Supercritical carbon dioxide extraction of volatiles from spices Comparison with simultaneous distillation–extraction[☆]

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Abstract

Supercritical fluid extraction (SFE) and simultaneous distillation–extraction (SDE) were used to extract the essential oils from three different spices (oregano, basil, and mint), and a comparative study of extracts obtained using SFE and SDE is presented. Temperature and pressure for the SFE extraction were optimized prior to the experimental extractions. The extracts obtained using the two methods were very similar in composition, but SFE yielded better relative standard deviations and avoided the thermal degradation or solvent contamination of samples. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Aroma in spices is the result of complex mixtures of volatile substances, ordinarily terpenes, sesquiterpenes, and oxygenated derivatives, generally present at low concentrations. The volatile fraction is designated the "essential oil". Before such substances can be analyzed, they have to be extracted and concentrated, and a number of different methods can be used for that purpose, e.g. hydrodistillation, Soxhlet extraction, and simultaneous distillation–extraction (SDE). Nevertheless, monoterpenes are well known to be vulnerable to chemical changes under steam distillation conditions, and even conventional solvent extraction is likely to involve losses of more volatile compounds during removal of the solvent.

Headspace techniques (purge-and-trap and solid-phase microextraction) could be a good option for qualitative analysis of the aroma from spices and herbs, but extraction yields are lower than those achieved using solvent extraction methods, and the profiles obtained with both methods are very different [1,2].

In recent years supercritical fluid extraction (SFE) has become an alternative to more conventional extraction procedures, chiefly because the dissolving power of supercritical fluids can be adjusted by regulating the pressure and temperature conditions

employed. CO_2 is the most widely used fluid for supercritical extraction, because supercritical conditions are readily attained (critical temperature: 31.05 °C; critical pressure: 72.9 atm; atm=101 325 Pa). Furthermore, CO_2 is non-toxic, non-flammable, chemically stable, and retains no solvent residue in the extract.

A number of workers have used supercritical CO_2 extraction of the volatile compounds from different spices and aromatic plants for purposes of analysis [3–7], or as a means of obtaining essential oils as flavor and fragrance ingredients in the food, flavoring and pharmaceutical industries [8–12]. Different aspects of extraction of essential oils by SFE are discussed in two reviews [13,14].

Selection of the extraction pressure and temperature range is an important factor affecting the final composition of the extract and process yield, since the solubility of every component in the fluid will depend on these parameters.

Using high CO_2 densities (pressure: 100–200 bar), terpenes and oxygenated terpenes are completely miscible in supercritical CO_2, but other non-volatile compounds such as fatty acids, waxes, and paraffins can appear in the extract. However, a knowledge of the solubilities of the pure compounds is not enough when compounds are adsorbed onto a cell wall matrix or are located inside complex plant structures. In this case an equilibrium can be established between the solid and fluid phases. Solute concentrations in both phases are related through an isotherm equilibrium, so a compound extracted from the vegetable matter will probably be lower than in the case of a pure substance. In addition different compound families display different diffusion times inside the vegetable matrix. For these reasons experimental optimization using the matrix is necessary [14].

In the present study SFE was used to extract the volatile aroma components from three spices, oregano, basil, and mint. Extracts were analyzed by GC–MS. Extraction conditions were adjusted in order to obtain the highest yields of volatile substances, and the influence of the extraction conditions and the precision of the method were examined. The results obtained were compared with the results obtained using SDE.

2. Experimental

Commercial samples of the three spices, oregano, basil, and mint were acquired, homogenized, and stored protected from light until analysis.

2.1. SDE

A microscale simultaneous distillation–extraction apparatus (Chrompack, Middelburg, Netherlands) was used as previously described [15]. An amount of 1 g of spice and 100 µl of internal standard were extracted for 2 h using dichloromethane as solvent, and the extract was concentrated with nitrogen. Four replications of the extraction and analysis procedure were performed for each of the spice samples.

2.2. SFE

A Hewlett-Packard 7680A extraction module with a 7-ml thick-walled stainless steel thimble was used to carry out supercritical fluid extraction. The extractor employed a variable restrictor to allow instant depressurization of the supercritical fluid and the decoupling of flow and pressure in order to control the pressure independently of the supercritical fluid flow rate.

An amount of 0.5 g of spice and 100 µl of internal standard were placed in the thimble. The SFE extract was collected on a solid-phase trap held at −5 °C. The trap consisted of octadecylsilane (ODS), which was flushed by dichloromethane, and the extract was collected in a 1-ml vial that was kept frozen until analysis by GC–MS.

Extraction time was 25 min, and four replications of the extraction and analysis procedure were performed for each of the spice samples. Density and temperature conditions of the extraction procedure were optimized before analysis.

2.3. Chromatographic conditions

An volume of 1 µl of extract was analyzed using a Hewlett-Packard G 1800 B GCD System with a mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The column was an SPB-1 methylsilicone (Supelco) (50 m×0.25 mm), with a

film thickness of 0.25 μm. The column temperature program was 70 °C (3 min), then 4 °C/min to 120 °C, and then 8 °C/min to 250 °C. Injector temperature was 250 °C. The transfer line temperature was 280 °C. Mass-selective detector conditions were: electron impact (EI) mode at 70 eV; source temperature, 178 °C; scanning rate, 1 scan/s; mass acquisition range, 35–350.

3. Results and discussion

3.1. Optimization of SFE conditions

The first step in SFE of the essential oils was to optimize the working pressure and temperature of the method in order to maximize recoveries of volatile substances while avoiding coextraction of unwanted substances, primarily waxes and high-molecular-mass compounds. Optimization was performed using oregano, testing CO_2 densities of between 0.25 and 0.90 g/ml and temperatures of between 40 and 60 °C. Higher temperatures were not used to prevent samples from being overheated and to ensure that thermally unstable substances would not be decomposed.

Fig. 1 graphically represents the yields (expressed as the sum of the peak areas of all the volatile compounds) obtained using each set of conditions. Optimum yields of volatile substances were obtained at a temperature of 40 °C and a CO_2 density of 0.72 g/ml (pressure: 120 bar).

Oxygenated monoterpenes decreased and non-volatile compounds progressively appeared in the extracts at increasing CO_2 densities, in agreement with findings published by other workers [16–21]. This fact could explain the drop in counts at 40 °C at 0.8 and 0.9 g/ml shown in Fig. 1, since there was a reduction in the yield of the volatile compounds quantified, but not in the total peak area, due to other non-volatile compounds extracted under these conditions.

Many researchers have recommended temperatures of between 40 and 50 °C and extraction pressures lower than 100 bar for obtaining essential oils, using the solubility data of the typical compounds extractable from vegetable matters [14]. In our case the optimum pressure was higher, 120 bar, probably because the matrix effect reduces the extraction of the compounds. Other authors have found an optimum pressure of 387 bar for the extraction of the essential oil of *Rosmarinus officinalis* [3].

3.2. Comparative analysis of spice aroma components using SDE and SFE

Samples of oregano, basil and mint were extracted by SFE and SDE using the optimized conditions described above, and the extracts were then analyzed. Fig. 2 presents the chromatograms for the chromatographic analysis of the oregano extracts produced by both methods.

Tables 1–3 list the substances identified in the extracts, together with their percentage concentrations and the relative standard deviations (RSDs) for each of the extraction methods considered.

In general, spice composition was quite similar for both extraction methods. Linalool and thymol were the main components in the oregano extract, with the SDE extract containing more terpene hydrocarbons, the most volatile components, and less linalool, thymol, and carvacrol than the extract obtained by SFE. Sesquiterpenes were similar in both extracts.
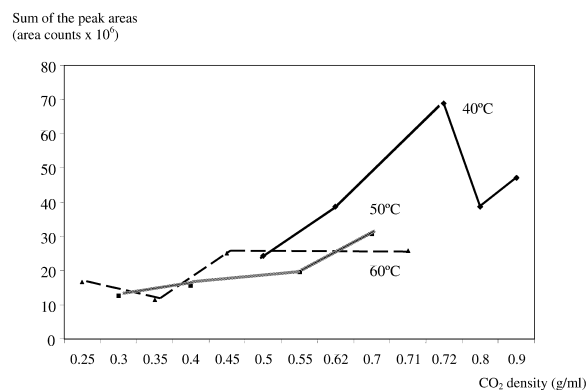

Fig. 1. Effect of temperature and CO_2 density on SFE yields for extractions performed using oregano.

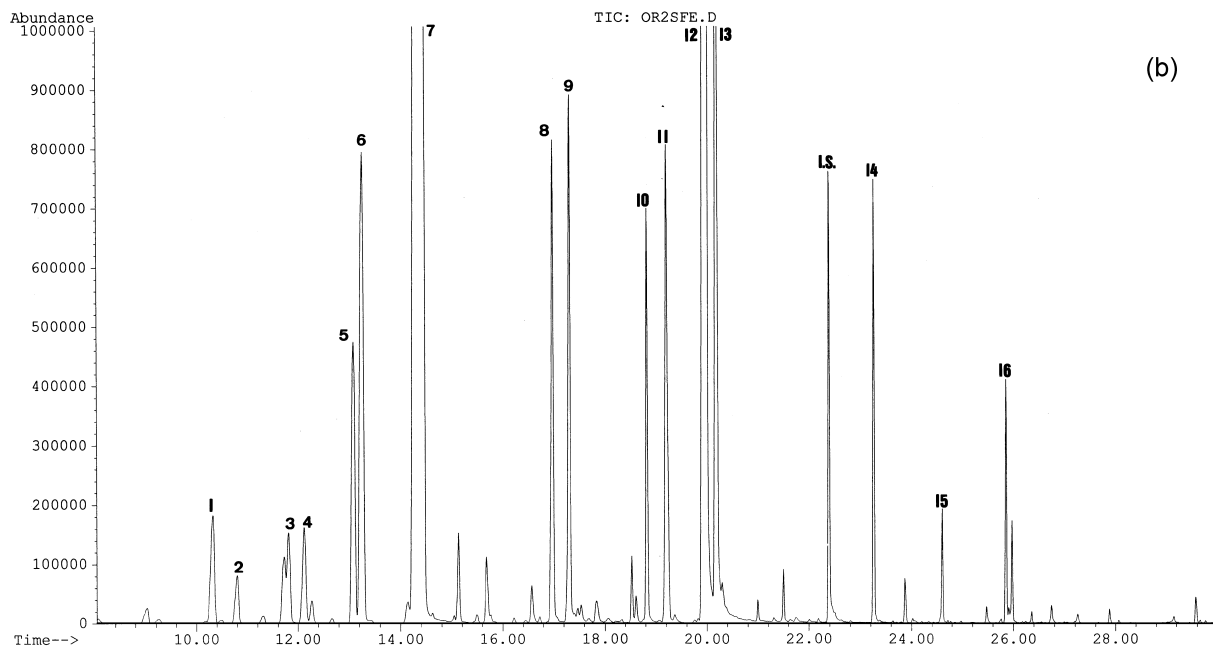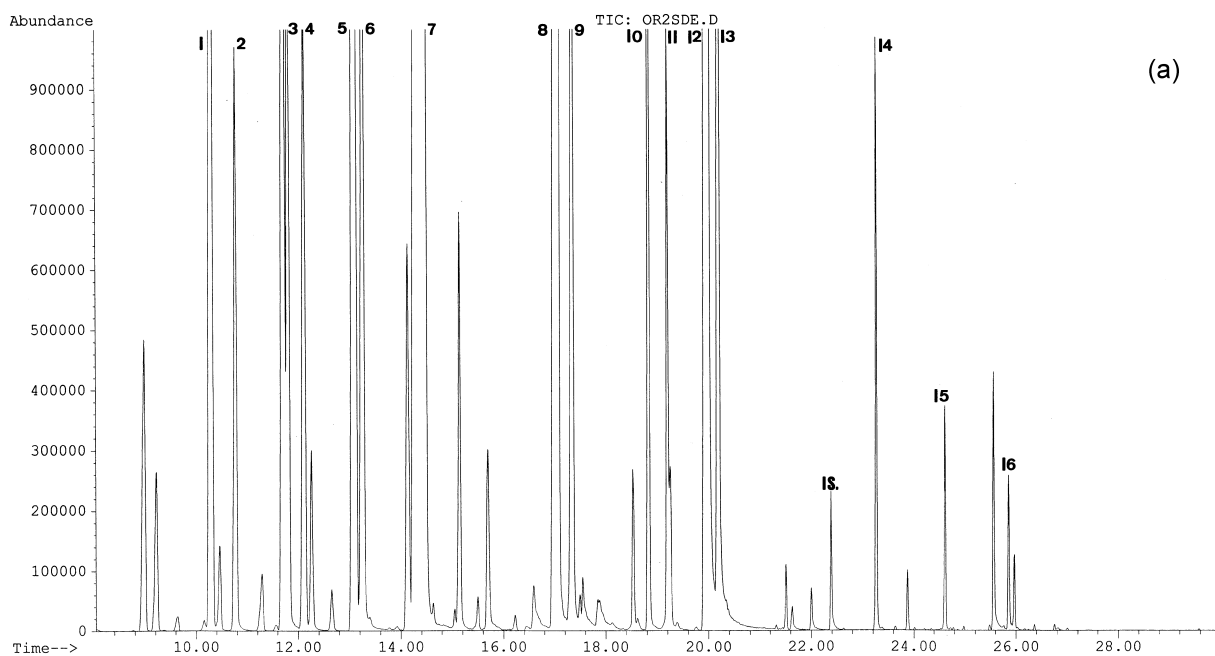Fig. 2. Total ion current chromatograms of the volatile components of oregano extracted by SDE (a) and SFE (b). Peak identification: 1: sabinene; 2: β-myrcene; 3: *p*-cymene; 4: β-phellandrene; 5: γ-terpinene; 6: *trans*-thujan-4-ol; 7: linalool; 8: 4-terpineol; 9: α-terpineol; 10: methylthymyl ether; 11: linalyl acetate; 12: thymol; 13: carvacrol; 14: *trans*-caryophyllene; 15: γ-elemene; 16: spathulenol. Time scale in minutes.

Table 1
Volatile composition and RSDs for SDE and SFE extracts of oregano

Component	SDE		SFE	
	Composition (%) (n=4)	RSD (%) (n=4)	Composition (%) (n=4)	RSD (%) (n=4)
α-Thujene	0.9	10.3	0.2	8.6
α-Pinene	0.5	10.9	Tr	12.4
Sabinene	4.9	9.2	1.2	7.7
β-Myrcene	1.7	16.8	0.5	12.1
α-Phellandrene	0.2	18.3	0.1	15.3
α-Terpinene	3.8	29.5	0.7	15.9
p-Cymene	2.6	15.7	0.8	13.1
β-Phellandrene	2.1	15.8	1.0	6.5
γ-Terpinene	7.7	25.1	2.5	5.2
trans-Thujan-4-ol	3.9	9.8	4.7	2.2
Linalool	31.7	7.6	43.6	4.7
endo-Borneol	0.3	9.5	0.3	2.3
4-Terpineol	10.7	26.3	3.0	8.3
α-Terpineol	3.3	9.4	3.2	2.1
Methyl thymyl ether	1.8	10.8	1.9	2.4
Linalyl acetate	1.5	5.3	3.1	2.4
Thymol	15.3	3.2	21.7	2.4
Carvacrol	4.6	2.8	6.4	2.4
trans-Caryophyllene	1.1	9.2	1.7	3.8
α-Humulene	0.1	7.6	0.2	4.1
γ-Elemene	0.5	8.8	0.4	4.0
Spathulenol	0.4	15.9	1.0	6.1

Linalool and estragol were the main components in the essential oil extracted from the basil and in fact are the character-impact components of basil and make up 50% of the total volatiles in that herb [22]. The percentage shares of these two components in the SDE and SFE extracts were very similar, and the same held true for the other oxygenated terpenes except for 1,8-cineole, which was more abundant in the SDE extract. Conversely, eugenol, *trans*-methyl cynamate, and many of the sesquiterpenes were present in higher proportions in the SFE extracts.

Carvone was the major component in the mint extracts (75–80%). SFE extracted slightly higher quantities of this compound and of the sesquiterpenes and very similar percentages of oxygenated terpenes, with the exception of 1,8-cineole, which was more abundant in the SDE extract.

In conclusion, SDE yielded higher amounts of extracted terpene hydrocarbons, whereas the SFE extracts contained higher amounts of oxygenated terpenes and high-boiling-point components (sesquiterpenes).

Other researchers have reported similar findings when comparing extracts of spices obtained using SFE with the extracts obtained using other, more traditional methods such as hydrodistillation [8,10,11,16,23–25], Soxhlet extraction [26], and SDE [3], using different spices or vegetable matter.

Since oxygenated terpenes are the main contributors to aroma of many essential oils, the SFE extracts retained more of the sensory attributes of the extracted spices than the SDE extracts, and as a result SFE extracts are suitable for organoleptic analysis of spices. In the extraction of chamomile essential oil, the SFE extract showed an odor indistinguishable from that of the starting vegetable matter [9].

The RSDs were lower for the supercritical CO_2 extraction than for the SDE, particularly in the case of oregano. This is a further advantage of using SFE

Table 2
Volatile composition and RSDs for SDE and SFE extracts of basil

Component	SDE		SFE (%)	
	Composition (%) ($n=4$)	RSD (%) ($n=4$)	Composition (%) ($n=4$)	RSD (%) ($n=4$)
Sabinene	0.37	8.1	0.09	16.3
β-Pinene	0.92	7.3	0.16	4.8
β-Myrcene	0.33	8.3	0.12	3.4
1,8-Cineole	10.94	5.7	5.85	6.0
trans-Thujan-4-ol	0.37	8.1	0.36	4.1
cis-Linalool oxide	0.59	3.1	0.30	7.0
trans-Linalool oxide	0.56	2.8	0.30	5.9
Linalool	35.99	1.9	30.73	4.9
Camphor	0.72	2.1	0.44	7.4
Borneol	0.21	8.9	0.29	5.2
4-Terpineol	0.98	6.5	0.94	12.3
α-Terpineol	1.07	1.3	0.82	6.1
Estragol	22.59	3.4	21.80	7.4
trans-Geraniol	0.12	27.2	0.15	10.4
trans-Anethole	0.09	11.6	0.11	4.9
Bornyl acetate	0.62	5.1	0.37	12.7
cis-Methyl cynamate	0.85	11.3	1.17	3.5
Eugenol	6.12	12.3	8.22	5.0
trans-Methyl cynamate	5.96	5.0	8.71	6.0
Methyl eugenol	1.07	13.9	1.18	17.5
α-Copaene	0.14	6.8	0.22	6.6
β-Bourbonene	0.12	8.2	0.20	7.3
β-Elemene	0.71	4.6	1.45	6.5
β-Caryophyllene	0.25	12.4	0.41	9.5
α-Bergamotene	2.35	7.5	5.67	4.1
α-Guaiene	0.26	6.7	0.61	5.1
β-Selinene	0.26	10.5	0.46	4.5
Epibicyclosesquiphellandrene	0.16	8.3	0.31	2.8
β-Cubebene	0.64	6.5	1.57	3.8
δ-Guaiene	0.28	10.2	0.73	5.6
γ-Cadinene	0.94	9.3	1.99	1.9
Spathulenol	0.33	18.4	0.37	4.5
trans-Cadinol	3.09	16.0	3.59	1.9

to extract the volatile components from spices, in addition to the other advantages of a lower extraction temperature and shorter extraction times.

4. Conclusions

SFE proved to be suitable for analyzing the volatile components of spices, but extraction conditions, primarily fluid pressure and temperature, should be optimized. The SFE extracts were comparable to the SDE extracts but had better RSDs. Additionally, supercritical CO_2 extraction requires less extraction time, does not produce thermal degradation or solvent contamination of samples, and preserves the natural character of the fresh product.

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Table 3
Volatile composition and RSDs for SDE and SFE extracts of mint

Component	SDE		SFE	
	Composition (%) ($n=4$)	RSD (%) ($n=4$)	Composition (%) ($n=4$)	RSD (%) ($n=4$)
α-Pinene	0.47	12.1	0.02	17.4
β-Phellandrene	0.18	9.7	0.03	19.8
β-Pinene	0.57	11.3	0.05	6.2
1,8-Cineole	7.98	12.0	3.65	9.6
trans-Thujan-4-ol	0.64	8.1	0.69	7.0
Linalool	0.29	7.8	0.29	11.8
p-Menthone	0.08	24.3	0.13	16.4
Borneol	0.80	22.3	0.84	8.8
4-Terpineol	0.82	16.4	0.56	6.1
Dihydrocarvone	1.76	13.3	1.91	7.0
Neodihydrocarveol	3.38	13.4	3.01	10.6
cis-Carveol	5.33	13.3	4.56	17.9
Carvone	74.49	14.8	81.85	5.3
Eugenol	0.01	16.6	0.16	8.8
trans-Carvyl acetate	0.14	15.1	0.22	9.9
cis-Jasmone	0.32	19.9	0.52	10.9
α-Cubenene	0.04	33.2	0.11	4.9
β-Bourbonene	1.06	21.5	2.31	3.1
β-Elemene	0.22	20.5	0.44	3.8
trans-Caryophyllene	0.80	21.9	1.39	2.5
β-Selinene	0.07	25.4	0.16	6.6
Epibicyclosesquiphellandrene	0.08	19.6	0.26	4.2
Spathulenol	0.46	19.9	0.60	6.4

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