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Continuous subcritical water extraction as a useful tool for isolation of edible essential oils

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Abstract

A method for extraction of oregano oil based on the use of subcritical water is proposed. Ground oregano (ca. 1.8 g accurately weighed) was extracted with water at 2.0 MPa, 125 °C and 1 ml min^{-1} for 24 min. The extracted compounds were removed from the aqueous extract by a single extraction with 4 ml hexane, determined by gas-chromatography-flame ionisation (GC-FID) and identified by mass spectrometry (MS). Hydrodistillation was performed on 30 g of ground oregano with 500 ml water for 3 h for comparison of the results with those provided by the proposed method. The subcritical water extraction-based method is clearly quicker and more efficient than the conventional: yields from the former for eight of the 11 major compounds in the extract are higher after 15-min extraction than after hydrodistillation for 3 h. This enables substantial cost savings of both energy and raw material. In addition, the proposed method has the possibility of manipulating the composition of the oil and thus its quality. The aqueous-oil extract was investigated for essential oil preparative purposes and several fast, cheap and clean alternatives for breaking the emulsion and separating the phases are proposed. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Continuous subcritical water extraction; Esential oil, oregano

1. Introduction

Continuous subcritical water extraction (SWE) is a technique based on the use of water as extractant, at temperatures between 100 and 374 °C (critical point of water, 22.4 MPa and 374 °C) and pressure high enough to maintain the liquid state. This technique is emerging as a powerful alternative for the extraction of solid samples (Luque de Castro & Jiménez, 1998). It has been used to extract pollutants with a wide range of polarities from environmental samples (Hawthorne, Yang, & Miller, 1994; Yang, Bowadt, Hawthorne, & Miller, 1995), and for the extraction of pesticides (Jiménez, Manclús, Montoya, & Luque de Castro, 1997) and polycyclic aromatic hydrocarbons (Kipp, Peyrer, & Kleiboehmer, 1998) from soils.

The use of SWE as a very promising alternative to conventional and supercritical CO_2 extraction-based methods for isolation of essential oils was proposed by Basile, Jiménez, and Clifford (1998). Since this date the technique has shown its applicability in the field of essences (Fernández, Jiménez, & Luque de Castro, 2000; Gámiz-Gracia & Luque de Castro, 2000; Jiménez, Ubera, & Luque de Castro, 1999; Jiménez & Luque de Castro, 1999) as compared with conventional techniques like steam distillation (Chalchat, Garry, & Michet, 1991; De-Pooter, Aboutabl, & El-Shabrawy, 1995) and solvent extraction (Verma, Uniyal, & Gupta, 1990), which have some well-known disadvantages; namely, low extraction efficiency, long extraction time and large amounts of toxic solvent waste.

Subcritical water enables rapid extraction and the use of low working temperatures, thus avoiding losses and degradation of volatile and thermolabile compounds. It has also the advantage of being selective, because by means of kinetic experiments, it is possible to manipulate the extract composition under given working conditions.

The aim of this research was to develop a method for the continuous SWE of oregano essential oil, and compare the results with those obtained by hydrodistillation. Oregano is a herbacean plant which has medical (anthelmintic, antiasthmatic and fungicide) properties

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and it is widely used in the preparation of a great variety of foods. The use of SWE applied to the oregano plant has been aimed at proposing a faster and cheaper way of obtaining its essential oil with better quality than that provided by the conventional method.

2. Experimental

2.1. Instruments and apparatus

The SWEs were performed using an extractor [a prototype designed and patented by Salvador & Merchán (US Pat, 1995)] which consisted of a 14.5 ml extraction cell (Keystone Scientific, Bellefonte, PA, USA) closed at both ends with screw caps enabling the circulation of the extractant. To keep the plant material inside the cell, the screw caps also contained steel filter plates (2 μm thick and $\frac{1}{4}$ inch in diameter). This chamber, together with a stainless steel preheater, was located in an oven, designed to work up to 300 °C and controlled using a Toho TC-22 temperature controller. A loop made from 1 m length stainless steel tubing and cooled with water at 25 °C, was used to cool the fluid, thus avoiding losses of volatile compounds.

The water was propelled through the system by means of a Merck Hitachi L-6200A high pressure pump.

Hydrodistillation was performed with a simple laboratory Quickfit apparatus, which consisted of a 2-l steam generator flask, a distillation flask, a condenser and a receiving vessel.

The extracts were analysed by means of a Varian Star 3400 gas chromatograph equipped with a flame ionisation detector (FID). A Fisons VG Autospec (Micromass Instruments) mass spectrometer was used to identify the compounds in the extracts.

A Beckman Optima^{TM} ultracentrifuge was also used.

2.2. Chemical and materials

Oregano (*lippia graveolens*) leaves were collected in the South of Spain (Córdoba), ground, homogenised and stored at −4 °C until use in order to avoid losses of volatile compounds. A stock standard solution of 17 000 μg ml^{-1} of nonane (Sigma, St. Louis, MO, USA) in HPLC grade hexane (Scharlau, Barcelona, Spain) was prepared and used as the internal standard. After leaching, the water-oil emulsion was extracted for removal of the extracted species into an organic solvent. For the liquid–liquid extraction step NaCl, Na_2SO_4 (both from Merck, Darmstadt, Germany) and HPLC grade hexane were used as demulsifier, drying agent and extractant, respectively. Bidistilled degassed water purified through a Milli-Q deionising unit (Millipore) was used as extractant.

All the gases were of 95% purity or higher (Carburos Metálicos, Barcelona, Spain).

2.3. SWE

The cell was filled with ground oregano (ca. 1.8 g accurately weighed). A glass-wool plug was inserted at the outlet of the chamber to prevent the frit from being plugged. Degassed Milli-Q water stored in a reservoir was pumped into the oven where it reached the preheater and the cell. At the outlet of the oven the aqueous extract was cooled to ca. 25 °C in a cooling loop and, after passage through a variable restrictor, it was collected in a vial. The kinetic experiments were carried out under the optimum working conditions by changing the vial every 5 min. After SWE, a liquid–liquid extraction step was performed, in which 4 ml hexane and 100 μl nonane stock standard solution were added to the extract in a separating funnel, and about 1 g NaCl was also added to facilitate the breaking of the emulsion. The hexane layer was then separated and dried with anhydrous sodium sulphate before gas chromatography (GC) analysis.

2.4. Hydrodistillation

Ground oregano (30 g) was placed in the steam generator flask and Milli-Q water (500 ml) was added. The distillate collected in the receiving flask after 3 h distillation was 0.75 ml.

2.5. Chromatographic separation and detection

Aliquots of 1 μl hexane after either SWE or hydrodistillation plus liquid–liquid extraction in both cases, were injected into an MFE-73 fused silica column (25m×0.25mm i.d., 0.25 μm film thickness). The carrier gas (helium) was delivered at a flow rate of 2 ml min^{-1} and the column head pressure was ca. 0.10 MPa. The detector temperature was 300 °C and the oven temperature was kept at 50 °C for 2 min after injection, then increased to 200 °C at 5 °C min^{-1}.

Table 1
Optimisation study

Step/parameter	Range studied	Optimum value
Subcritical-water extraction		
Temperature (°C)	100–175	125
Pressure (MPa)	1.0–5.1	2.0
Flow-rate (ml min^{-1})	1.0–4.0	1.0
Liquid–liquid extraction		
Extractant volume (ml)	2.0–5.0	4.0
Number of extraction steps	1–2	1
Chromatography		
Carrier gas	–	Helium
Injection volume (μl)	–	1
Column	–	MFE - 73
Split ratio	1:5–1:50	1:11
Carrier gas flow-rate (ml min^{-1})	1.0–2.0	2.0
Rate of temperature gradient (°C min^{-1})	2.0–5.0	5.0

3. Results and discussion

3.1. Optimisation study

First, the variables that influence the chromatographic step were optimised and then, those affecting the extraction in order to maximise the yield of essential oil in a time as short as possible. Both types of variables were studied by the univariate method, and the optimum values found in both cases are shown in Table 1, together with those of the liquid–liquid extraction step.

3.2. Optimisation of SWE

The variables affecting SWE are temperature, pressure and flow-rate of the extractant.

3.2.1. Effect of temperature

The temperature of the extraction chamber is a key variable of the extraction process. Its influence was studied between 100 and 175 °C at 25 °C intervals, at a constant flow-rate of 1.0 ml min^{-1} and a pressure of ca. 2.0 MPa. The chromatograms obtained from the extract revealed that the yield of all the compounds present in the extract increased with increased temperature up to 125 °C and then dropped as the temperature was increased to 175 °C, meanwhile a brown colour of the extract indicated degradation of the extracted species. Because the best yield was obtained at 125 °C, this temperature was selected as optimum.

3.2.2. Effect of pressure

By means of the variable restrictor, the effect of the pressure at a constant flow-rate of 1.0 ml min^{-1} and a temperature of 125 °C was studied. It was found that this variable has little influence on the yield, because there were no significant differences in the chromatograms obtained from the corresponding extracts. Pressures tested from 1.0 to 5.1 MPa showed the null influence of this variable above the value sufficient to keep the liquid state at the working temperature. It is worth mentioning that the saturation pressure of water at 125 °C is ca. 0.3 MPa, so a higher pressure keeps the water as compressed liquid. A pressure of 2.0 MPa was selected for further experiments as this pressure was almost set up by the extraction chamber during its operation.

3.2.3. Effect of flow-rate

The flow-rate was studied in the range 1.0–4.0 ml min^{-1} at a constant temperature of 125 °C and pressure of ca. 2.0 MPa. The yield of all compounds was better at 1.0 ml min^{-1} than at other volumetric flows. At 2.0 ml min^{-1}, only two compounds gave higher yields than those obtained at 1.0 ml min^{-1}. Thus, this value was selected as optimum.

3.3. Conditions used for liquid–liquid extraction

Both the extractant volume and number of extraction steps were studied.

3.3.1. Extractant volume

Volumes of hexane between 2.0 and 5.0 ml were investigated for removal of the compounds from the aqueous-oil emulsion extract and preconcentration before GC analysis. The optimum value found was 4.0 ml.

3.3.2. Number of extraction steps

To ensure that no compounds remained in the aqueous extract, a second extraction with hexane using the same volume as in the first extraction was carried out. A flat chromatogram was obtained with the hexane extract from the second extraction, thus showing that a single extraction with hexane was enough for total removal of the compounds.

3.4. Conditions used for chromatography

The main conditions studied were: split ratio, carrier flow-rate and temperature programming.

3.4.1. Split ratio

Splitless injection gave rise to off-scale peaks, making it mandatory to work in the split mode. From the different ratios tested, an 1:11 value gave the highest peaks. Thus, this value was selected as optimum.

3.4.2. Carrier flow-rate

The split ratio selected gave a helium flow-rate of ca. 2.0 ml min^{-1} (measured at the column outlet) with a column head pressure of 0.1 MPa. This resulted in well-resolved chromatographic peaks.

3.4.3. Temperature programming

A temperature program comprising an initial temperature of 50 °C, held for 2 min after injection, then increased to 200 °C at 5 °C min^{-1} provided separation of the major compounds in 25 min.

3.5. Kinetics of the leaching process

The kinetics of SWE under the optimum working conditions was studied according to the following experiment: ca. 1.8 g of ground oregano was subjected to SWE and six aqueous extracts of 5 ml each were successively collected from the beginning of the extraction. The rate of extraction for each compound can be quantitatively evaluated from Fig. 1. As a consequence of the optimum conditions chosen, the kinetics of the SWE is fast for most compounds. Thus, within the first 15 min, the extraction is almost complete.

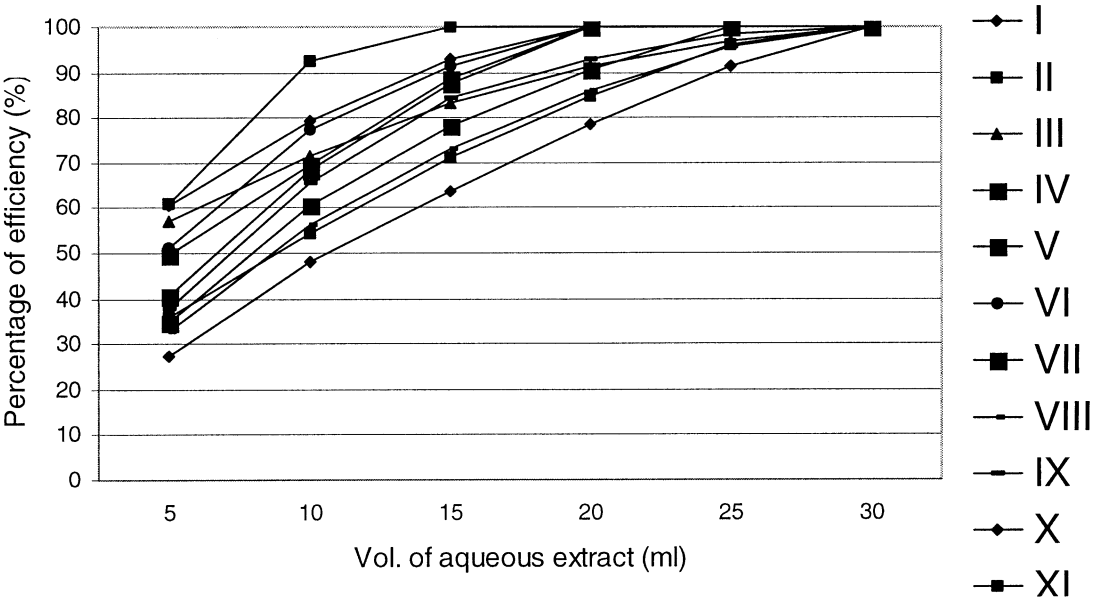

Fig. 1. Kinetic curves obtained by subcritical-water extraction (SWE) under the optimum working conditions for 1,3- cyclohexadiene, 1-methyl-4-(1-methylethyl) (**I**), α-phellandrene (**II**), 3-carene (**III**), unidentified (**IV**, **V**, **VI**, **VII**), 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) (**VIII**), 3-cyclohexene-1-methanol, β...β...,4-trimethyl (**IX**), thymol (**X**), phenol, 2-methyl-5-(1-methylethyl) (**XI**). Note: The following compounds were completely extracted in the first aliquot: caryophyllene; cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylidene) and diethyl phthalate.

3.6. *Obtainment of essential oils for food uses*

In previous sections the extraction step has been optimised. In order to propose an overall process to obtain edible essential oils, a step which enabled the removal of the extracted oil free either from water and/or organic solvent was investigated. Experiments were carried out in order to break the emulsion thus obtaining a two immiscible phases system. Three strategies were assayed; namely, centrifugation, lyophilisation and addition of chemical reagents.

3.6.1. *Centrifugation*

The water–oil emulsion was subjected to centrifugation during 30 min at 330 000 ***g*** and 4 °C. No phase separation was detected despite the drastic working conditions used.

3.6.2. *Lyophilization*

A sample of water–oil emulsion was subjected to lyophilization in order to eliminate water. The fact that water extracted not only oil, but also other soluble substances in the extractant under the working conditions, was demonstrated by the composition of the lyophilised mixture consisting of powder impregnated with the essential oil.

3.6.3. *Chemical reagents*

It is known that salts can break emulsions formed between oil and water by a salting out effect. Two samples of water–oil emulsion were used to observe this effect. NaCl was added to one of them, shaken for 1 min and then allowed to stand for a while. Separation of water–oil phases was observed. When the experiment was carried out with hydrochloric acid, the same result was obtained. Any of these two reagents can be used for obtaining the essential oil from the extract.

3.7. *Comparison of SWE and hydrodistillation*

Hydrodistillation was carried out under the working conditions described in Section 2. The comparison of both methods shows that the method based on SWE is quicker than its hydrodistillation-based counterpart. Thus, the time required for the overall process is clearly reduced by the proposed method (less than 30 min for SWE versus 3 h for hydrodistillation). The proposed method is more efficient than hydrodistillation as can be inferred from Table 2, where the areas ratio for the majority of the compounds, from both methods, are shown. Such ratios are higher than unity for eight of the 11 major compounds after 15 min extraction than after hydrodistillation for 3 h.

The cost of the extraction is clearly advantageous for the proposed method. The energy cost required to perform hydrodistillation was ca. 20 times higher than that required for SWE. This feature is of fundamental importance for the potential future implementation of this technique at an industrial scale.

SWE allows the manipulation of the composition of the extract by changing extraction parameters such as temperature and flow-rate.

A positive aspect of hydrodistillation is the spontaneous separation of the two immiscible phases; meanwhile the

Table 2
Subcritical-water extraction peak area/hydrodistillation peak area ratio (R) for gas chromatography (GC) of extracts obtained under the optimum working conditions

Compound	R
1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl)	0.80
α - Phellandrene	1.33
3 - Carene	0.70
Isopulegol	0.47
Unidentified	1.50
Unidentified	2.40
Unidentified	2.10
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	6.41
3-Cyclohexene-1-methanol,. . .β. . .β. . .,4-trimethyl	5.82
Thymol	5.83
Phenol, 2-methyl-5-(1-methylethyl)	5.55

SWE extract is an emulsion which requires to be broken by an external agent.

4. Conclusions

A study of the features of the oil extracted by subcritical water has been developed combining the dynamic extraction with gas-chromatography-flame ionisation (GC-FID) and gas chromatography–mass spectrography (GC–MS) for the determination and identification of the compounds, respectively.

The method proposed for the isolation of oregano essential oil is quicker, cheaper and more effective than hydrodistillation. It has also the possibility of manipulating the composition of the oil by changing the parameters of the extraction (temperature and flow-rate, mainly), doing this method more selectively than the conventional method. These reasons make SWE a promising tool for the extraction of essential oils from plants, of great concern in the food industry.

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