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Superheated Water Extraction of Essential Oils of *Origanum micranthum*

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Abstract

Superheated water extraction is used to extract essential oil of leaves of *Origanum micranthum*. The effect of different temperatures on the essential oil profile and rate of extraction as a function of time is investigated. The components of essential oil of *Origanum micranthum* are removed from the aqueous extract by C18 solid-phase extraction. The identification of components is carried out using comprehensive gas chromatography–time of flight-mass spectrometry. The number of extracted components is almost the same; however, the concentrations change with changing temperature. The highest yield (0.64%) is found at a temperature of 150°C, 2 mL/min and 60 bar for 30 min. The increasing temperature from 100°C to 175°C increased the rate of extraction of six selected components of essential oil of *Origanum micranthum*. cis-Sabinenehydrate exhibits the fastest rate of extraction at all temperatures studied. Some degradation products are observed at a temperature of 175°C.

Introduction

The genus *Origanum* is characterized by a large morphological and chemical diversity. Forty-nine taxa, divided into 10 sections, belong to this genus. Twenty one of them are local to Turkish endemics. The essential oils of the members of the *Origanum* genus vary in respect to the total amount produced by plants, as well as in their qualitative composition. *Origanum* essential oils are characterized by a number of main components, which are implicated in the various plant odors. *Origanum micranthum* Vogel is in the Chilocalyx (Briquet) Ietswaart section of the genus *Origanum* and is one of the endemic species of Turkey (1). Origanum plants are widely used all over the world as a very popular spice under the vernacular name "oregano". They are of great economic importance, which is related not only to their use as a spice. In fact, oregano is used traditionally in many other ways as their essential oils have antimicrobial, cytotoxic, and antioxidant activity.

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The common methods used currently for the isolation of essential oils from natural products are steam distillation and solvent extraction. Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects, and toxic solvent residue in the extract may be encountered with these extraction methods (2). Recently, more efficient extraction methods, such as supercritical fluid extraction (3,4) and accelerated solvent extraction (5), have been used for the isolation of organic compounds from various plants.

The recent analytical interest in superheated water as an extraction solvent began with the work of Hawthorne et al. (6) with the extraction of polar and nonpolar analytes from soil samples. Recently, a continuous superheated water extraction (SWE) technique has been used for the extraction of solid samples in a number of studies (7–10). More recently, a superheated water extraction technique has been reviewed by Smith (11). The term, superheated water, is used to denote the region of the condensed phase between 100°C and the critical point (374°C). The pressures required to maintain a condensed state of water are moderate: 15 bar at 200°C and 85 bar at 300°C. Previous researchers (7–11) have reported that superheated water for the extraction of essential oils is a powerful alternative because it enables a rapid extraction and the use of low working temperatures. This avoids the loss and degradation of volatile and thermo labile compounds. Additional positive aspects of the use of SWE are its simplicity, low cost, and favorable environmental impact.

The aim of this study was to determine the optimum temperature and the time of extraction at various temperatures for the continuous SWE of essential oils of *Origanum micranthum* leaves and to investigate the effects of temperature on the composition of extracted essential oils.

Experimental

Materials

Origanum micranthum Vogel was harvested at the preflow-

ering stage (May 2003) from an experimental field at Cukurova University, Adana (mid-Southern Turkey). The leaves were separated from the branches and the air-dried leaves were stored in a polyethylene bag until extraction.

Hexadecane (as an internal standard) was provided by Aldrich (Gillingham, Dorset, U.K.). Hexane, methanol, ethyl acetate, and water were of high-performance liquid chromatographic (HPLC) grade, supplied by Fisher Scientific (Loughborough, U.K.). DSC-18 solid-phase extraction (SPE) cartridges (500 mg/3 mL tube) were purchased from Supelco (Gillingham, Dorset, U.K.).

SWE

A detailed description of the laboratory-built SWE apparatus has been given elsewhere (7). The water was purged with nitrogen to remove dissolved oxygen prior to the extraction. Deoxygenated water was used in an HPLC pump programmed for a constant flow of 1–3 mL/min. A Carlo Erba series 4200 gas chromatography (GC) oven heated the extraction system. A 3-m (length) preheated coil (0.76-mm i.d. × 1.6-mm o.d.) was used to equilibrate the water to the desired temperatures. A 10.4-mL extraction cell (Keystone Scientific, Bellefonte, PA), equipped with 0.5-µm frit at the inlet and outlet, was connected to a 1-m cooling loop (in iced water; 0.76-mm i.d. × 1.6-mm o.d.) outside of the oven. A pressure control valve was placed between the cooling loop and the collection vial.

SWE was performed using 1.0 g of *Origanum micranthum* leaves, an extraction cell that contained a stainless steel filter and glass wool at both ends, 1–3 mL/min flow rate, temperatures of 100–175°C, pressures of 40–80 bar, and 30 min of extraction time. The collection vial was replaced every 5 min during the kinetic studies.

SPE

A DSC-18 SPE cartridge was used to re-extract the analytes with hexane–ethyl acetate (9:1). The DSC-18 cartridge was first washed with 4 mL of methanol, followed by 4 mL of water. The sample was then loaded at an approximately 2-mL/min flow rate and washed with 4 mL of water. Then it was dried for 5 min using a vacuum and 5 min using a nitrogen gas flow. Following the drying, the DSC-18 cartridge was eluted with 4 mL hexane–ethyl acetate (9:1) mixture. The collected eluent was concentrated under a nitrogen stream to an approximate volume of 0.5 mL. An appropriate amount of hexadecane was added into concentrate as an internal standard. The mixture (1 µL) was directly injected into the comprehensive GC (GC×GC)–time of flight (TOF) mass spectrometry (MS) system.

Chromatographic analysis

The GC×GC–TOF-MS system consisted of an HP 6890 (Agilent Technologies, Palo Alto, CA) GC and a Pegasus III TOF-MS (LECO, St. Joseph, MI). The first column was a nonpolar DB5 (10-m × 0.18-mm i.d., 0.18-µm film thickness) and the second column a polar DB17 (1.6-m × 0.18-mm i.d., 0.18-µm film thickness). Both columns were purchased from J&W Scientific (Folsom, CA). The columns were connected by means of a press-fit-connector. The second-dimension column was installed in a separate oven, which was maintained in the main GC oven. The separate oven provided a more flexible system because it allows fine-tuning of the retention in the second column using a higher or lower temperature relative to the first dimension column. In this particular system, the need to use a two-oven system was driven by detector stability considerations, requiring accurate and stable control of the secondary column temperature. This temperature control of both ovens enables more rapid and higher resolution separations.

The system does not require any valving or switching facilities. The modulator is the key to the performance of the GC×GC experiment. Cryogenic modulation was performed using a jet-type modulator that was installed at the top of the second dimension column. This consists of two cold and two hot jets, with the nozzles providing the cold jets mounted orthogonal to the hot jets. Nitrogen gas is cooled by heat exchange through copper tubing immersed in liquid nitrogen outside the GC system and delivered through vacuum-insulated tubing to the cold jets, which provide two continuous jets of approximately 10 L/min of cold nitrogen gas. The modulation time was 10 s. When the hot downstream pulse is fired, the analytes are effectively injected into the second-dimension column. A detailed description of the setup is given elsewhere (12).

Helium was used as a carrier gas. The initial temperature of the first dimension column was 35°C for 30 s and the subsequent temperature program was a heating rate of 5°C/min until 250°C was reached. The initial temperature of the second-dimension column was 50°C for 30 s, and a 5°C/min heating rate was used until 265°C. Peak identification was made using TOF-MS with electron impact ionization. The MS used a push plate frequency of 5 kHz, with transient spectra averaging to give unit resolved mass spectra between 45 and 350 amu at a rate of 50 spectra/s. Mass spectra were compared against the National Institute of Standards and Technology '98 (NIST, Gaithersburg, MD) mass spectral library.

Results and Discussion

It has been discussed earlier that the separation of the compounds from the aqueous extract obtained by SWE is the critical stage (7). The SPE has been applied in many cases (13,14). Recently, SPE was used by Rovio for the separation of essential oils of clove (15) and by Perez & Castro for the separation of cholesterol from the aqueous extract (16). They reported that both essential oils of clove and cholesterol were successfully removed from aqueous extract by SPE with a packing material of C18.

The efficiency of C18 material for the separation of essential oils of *Origanum micranthum* was tested with a steam distilled sample of the essential oils *of Origanum micranthum* with a known composition. It was found that the efficiency of the C18 material changed in a range of 93–100% for the components of the essential oils of *Origanum micranthum*. The final concentrations of the components were correlated according to their efficiencies found from these preliminary studies.

The pressure was studied in the range of 40–80 bar. There was very little effect of pressure studied on the yield of essential oil as long as the extractant water was kept in its liquid state. The pressure was maintained at 60 (± 5) bar throughout all extractions. As

described in earlier studies (7,8), the lower pressures were possible to use. However, higher pressures with lower percentage errors were preferred.

The extractant water flow rate was studied in the range 1.0–3.0 mL/min at a constant pressure of 60 bar and a temperature of 150°C. It was found that 2 mL/min was optimum from the standpoint of both time and yield. A optimum flow rate of 2 mL/min was found by Ozel et al. for the essential oils of *Thymbra spicata* (7), Gamiz-Gracia and Luque de Castro for the essential oils of fennel (8), and Fernandez-Perez et al. for the essential oils of laurel (17).

Yields of essential oils at temperatures of 100°C, 125°C, 150°C, and 175°C for a 30-min extraction at a flow rate of 2 mL/min and 60 bar were 0.51, 0.60, 0.64, and 0.49, respectively. The yields are given as weight percent. They are the means of the three experiments and the relative standard deviation was in a range of 1–4%. Baser et al. (18) found that the yield of essential oils of *Origanum micranthum* by hydrodistillation is 0.5%. This value is slightly lower than the yield obtained by SWE at optimum conditions. The yield increased with temperature up to 150°C. A further increase to 175°C resulted in an important decrease in yield. At 175°C, superheated water extracts were dark brown with a burning smell after the start because of high temperature. Therefore, the optimum temperature for the extraction of essential oils of leaves of *Origanum micranthum* was chosen as 150°C. It was found by Ozel et al. (7) for *Thymbra spicata* and Gamiz Gracia and Luque de Castro (8) for fennel extraction by SWE that the overall yield of essential oils reached their maximum at 150°C

Table I. Compounds, Retention Times, Percentage Compositions of *Origanum micranthum* Essential Oil Constituents for Various Temperatures of Superheated Water Extraction

Compound*	^1t_R† (s)	^2t_R† (s)	(%)‡ 100°C	125°C	150°C	175°C	Compound*	^1t_R† (s)	^2t_R† (s)	(%)‡ 100°C	125°C	150°C	175°C
ethyl Propionate	100	7.54	2.15	1.76	1.40	1.24	Dodecane	710	3.48	tr	tr	tr	tr
Tropilidene	120	8.08	tr§	tr	0.11	nd**	α-Terpineol	710	6.74	43.63	42.95	47.28	44.14
3-Hexanone	160	2.22	nd	nd	nd	0.12	cis-Piperitol	730	6.06	0.84	0.74	0.33	nd
p-Xylene	220	3.88	tr	0.25	0.70	1.18	Perillol	780	6.32	0.15	0.30	nd	nd
[8] Annulene	240	4.76	tr	0.11	0.15	0.35	1-Carvone	790	7.54	tr	tr	tr	nd
Furfural	240	4.84	nd	nd	nd	0.73	Linalyl anthranilate	810	5.24	0.55	0.75	0.93	1.65
3-Furaldehyde	260	4.62	nd	nd	nd	0.65	Nerol	820	5.92	1.74	1.06	0.68	0.21
α-Thujene	290	3.26	nd	nd	nd	tr	Epoxylinalol	920	7.44	tr	0.18	nd	nd
α-Pinene	300	4.28	nd	tr	tr	0.27	Nonanoic acid	970	3.94	0.41	0.30	0.26	nd
Camphene	320	3.70	nd	nd	nd	0.12	Tetradecane	1030	3.48	tr	tr	0.15	0.11
5-methyl-3-Heptanone	340	4.56	0.12	0.29	tr	nd	Caryophyllene	1040	5.58	nd	nd	0.39	0.46
Benzaldehyde	350	7.10	nd	nd	nd	0.40	8-Hydroxylinalool	1060	7.06	tr	nd	nd	nd
1-Octen-3-ol	380	4.16	0.31	0.27	0.18	tr	8-Hydroxycarvotan-acetone	1090	9.32	0.33	0.39	0.34	0.14
Decane	400	2.88	tr	0.10	0.22	0.59							
3-Octanol	400	4.04	0.42	0.71	0.31	0.29	(D)-Germacrene	1130	5.90	nd	nd	0.16	0.36
α-Phellandrene	430	4.52	tr	0.15	0.23	0.43	Bisabolene	1180	5.26	nd	nd	0.24	0.21
m-Cymene	430	4.94	tr	0.17	0.18	0.19	cis-Farnesol	1190	5.84	0.34	0.74	1.20	2.45
Eucalyptol	440	4.80	tr	0.22	nd	nd	α-Cadinene	1200	5.92	nd	nd	tr	0.53
Linalool oxide	510	5.06	0.22	0.37	0.16	0.10	cis-3-Hexenyl benzoate	1260	8.08	nd	nd	tr	tr
Limonene	520	6.88	tr	0.11	0.36	0.44							
terpinolene	540	4.70	0.60	1.21	2.04	2.83	Spathulenol	1270	7.16	0.15	0.40	0.62	1.14
(Z)-α-Terpineol	550	5.96	22.31	19.17	17.40	13.11	Caryophyllene oxide	1270	7.32	tr	0.14	0.24	0.30
γ-Terpinene	550	6.10	1.36	0.87	0.63	0.21	3-Hexen-1-ol	1270	7.84	tr	tr	tr	nd
Undecane	560	3.12	0.40	0.46	0.51	0.93	1-Octadecene	1300	3.82	tr	tr	0.43	tr
Linalool	560	5.14	4.80	5.96	3.95	3.89	Eicosane	1370	3.56	0.27	0.21	0.38	1.17
cis-Sabinenehydrate	560	5.40	3.75	3.91	2.31	1.75	α-Cadinol	1370	7.26	nd	nd	1.09	2.05
Camphore	610	7.42	1.53	1.65	0.99	1.02	Pentacosane	1390	3.58	tr	tr	tr	0.43
(S)-cis-Verbenol	630	6.04	tr	tr	nd	nd	Dodecanoic acid	1390	4.10	tr	nd	nd	nd
Sabina ketone	640	8.04	tr	0.10	tr	nd	Lauric anhydride	1440	3.72	0.10	tr	tr	tr
Borneol	660	6.32	2.37	2.78	2.54	2.49	methyl Linolenate	1970	6.82	nd	tr	0.16	0.61
Terpinen-4-ol	680	5.96	6.39	7.26	7.54	5.51	Unknown			3.79	3.37	2.66	4.74
Naphthalene	680	8.88	tr	tr	tr	nd	Total			100.00	100.00	100.00	100.00

* As identified by GC×GC–TOF-MS software; names according to NIST mass spectral library.

† ^1t_R and ^2t_R, retention times in the first and second dimension, respectively. In the case of multiple identification, the retention time of the best spectral matching peak is shown. If the component is present in more than one temperature application, retention times were given for 150°C; all first dimension retention times were within ± 10 s and second-dimension retention times were within 0.12-s agreement.

‡ Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100. In the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte.

§ Trace (less than 0.1 %).

** Not detected.

over studied temperature ranges of 50–200°C.

Table I is a list of the compounds identified in *Origanum micranthum*, with their retention times and relative abundances. It was seen that the change in temperature resulted in a change of components and compositions of the extracted essential oils. The number of components extracted at 100°C, 125°C, 150°C, and 175°C was 46, 46, 48, and 48, respectively. The actual number of components for each temperature studied was more than 80 in this study. However, even if they were the components defined definitely by GC×GC–TOF-MS, the components with a percentage of less than 0.05 were not shown in this paper. Baser et al. (18) found 43 components for the essential oils of hydrodistilled herbal parts of *Origanum micranthum* Vogel. As seen in Table I, the major components of essential oils of leaves of *Origanum micranthum* Vogel are α-terpineol, (Z)-α-terpineol, and terpinen-4-ol for all temperatures studied. Baser et al. (18) found that linalyl acetate, cis-sabinenehydrate, α-terpineol, linalool, and terpinen-4-ol were the major components. Even if there is a similarity between the major components, their concentrations are quite different from each other.

The number of components common at all temperatures is 33. Some of the components are available only at low temperatures and some available only at high temperatures in essential oils obtained by SWE. Some of the components that appear at high temperatures might be browning reaction products (furfural, 3-furaldehyde); however, some of them are most likely browning components that are not soluble at low temperatures. Miller and Hawthorne (19) also reported that the solubilities of pure forms of d-limonene, carvone, and eugenol increased with increasing temperature from 25°C to 200°C. They found that nerol also increased with increasing temperature but was not determined at 200°C because of degradation, and eucalyptol showed a decrease at up to 100°C and then a slight increase with further increase in temperature. It was also found that both of the components (eucalyptol and nerol) decreased with increasing temperature in this study. This could be explained with the possible degradation of these components. Z-α-terpineol, γ-terpinene, cis-sabinenehydrate, and ethyl propionate also showed similar patterns of decrease with increasing temperature.

Conventional one-dimensional GC generally does not provide sufficient separation for complex mixtures. Because essential oils contain numerous components, it is possible that some components can obscure the analytes of interest (20). As can seen in Table I, there are some components that can only be separated on the second column. For example, even if the first dimensions of undecane (560, 3.12), linalool (560, 5.14), cis-sabinenehydrate (560, 5.40), dodecane (710, 3.48), and α-terpineol (710, 6.74) are the same, they have different second dimensions that make their separation from each other possible. The essential oil contains around 47 compounds, and it is easy to see all of them here.

The kinetics of SWE under optimum pressure and extractant flow-rate conditions were studied at temperatures of 100°C, 125°C, 150°C, and 175°C to observe the effect of temperature on the extraction time of selected components. The experiments were carried out for 30 min to obtain a value for the 33 components common at all temperatures studied. The rate of extraction for six main common compounds at 100°C can be quantitatively inferred from Figure 1. The extraction of all components completed in 25 min at 100°C. The cis-sabinenehydrate was extracted in only 15 min at the same temperature. The increase in temperature from 100°C to 125°C increased the rate of extraction and the extraction completed in 20 min for all components. Gamiz-Gracia and Luque de Castro (8) also reported that 20 mL of superheated water was enough to complete the extraction of monoterpene compounds of essential oil of fennel in 10 min at 150°C. All of the components were extracted completely in first 5 min at 175°C, except for linalool and terpinen-4-ol.

cis-Sabinenehydrate, (Z)-α-terpineol, and α-terpineol were extracted more rapidly than the other components (linalool, borneol, and terpinen-4-ol) at all temperatures studied. The rate of extraction for all components increased with increasing temperature. This increase was more apparent for cis-sabinenehydrate, (Z)-α-terpineol, and α-terpineol. Figure 2 shows the effect of temperature on the extraction time of α-terpineol. Rovio et al. (15) also found a similar pattern of increase for eugenol and eugenyl acetate in a temperature range of 125–300°C. The kinetic study performed here clearly demonstrated SWE to be a faster technique than conventional essential oil production techniques.

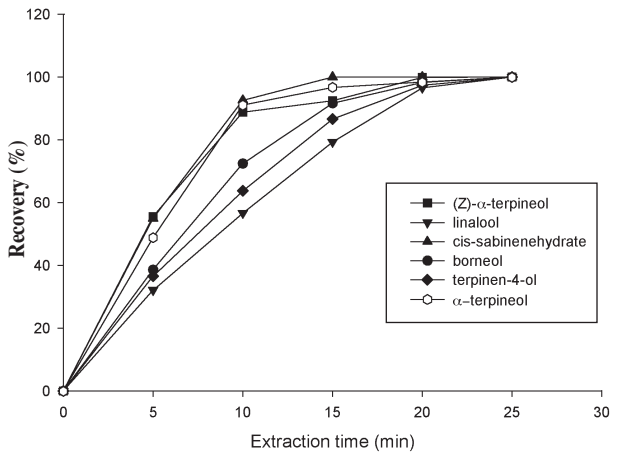

Figure 1. Effect of extraction time on the extraction efficiency of some compounds of essential oil of *Origanum micranthum* using SWE at a temperature of 100°C (pressure, 60 bar; water flow rate, 2 mL/min).

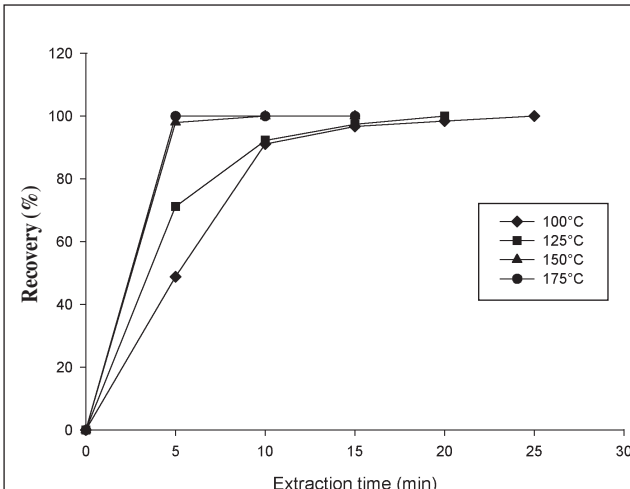

Figure 2. Effect of temperature on the superheated water extraction time of α-terpineol (pressure, 60 bar; water flow rate, 2 mL/min).

Conclusion

Extraction of essential oil of *Origanum micranthum* leaves was studied by using superheated water at various temperatures. The collection of the components from the aqueous extract was achieved using a C18 SPE material. Compherensive GC×GC–TOF-MS successfully achieved the separation and identification of the components that cannot be separated by a one-dimensional technique. Superheated water extract at 150°C gave a yield of 0.64%, which is almost 30% higher than that of water distillation (18). The essential oil composition changed with changing temperature. The browning reaction products were observed at a temperature of 175°C. Extraction kinetic studies showed that the extraction is very fast at high temperatures (175°C), giving 100% recovery after 5 min compared with the extraction at 100°C in which the same recovery was achieved only after 25 min extraction. A superheated water extraction technique is a notably faster extraction method than conventional essential oil production techniques, as well as being clean, cheap, and environmentally friendly.

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