and HD have approximately similar chemical compositions. The essential oil composition of many aromatic plants can be influenced by harvest time, ecological and climatic conditions (Kastner, 1969; Cabo *et al.*, 1987; Piccaglia and Marotti, 1991; Özgüven and Tansi, 1998; Baranauskiene, 2003). Many reports have already confirmed the major components reported in this survey to be the most abundant components in *Mentha piperita* L. essential oil (i.e. Clark *et al.*, 1980; Tabatabaie and Nazari, 2007; Khorasaninejad *et al.*, 2010)

Conclusion

In this study, SD resulted in a similar extraction time to the conventional HD technique. SEM images of mint leaves having undergone SD and HD indicated that both extraction methods resulted in significant changes in glands and the amount of changes in HD

were slightly greater than SD. Essential oils obtained by HD and SD were almost similar in their physical properties and chemical compositions. These similarities can suggest SD as an alternative reference method for qualitative extraction of essential oils.

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