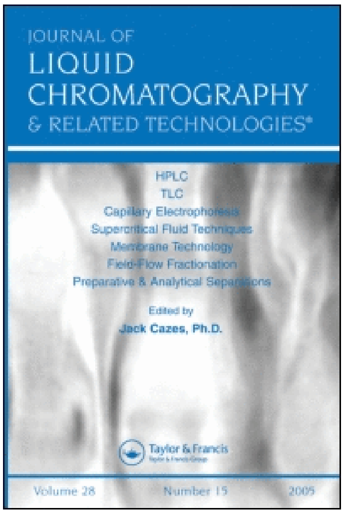

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Comparative Evaluation of SFE and Solvent Extraction Methods on the Yield and Composition of Black Seeds (*Nigella Sativa*)

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Comparative Evaluation of SFE and Solvent Extraction Methods on the Yield and Composition of Black Seeds (*Nigella Sativa*)

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Abstract: Supercritical Fluid Extraction (SFE) conditions (temperature, pressure, and volume of CO_2) were optimized to obtain high quality black seed oil rich in antioxidants. The highest extraction yield (31.7%) was obtained under the SFE condition (50°C, 400 bar, and 100 mL), whereas SFE condition (50°C, 100 bar, 200 mL) gave a low yield (0.84%) as lipids were not extracted. HPLC characterization of compounds in the SFE extracts indicates the presence of a large number of

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compounds in high concentrations in the extract with a low yield. The yield and composition of SFE extracts were compared with the extracts obtained by the soxhlet extraction method and the SFE extract with low yield was found to be superior. Selected SFE extracts were also subjected to GSH recovery tests, and maximum recovery (84.6%) was obtained for the extract with low yield confirming the presence of antioxidant compounds.

Keywords: Black seeds, *Nigella sativa*, Antioxidant, SFE, HPLC characterization

INTRODUCTION

The black seed (*Nigella sativa*) extract, commonly known as Habbat El Baraka in the Arab world, has been in use for generations in various parts of the world, including most of the Arab population. Recent investigations of black seedSand many other herbs used for culinary, as well as medical purposes, have been shown to contain high levels of antioxidants.[1–2] The yield and chemical composition of black seed oil has been investigated by several researchers.[3–8] The antioxidant properties of black seed oil are recently reviewed.[1] Antioxidant and antimicrobial properties of black cumin are also studied.[9] It has been shown that some of the compounds isolated from black seeds have appreciable free radical scavenging properties.[10] This antioxidant property has also been reported by other investigators.[11,12] Thus, it is hypothesized that the beneficial effects of black seeds and other herbs are most likely due to their protection against cellular damage caused by oxidative stress.

Supercritical fluid extraction (SFE) has received increasing attention by several authors, as this technology uses supercritical carbon dioxide (SC CO_2), and, thus, no solvent residues are left behind in the product.[13–16] This technique has the added advantage of recovering the volatile compounds and does not alter the delicate balance of components in natural products. Very limited numbers of studies have been reported on the use of supercritical fluids for the extraction of black seeds. Fullana et al.[14] have used statistical, kinetic modeling, and simulation studies for the extraction of black seed oil using supercritical carbon dioxide. The study aimed at obtaining higher yields of oil rather than compositional quality of the extract. In another study, deacidification of black seed oil extracted by supercritical carbon dioxide was investigated.[17] These studies lack a thorough optimization of the SFE process in order to achieve black seed extract rich in antioxidant principles (with high quality and yield). Therefore, it is desirable to investigate the compositional quality of black seeds extracted by supercritical carbon dioxide.

Pharmacologically active principles (thymoquinone, dithymoquinone, thymo-hydroquinone, and thymol) of black seed oil extracted using conventional solvent extraction techniques have been isolated by solid phase extraction (SPE) and HPLC separation.[18] In addition, four novel alkaloids, namely nigellicine, nigellidine, nigellimine, and nigellimine N-oxide have also been isolated from black seeds.[19] Thymoquinone content fixed oil of *Nigella sativa* obtained from different sources has been estimated by gas chromatography.[20]

Oxidative stress caused by reactive oxygen species (ROS) deplete intracellular Glutathione (GSH) levels.[21–23] Recently, Ashraf et al. have shown that diverse environmental pollutants including xylene, redox cycling metals, and UV radiation can cause oxidative stress in skin fibroblasts, leading to GSH depletion and causing S-thiolation of intracellular proteins.[24] GSH is recovered by the addition of extracts containing antioxidant compounds. Optimum GSH recovery indicates the maximum concentration of antioxidant compounds in the extract, which shows its protective effect against oxidative stress. In this study, intracellular GSH was measured following the method published by Coleman et al.[25]

The present study focuses on optimizing SFE conditions for extraction of black seeds, aiming at obtaining extract rich in antioxidants through comparison of their composition with those obtained by traditional soxhlet extraction methods.

EXPERIMENTAL

Supercritical Fluid Extraction

The experimental apparatus consisted of a 260 mL capacity syringe pump and controller system (ISCO 260D), and an ISCO series 2000 SCF extraction system (SFX 220) consisting of a dual chamber extraction module with two 10 mL stainless steel vessels. Temperature and pressure within the vessels were measured and could be independently adjusted. The 10 mL stainless steel cell was filled with about 5 g of ground black seeds. The cell was pressurized and heated to the desired pressure (100–400 bar) and temperature (40–70°C) and kept for 15 minutes to reach equilibrium. A known volume of SC CO_2 (50–400 mL) was passed through the cell at a flow rate of 1 mL/min. The extract was collected in a cold trap after depressurization of the gas. The collected sample was stored at −18°C until analysis.

Soxhlet Extraction

A known quantity of ground black seeds (about 5 g) was placed in a cellulose thimble and extracted with about 300 mL of either hexane or methanol for 12 hrs. Solvent was removed using a rotary evaporator operated at 45°C and the final traces of solvent were removed under a stream of nitrogen.

Characterization of Extracts

The compounds in the extracts obtained under different SFE conditions and solvent extraction were separated by high performance liquid chromatography on Sphereclone C8 column (250 mm × 4.6 mm id, 5 μm, Phenomenex) using water:methanol:2-propanol (50:45:5), at a flow rate of 1 mL/min. A 20 μL of extract solution [0.1 g of oil dissolved in methanol: 2-propanol (1:1) and made up to 10 mL] was injected and the compounds were separated. Four compounds (trans-anethole, thymoquinone, thymol, and carvacrol) were identified using commercially available standards. The relative percentage of compounds in the extract was calculated from the normalized peak areas and concentration of known compounds was calculated using the standards.

RESULTS AND DISCUSSION

Yields and Composition of Extracts (SFE and Soxhlet)

The extraction yield is used to evaluate the performance of the SFE process for the extraction of black seeds at different pressures, temperatures, and CO_2 volumes. The extract weight is used to calculate the extraction yield, which is defined as the ratio of the extract weight to the sample weight. SFE conditions (temperature, pressure, and volume of CO_2) were optimized to obtain high quality black seed oil which is rich in antioxidants.

SFE yields ranged from 0.84 to 31.7% under different conditions, where as soxhlet extraction with hexane and methanol gave 28.1% and 29.2%, respectively (Table 1). The highest SFE yield was obtained at SFE condition (50°C, 400 bar, and 100 mL) while the lowest SFE yield was obtained at 100 bar, 200 mL CO_2 volume, and the same temperature. The higher yield obtained under high pressure condition is mainly due to the higher extraction of fats and lipids.

Effect of Pressure

Figure 1 shows the influence of pressure on the yield of black seeds extracted by the SFE process at 40, 50, and 60°C, when 100 mL of CO_2 was passed through the sample at 1 mL/min. As it can be seen in the figure, at a constant temperature, higher pressures led to greater extraction yields. The extraction yield increased drastically (by about 150%) when the pressure was increased from 200 to 300 bar, however, the increase was only about 30% with further increases in pressure (300 to 400 bar). This was expected since an increase in pressure leads to an increase in CO_2 density, resulting in a higher solubility and, hence, higher extraction yield. The increase in

Table 1. Experimental conditions and yield obtained during SFE & solvent extraction methods

Run number	Temperature (°C)	Pressure (bar)	CO_2 volume (mL)	Yield (%)
1	40	200	100	9.20
2	40	300	100	24.0
3	40	400	100	30.3
4	40	200	400	27.7
5	50	100	200	0.84
6	50	200	100	9.03
7	50	200	200	15.0
8	50	300	50	12.0
9	50	300	100	24.5
10	50	300	150	29.0
11	50	300	200	29.5
12	50	300	300	29.6
13	50	400	100	31.7
14	60	200	100	8.49
15	60	200	400	14.3
16	60	300	100	22.0
17	60	400	100	30.1
18	70	400	250	30.3
19	Soxhlet extraction using hexane			28.1
20	Soxhlet extraction using methanol			29.2

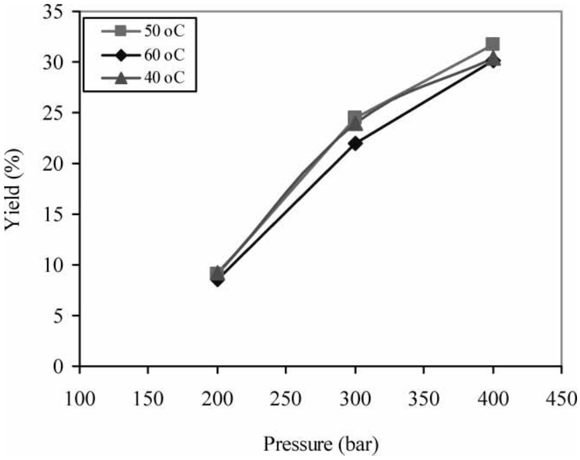

Figure 1. Effect of pressure on the yield of black seed extract, CO_2 volume = 100 mL, CO_2 flow rate = 1 mL/min.

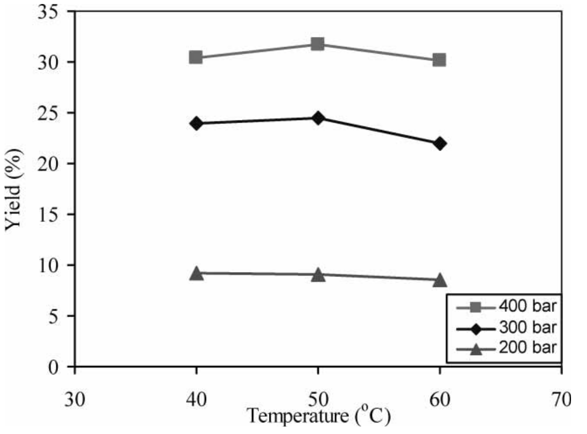

Figure 2. Effect of temperature on the yield of black seed extract, CO_2 volume = 100 mL, CO_2 flow rate = 1 mL/min.

density is more significant at lower pressures since the condition is closer to the critical point of CO_2. Figure 1 also shows that extraction yield was only slightly affected by the temperature.

Effect of Temperature

The influence of temperature on the yield of black seeds extracted by the SFE process is shown in Figure 2. The effect of temperature on the extraction yield was very small, especially at 200 bar. At the higher pressures (300 and 400 bar), extraction yield increased when the temperature was increased from 40 to 50°C, while the yield decreased when the temperature was raised from 50 to 60°C. However, at the lower pressure of 200 bar, extraction yield slightly decreased with an increase in temperature.

Solubility of solutes in SC CO_2 is affected by two competing factors (density of the SC CO_2 and volatility of the solute), which depend on the temperature in opposite ways. Higher temperatures increase the volatility of solutes and improve their solubility and extraction. On the other hand, density of supercritical CO_2 decreases with increasing temperature, reducing the solvating power of CO_2 and thus reducing the solubility and extraction efficiency. This may be the reason for the varying effect of temperature on the extraction yield.

Effect of CO_2 Density

Figure 3 shows the relation between the extraction yield and the density of CO_2. As it can be seen in the figure, at a constant temperature the yield increases

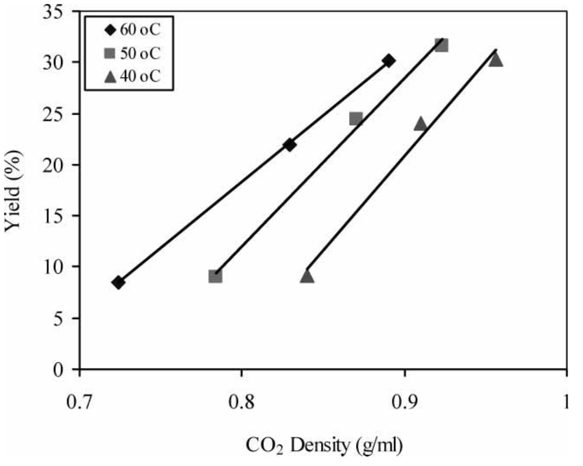

Figure 3. Effect of CO_2 density on the yield of black seed extract, CO_2 volume = 100 mL, CO_2 flow rate = 1 mL/min.

linearly with density ($r^2 > 99\%$). The increase in yield with an increase in CO_2 density is a result of the increase in the solvent power of CO_2 at higher densities.

Effect of CO_2 Volume

Effect of CO_2 volume on the yield of black seeds extracted by the SFE process at 50°C, 300 bar, and flow rate of 1 mL/min is presented in Figure 4. As

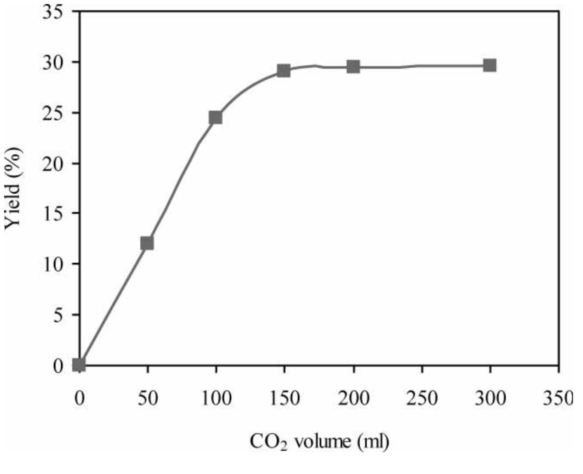

Figure 4. Effect of CO_2 volume on the yield of black seed extract, P = 300 bar, T = 50°C, CO_2 flow rate = 1 mL/min.

Table 2. HPLC characterization of active principles in the SFE and solvent extracts

	Peak 1	Peak 2	Peak 3	Peak 4 (t-Anethole)		Peak 5	Peak 6	Peak 7	Peak 8 (Thymoquinone)		Peak 9	Peak 10 (Carvacrol)		Peak 11
Run number[a]	Normalized area			Normal-ized area	Conc. (mg/g)	Normalized area			Normal-ized area	Conc. (mg/g)	Normal-ized area	Normal-ized area	Conc. (mg/g)	Normal-ized area
1	843628	348967	161033	418903	4.12	52856	116524	72009	1189016	2.33	—	142679	0.046	—
2	629543	1833450	224251	176417	1.92	50263	114425	62042	5626146	11.3	—	162902	0.53	—
3	576391	859689	62056	63973	0.93	15068	62021	26241	1949514	38.9	—	49521	0.21	42757
5	5402880	12620435	6268210	1697690	16.7	114429	963125	886848	3648451	72.8	3054848	838478	2.20	107174
9	399562	714529	98142	38282	0.56	11987	39218	24616	1988219	3.67	—	69534	0.24	50067
10	479942	794133	104717	44486	0.65	13500	42457	26935	2036113	3.76	—	72996	0.25	61076
12	528813	874136	2856866	43654	0.63	12956	43278	24518	2074734	3.83	—	68536	0.23	—
12	95008	502848	1159447	47939	0.70	39258	47079	19842	1911104	3.53	—	15980	0.12	—
14	813931	328991	169233	461706	4.54	54351	122829	78217	1249078	2.45	—	153973	0.50	—
17	529288	707695	75926	82690	0.82	18859	45520	17683	2017271	3.95	—	38434	0.16	—
18	45604	10537172	81581	—	—	9275	29231	38094	1781142	3.44	—	68,676	0.29	47466
19	703413	1069196	258680	159236	1.72	22072	32186	208659	911253	1.76	1721	10631	0.08	54833
20	694862	1329217	212806	160298	1.74	164036	50370	218766	1012366	1.96	14238	14589	0.11	78468

[a]Refer Table 1for SFE conditions.

shown in the figure, the yield increased as more CO_2 was passed through the sample. However, the extract amount approached a maximum value as the CO_2 amount was increased, indicating that no more extract could be obtained by passing additional CO_2 through the sample.

Characterization of Active Principles

HPLC separation of various compounds in the extracts obtained under different SFE conditions, as well as soxhlet extracts, is given in Table 2. t-Anethole, thymoquinone, and carvacrol were identified and quantitated using standards. A large number of compounds in high concentrations were present in the extract for the conditions (temperature, pressure, and CO_2 volume: 50°C, 100 bar, 200 mL). This may be due to the relative increase in the concentration of active principles, as lipids were not extracted under this condition. Therefore, the SFE process should be operated at the lower pressure of 100 bar to obtain extracts rich in antioxidants. Moreover, SFE extraction gave better antioxidant composition as compared to the soxhlet extraction. Since extraction of polar and non-polar compounds depends on the polarity of the extraction solvent, it is difficult to get extracts rich in antioxidants and low in fats and lipids, using common solvents such as hexane or methanol, where as optimized conditions in the SFE process provide selective extraction. Moreover, the SFE process is more flexible as compared to soxhlet extraction, since the solvent power of the CO_2 can be changed by simply changing the temperature and pressure.

The GSH recovery of some SFE extracts was carried out and the recovery ranged from 27.8–84.6% (manuscript in preparation). The highest GSH recovery was found in the SFE extract obtained under the condition (50°C, 100 bar, 200 mL CO_2). Thymoquinone (marker compound) was found to be the highest (72.8 mg/g) in the extract obtained under this condition. The high concentration of marker compound, as well as other compounds in the extract, clearly substantiates the highest GSH recovery in the extract.

CONCLUSIONS

SFE conditions (temperature, pressure, and volume of CO_2) were optimized to obtain quality black seed oil, which is rich in antioxidants. Extraction efficiency drastically increased with the increase in pressure and was only slightly affected by the temperature. The highest extraction yield was obtained at 50°C, 400 bar, and 100 mL of CO_2 volume. However, the highest antioxidants concentration was found for the condition at 50°C, 100 bar, 200 mL, suggesting that the SFE process should be operated at lower pressure for obtaining higher quality extracts. Moreover, SFE extraction gave better antioxidant composition as compared to the soxhlet extraction.

Our current work focuses on separation of individual compounds from SFE extract to investigate their antioxidant ability (through in vitro and in vivo studies) in reducing oxidative stress caused by petrochemical pollution.

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