

Supercritical CO₂ and Subcritical Propane Extraction of Spice Red Pepper Oil with Special Regard to Carotenoid and Tocopherol Content

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Abstract

A study is conducted to optimize the supercritical fluid extraction of spice paprika oil (oleoresin) using supercritical (SF) CO₂ and subcritical propane as the solvents. The main focus is on carotenoid and tocopherol composition as the quality attributes of technological and nutritional interest. The maximum oil yield is 7.2 and 8.8 g per 100 g paprika with SF-CO₂ and subcritical propane, respectively. For the implementation of complete extraction at the given conditions, the ratio of solvent to solid is found to be 6.0 and 1.0 using SF-CO₂ and propane, respectively. The carotenoids, particularly fatty acid esters of red xanthophylls, are hardly solubilized by SF-CO₂, whereas with subcritical propane, 80–94% of such components could be mildly extracted. Tocopherols were easy to extract using either fluid, with propane being the most efficient solvent. The result of the impact of extraction conditions on the major carotenoids (such as capsanthin monoester, β-carotene, capsorubin diesters, and capsanthin diesters, as well as α-, β- and γ-tocopherol) are tabulated with special focus on the degree of loss during extraction by the two solvents.

Introduction

Carotenoid-rich oleoresin of spice red pepper (*Capsicum annuum* L.) is of nutritional, pharmaceutical, and industrial value. In addition to the vital carotenoids, spice red pepper contains considerable amounts of α-tocopherol and other analogues that have chemical and biological roles as bioantioxidants (1). The traditional extraction of spice paprika by acetone (in Hungary) or cyclohexane (in Spain) is no longer recommended because of the risk of organic solvent residues and loss of aroma compounds as a normal consequence of solvent

evaporation.

Recently, supercritical fluid extraction (SFE) has been widely used in the nontoxic extraction of plant oils. The mode in which oil can be solubilized in a supercritical fluid (SF) is either static or dynamic (2,3). The most frequently used SF is carbon dioxide (SF-CO₂), alone or with solubility modifiers (4–6). Other compressed gases (such as ethane, propane, ethylene, dinitrogen oxide, etc.) can be applied under sub- or supercritical conditions (7–9). As a general principle of extraction of vegetable oils by nonsequential SFE, the solubilization power of the solvent should be maximized by performance at the optimum conditions (gas density, temperature, pressure, etc.) (10,11).

In the present work, SF-CO₂ and subcritical propane were applied to extract oleoresin from ground spice red pepper at different conditions. The changes in quality components (carotenoids and tocopherols) as a function of SFE conditions were also studied using recent high-performance liquid chromatographic (HPLC) methods in the analysis.

Experimental

Technical-grade CO₂ was obtained from Repcelak (Répcelak, Hungary), and 99.9% pure propane was from MÁFKI Institute (Veszprém, Hungary). Other analytical- or HPLC-grade organic solvents were from Reanal (Budapest, Hungary). All the standard materials used in the qualitative and quantitative analyses of quality components were purchased from Sigma (St. Louis, MO).

Ground spice red pepper was obtained from the Research Station for Spice Red Pepper (Kalocsa, Hungary). The powder was of approximately 200-μm particles and contained about 20% seeds.

The sub- and supercritical extractions were performed with a previously described high-pressure, flow-up stream extraction

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apparatus (9). The solvents were pumped to the extractor by a membrane pump (LEVA EL-1, Tip, Hamburg, Germany). The pressure was adjusted between 30 and 400 bar by a backpressure regulator. Samples (60 g) of ground spice red pepper were put in the extractor, through which the solvent was compressed with a flow rate of 1.0–1.5 L/min. The solvent that passed through the extractor was then expanded to atmospheric pressure through a heated needle valve. The extract was received in a cooled container and left to stand for 20 min to warm to room temperature, allowing CO₂ or propane to release. The expanded propane and CO₂ were safely guided out through a metal exhaust pipe. The stable weight of the extract was measured in time intervals. The mass of used solvent was calculated with the Peng-Robinson equation (12) after the normalization of the volume. The density of SF-CO₂ was determined by applying the Bender equation (13).

Extraction studies were conducted to extract the ground spice red pepper with SF-CO₂ at 35°C and 55°C over a pressure range of 100–400 bar. In the case of propane, subcritical extraction was performed at 25°C and 30, 50, and 80 bar.

Solvent extraction using mixture of dichloroethane–acetone–methanol (2:1:1) was performed to prepare the control sample. The solvent mixture was strong enough to extract all pigments and tocopherols from the powder. After mechanical shaking for 15 min, the mixture was filtered through filter paper, and the solvent was evaporated under a vacuum at 35–40°C using a rotary evaporator.

Carotenoid content and composition was determined using HPLC (14). The analysis included the extraction of carotenoids from oils by shaking 50–100 mg with 5–10 mL of the HPLC eluent. In the case of greasy oils, the sample should first be dissolved in 1 mL chloroform, and the final volume should be adjusted with the HPLC eluent. Carotenoid extract was fractionated and determined by HPLC using a Beckman (Fullerton, CA) chromatograph consisting of a model 114 solvent delivery pump, a model 420 controller, and a model 165 variable wavelength ultraviolet–visible detector. The separation was carried out on a column (25 cm × 4.6-mm i.d.) packed with a Chromsil C-18 (6 nm) using acetonitrile–isopropanol–methanol (2:1.75:1.25) as the mobile phase at a flow rate of 0.9–1.2 mL/min. The separated carotenoids were monitored at 480 nm. The signals of the detector were recorded and integrated by a Shimadzu (Tokyo, Japan) C-R3A integrator.

The peaks of carotenoid profile were identified on the basis of the comparison of retention times and scanned spectra of the samples with those of authentic standards prepared by thin-layer chromatography (TLC) according to Vinkler and Richter (15). Standard β-carotene and lutein (Sigma) were also applied in the quantitation of the individual pigments, taking into consideration the response factor of red/yellow at the monitoring wavelength of 480 nm.

To analyze the tocopherol content of the extracts, 50–100 mg of each sample were saponified by refluxing with 5 mL of saturated methanolic KOH and 20 mL of methanol at the boiling point of methanol for 40 min in the presence of 0.5 g ascorbic acid as an antioxidant. Following cooling, 15 mL of saturated NaCl solution was added, and the tocopherols were extracted by gentle shaking in a separatory funnel with 40 mL petroleum ether. The lower layer was separated and extracted with an addi-

tional 40 mL of petroleum ether. The petroleum ether fractions were combined, washed twice with double-distilled water, dried on anhydrous Na₂SO₄, and evaporated under a vacuum using a rotary evaporator. The residues were dissolved in 10 mL HPLC-grade *n*-hexane and injected on the normal-phase HPLC column (240 × 4.6-mm i.d., 5-μm Si-60). The mobile phase consisted of *n*-hexane–absolute alcohol (99.5:0.5) (16). The separated compounds were detected fluorimetrically using a Shimadzu RF-535 fluorescence detector at 295 nm (excitation) and 320 nm (emission).

Results and Discussion

At the given temperature and pressure and during the extraction process, the flow of super- or subcritical gases was stopped for short time intervals to weigh the extract collection container.

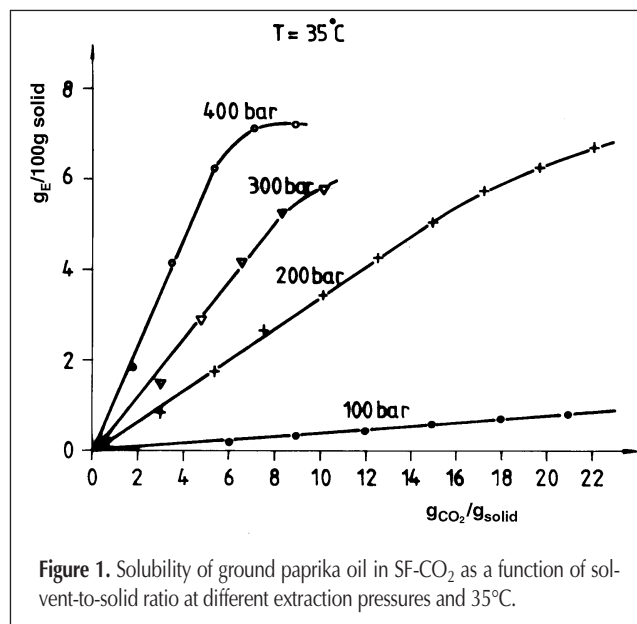


Figure 1. Solubility of ground paprika oil in SF-CO₂ as a function of solvent-to-solid ratio at different extraction pressures and 35°C.

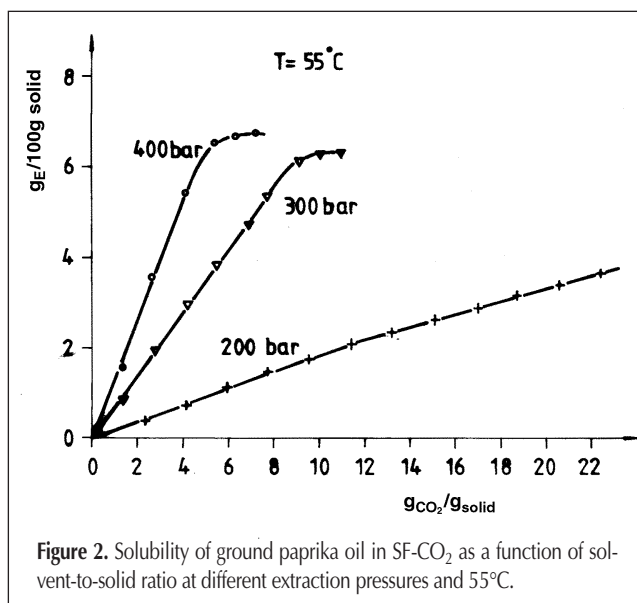


Figure 2. Solubility of ground paprika oil in SF-CO₂ as a function of solvent-to-solid ratio at different extraction pressures and 55°C.

During this period, no shift was recorded in temperature or pressure. In this way, the cumulative extraction curves (oil solubility curves) were determined.

Figures 1 and 2 show the extraction curves of paprika oil by SF-CO₂ at 35°C and 55°C, respectively. The quantity of recovered oil from 100 g spice paprika powder was plotted versus the amount of solvent used. At constant pressure, the extraction curves were characterized by an initial steep linear increase of oil solubility as a function of solvent used. The linear parts of the

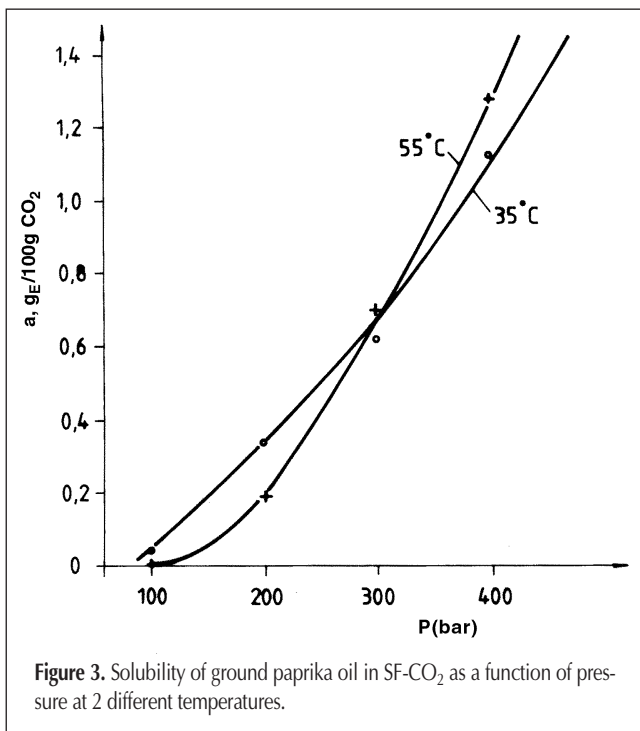


Figure 3. Solubility of ground paprika oil in SF-CO₂ as a function of pressure at 2 different temperatures.

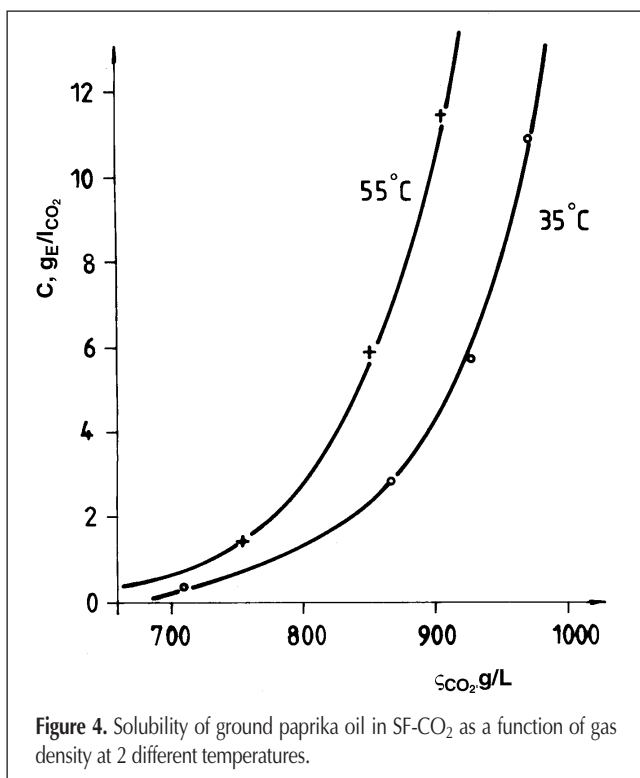


Figure 4. Solubility of ground paprika oil in SF-CO₂ as a function of gas density at 2 different temperatures.

extraction curves (tangent of the straight phase) give the equilibrium solubility of paprika oil in SF-CO₂ expressed as grams of extract per 100 g CO₂. It is noticeable that at higher pressure, the curves reach a plateau and approach the maximum extraction. At lower pressures, the maximum yield of oil could be achieved only with a greater ratio of solvent to solid. The maximal yield was 7.2 and 6.8 g per 100 g of solid at 35°C and 55°C, respectively, when the extraction pressure was kept constant at 400 bar. To attain such a yield, the ratio of SF-CO₂ to ground paprika was found to be 6 and 8 at 55°C and 35°C, respectively. It is also of interest that at 55°C and 100 bar, the solubility of paprika oil in SF-CO₂ was almost zero.

The relationship between the extraction pressure and equilibrium solubility of spice paprika oil in SF-CO₂ at 2 different temperatures is shown in Figure 3. It can be observed that at low pressure, the solubility of oil in SF-CO₂ is very low, and at 100 bar and 55°C, no extract was obtained. The density of SF-CO₂ under the latter conditions was 328 g/L, which is much lower than the 600 g/L needed for compressed CO₂ to solubilize the oils from plant materials (9,10). Proportional to the increased pressure, equilibrium solubility increased exponentially. In agreement with previously published results on the SFE of hiprose oil, the increase of oil solubility as a function of increasing pressure was higher at a high extraction temperature than that observed at low temperature. Also, between 100 and 300 bar, there was a noticeable inversion in the relationship between oil solubility and temperature. This can be attributed to the density of SF-CO₂ (which affects solubilization) and the vapor pressure of the solute as a result of temperature change.

The relationship between the equilibrium solubility of oil in SF-CO₂ and the solvent density is described in Figure 4. It is clear that with a density less than 600 g/L, the solubilization capacity of SF-CO₂ is extremely low. This capacity could be exponentially increased by increasing the density of solvent (normally achieved by increasing the extraction pressure). At higher temperature (55°C), substantially higher pressure is required to ensure the high solvent density that is needed to get the same solubility value obtained at 35°C.

To describe the isotherms of equilibrium solubility curves shown in Figure 3 and 4, the Chrastil equation (17) was followed.

$$C = \rho^k \exp(D/T + E) \quad \text{Eq. 1}$$

where C is the concentration (g/L) of solute at equilibrium, ρ is the solvent (CO₂) density (g/L), and k , D , and E are empirical constants.

The empirical constants of Equation 1 were determined on the basis of the data from experiments carried out at 2 different temperatures (T) and the tangent (k) of the straight lines shown in Figure 5 as follows:

$$-D = (kT_1T_2 \ln \rho_1/\rho_2)/(T_2 - T_1) \quad \text{Eq. 2}$$

$$E = -k \ln \rho_1 - (D/T_1) = -k \ln \rho_2 - (D/T_2) \quad \text{Eq. 3}$$

where ρ_1 and ρ_2 are intercepts of the $\ln c$ and $\ln \rho$ isotherms with the $\ln \rho$ axis at $c = 1$ g/L.

From the aforementioned experimental data, the estimated

values of the constants are as follows: $k = 11.067$, $D = -4364.720$, $E = -59.631$. These values are significantly lower than those obtained from hiprose oil extraction using the same technique and similar conditions (9), indicating that spice paprika oil is more difficult to extract than hiprose oil.

Figure 6 shows the curves of cumulative extraction of paprika oil at 35°C and 200–300 bar. In all three extraction experiments, the extract was fractionated into 3–4 subfractions according to the extraction time and ratio of solvent to solid. The goal was to test the fractionation ability of the applied technique with the increasing extraction degree. The results will be discussed later in the evaluation of the recovered extracts.

The extraction of spice paprika with propane is shown in

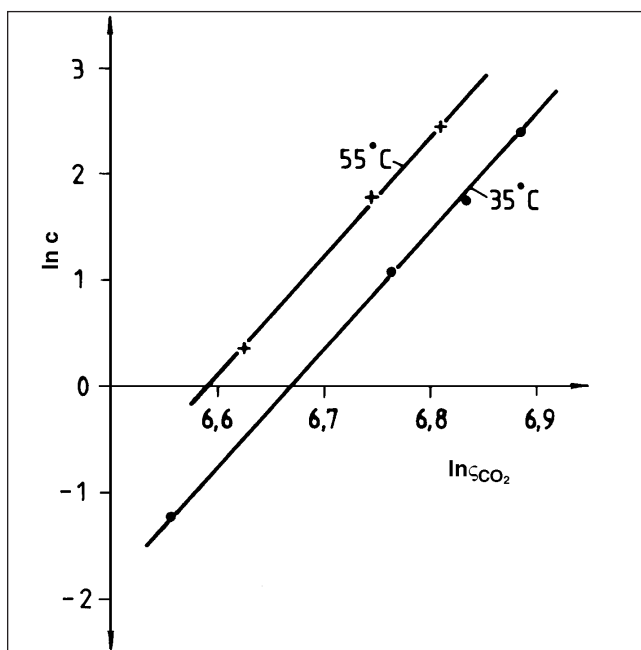


Figure 5. Chrastil plot of paprika oil solubility in SF-CO₂ at 35°C and 55°C.

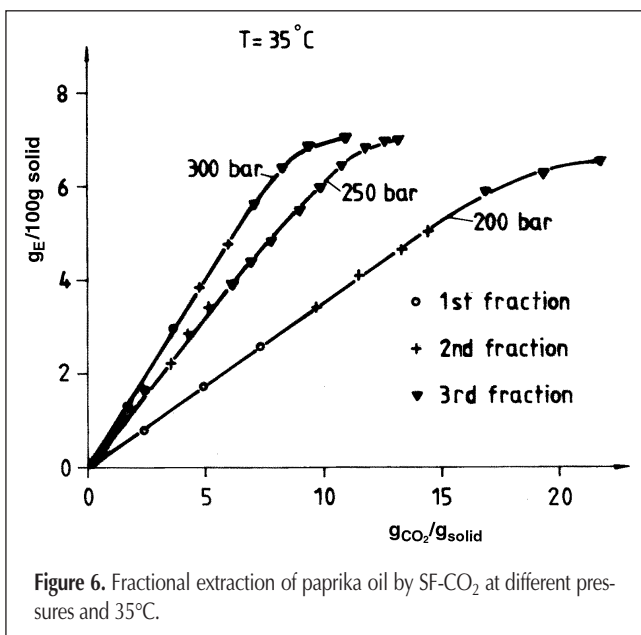


Figure 6. Fractional extraction of paprika oil by SF-CO₂ at different pressures and 35°C.

Figure 7. The extraction was performed at subcritical conditions (25°C and 30, 50, and 80 bar). The amount of extract was plotted versus the propane-to-solid ratio. As with SF-CO₂, there was an initial steep increase in the oil extraction, which then reached a

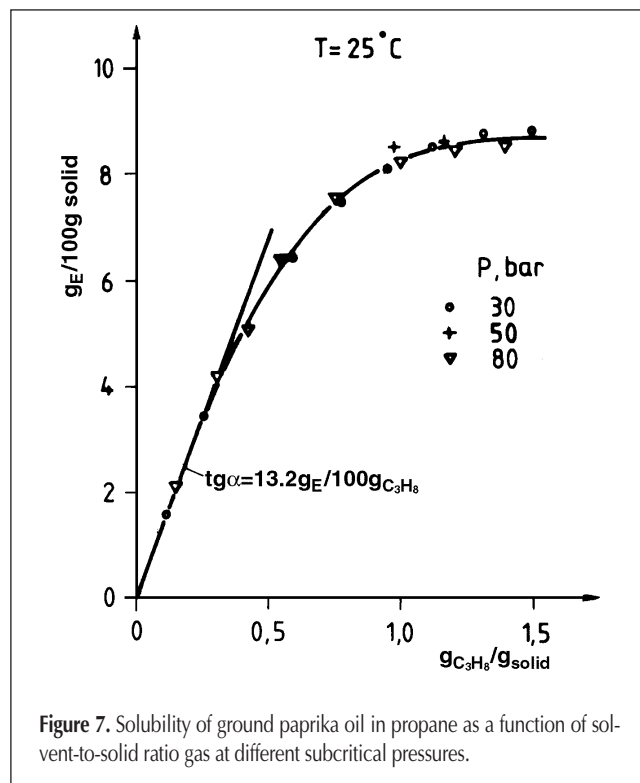


Figure 7. Solubility of ground paprika oil in propane as a function of solvent-to-solid ratio gas at different subcritical pressures.

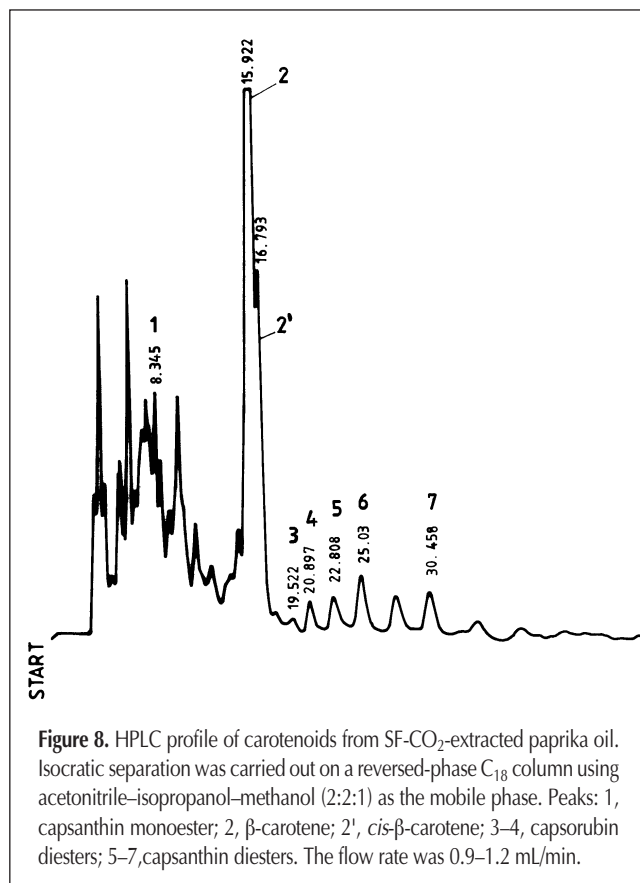
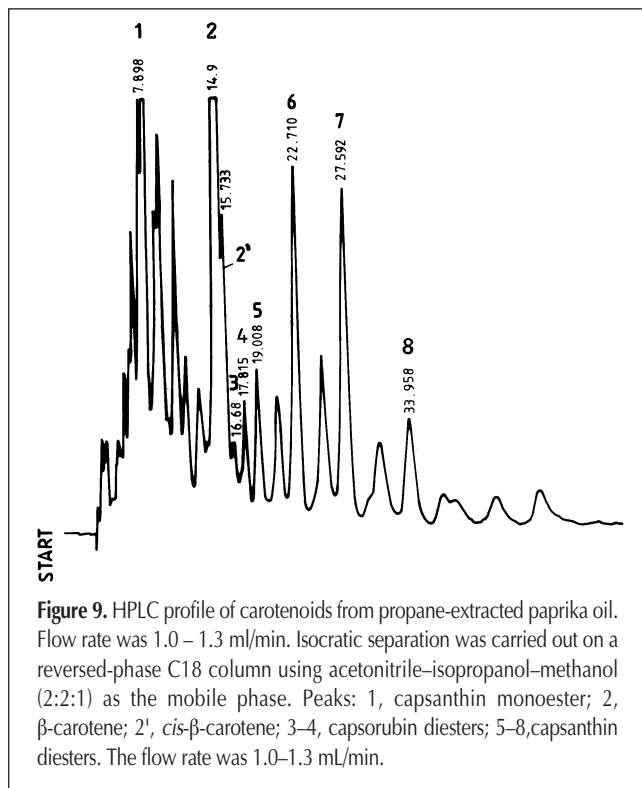


Figure 8. HPLC profile of carotenoids from SF-CO₂-extracted paprika oil. Isocratic separation was carried out on a reversed-phase C₁₈ column using acetonitrile–isopropanol–methanol (2:2:1) as the mobile phase. Peaks: 1, capsanthin monoester; 2, β -carotene; 2', *cis*- β -carotene; 3–4, capsorubin diesters; 5–7, capsanthin diesters. The flow rate was 0.9–1.2 mL/min.

plateau and approached the maximum. It is interesting that the complete extraction of oil could be implemented even at 30 bar at subcritical temperature (25°C). The extraction curves obtained at 30, 50, and 80 bar coincided with each other, and 30 bar is enough to attain the complete extraction of paprika oil using propane as the solvent. Additionally, the maximum extrac-



tion was achieved using a solvent-to-solid ratio of approximately 1.0, which is much lower than the 6–8 recorded with SF-CO₂ at 200–400 bar and 35–55°C, revealing the high solubility of paprika oil in propane.

The maximal yield using subcritical propane was 8.8 g per 100 g of ground paprika. This value is well above the 7.2 g per 100 g obtained with SF-CO₂ at 400 bar and 35°C. From the tangent of the first pattern of the extraction curve (straight line), the capacity of equilibrium solubility could be estimated to be 13.2 g extract per 100 g propane. This value is well above the 1.125 g extract per 100 g CO₂ at supercritical conditions.

Quality of the extracts

The quality of spice paprika products is usually evaluated on the basis of their content of aroma compounds, pigments, and natural antioxidants. The present work does not deal with aroma components, because first, they need special analysis using GC–MS, and second, spice paprika oil is very often used as a source of natural colors in the food, pharmaceutical, and cosmetic industries. The HPLC method of analysis provided the satisfactory separation of more than 30 carotenoid components, of which capsanthin monoester, all *trans*- β -carotene, *cis*- β -carotene, capsorubin, and capsanthin diesters are of special interest from the technological and biological points of view. Different forms of capsanthin and capsorubin are the major attributes of red color and determine the coloring capacity of paprika oil, whereas β -carotene is the main Vitamin A precursor and has a high biological activity as an unusual bioantioxidant (18).

Figures 8 and 9 show the HPLC separation of carotenoids from oils extracted by SF-CO₂ and subcritical propane, respectively. It was evident that SF-CO₂ at all the given conditions has less solvation capacity than propane toward mono- or diesters of

Table I. Effect of Type and Conditions of SFE on the Yield and Quality of Spice Paprika Oil

Type and fractions	Extraction parameters			degree (%)	Concentration of carotenoids ($\mu\text{g/g}$)		
	Pressure (bar)	T ($^{\circ}\text{C}$)			Extraction (gE/100 g powder)	Yield β -carotene	Capsorubin DE
Capsanthin DE							
Control*	1	25			100		
9.0	319.3	436.5	100.7	670.4			
SF-CO ₂	100				35		14
	1.1	0.69	7.1	0.1	0.5		
Cumulative	200		35		100	7.3	13.5
231.7	3.1	22.4					
	300		35	82	5.9	18.3	282.6
5.2	21.9				7.4	30.5	313.9
	400	35		100			
8.6	33.9				5.8	12.2	149.1
	200		55	62			
1.5	4.0				6.4	29.8	364.1
	300	55		93			
6.5	25.5						
	400		55		100	15.5	236.0
6.6	29.9						

carotenoids (the most stable pigments of spice paprika extract) (1). Inversely, polar unesterified xanthophylls such as violaxanthin, capsanthin, and lutein were much more soluble in SF-CO₂ than in subcritical propane. But even with the former solvent, they are not completely recovered from the matrix of ground paprika samples, because their content in organic solvent extracted oil is still higher.

The effect of different extraction conditions on the carotenoid content of of spice paprika oil is shown in Table I. The oil samples were compared to those recovered by a mixture of organic solvent consisting of dichloroethane–acetone–methanol (2:1:1) (control). At 100 bar and 35°C (where only 13.9% extraction could be approached), SF-CO₂ solubilized small quantities of the major carotenoids. Better solubility of the carotenoids was observed when the extraction pressure was raised from 100 bar to 400 bar. However, the pigments extracted at these conditions were much less than those extracted by a mixture of organic solvents. For instance, the best recovery of capsanthin monoester, β -carotene, capsorubin diesters, and capsanthin diesters did not exceed 9.5%, 71.9%, 8.5%, and 5.1%, respectively, of that in the

control extraction. To improve the solubility of pigments, the temperature was raised to 55°C. This temperature did not amend pigment solubility and, moreover, at 400 bar, the extraction of capsanthin monoesters and β -carotene was worsened.

In the case of extraction using subcritical propane, the highest oil yield with the highest carotenoid content could be achieved at 30–50 bar. By increasing the pressure to 80 bar, the carotenoid content of the extract could be noticeably decreased. Compared with the control extraction, subcritical propane recovered 58.6%, 90%, 93%, 87.8%, and 93.6% of the initial content of capsanthin monoester, β -carotene, *cis*- β -carotene, capsorubin diesters, and capsanthin diesters, respectively. Additionally, the total carotenoids extracted from 1 g ground paprika was 970–1350 μ g. With SF-CO₂ at the best extractions, only 290–390 μ g could be recovered from 1 g paprika. These results confirmed that propane is superior to and more cost-effective than SF-CO₂ in the extraction of spice paprika oil. It is also evident that SF-CO₂ alone is not able to break the chemical association between diesters of red xanthophylls and the matrix. Therefore, it seems indispensable to use modifiers having lower polarity to

Table II. Distribution of Carotenoids Between Recovered Oil and Residues and Their Loss During Extraction at Different Conditions*

Pigments	SF-CO ₂ at 35°C			Subcritical propane
	100 bar (14%)	200 bar (100%)	300 bar (93%)	30 bar (100%)
Capsanthin ME				
in oil (mg)	0.69	13.46	18.28	187.15
in residues (mg)	220.92	33.60	121.92	91.84
total (mg)	221.61	47.06	140.20	278.99
found (%)	69.41	14.74	43.91	87.38
lost (%)	30.59	85.26	56.09	12.62
β-Carotene				
in oil (mg)	7.03	231.73	282.57	374.86
in residues (mg)	353.21	47.36	48.00	18.56
total (mg)	360.24	279.09	330.57	393.42
found (%)	82.54	63.94	75.74	90.14
lost (%)	17.46	36.06	24.26	9.86
Capsorubin DE				
in oil (mg)	0.14	3.07	5.16	88.52
in residues (mg)	39.18	8.42	20.80	13.38
total (mg)	39.32	11.49	25.96	101.90
found (%)	39.04	11.41	25.77	101.16
lost (%)	60.96	88.59	74.23	—
Capsanthin DE				
in oil (mg)	0.50	22.37	21.99	526.32
in residues (mg)	360.18	123.20	114.56	trace
total (mg)	360.68	145.57	136.55	526.32
found (%)	53.80	21.72	20.37	78.51
lost (%)	46.20	78.28	79.63	21.49

* Quantities of carotenoids are in μ g recovered from 1 g ground paprika.
† The values of extraction degree under the used conditions are in parentheses.

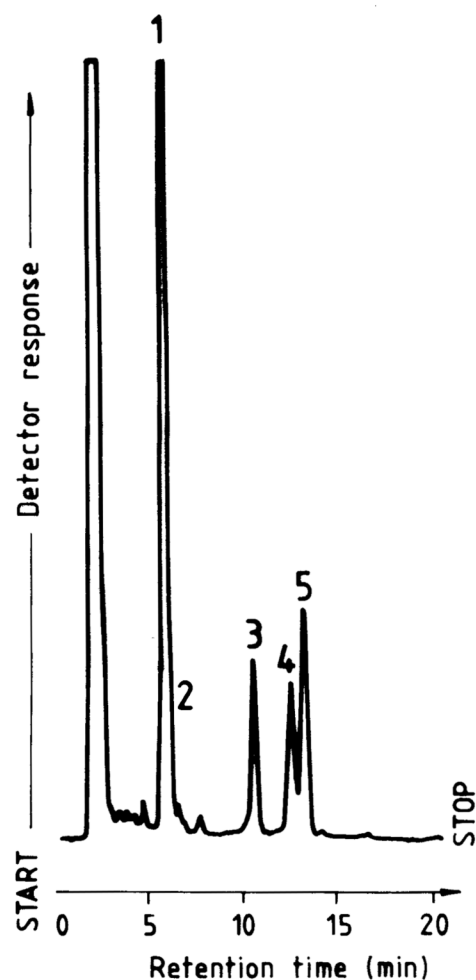


Figure 10. HPLC profile of tocopherols from paprika oil. Isocratic separation was carried out on a normal-phase silica column using *n*-hexane–ethanol (99.5:0.5) as the mobile phase. Peaks: 1, α -tocopherol; 2, α -tocotrienol; 3, α -tocopherolquinone; 4, β -tocopherol; 5 γ -tocopherol.

Table III. Effect of Type and Conditions of Super- and Subcritical Extraction on the Tocopherol Content of Spice Paprika Oil

Type and fractions	Extraction parameters			Concentration of tocopherol ($\mu\text{g/g}$ oil)		
	Pressure (bar)	T ($^{\circ}\text{C}$)	Extraction degree (%)	α -tocopherol	β -tocopherol	γ -tocopherol
Control*	1	25	100	369	13	41
SF-CO₂						
Cumulative	100	35	13.9	25	6	5
	200	35	100	249	20	34
	300	35	81.9	228	14	29
	400	35	100	241	12	28
	200	55	61.8	314	10	27
	300	55	92.6	320	10	29
	400	55	100	331	10	30
Fractional	200	35	36.8	137	6	14
	200	35	71.1	109	5	14
	200	35	92.9	12	0.3	0.6
	300	35	41.9	94	4	12
	300	35	67.8	90	3	9
	30	35	100	53	3	10
Subcritical propanol						
	30	25	100	391	12	41
	50	25	100	350	12	38
	80	25	100	375	13	40

* Control oil was prepared by organic solvent extraction using dichloroethane-acetone-methanol (2:1:1).

amend the solubilization ability of SF-CO₂ in a sequential extraction. As mentioned earlier (Figure 6), the extracts of SFE were fractionated into 3–4 subfractions to investigate whether paprika pigment can be separated into different carotenoid classes under the given conditions. The data in Table I showed that there is no selectivity in carotenoid extraction as a function of time or solvent-to-solid ratio, but most of the recovered diesters of capsorubin and capsanthin were found in the first subfraction. This was not observed with the other pigments.

To evaluate the low recovery of carotenoids by SF-CO₂ extraction, the residues (ground paprika samples after extraction) were analyzed for their carotenoid and tocopherol content. Table II summarizes the results obtained. It was repeatedly evident that in addition to the considerable amounts of pigments retained by the powder of paprika, there was a remarkable loss due to the degradation taking place on the carotenoid during SFE, particularly when CO₂ was the solvent. The loss was recorded with all the examined carotenoids, β -carotene being the least susceptible one. The percent loss for capsorubin and capsanthin ranged between 30 and 89, depending on the extraction pressure and type of carotenoid pigment. In the case of subcritical extraction using propane, the degree of carotenoid loss ranged between 0 and 22%, revealing the less-detrimental effect propane has on carotenoids in comparison with that of SF-CO₂.

The second important quality component is the tocopherol content of paprika oil. The HPLC analysis provided excellent separation of all the tocopherol analogues, including minute quantities of tocotrienols (Figure 10). α -Tocopherol (Vitamin E) was dominant in the extracts. Data in Table III indicate that 200 bar

is the lowest level of pressure needed to recover tocopherols from ground paprika by SF-CO₂. When the pressure was raised from 100 to 200 bar, α -, β -, and γ -tocopherol increased 9.96, 3.3, and 6.8 times in the recovered oil. Higher pressure levels did not improve the solubility of tocopherol in SF-CO₂. Increasing the temperature to 55 $^{\circ}\text{C}$ caused the recovered quantity of α -tocopherol to be 1.26-, 1.40-, and 1.37-times higher than at 35 $^{\circ}\text{C}$ when the pressure was 200, 300, and 400 bar, respectively. However, the gained amounts of tocopherols comprise only 85–89% in relation to the control. Fractionation of the extract of the subfractions implied that 95–98% of tocopherols can be recovered by the first and second subfraction (37–71% extraction) together with the bulk of carotenoids. Therefore, this procedure is not useful in carrying out fractional extraction of either carotenoids or tocopherols. In the case of propane extractions, 95–106% recovery of tocopherols was achieved. Raising the extraction pressure did not affect the solubility of tocopherols in propane. The best recovery was obtained at 30 bar, making this type of application very cost-effective in producing high quality oleoresins from spice red pepper.

Conclusion

The results of the present work indicated that SFE using CO₂ as the solvent has serious problems with the solubilization of fatty acid esters of red xanthophylls, the most important constituents of spice paprika color. In addition, a considerable quan-

tity of pigment is lost during the extraction process as a function of degradation. By the manipulation of the extraction conditions (such as temperature and pressure), a slight modification in pigment solubility can be obtained.

With propane as the solvent, the oil fraction (including most of carotenoids and all tocopherols) can be efficiently extracted at very mild (subcritical) conditions. From the economic point of view, propane is more cost-effective than SF-CO₂ because of the lower solvent-to-solid ratio to be used for the complete extraction of spice paprika oil.

Acknowledgments

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References

1. H.G. Daood, M. Vinkler, F. Márkus, E.A. Hebshi, and P.A. Biacs. Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and variatal factors. *Food Chem.* **55**: 365–72 (1996).
2. M.A. McHugh and V.J. Krukoni. *Supercritical Fluid Extraction, Principles and Practice*. Butterworths, Boston, MA, 1986.
3. C. White and V. Berry. A primer on supercritical fluids. *LC-GC* **8**: 734–38 (1990).
4. E. Stahl, E. Schütz, and H.K. Mangold. Extraction of seed oils with liquid and supercritical carbon dioxide. *J. Agric. Food Chem.* **28**: 1153–58 (1980).
5. F. Favati, J.W. King, and M. Mazzanti. Supercritical carbon dioxide extraction of evening primrose oil. *J. Am. Oil Chem. Soc.* **68**: 422–27 (1991).
6. C.R. Blatt and R. Ciola. Analysis of vetiver essential oil by supercritical fluid extraction and on-line capillary gas chromatography. *J. High Resolut. Chromatogr.* **14**: 775–77 (1991).
7. E. Stahl, K.W. Quirin, and P. Gerard. *Verdichtete gaze zur extraktion und raffination*. Spriger, Heidelberg, Germany, 1987.
8. T. Klein. Phasengleichgewichte in gemischen aus pflanzlichen ölen und kohlendioxid. *Fortscher.-Ber. VDI-Z. Reihe* **3(159)** (1988).
9. V. Illés, O. Szalai, M. Then, H.G. Daood, and S. Perneczki. Extraction of hiprose fruit by supercritical CO₂ and propane. *J. Supercrit. Fluids* **10**: 209–218 (1997).
10. C.D. Bevan and P.S. Marshal. The use of supercritical fluids in the isolation of natural products. *Nat. Prod. Rep.* **11**: 451–66 (1994).
11. J.L. Hedrick, L.J. Mulcahey, and L.T. Taylor. Fundamental review: supercritical fluid extraction. *Mikrochem. Acta* **108**: 115–32 (1992).
12. D.Y. Peng and D.B. Robinson. A new two-constant equation of state. *Ind. Eng. Chem. Fund.* **15**: 59–63 (1976).
13. E. Bender. Equation of state exactly representing phase behavior of pure substances. *Proceedings of the 5th Symposium on Thermophysical Properties*. American Society of Mechanical Engineers, New York, NY, 1970, pp 227–35.
14. P.A. Biacs and H.G. Daood. High-performance liquid chromatography and photodiode-array detection of carotenoids and carotenoid esters in fruits and vegetables. *J. Plant Physiol.* **143**: 520–25 (1994).
15. M. Vinkler and K.A. Richter. Thin layer chromatographic method to determine the pigment content in the pericarp of paprika. *Acta Aliment.* **1**: 41–58 (1972).
16. A.G. Speek, F. Schrijver, and H.P. Shreurs. Vitamin E composition of some oils as determined by HPLC with fluorimetric detection. *J. Food Sci.* **50**: 121–24 (1985).
17. I. Chrastil. Solubility of solids and liquids in supercritical gases. *J. Phys. Chem.* **8**: 3016–20 (1982).
18. G.W. Burton and K.V. Ingold. β -carotene: an unusual type of lipid antioxidant. *Science* **224**: 569–73 (1984).
19. P.A. Biacs, B. Czinkotai, and A. Hoschke. Factor affecting stability of colored substances in paprika powders. *J. Agric. Food Chem.* **40**: 363–67 (1992).

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