

Supercritical CO₂ and Subcritical Propane Extraction of Pungent Paprika and Quantification of Carotenoids, Tocopherols, and Capsaicinoids

M. H. Gnayfeed,[†] H. G. Daood,^{*,†} V. Illés,[‡] and P. A. Biacs[†]

Central Food Research Institute, Herman Ottó u. 15, 1022 Budapest, Hungary, and Department of Chemical Engineering, University of Veszprém, Egyetem u. 10, 8201 Veszprém, Hungary

Ground paprika (*Capsicum annuum* L.) was extracted with supercritical carbon dioxide (SC-CO₂) and subcritical propane at different conditions of pressure and temperature to estimate the yield and variation in carotenoid, tocopherol, and capsaicinoid contents and composition. The yield of paprika extract was found to be affected by the extraction conditions with SC-CO₂ but fairly constant at different conditions with subcritical propane. The maximum yields of oleoresin were 7.9 and 8.1% of ground paprika by SC-CO₂ and subcritical propane, respectively. The quantitative distribution of carotenoids, tocopherols, and capsaicinoids between paprika extract and powder was influenced by extraction conditions. SC-CO₂ was inefficient in the extraction of diesters of xanthophylls even at 400 bar and 55 °C, whereas tocopherols and capsaicinoids were easy to extract at these conditions. Under mild conditions subcritical propane was superior to SC-CO₂ in the extraction of carotenoids and tocopherols but less efficient in the extraction of capsaicinoids.

Keywords: *Supercritical extraction; paprika; tocopherols; carotenoids; capsaicinoids*

INTRODUCTION

Hot spice red pepper (paprika) is heavily consumed throughout the world and valued for its colorants, flavors, and pungency principles. The pigment of paprika consists of red and yellow carotenoids, of which the biologically active β -carotene and lutein are of special interest from a nutritional point of view. The coloring capacity and color stability of paprika products determined by the content of mono- and diesters (1, 2). Also paprika distributes considerable amounts of fat-soluble antioxidants such as tocopherols (mainly α -tocopherol) (1). Another quality attribute in paprika is the hot flavor caused by capsaicinoids, the pungency principles. These alkaloids have been intensively investigated for their physiological and pharmaceutical importance (3, 4). Occurrence of such bioactive nutrients at high concentration in paprika makes it an important functional food or food ingredient.

Due to some advantages such as high solvating capacity, selectivity, and cost-effectiveness, extraction by compressed gases has become, in some cases, superior to traditional methods using organic solvents. Because of its critical temperature and its nontoxic, nonflammable, and environmentally preferred qualities, supercritical carbon dioxide (SC-CO₂) is widely used as solvent in the supercritical fluid extraction (SFE) of a wide variety of compounds, particularly thermally labile ones. Few studies have been reported for the extraction of spice paprika (5–8) using SC-CO₂ alone or with modifiers. The target of recent studies was the recovery of capsaicinoids from pungent peppers with no interest given to the carotenoid and tocopherol content of the extracts (9–11).

In a previous work (12) SC-CO₂ and subcritical propane extraction of nonpungent paprika has been studied with special focus on the behavior of carotenoid and tocopherols. The present study was undertaken to investigate the effect of different conditions of SC-CO₂ and subcritical propane on the recovery of capsaicinoids, carotenoids, and tocopherols from pungent paprika.

MATERIALS AND METHODS

Materials. Ground paprika (*Capsicum annum* L. var. SZ-178) was obtained from the Paprika Research Developing Co. (Kalocsa, Hungary). The particle size of the powder was between 400 and 600 μ m. Standard capsaicinoids, β -carotene (95%), and tocopherols were from Sigma (St. Louis, MO). All organic solvents and chemicals used in the extractions were of analytical grade and from Reanal, whereas those used for chromatographic analyses were of HPLC grade from Merck (Darmstadt, Germany). Technical grade CO₂ and propane of 99.9% purity were purchased from Messier Griesheim Hungaria (Budapest, Hungary).

Supercritical Fluid Extraction. The extractions were performed in a high-pressure flow-up apparatus, in which oil solubilization by the different solvents takes place in a dynamic mode. A detailed description of the apparatus can be found in a previously published paper (13). A model EL-1 membrane pump (from LEWA Herberott, Leonberg, Germany) pumped the liquid solvent through a buffer vessel into the thermostated extraction column. The pressure of the solvent was controlled with a back-pressure valve. The solute-rich compressed gas was expanded through a heated needle valve to atmospheric pressure. The extract was trapped in a cooled vessel and the amount of solvent gas also measured.

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

High-Performance Liquid Chromatographic (HPLC) Apparatus and Conditions. The capsaicinoid, carotenoid, and tocopherol composition of the extracts was analyzed by

* Author to whom correspondence should be addressed (fax +361-212-9853; e-mail h.daood@cfri.hu).

[†] Central Food Research Institute.

[‡] University of Veszprém.

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. For the analysis of capsaicinoids and tocopherols, the HPLC apparatus consisted of a Beckman model Gold System 116 pump and a Shimadzu (Tokyo, Japan) model RF-535 fluorescence detector. The signals of the detectors were recorded and integrated by a Shimadzu C-R3A integrator.

The carotenoids were separated on Lichrosorb C-18, 6 μm , 250 \times 46 mm column using a mobile phase consisting of 39:52:5:4 acetonitrile/2-propanol/methanol/water at a flow rate of 0.9–1.2 mL/min (14). The effluents were monitored at 470 nm. The separation of tocopherols was performed on Lichrosorb, 10 μm , 250 \times 46 mm column with 99.5:0.5 *n*-hexane/ethanol as the mobile phase at a flow rate of 1.2 mL/min. The detection was carried out at 295 nm for excitation and 320 nm for emission (15).

In the analysis of capsaicinoids, the method of Chiang (16) was slightly modified. Instead of an Altex Ultrasphere ODS, a Chromsil C₁₈, 6 μm column was used with a modified mobile phase consisting of 1:1 acetonitrile/3% KH₂PO₄. The flow rate was 1.4 mL/min. The effluents were fluorometrically detected at 288 nm for excitation and 370 nm for emission.

Peak Identification and Quantification. The peaks of the carotenoid profile were identified on the basis of comparison of retention times and scanned spectra of the samples with those of authentic standards prepared by thin-layer chromatography (17). Standard β -carotene, lutein, capsaicinoids, and tocopherols (Sigma) were also applied in the qualitative and quantitative analysis of the individual pigments, pungency materials, and vitamin E components.

To validate the applied methods, stock solutions of standard materials were diluted to make working solutions of different concentrations for testing the calibrations, recoveries, and detection limits. In the recovery test, 200 μg of each standard material was dissolved in a suitable solvent and added to 1 g of paprika powder or oil. The powder was thoroughly mixed and then stored for 2 h, in the dark, to evaporate the solvent. In the case of capsaicinoids the standard material was added to sweet paprika (free of capsaicinoids). The extractions were carried out as described later. The original quantities of each material in unspiked (control) samples were subtracted from the total in spiked samples to calculate the recovered amounts. A precision test was based on the relative standard deviation for five replications of the same sample. Limit of detection (LOD) and limit of quantification (LOQ) were the concentrations of the solutes that provided signal/noise ratios of 3:1 and 10:1, respectively.

Extractions. Capsaicinoids were extracted from the ground paprika by methanol according to the method of Chiang (16). A half gram of paprika was transferred to a 10-mL volumetric flask, and 5 mL of methanol was added. The mixture was homogenized for 1 min by means of an ultrasonication water bath (Tesla). The volume was then adjusted to 10 mL with methanol. After shaking for 5 min, the mixture was filtered through an MN-640 filter paper, and an aliquot of this was passed through a 0.45 μm syringe filter. In the case of oleoresin (SFE-extracted), 50–100 mg was taken in a 10 mL volumetric flask and 10 mL of acetone was added with hand shaking and mixing on a Vortex mixer to ensure complete solubility of the oleoresin. Before injection onto the HPLC column, an aliquot was filtered through a 0.45 μm syringe filter.

Extraction of carotenoids from ground paprika was carried out as previously reported (2). A half gram of ground paprika was gently shaken with 50 mL of 2:1:1 dichloroethane/acetone/methanol for 15 min. The mixture was then filtered through an MN-640 filter paper and the solvent evaporated to dryness under vacuum at not higher than 40 °C.

To analyze carotenoids from paprika oleoresin, 50–100 mg was directly dissolved in 10–25 mL of the HPLC eluent, which was capable of completely dissolving the oleoresin. Nevertheless, it was filtrated through a 0.45 μm filter to provide clear solutions for HPLC analysis.

To simplify the tocopherol extract and to remove the glycerol lipids that may interfere with tocopherols in the HPLC

separation and fluorescence detection, ground paprika or oleoresin was saponified. A half gram of ground paprika or 50 mg of oleoresin was put in a brown round-bottom flask, and 20 mL of methanol, 0.5 g of ascorbic acid, and 5 mL of saturated methanolic KOH were added. The mixture was refluxed at the boiling point of methanol for 40 min. Following rapid cooling, 15 mL of salted water was added, and tocopherols were extracted twice with 40 mL of *n*-hexane. The hexane fractions were collected, washed three times with distilled water, and dried over anhydrous Na₂SO₄. The solvent was evaporated under vacuum at 30 °C, and the residues were dissolved in 5 mL of HPLC grade *n*-hexane.

RESULTS AND DISCUSSION

By the applied HPLC procedure paprika pigment could be fractionated to four carotenoid classes (unesterified xanthophylls, monoesters, carotenes, and diesters). Because these classes vary in their fatty acid moieties and polarities (14, 18) it is expected that they have different solubilities in SC-CO₂ or subcritical propane. Variations between these classes in their chemical association with the cell membranes and their stability during extraction process are also expected.

Paprika extracts contained α -, β -, and γ -tocopherols as the major components of vitamin E with small quantities of other minor compounds such as α - and β -tocotrienols. The tocopherol composition of pungent paprika extract is close to that of sweet paprika (12).

The modified HPLC method provided good separation of pungency principles. The capsaicinoid profile consisted of nordihydrocapsaicin (NDC), capsaicin (C), dihydrocapsaicin (DC), homocapsaicin (HC), and homodihydrocapsaicin (HDC), with C and DC being dominant.

On the basis of recovery, precision, detection limit, and range of linearity tests the applied methods were validated. From five replicate extractions, recoveries of 95–98, 93–95, and 94–96% were recorded for carotenoids (β -carotene), tocopherols (α -tocopherol), and capsaicinoids, respectively. The values of the relative standard deviation were 4.4–5.2 for carotenoids, 3.0–3.8 for tocopherols, and 4.6–5.0 for capsaicinoids when four or five extractions were carried out for the same sample. LOD values of 0.05, 0.02, and 0.04 and LOQ values of 0.17, 0.09, and 0.11 were obtained for carotenoids, tocopherols, and capsaicinoids, respectively. Calibration data indicated the straight relationship between peak area and concentration in a wide range of 0–60, 0–50, and 0–200 $\mu\text{g}/\text{mL}$ with correlation coefficients of 0.966, 0.996, and 0.988 for carotenoids, tocopherols, and capsaicinoids, respectively. These data indicated an acceptable precision, accuracy, and sensitivity of the applied methods of analysis.

The extracts with SC-CO₂ were light red to red in color, whereas the extract with subcritical propane was of highly intense red color (appears to be black in color). Increasing the volume of SC-CO₂ (Figure 1) increased the yield of oleoresin from pungent paprika. At constant pressure, the extraction curve can be characterized by an initial steep linear increase of oleoresin solubility as a function of solvent volume (solvent/solid ratio). Then the solubility curve reaches a plateau and approaches the maximum yield of oleoresin. The volume of supercritical solvent needed to achieve the maximum yield of oleoresin depended on the applied pressure. At low pressure, the solubility of paprika oil in SC-CO₂ was low and remarkably affected by the extraction temper-

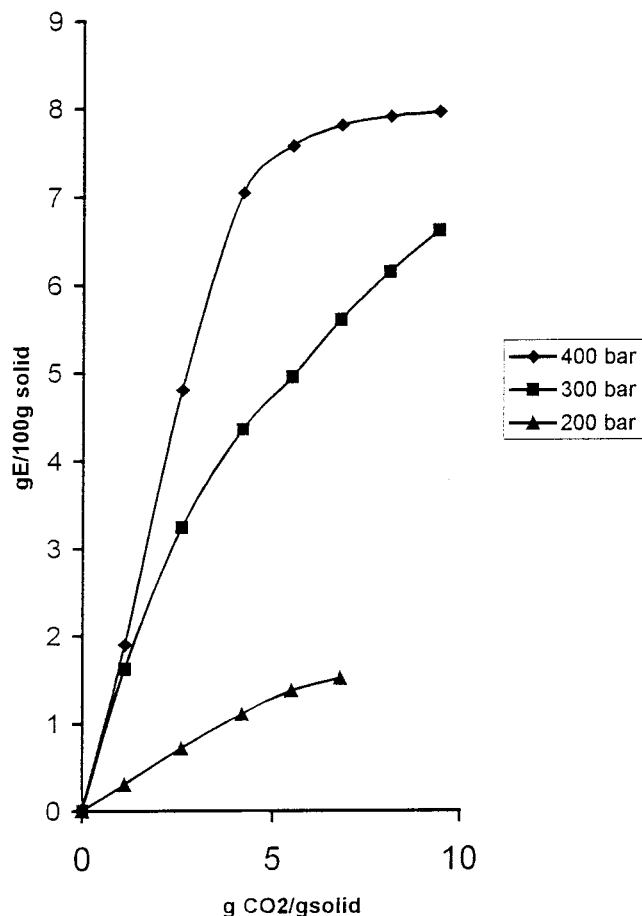


Figure 1. Solubility of ground paprika oil in SC-CO₂ as affected by solvent/solid ratio at different extraction pressures and 35 °C.

ature. For instance, at 200 bar and a ratio of solvent/solid of 12 the yields of oleoresin were 1.51 and 4.42 g from 100 g of paprika when the extraction was performed at 55 and 35 °C, respectively. At a pressure of 100 bar, no oleoresin could be obtained under the conditions used. The temperature had a slight effect on the oil solvating capacity of SC-CO₂ at pressures >300 bar. At a constant pressure of 400 bar 7.95 and 7.57 g could be delivered from 100 g of paprika, as the maximum yield, when the extraction was performed at 55 and 35 °C, respectively. The impact of extraction conditions on the solubilization of oleoresin from pungent paprika, by SC-CO₂, was similar to that noticed in the SFE of sweet (nonpungent) paprika (12). From these results it can be concluded that the presence of capsaicinoids in paprika oil has no substantial effect on the solubility equilibrium of paprika oil in SC-CO₂.

The extraction of paprika oil with subcritical propane is shown in Figure 2. The solubility curve was obtained by plotting the amount of extract versus the propane/solid ratio. The relationship between solvent used and recovered oleoresin was similar to that found with SC-CO₂, but the complete extraction of oil could be implemented at a solvent/solid ratio between 1.0 and 1.5 and substantially lower pressure (50 bar). These values are much lower than the solvent/solid ratio of 10 needed for the complete extraction with SC-CO₂ at 400 bar. The maximum quantity of oil recovered by subcritical propane was 8.1 g/100 g of paprika. This value is very close to the 7.95 g/100 g of paprika obtained with SC-CO₂ at

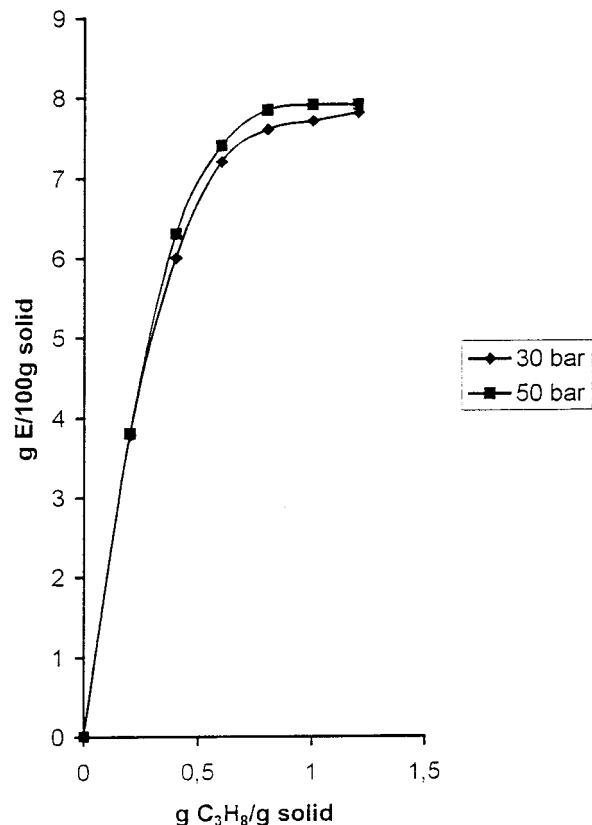


Figure 2. Solubility of ground paprika oil in subcritical propane as affected by solvent/solid ratio at different extraction pressures.

the best conditions. Increasing the pressure of subcritical extraction with propane from 30 to 80 bar had no significant effect on the yield of oleoresin.

Evaluation of the Extracts. Table 1 shows the effect of extraction conditions on the carotenoid content of paprika oil. It is evident that SC-CO₂, particularly at low pressures, is not capable of extracting the carotenoids from ground paprika. As previously reported (12) mono- and diesters of xanthophylls can hardly be solvated by SC-CO₂. At 200 bar and 35 °C only 0.8% of the total carotenoids could be extracted by SC-CO₂. The solubility of pigments in SC-CO₂ and their recovery in the oleoresin was increased by increasing the pressure to 400 bar. Nevertheless, the maximum pigment recovery did not exceed 11% even though the temperature was raised to 55 °C. These results support the conclusion that oil and pigment extraction by SC-CO₂ is pressure-dependent (18). When the pigment concentration in the remaining powder (after extraction) was related to that in the starting material, pigment retention (percent) was obtained. A 93% retention could be recorded in powders extracted by SC-CO₂ at 200 bar. The lowest retention (44%) was found in a powder extracted at 400 bar and 55 °C. The total of solubilized and retained pigment was almost 55% of the initial pigment content of paprika. These results point to the degradation of carotenoid during SFE (in-extraction loss). The magnitude of loss increased with the increase of extraction pressure and temperature. The different carotenoid classes showed different susceptibilities to degradation. The highest stability was found with unesterified (free) pigments, which were either soluble in oil or retained by the powder. However, extraction at 55 °C and pressure >300 bar caused marked loss of

Table 1. Effect of Extraction Conditions on the Carotenoid Content of Oleoresin from Pungent Paprika

SFE conditions			carotenoids					total	found (%)
pressure (bar)	temp (°C)	solvent	free pigment	monoesters	β -carotene	diesters			
in-oil ($\mu\text{g/g}$ of oil)									
200	35	CO ₂	211 (3.9) ^a	194 (0.9)	57 (1.7)	15 (0.1)	484	1	
300	35	CO ₂	358 (11)	661 (5.2)	258 (13)	147 (1.0)	1515	5	
400	35	CO ₂	394 (12.5)	824 (6.6)	454 (24)	570 (4.1)	2391	7	
200	55	CO ₂	189 (1.12)	301 (0.5)	217 (2.3)	135 (0.2)	914	1	
300	55	CO ₂	358 (11)	1024 (8.1)	420 (22)	798 (5.6)	2759	9	
400	55	CO ₂	526 (17.5)	1468 (12.4)	595 (33)	854 (6.4)	3676	11	
50	25	propane	372 (13)	7490 (65)	1381 (78)	10830 (83)	20461	70	
residual ($\mu\text{g/g}$ of powder)									
200	35	CO ₂	236 (99)	906 (96)	117 (81)	1036 (97)	2329	93	
300	35	CO ₂	211 (88)	796 (85)	66 (46)	947 (89)	2026	82	
400	35	CO ₂	220 (92)	740 (79)	44 (31)	879 (83)	1888	76	
200	55	CO ₂	250 (94)	939 (99)	128 (89)	1146 (98)	2316	93	
300	55	CO ₂	161 (67)	608 (65)	61 (42)	676 (64)	1512	61	
400	55	CO ₂	155 (65)	449 (48)	19 (13)	338 (32)	1083	44	
50	25	propane	166 (69)	236 (25)	13 (9)	84 (8)	500	20	
starting material	25	organic solvents	239 (100)	940 (100)	144 (100)	1062 (100)	2482	100	

^a Within parentheses is the proportion of each component as related to its content in starting material.

Table 2. Effect of Extraction Conditions on the Tocopherol Content of Oleoresin from Pungent Paprika

SFE conditions			tocopherols			
pressure (bar)	temp (°C)	solvent	α -tocopherol	γ -tocopherol	found %	
					α -tocopherol	γ -tocopherol
in-oil ($\mu\text{g/g}$ of oil)						
200	35	CO ₂	3066	298	49	22
300	35	CO ₂	2606	354	68	43
400	35	CO ₂	3240	368	88	47
200	55	CO ₂	4740	344	26	8
300	55	CO ₂	2454	416	65	52
400	55	CO ₂	3288	358	93	47
50	25	propane	3088	534	94	75
residual ($\mu\text{g/g}$ of powder)						
200	35	CO ₂	21	28	8	47
300	35	CO ₂	13	28	5	47
400	35	CO ₂	13	27	5	45
200	55	CO ₂	75	28	27	47
300	55	CO ₂	7	25	3	42
400	55	CO ₂	5	12	2	20
50	25	propane	10	12	4	20
starting material	25	organic solvents	280	60	100	100

Table 3. Effect of Extraction Conditions on the Capsaicinoid Content of Oleoresin from Pungent Paprika

SFE conditions			capsaicinoids						
pressure (bar)	temp (°C)	solvent	oil yield (g)	NDC	C	DC	total	$\mu\text{g/g}$ of paprika	found (%)
200	35	CO ₂	2.3870	951	7888	7766	17204	760	62
300	35	CO ₂	3.9601	655	5386	5261	11644	854	70
400	35	CO ₂	4.0893	679	5644	5400	12090	915	75
200	55	CO ₂	0.8166	1191	9667	9184	20601	312	26
300	55	CO ₂	4.9944	663	5542	5366	11954	884	72
400	55	CO ₂	4.2907	785	6561	6452	14270	1134	93
50	25	propane	4.3900	129	1158	1197	2484	202	17
residual ($\mu\text{g/g}$ of powder)									
200	35	CO ₂		11	68	65	143	143	12
300	35	CO ₂		10	61	56	134	134	11
400	35	CO ₂		11	71	63	127	127	10
200	55	CO ₂		27	204	203	440	440	36
300	55	CO ₂		5	28	23	74	74	6
400	55	CO ₂		6	34	31	55	55	5
50	25	propane		55	411	386	875	875	72
starting material	25	organic solvents		76	551	588	1222	1222	100

these pigments, but not as much as what happened with the other classes. Monoesters of xanthophylls ranked second among carotenoid classes in their loss during extraction. The greatest loss was recorded for β -carotene at 55 °C and 400 bar (65% loss) followed by diesters, which lost 62% of their content under the same conditions. The high susceptibility of β -carotene to SFE

explains why, despite the relatively high solubility of β -carotene in SC-CO₂ (18, 19), only 33% of its initial content could be recovered in the oleoresin under the conditions used.

Extraction by propane at subcritical conditions yielded oleoresin of high color intensity. The relatively low polarity of propane, as compared to that of SC-CO₂,

assisted in the rapid solubilization of both oil and fat-soluble carotenoids. The solvent recovered efficiently the diesters followed by β -carotene and monoesters. The lowest recovery (13%) was recorded for the highly polar, unesterified xanthophylls. After propane extraction, the remaining paprika powder contained the lowest level of carotenoid pigments. The highest retention, by powder, was found with unesterified xanthophylls, and the lowest was with diesters. Because propane solvates the pigment in a substantially low ratio of solvent/solid, the time needed for the complete extraction of the oleoresin was shorter than with SC-CO₂. Therefore, the in-extraction loss of pigments with propane was less than that with SC-CO₂. The lost proportion of diesters and unesterified xanthophylls accounted for 9 and 18% of the initial content (in raw material), respectively.

Table 2 shows the content of the major tocopherols (α - and γ -) as micrograms per gram of oil and micrograms per gram of powder. At various extraction volumes, increasing the pressure increased the solubility of both α - and γ -tocopherols in SC-CO₂, with the former being more efficiently recovered. The recovery of α -tocopherol increased from 49 to 88%, whereas the recovery of γ -tocopherol could be increased only from 22 to 47% when the pressure was raised from 200 to 400 bar. It seems that at high temperature and low pressure both α - and γ -tocopherols undergo substantial degradation, and therefore performance of SFE at higher pressure is necessary to increase in-oil recovery of tocopherols and to minimize their degradation. The best recovery of α -tocopherol (93%) was achieved with SC-CO₂ at 400 bar and 55 °C. The behavior of tocopherols in SFE extraction of pungent paprika is somewhat different from that observed in the extraction of nonpungent paprika (13). This variation may be due to the fact that tocopherols exist in pungent paprika extract in a more diverse system than that of nonpungent paprika. The presence of capsaicinoids, as highly soluble solids in SC-CO₂, can alter the solubility isotherm of tocopherol in SC-CO₂, particularly at high temperatures and pressures (20).

With propane at subcritical conditions almost 94 and 75% of α - and γ -tocopherols could be recovered in the oil, respectively, with minimal degradation (2–5%).

The paprika oleoresin obtained with SC-CO₂ extraction contained capsaicinoids with a composition similar to that found in starting material extracted with methanol (Table 3). The highest level of pungency principles was estimated in oil extracted at 200 bar and 55 °C. This was due to the small quantity of oil recovered at the given conditions. On the basis of the capsaicinoid quantity extracted from 1 g of powder, the recovery of these compounds, by SC-CO₂, could be achieved at 400 bar and 55 °C. At these conditions 93% of capsaicinoid content could be recovered in oil with 5% being retained by the powder. A similar recovery was achieved by Yasumoto and co-worker (9), who reported that the use of modifier was not effective in increasing the value of capsaicin recovery.

By increasing the pressure from 200 to 400 bar the solubilities of capsaicinoids in SC-CO₂ were increased 3.6 and 1.2 times at 55 and 35 °C, respectively. The high pressure and temperature of extraction were necessary not only for the highest recovery of such compounds but also for the minimization of their loss (degradation) during the extraction process.

Extraction with propane yielded oleoresin with a considerably low level of capsaicinoids. Only 17% of the original capsaicinoids could be recovered in the oleoresin, whereas the ground paprika retained 73%. The different constituents of pungent material did not vary in their quantitative distribution between oleoresin and remaining powder at all of the conditions used in the present study.

It can be concluded that although SFE using CO₂ produces paprika oleoresin with relatively high levels of pungency principles and tocopherols, it hardly extracts the red xanthophylls, particularly the diesters of capsanthin. Unlike SC-CO₂, propane at subcritical conditions is an excellent solvent for the recovery of carotenoid pigments and tocopherols, but hardly solvates capsaicinoids from paprika. Therefore, the use of mixtures of CO₂ and propane is suggested to produce paprika oleoresins with different color intensities, pungency levels, and antioxidant contents.

LITERATURE CITED

- (1) Biacs, P. A.; Czinkotai, B.; Hoschke, A. Factors affecting stability of colored substances in paprika powders. *J. Agric. Food Chem.* **1992**, *40*, 363–367.
- (2) Daood, H. G.; Vinkler, M.; Márkus, F.; Hebshi, E. A.; Biacs, P. A. Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. *Food Chem.* **1996**, *55*, 365–372.
- (3) Govindarajan, V. S.; Sathyanarayana, M. N. Capsicum production, technology, Chemistry and quality. Part V. Impact on physiology, pharmacology, nutrition and metabolism: structure, pungency, pain and desensitization sequences. *CRC Crit. Rev. Food Sci. Nutr.* **1991**, *29*, 435–474.
- (4) Surh, Y.; Lee, S. S. Capsaicin in hot chili pepper: Carcinogen, co-carcinogen or anti-carcinogen? *Food Chem. Toxicol.* **1996**, *34*, 313–316.
- (5) Coenene, H.; Kriegel, E. Application of extraction with supercritical gases in food industry. *Chem. Ing. Tech.* **1983**, *11*, 890–891.
- (6) Coenene, H.; Hagen, R. natural colorant from paprika with gas extraction. *Gordian* **1983**, *9*, 164–170.
- (7) Knez, Z.; Posel, F.; Hunek, J.; Golob, J. Extraction of plant materials with supercritical CO₂. In *Proceeding of the 2nd International Conference on Supercritical Fluids*; McHugh, M., Ed.; John Hopkins University: Baltimore, MD, 1991; pp 101–104.
- (8) Knez, Z.; Skerget, M. Application of supercritical CO₂ for food processing. In *Proceeding of the 6th International Congress on Engineering and Food*; Yano, T., Matsuno, R., Nakamura, K., Eds.; Blackie Academic and Professional: Chiba, Japan, 1994; Part 2, pp 826–828.
- (9) Yao, J.; Nair, M. J.; Chandra, A. Supercritical carbon dioxide extraction of Scotch Bonnet (*Capsicum annum*) and quantification of capsaicin and dihydrocapsaicin. *J. Agric. Food Chem.* **1994**, *42*, 1303–1305.
- (10) Yasumoto, M.; Inakuma, T.; Okazaki, T.; Kobayashi, T. Extraction of capsaicin from red pepper with liquid carbon dioxide. *J. Jpn. Soc. Food Sci.* **1994**, *41*, 565–567.
- (11) Peusch, M.; Mueller, S. E.; Petz, M.; Mueller, A.; Anklam, E. Extraction of capsaicinoids from chillies (*Capsicum frutescens* L.) and paprika (*Capsicum annum* L.) using supercritical fluids and organic solvents. *Food Res. Technol. (Germany)* **1997**, *204*, 351–355.
- (12) Illés, V.; Szalai, M.; Then, M.; Daood, H. G.; Perneckzi, S. Extraction of hiprose fruit by supercritical CO₂ and propane. *J. Supercrit. Fluids* **1997**, *10*, 209–218.

- (13) Illés, V.; Daood, H. G.; Biacs, P. A.; Gnayfeed, M. H.; Mészáros, B. Supercritical CO₂ and subcritical propane extraction of spice red pepper oil with special regard to carotenoid and tocopherol content. *J. Chromatogr. Sci.* **1999**, *37*, 345–352.
- (14) Biacs, P. A.; Daood, H. G. High-performance liquid chromatography with diode-array detection of carotenoids and carotenoid esters in fruits and vegetables. *J. Agric. Food Chem.* **1994**, *42*, 520–525.
- (15) Speek, A. J.; Schrijver, F.; Shreurs, H. P. Vitamin E composition of some oils as determined by HPLC with fluorometric detection. *J. Food Sci.* **1985**, *20*, 121–124.
- (16) Chiang, G. H. HPLC analysis of capsaicin and simultaneous determination of capsaicins and piperine by HPLC-ECD and UV. *J. Food Sci.* **1986**, *51*, 499–503.
- (17) Vinkler, M.; Richter, K. A. A thin layer chromatographic method to determine the pigment content (components) in the pericarp of paprika. *Acta Aliment.* **1972**, *1*, 41–58.
- (18) Galán-Járen, M.; Nienaber, U.; Schwartz, S. J. Paprika (*Capsicum annuum*) oleoresin extraction with supercritical carbon dioxide. *J. Agric. Food Chem.* **1999**, *47*, 3558–3564.
- (19) Vega, P. J.; Bakaban, M. O.; Sims, C. A.; O'keefe, S. F.; Cornell, J. A. Supercritical carbon dioxide extraction efficiency for carotenes from carrots by RSM. *J. Food Sci.* **1996**, *61*, 757–759.
- (20) Skerget, M.; Knez, Z. Solubility of binary mixture β -carotene-capsaicin in dense CO₂. *J. Agric. Food Chem.* **1997**, *45*, 2066–2069.

Received for review October 26, 2000. Revised manuscript received January 18, 2001. Accepted February 21, 2001. The financial support of the National Committee of Technical Development (OMFB) under Neodiet Project (1999–2001) and the National Scientific Research Fund (OTKA) under Grants T029365 and T025814 is gratefully acknowledged.

JF001292Q