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Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods

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Abstract

Essential oil of *Carum copticum* cultivated in Iran was obtained by hydrodistillation and supercritical (CO_2) extraction (SFE) methods. The oils were analysed by capillary gas chromatography, using flame ionization and mass spectrometric detection. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters, such as pressure, temperature, modifier volume and extraction time, on the supercritical fluid extraction of *C. copticum* oil were investigated. The results showed that, under pressure of 30.4 MPa, temperature 35 °C, methanol 0% and dynamic extraction time of 30 min, the method was most selective for the extraction of thymol. Eight compounds were identified in the hydrodistilled oil. The major components of *C. copticum* were thymol (49.0%), γ-terpinene (30.8%), *p*-cymene (15.7), β-pinene (2.1%), myrcene (0.8%) and limonene (0.7%). However, by using supercritical carbon dioxide under optimum conditions, only three components constituted more than 99% of the oil. The extraction yield, based on hydrodistillation was 2.8% (v/w). Extraction yield based on the SFE varied in the range of 1.0–5.8% (w/w) under different conditions. The results show that, in Iranian *C. copticum* oil, thymol is a major component.

Keywords: *Carum copticum*; Supercritical; Carbon dioxide; Hydrodistillation; Essential oil; Thymol

1. Introduction

Carum copticum is a grassy, annual plant, which grows in the east of India, Iran, and Egypt, with white flowers and small, brownish seeds. The seeds of *C. copticum* have several therapeutic effects, including diuretic, antivomiting, analgesic, antiasthma, and antidyspnea effects. They also have a therapeutic effect on some cutaneous, neural, and urinary tract disorders. *C. copticum* is, therefore, used in household remedies. A watery extract of this plant is widely used to relieve flue in children (Boskabady & Shaikhi, 2000).

The essential oil of plants has usually been isolated by either hydrodistillation or solvent extraction. The disadvantages of all these techniques are: low yield, losses of volatile compounds, long extraction times, toxic solvent residues, and degradation of unsaturated compounds, giving undesirable off-flavour compounds, due to heat (Doneanu & Anitescu, 1998; Ebrahimzadeh, Yamini, Sefidkon, Chaloosi, & Pourmortazavi, 2003; Illes, Daood, Perneczki, Szokonya, & Then, 2000; Oszagyan et al., 1996; Poiana, Sicari, & Mincione, 1998).

Supercritical fluid extraction (SFE) is an interesting technique for the extraction of flavouring compounds from vegetable materials. It can constitute an industrial alternative to solvent extraction and steam distillation processes. SFE allows a continuous modification of solvent power and selectivity by changing the solvent density. Nevertheless, the simple SFE process, consisting

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of supercritical CO_2 extraction and a one-stage subcritical separation, in many cases does not allow a selective extraction because of the simultaneous extraction of many unwanted compounds. This situation is typical of CO_2 SFE of essential oils from herbaceous material in which, even when the process is conducted under conditions that produce the optimum oil composition, cuticular waxes are co-extracted because of their lipophilic character and their localization on the leaf surface. SFE, followed by fractional separation of the extract in multiple-stage separators, overcomes these limitations and produces high-quality essential oils (Anitescu, Doneanu, & Radulescu, 1997; Baysal & Starmans, 1999; Devittori et al., 2000; Eikani, Goodarznia, & Mirza, 1999; Kohler, Haerdi, Christen, & Veuthey, 1997; Nieves, Bartley, & Schwede, 1994; Reverchon, Ambruosi, & Senatore, 1994; Yamini, Sefidkon, & Pourmortazavi, 2002).

Often SFE methods involve the investigation of many variables, which may affect the efficiency of extraction. Selection of these variables and their levels is critical. Several statistical techniques, such as simplex optimization and factorial design, were employed for the optimization of analytical methods. Factorial design has some advantages over simplex optimization in that a global optimum can be provided, large amounts of quantitative information can be extracted and both discrete and continuous factors can be estimated. One obvious disadvantage of the factorial design is the large number of experiments required when several variables are examined. However, the number of the experiments can be considerably reduced by the use of an orthogonal array design (Lan, Wong, Chen, & Sin, 1994; Lan, Wong, Lee, & Sin, 1995).

The aim of the present work is the investigation of the effects of different parameters, such as pressure, temperature, modifier volume and dynamic extraction time, on the supercritical fluid carbon dioxide extraction of *C. copticum*. The essential oil obtained by hydrodistillation was used for comparison. Steam distillation of *C. copticum* has already been reported (Masoudi, Rustaiyan, & Amiri, 2002).

2. Materials and methods

2.1. Plant material

The plant materials were collected from the mountains in the city of Damgan-Iran in July 2002. Immediately prior to SFE, the sample was ground in a blender to produce powder.

2.2. Reagents

HPLC grade dichloromethane and methanol were purchased from Aldrich. Carbon dioxide (99.99% purity), contained in a cylinder with an eductor tube, was obtained from Sabalan Co. (Tehran, Iran).

2.3. Hydrodistillation

The plant (80 g of dried material were charged with a particle size of about 500 μm) was submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus, according to the *European Pharmacopoeia* (European Pharmacopoeia, 1975). The volatile distillate was collected over anhydrous sodium sulphate and refrigerated prior to analysis. The yield of the oil was 2.8% (v/w), based on dry plant weight.

2.4. Supercritical fluid extraction

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions. The extraction vessel was an 8 ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 10.1, 20.3 and 30.4 MPa and temperatures of 35, 45 and 55 °C for a duration of 20 min, static, followed by 10, 20 or 30 min, dynamic. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restrict point was warmed electrically. The supercritical CO_2 flow rate through the Duraflow restrictor was approximately 0.3–0.4 ml/min (compressed). Plant powder (3.0 g) mixed well with 2 mm diameter glass beads, and was then charged into the 8-ml extraction vessel. The essential oil was extracted from the plant using supercritical CO_2 under various conditions according to the Taguchi method (Roy, 1990). Table 1 shows the experimental conditions for each of the SFE runs. The extracted analytes were collected in dichloromethane in a 5.0 ml volumetric flask. The final volume of the extract was adjusted to 5.0 ml with dichloromethane at the end of the extraction. In order to improve the collection efficiency, the 5.0 ml volumetric flask was placed in an ice bath during the dynamic extraction

Table 1
SFE experimental conditions and extraction yields for *C. copticum*

Run no.	Pressure (MPa)	Temperature (°C)	Dynamic time (min)	Modifier volume (μl)	Extraction yield (w/w) (%)
1	10.1	35	10	0	0.96
2	10.1	45	20	80	1.10
3	10.1	55	30	400	1.20
4	20.3	35	20	400	3.90
5	20.3	45	30	0	4.90
6	20.3	55	10	80	1.96
7	30.4	35	30	80	5.80
8	30.4	45	10	400	4.10
9	30.4	55	20	0	4.96

stage. For all the modifier studies, methanol was spiked directly into the extraction vessel with charged sample prior to the extraction.

Four ml of solution was poured into a 20 ml beaker. Bubbling of the solution was done by using argon gas to evaporate the solution. Then the weight of essential oil was measured. Finally the extraction yield was calculated.

2.5. GC and GC/MS analyses

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a FID and a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed to 50 °C for 5 min, and then increased to 250 °C at a rate of 4 °C/min. Injector and detector temperatures were 250 and 265 °C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The SFE samples (1 μl) were injected into the GC (without any further dilution) using the split mode with a split ratio of 1/60. Hydrodistilled extracts were diluted 30 times and 1 μl of diluted solution was injected into the GC with the same split ratio. The GC/MS analysis was carried out on a Varian 3400 equipped with a DB-1 column with the same characteristics as the one used in GC. The transfer line temperature was 260 °C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of oil were identified by comparison of their mass spectra with those assembled via a Wiley 5 mass spectra computer library or with authentic compounds. Data obtained were confirmed by comparison of their retention indices, either with those of authentic compounds or with the data published in the literature (Sandra & Bicchi, 1987).

3. Results and discussion

3.1. General

The aim of this work was to find the conditions providing the highest SFE (static-dynamic approach) recoveries of *C. copticum* inside the experimental domain explored, and the results were compared with essential oil composition obtained through hydrodistillation. A static extraction period was employed in order to increase the sample-extractant contact duration. This was followed by a dynamic extraction period in which extractant passed continuously through the extraction chamber thus shifting the equilibrium toward quantitativeness.

3.2. Optimization of the experimental conditions

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the development of a SFE method. In fact, pressure and temperature of the fluid, percentage of the modifier and the extraction times are generally considered as the most important factors. The optimization of the method can be carried out, step-by-step, or by using an experimental design. Table 1 shows different conditions of experiments carried out with SFE for extractions of *C. copticum* according to the Taguchi experimental design. All the selected factors were examined by using a three-level orthogonal array design with an OA_9 (3^4) matrix. In this study, interactions among variables were not incorporated in the matrix, and emphasis was placed on the main effects of the four most important factors. The results of the SFE experiments, based on the extraction yields, are given in Table 1. The mean values of the extraction yields for the corresponding factors at each

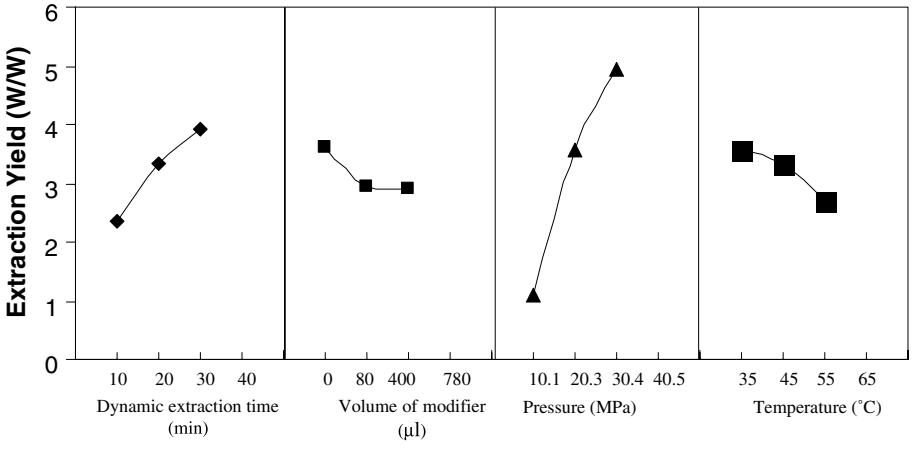

Fig. 1. Effects of temperature, pressure, dynamic extraction time and volume of modifier on extraction yield.

level were calculated according to the assignment of the experiment (Fig. 1). For example, the extraction yields of the three trials at 30.4 MPa were evaluated as a mean value of the corresponding three runs. The mean values of the three levels of each factor (e.g. pressure) reveal how the extraction yield will change when the level of that factor is changed. Fig. 1 shows the variations in extraction yield as a function of change in different levels of the factors studied. For the complete recovery of the main components of the plant, higher pressures are necessary. This is because raising the extraction pressure, at constant temperature, leads to higher fluid density, which increases the solubility of the analytes. To obtain quantitative recovery of analytes, they must be efficiently partitioned from the sample matrix into the supercritical fluid. The influence of temperature on the composition of the extracts was studied. For all the analytes, the temperature of the supercritical fluid was found not to be significant as the main effect. The influence of the dynamic extraction time on the composition of the extracts was studied. Extraction was performed with supercritical carbon dioxide at the static extraction step of 20 min, followed by 10, 20 and 30 min of dynamic extractions. Results showed that increased dynamic extraction time to 30 min enhanced the extraction of most components. In the present work, the modifier did not influence the extraction efficiency of the main components.

The SFE and hydrodistillation extracts from *C. copticum* showed a relatively simple GC–MS chromatographic pattern. Detailed identification and quantitation of the compounds found in *C. copticum* seed oil, produced by SFE under different conditions, were performed by GC–MS, as shown in Table 2. Products obtained by hydrodistillation were also analysed by GC–MS. The results are also shown in Table 2, for comparison. The major compounds were: α-thujene (0.4%), β-pinene (2.1%), γ-terpinene (30.8%), myrcene (0.8%), *p*-cymene (15.7%), limonene (0.7%), α-terpinene (0.5%) and thymol (49.0%). It is noteworthy that the oil extracted by SFE, under Run 1 conditions, has a composition similar to that of the oil obtained by hydrodistillation, but a marked difference in the thymol content between the SFE (Runs 4 and 5) and the hydrodistillation product can be noted in Table 2. As shown in Table 2, SFE offers a rapid method for the extraction of *C. copticum* oil, as well as high selectivity for thymol, depending on the extraction conditions (Runs 4 and 5). These extracts are richer in thymol. However, the recovery of thymol by SFE is better than that by hydrodistillation. The major disadvantage of the oil obtained by SFE is the presence of co-extracted cuticular waxes.

Table 3
ANOVA table for the experiments (at 95% confidence)

Source of variance	Sum of square	Degree of freedom	Mean square	F^a value
Pressure	23.14	2	11.57	33.1
Temperature	1.21	2	0.61	1.7
Dynamic extraction time	3.93	2	1.97	5.6
Methanol percentage	0.96	2	0.35	
Pooled error	5.82	6	2.91	
Total	35.06	14		

[a] $F_{critical} = 19$.

Table 3 shows analysis of variance (ANOVA) results for calculated models. The ANOVA results of this experiment indicate that the pressure of the SFE plays an important role in the SFE of *C. copticum*. In fact, it appeared to be significant for all the analytes. This means that extraction recovery is enhanced as the pressure increases. The pressure increase causes an increase of the fluid density and thus it could have an important effect: increase of the solvating power of the supercritical fluid, responsible for quantitative recoveries.

4. Conclusion

In the investigation of the effects of the four tested parameters on the SFE recoveries of α-thujene, β-pinene, γ-terpinene, myrcene, *p*-cymene, limonene, α-terpinene and thymol from *C. copticum* sample, the use of an experimental design approach led to polynomial

Table 2
Composition (%) of *C. copticum* oils obtained by SFE and hydrodistillation (the compounds were listed in order of elution time from a DB-1column)

No.	Compound	R.I.[a]	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	HD[b]
1	α-Thujene	404	–	–	–	–	–	–	–	–	–	0.4
2	β-Pinene	500	1.6	1.5	1.2	–	–	0.9	–	–	–	2.1
3	γ-Terpinene	680	32.4	41.9	39.7	14.2	14.8	14.1	14.7	7.6	9.3	30.8
4	Myrcene	523	0.6	0.7	0.7	–	–	–	–	–	–	0.8
5	*p*-Cymene	598	17.4	19.9	19.9	23.1	25.2	35.9	45.1	73.4	64.6	15.7
6	Limonene	610	–	–	–	–	–	–	–	–	–	0.7
7	α-Terpinene	582	0.4	–	–	–	–	–	–	–	–	0.5
8	Thymol	1289	47.9	35.8	38.7	62.8	60.1	49.4	40.4	19.1	26.2	49.0

[a] Kovats retention indices on DB-1 column.
[b] Hydrodistillation.

functions describing the relationships between variables and responses and elucidation, for each compound, of the best experimental conditions for the supercritical fluid extraction of these substances within the experimental domain considered.

The supercritical fluid extraction of *C. copticum* was studied, and the results were compared with essential oil composition obtained by hydrodistillation. The SFE method offers many important advantages over hydrodistillation. SFE requires shorter extraction times (30 min vs. 4 h for hydrodistillation). Energy cost is higher in performing hydrodistillation than that required for achieving SFE conditions.The possibility of manipulating the composition of the oil, by changing the parameters of the extraction (pressure, temperature, modifier volume and dynamic extraction time), is more attainable in SFE. Although compositions of the oils obtained by SFE and hydrodistillation are not qualitatively different, they do differ quantitatively. We obtained a higher selectivity at high pressure in SFE than that by the hydrodistillation method.

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