CHEMIJA. 2012. Vol. 23. No. 2. P. 113–117

Salt effects on the quality and recovery of *Mentha pulegium* L. essential oil

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Effects of four different salts, sodium chloride, sodium carbonate, calcium chloride and calcium carbonate used as additives in the water distillation process, on the essential oil quality and recovery of *Mentha pulegium* L. were evaluated. GC/MS analysis of oils revealed that salts application had considerable effects on the chemical constituents of distilled oil. Chemical constituents proportional variation was salt-dependant. Pulegone (39.1–45.3%) was the main common component of the oils with its high content detected in the control oil (hydrodistillation without any salt addition). Menthone (13.8–20.9%) was another major component with the highest amount related to calcium chloride. Compositional variation of other major components such as piperitenone oxide, isopulegol, 1,8-cineole, cis-piperitone epoxide and (E)-caryophyllene was greatly salt-dependant.

Key words: *Mentha pulegium* L., Lamiaceae, salt, floral water, GC/MS, pulegone, menthone

INTRODUCTION

Mentha pulegium L. (Fam. Lamiaceae or Labiatae) is an aromatic perennial herbaceous plant that wildly grows in humid and damp areas and water banks in many parts of the world mainly Mediterranean countries [1, 2]. Fresh or dried leaves and flowering tops are of common use for their healing and culinary properties [1].

For therapeutic applications, *M. pulegium* and its preparations have long been used as antispasmodic, carminative, diaphoretic, sedative, anti-tussive, cholagogue, expectorant, antiseptic, anti-bronchitis and digestive materials [1–3]. Economic and pharmaceutical significance of *M. pulegium* L. is due to high occurrence of monoterpenes, mainly oxygenated monoterpenes in essential oil extracted from flowering aerial parts. Three different chemotypes have been reported for this plant: pulegone, piperitenone / piperitone and isomenthone / neoisomenthone types [4].

Steam distillation is the principal volatile oil extraction method commonly used in the food and pharmaceutical industries. During distillation, a certain proportion of essential oil becomes dissolved in distillation or condensate water (hydrosol or floral water). The floral water is discarded, leading to loss of dissolved volatile oil components [5]. Losses up

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to 25% were reported for *Pelargonium* species [5]. Attempts were made to recover the dissolved volatile oil components from floral water. The methods experienced to recover oil components from floral water were cohobation, extraction with some organic solvents such as diethyl ether and hexane, adsorption of oil components on to an adsorbent such as XAD-4 and protoplast techniques [5, 6].

Salts are known to improve the recovery of some volatile constituents from floral waters. The magnitude of this salt effect depends upon the nature and concentration of salt applied [7, 8]. Salt effects may be employed to enhance the concentration of certain components in essential oil. Nagvi et al. [7] reported calcium chloride as the salt of choice for improving the recovery and quality of the essential oil of *Cymbopogon winterianus* Jowitt from India.

In the present investigation we report the effects of some salts used during distillation on the composition and oil recovery in the case of *Mentha pulegium* L. from Iran.

EXPERIMENTAL

Plant material

The flowering aerial parts of a local population of spontaneously growing *Mentha pulegium* L. from Northwest Iran (Ahar District) were harvested in July 2008. A sample of specimen was deposited in the Herbarium of Maragheh University, Iran. The harvested materials were air-dried in a shaded place at ambient temperature of about 30 °C. The air-dried materials were mixed and pulverized to obtain a homogenous fine grade powder.

Isolation of volatile oil

50 grams of air-dried and grinded plant materials were subjected to hydrodistillation by an all-glass Clevenger type apparatus for 3 hrs, using 500 ml of distilled water. The oil was dried over anhydrous sodium sulphate and labeled as a control. The whole extraction process was accomplished with 500 ml solutions (3% each) of sodium carbonate, calcium carbonate, sodium chloride and calcium chloride. The oils were refrigerated in sealed glass vials until analysis. Essential oil content was expressed as ml/100 g dry weight of plant material. The extraction process was repeated in triplicate for each salt, and the equally mixed oils from their replicates were subjected to analysis.

GC/MS analysis

The compositional analysis of volatile oils were carried out by GC (Agilent Technologies 6890N) interfaced with a mass selective detector (MSD, Agilent 5973B) equipped with an apolar Agilent HP-5ms (5%-phenylmethylpolysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with a constant flow rate of 1 ml/min. Oven temperature was set at 50 °C for 2 min, then programmed to 110 °C at a rate of 10 °C/min, then heated to 200 °C at the 20 °C/min rate and finally increased at the rate 10 °C/min to 280 °C, isothermal at the temperature for 2 min. Injector and detector temperatures were 300 °C and 200 °C, respectively. Injection mode, split: split ratio 1 : 100, volume injected, 4 µl of the oil. The MS operating parameters were as follows: ionization potential: 70 eV, interface temperature: 200 °C and acquisition mass range: 50–800.

Identification and quantification of constituents

Relative percentage amounts of the volatile oil components were evaluated from the total peak area (TIC) by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature [3, 4, 9, 10–15].

RESULTS AND DISCUSSION

Hydrodistillation of the aerial parts of *M. pulegium* L. gave a pale yellow (in case of calcium chloride and carbonate) or whitish (in case of sodium chloride and carbonate) colored liquid with a yield of 0.4–0.45 (v/w) based on dry weight. There was no significant difference between control and salt treatments regarding the essential oil content. The effects of salt on the essential oil constituents and oil recovery of *M. pulegium* are presented in Table. Fifty-seven components were identified in the essential oils accounting for 93.7–98.9% of total identified components. Pulegone (39.1–45.3%) and menthone (13.8–20.9%) were characterized as the major components of volatile oils consistent with previous reports. The effect on the oil composition was found to be meaningful with some salts. This effect was quite salt and constituent dependant. The pulegone content decreased from 45.3% in the control to 39.1% with calcium carbonate. Menthone content was greatly influenced by salt so that the highest (20.9%) and the lowest (13.8%) amount for this compound were determined in the oils added with calcium chloride and sodium chloride, respectively. The content of isopulegol, an alcoholic oxygenated monoterpene, weakly increased (5.6–6.3%) by salt application compared with the control (5.1%). *Cis*-piperitone epoxide was another main component; its content was considerably influenced by salt application. This compound was absent under sodium carbonate application. Meanwhile, its content was about twice higher in other salts (5.3–5.8%) compared to the control (2.6%). Piperitenone oxide content was considerably affected by salts. Sodium carbonate (0.2%) had the lowest amount for this compound in spite of its high (8.9%) content in calcium carbonate. Calcium carbonate (2.7%) and calcium chloride (8.7%) had the highest and the lowest range for 1,8-cineole. (E)-Caryophyllene was the main sesquiterpenoid compound and its content was influenced by salt application. Sodium salts (4.2–4.3%) had increasing effects on its content. (Z)-dihydrocarvone was not present in the control but this compound was efficiently (0.8–2.2%)

Table. **Effects of salts in hydrodistillation on the quality and recovery of *Mentha pulegium* L. essential oil**

Compound	RI	Distilled water	Sodium carbonate	%	Sodium chloride	Calcium chloride
				Calcium carbonate		
α-Pinene	0 939	0.2	0.2	0.2	0.3	0.4
Sabinene	0 975	0.4	0.4	0.2	0.3	0.7
β-Pinene	0 979	0.4	0.5	0.3	0.4	0.8
Myrcene	0 991	0.3	0.3	0.2	0.2	0.4
3-Octanol	0 991	–	0.7	0.5	0.6	**1.1**
α-Phellandrene	1 003	–	–	–	–	0.1
α-Terpinene	1 017	0.1	–	–	0.1	0.2
p-Cymene	**1 025**	**1.5**	**1**	**0.4**	**0.5**	**0.8**
Limonene	1 029	0.8	**1.1**	0.5	0.7	–
1,8-Cineole	**1 031**	**3.8**	**5**	**2.7**	**3.8**	**8.7**
δ-3-Carene	1 031	0.3	–	–	–	0.1
(Z)-β-Ocimene	1 037	0.3	**1.1**	0.2	0.2	–
(E)-β-Ocimene	1 050	–	0.6	–	–	–
γ-Terpinene	1 060	**1.5**	0.3	0.1	0.2	0.4
cis-Sabinene hydrate	1 070	–	–	–	–	0.4
Terpinolene	1 089	0.1	–	0.1	0.1	0.1
Linalool	1 097	0.6	0.5	0.5	0.4	0.8
trans-Sabinene hydrate	1 098	0.5	**1.4**	0.2	0.2	–
cis-Thujone	1 102	0.1	–	–	–	–
Alloocimene	1 132	0.1	0.1	0.1	0.1	0.2
Terpineol <1->	1 134	0.3	–	–	0.2	0.1
trans-Pinocarveol	1 139	–	0.3	0.5	0.3	0.3
trans-p-Menth-2-en-1-ol	1 141	0.2	–	–	–	–
(Z)-Verbenol	1 141	0.2	–	–	–	–
Neoalloocimene	1 144	–	–	0.1	–	–
Isopulegol	**1 150**	**5.1**	**5.9**	**6.2**	**5.6**	**6.2**
Menthone	**1 153**	**16.1**	**18**	**14.6**	**13.8**	**20.9**
δ-Terpineol	1 166	0.2	–	0.3	–	0.1
Terpinene-4-ol	1 177	**2.8**	–	–	–	–
α-Terpineol	1 189	0.6	–	0.3	0.2	–
(Z)-Dihydrocarvone	**1 193**	**–**	**1.2**	**2.2**	**0.8**	**1.1**
Dihydrocarveol	1 194	–	0.1	0.2	–	
(E)-Carveol	1 217	–	–	–	–	0.1
Nerol	1 230	–	0.2	–	–	
Pulegone	**1 237**	**45.3**	**42.3**	**39.1**	**41.7**	**39.6**
cis-Piperitone epoxide	**1 254**	**2.6**	**–**	**5.8**	**5.3**	**5.5**
(E)-Anethole	1 285	0.2	0.2	0.2	0.1	0.1
Thymol	1 290	0.8	**1.1**	**1.2**	**1.3**	0.4
Menthyl acetate	1 295	–	–	–	–	0.2
Carvacrol	1 299	0.1	0.2	0.2	0.2	–
Piperitenone	1 343	0.6	**1**	0.8	0.8	0.3
α-Terpinyl acetate	1 349	–	0.3	0.2	0.2	0.2
Piperitenone oxide	**1 369**	**3.9**	**0.2**	**8.9**	**7.2**	**3.4**
α-Copaene	1 377	0.2	0.2	0.2	0.2	0.1
β-Bourbonene	1 388	0.1	0.1	0.1	0.2	0.1
β-Cubebene	1 388	–	0.1	0.1	0.1	0.1
β-Elemene	1 391	0.1	0.1	0.1	0.2	0.1
(E)-Caryophyllene	**1 419**	**3.8**	**4.3**	**3.5**	**4.2**	**2.3**
α-Humulene	1 455	0.8	**1**	0.8	**1**	0.5
Germacrene D	**1 485**	**1.3**	**1.4**	**1.2**	**1.6**	**0.9**
Bicyclogermacrene	1 500	0.2	0.2	0.2	0.3	0.2
β-Bisabolene	1 506	–	0.1	0.1	0.1	0.1
Δ-Cadinene	1 523	–	–	0.2	0.2	–
α-Cadinene	1 539	0.2	0.2	–	–	0.1
Spathulenol	1 578	–	0.1	0.1	0.6	0.1
Caryophyllene oxide	**1 583**	**1.1**	**1.7**	**1.6**	**1.7**	**0.6**
α-Cadinol	1 654	–	–	0.1	0.1	–
Total		97.8	93.7	95.3	96.3	98.9

Compounds are reported according to their elution order on apolar column.

recovered by salts with its highest recovery belonging to calcium carbonate. Sodium carbonate enhanced the recovery of some minor components such as limonene, (Z)-β-ocimene, trans-sabinene hydrate, piperitenone and α-humulene. Noticeably, all studied salts lacked terpinen-4-ol despite of its substantial (2.8%) amounts in the control. Furthermore, the control had a higher amount of γ-terpinene compared to salt treatments. Figure shows the GC/MS chromatograms of treatments. Salts may affect the extraction process of volatiles due to several chemical properties of ions from their dissipation. Mainly, the charge balance, hydration potential, solubility and the ratio and types of ions are of special attention. Also,

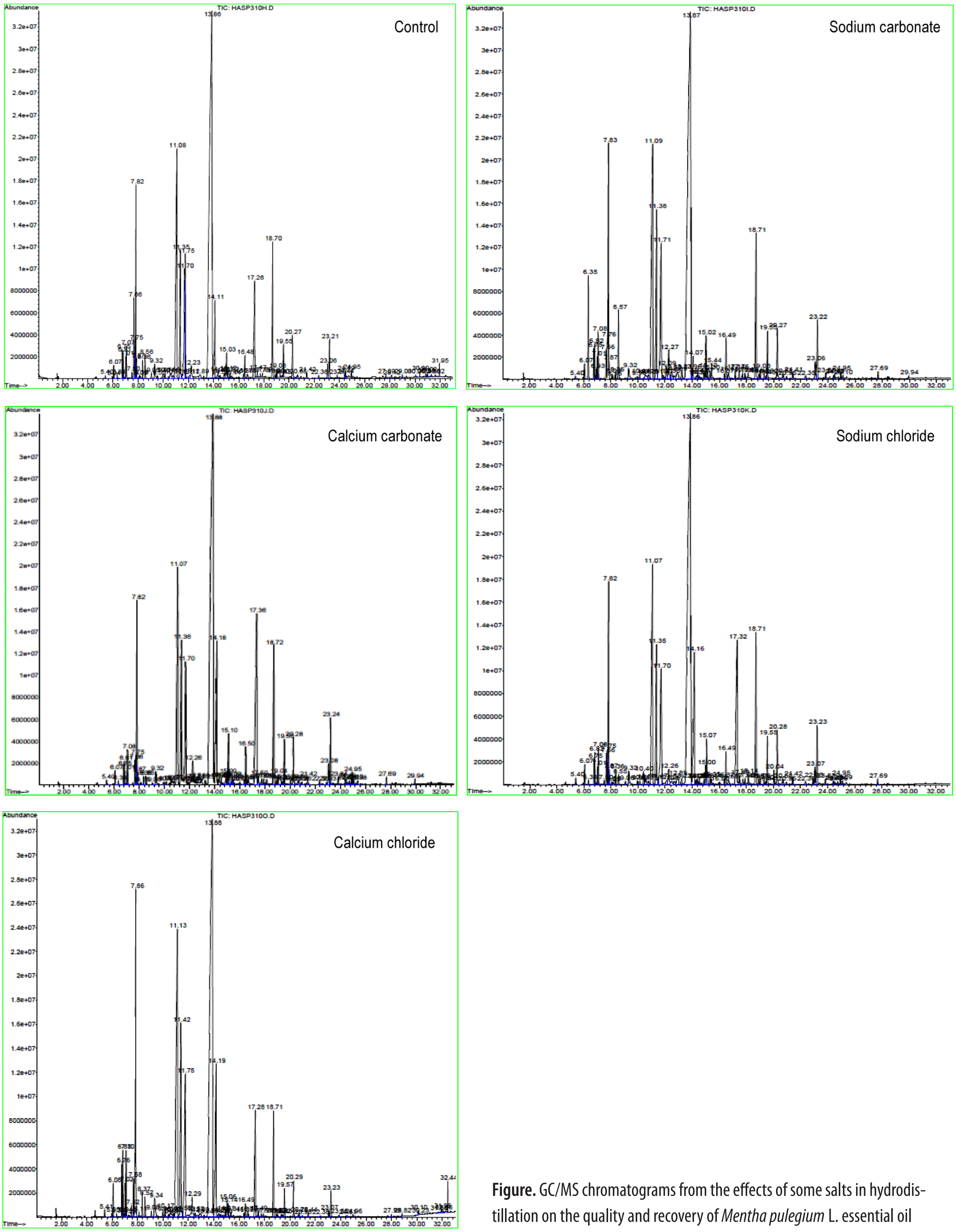

Figure. GC/MS chromatograms from the effects of some salts in hydrodistillation on the quality and recovery of *Mentha pulegium* L. essential oil in a defined concentration, diverse salt qualities undoubtedly affect solution characteristics. Moreover, owing to divergent properties (charge balance, solubility, spatial conformation, chemical structure, etc.) of oil components, their variant response to several salts in the extraction solution is greatly predictable. Salts ions in the aliquot form affect the tissue extraction and solubility of volatile components and besides other possible factors, this is principally salt and the related ions dependant. In the context of volatile constituents, surely, their proportional amounts and recovery potential may be influenced by the salt nature, ionic forms, ions solubility and other solutes related characteristics of the entire salt solution. In total, considering the proportional amounts of predominant constituents (pulegone, menthone, piperitenone oxide, isopulegol, 1,8-cineole, cis-piperitone epoxide and (E)-caryophyllene) of the volatile oil, it seems that a commercial large scale application of salt treatment for enhancement of volatile oil components recovery is highly dependent on the desirable constituents and / or the type and nature of salt employed. Furthermore, diverse concentrations of studied and other salts need to be explored for optimum decision making on a commercial basis.

Received 2 January 2012
Accepted 25 January 2012

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DRUSKŲ ĮTAKA *MENTHA PULEGIUM* L. ETERINIO ALIEJAUS KOKYBEI IR IŠEIGAI

Santrauka

Buvo tiriamas keturių druskų: natrio chlorido, natrio karbonato, kalcio chlorido ir kalcio karbonato įtaka *Mentha pulegium* L. eterinio aliejaus, gaunamo hidrodistiliacijos būdu, kokybei ir išeigai. Nustatyta, kad minėtos druskos turi akivaizdų poveikį distiliuoto aliejaus cheminei sudėčiai.