Paprika (*Capsicum annuum*) Oleoresin Extraction with Supercritical Carbon Dioxide

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Paprika oleoresin was fractionated by extraction with supercritical carbon dioxide (SCF-CO_2). Higher extraction volumes, increasing extraction pressures, and similarly, the use of cosolvents such as 1% ethanol or acetone resulted in higher pigment yields. Within the 2000−7000 psi range, total oleoresin yield always approached 100%. Pigments isolated at lower pressures consisted almost exclusively of β-carotene, while pigments obtained at higher pressures contained a greater proportion of red carotenoids (capsorubin, capsanthin, zeaxanthin, β-cryptoxanthin) and small amounts of β-carotene. The varying solubility of oil and pigments in SCF-CO_2 was optimized to obtain enriched and concentrated oleoresins through a two-stage extraction at 2000 and 6000 psi. This technique removes the paprika oil and β-carotene during the first extraction step, allowing for second-stage oleoresin extracts with a high pigment concentration (200% relative to the reference) and a red:yellow pigment ratio of 1.8 (as compared to 1.3 in the reference).

Keywords: *Paprika (Capsicum annuum); oleoresin; supercritical carbon dioxide; carotenoids; color*

INTRODUCTION

The use of supercritical fluids (SCF) for extraction purposes was introduced in the late nineteenth century. A supercritical fluid is a substance at temperatures and pressures beyond its critical point at which the liquid phase of the substance will not exist. At these temperatures and pressures, the supercritical fluid has properties between gas- and liquid-phase characteristics. These properties make supercritical fluids efficient extraction solvents with high mass transfer characteristics (McHugh and Krukonis, 1986; Krukonis, 1988).

In food technology, the use of supercritical fluids is essentially limited to supercritical carbon dioxide (SCF-CO_2) extraction since carbon dioxide has the advantages of being inexpensive and nontoxic and because its critical point is easily reached. Many studies have been conducted to investigate the applicability of SCF-CO_2 for the extraction of compounds significant to food industries (Reverchon and Senatore, 1994; Gopalakrishnan, 1994), and several authors have focused their research more specifically on the extraction of carotenoids by SCF-CO_2 from leaf protein concentrates (Favati et al., 1988), annatto seeds (Degnan et al., 1991; Chao et al., 1991), and sweet potatoes (Spanos et al., 1993).

Carotenoids are of interest in the food technology field because of their nutritional, pharmacological, and colorant properties. Paprika oleoresin has recently been commercialized and has one of the highest carotenoid pigment concentrations of products derived from natural sources. Paprika is defined as the powder of dry red pepper (*Capsicum annuum*). Hexane extraction of paprika and subsequent solvent removal produces paprika oleoresin, which is composed of carotenoid pigments and lipids. The main red carotenoids are capsanthin and capsorubin, characterized by pentagonal ring structures (Curl, 1962; Davies, 1970). During fruit maturation these hydroxylic carotenoids are esterified, which does not affect their chromatic properties but modifies their polarity (Mínguez-Mosquera and Hornero-Méndez, 1994). The nonesterified molecules show low solubility in hexane, but esterification makes them highly lipophilic and highly soluble in nonpolar solvents. This change in polarity allows the extraction process with organic solvents to produce the oleoresin.

The extraction solvent most commonly employed in the industry is hexane. Generally for complete extraction of dehydrated tissue it is necessary to repeat the process several times in order to obtain an acceptable yield. The system most employed is the discontinuous extraction with recycled extraction solvent. The main disadvantage of this method is that, after the extraction, the solvent needs to be evaporated, which causes heat degradation of carotenoids. At the end of the process the solvent residue concentration has to be reduced below legal limits, requiring even more drastic conditions.

The extraction of carotenoids by traditional methods is often complicated by their susceptibility to isomerization and degradation. At room temperature and exclusion of light, cis−trans isomerization of these compounds easily occurs, resulting in a partial loss of provitamin A activity (Sweeney and Marsh, 1973). Under light exposure and room temperature, carotenoid structures are broken down, and the molecules lose all their nutritional, medical and colorant properties (Mínguez and Jarén, 1995).

Therefore, new extraction procedures offering advantages over traditional methods are of interest. The

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10.1021/jf9900985 CCC: $18.00
Published on Web 08/31/1999

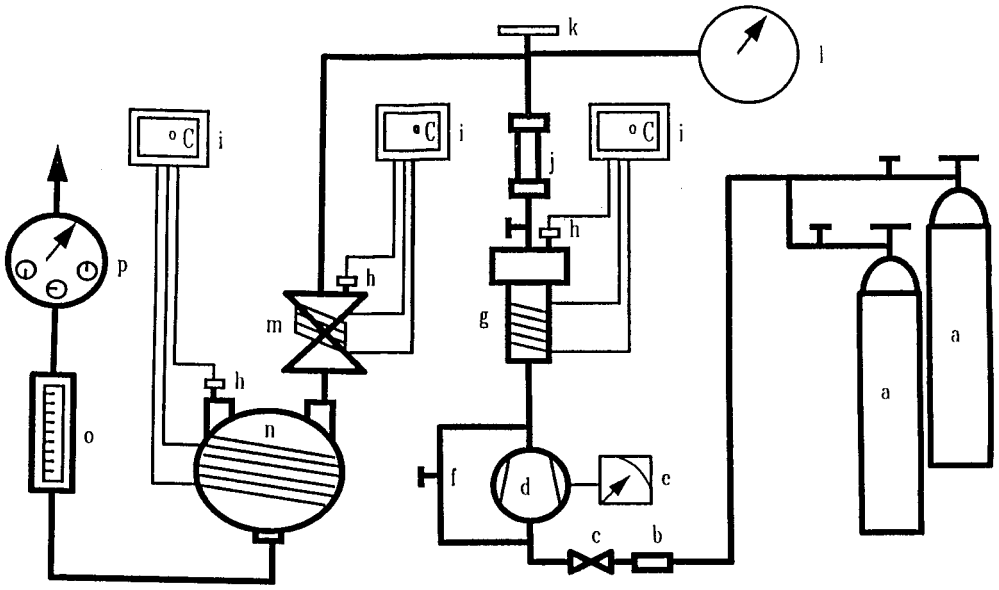

Figure 1. Scheme of supercritical extraction system: a, cylinders; b, one-way filter; c, one-way valve; d, compressor; e, compressor speed regulator; f, diversification valve; g, heat exchanger; h, thermocouple; i, temperature controller; j, extraction vessel; k, rupture disk; l, manometer; m, back pressure regulator; n, expansion vessel; o, fluxometer; p, flow totalizer.

lipophilic nature of carotenoids makes SCF-CO_2 a theoretical alternative to the traditional solvent extraction methods. One advantage that SCF-CO_2 presents with respect to the traditional organic solvent extraction is that it is performed at a low temperature, allowing for a reduction of carotenoid loss due to heat degradation. Another advantage is that the need for organic solvents is eliminated and that the carbon dioxide, after decompression and isolation, may be collected and recycled, thereby reducing environmental contamination. In addition, by eliminating organic solvents from the process, the extracted compound may be suitable for direct human consumption. There are no published studies utilizing SCF-CO_2 for the extraction of paprika oleoresin.

The objective of this study was to establish appropriate conditions for the preparation of paprika oleoresin using SCF-CO_2. To optimize the extraction, different pressures and volumes were tested. In addition, the use of cosolvents was investigated and a procedure employing a two-stage extraction was developed in order to provide unique SCF fractions differing in pigment composition and colorant properties. The oleoresins obtained by the various SCF-CO_2 paprika extractions were compared both quantitatively and qualitatively to oleoresins obtained by performing traditional extractions with either hexane or acetone on the same paprika starting material.

MATERIALS AND METHODS

Materials. The raw sample was industrial paprika provided by Dr. Mínguez-Mosquera, Instituto de la Grasa y sus Derivados, Seville, Spain. All solvents used were HPLC grade.

Pigment Extraction System. The extractor (Super Critical Fluid Extraction System, Cat. No. 46-19345, Newport Scientific, Inc., Jessup, MD) consists of a double-headed reversible pump and the extraction vessel, which is thermostatically controlled by a heater and a thermocouple. The back pressure regulator is located after the extraction vessel, followed by the expansion vessel. The fluxometer and totalizer, which record the flow and consumption of CO_2, are located at the end of the system. The design of the extractor is schematically depicted in Figure 1.

The original design of the extractor was modified to allow the extraction of small sample sizes and at the same time guarantee the control of the extraction fluid temperature. The original extraction vessel (850 mL inner volume) was filled with glass beads to minimize the dead volume. Under these conditions, the extraction vessel together with the heater system acts as a heat exchanger. A stainless steel column (length 10.16 cm, 0.47 cm i.d., and 1.47 cm o.d.) was used as the actual extraction vessel. To reduce process time and CO_2 consumption, a valve was placed before the extraction column. At the end of the extraction process, the valve was closed, and the final part of the line was depressurized, including the extraction column and the connection lines with the back pressure regulator. This modification is very useful for consecutive extractions.

Extraction Conditions. The following extraction procedures were used to investigate the effect of several variables on the extraction, including extraction pressure and CO_2 volume, use of a cosolvent, and continuous versus discontinuous extraction. All experiments were performed on paprika samples weighing 1 g at 40 °C with a CO_2 flow rate of 3 L/min (5.8 g/min). All extractions were performed at least twice.

Extraction with Organic Solvents. To compare the extraction strength of SCF-CO_2 to the traditional organic solvent extractions with acetone or hexane, several different extractions were performed using these solvents. Continuous extractions were performed by placing the sample in the SCF extraction column and pumping 100 mL of either acetone or hexane through the sample at a flow rate of 5.9 g/min to simulate the dynamic conditions of SCF-CO_2 extraction. Typical Soxhlet solvent extractions were performed with either 100 mL of acetone at 60 °C for 7 h or 100 mL of hexane at 70 °C for 13 h. Acetone and hexane extractions were also performed by homogenization and mixing of the tissue for 3 h with 100 mL of solvent followed by filtration.

Continuous SCF-CO_2 Extraction at Different Pressures and Volumes. The pressures tested were 2000, 3000, 4000, 5000, 6000, and 7000 psi. The experimental CO_2 extraction volumes were 50, 100, and 200 L. The use of cosolvents was evaluated at the pressures and extraction volumes stated above. The cosolvents employed were acetone and ethanol, which were used in a 1% (w/w) proportion to the SCF-CO_2.

Discontinuous Extraction. The discontinuous extractions consisted of a two-stage process, the first at a low pressure and the second at a higher pressure. All combinations of low and high pressures were tested within the range of 2000−7000 psi. During both stages, the extraction volume of CO_2 was 50

Table 1. Gradient Elution Program for HPLC Analysis Using Acetone (A) and a Mixture of 1 Part Methanol and 1 Part Water (B) as Eluents

run time (min)	% A	% B	gradient curve
0	78	22	
30	78	22	6
32	85	15	2
43	90	10	
57	90	10	6
62	100	0	
70	100	0	6
75	78	22	2

L. Cosolvents were not used in these experiments as it would introduce too many variables for comparison.

Oleoresin Analysis. The yields of oleoresins extracted by SCF-CO_2 or organic solvents were analyzed indirectly by determining the weight loss of the extracted samples.

For color quantification, the extracted oleoresin was dissolved in 100 mL of acetone. One milliliter was diluted to 10 mL with acetone, and the absorption was measured at 460 nm (UV−Vis spectrophotometer Spectronic 1001, Bausch & Lomb, Rochester, NY). To calculate the percentage of extracted color, the oleoresin obtained by Soxhlet extraction with acetone was adopted as a reference. This oleoresin showed the highest absorbance per gram of extracted sample and was assigned a value of 100% extracted color.

Pigment concentration in the oleoresins was calculated by using the extinction coefficient in acetone of the major pigment capsanthin ($^{1\%}E_{460nm} = 2300$). The results are expressed in grams of capsanthin per kilogram of oleoresin.

High-Performance Liquid Chromatography. The chromatographic system consisted of a Waters U6K Universal Injector for liquid chromatography, two Waters 501 pumps, a Waters 680 gradient controller, and a Waters 990 photodiode array detector (Waters, Milford, MA). The separation was performed on a 250 mm × 4.6 mm i.d. Spherisorb ODS-2 column (Alltech, Deerfield, IL), 5 μm, protected by a 20 mm × 2 mm i.d. guard column, packed with the same stationary phase. Data acquisition and calculation was performed by Waters 991 software.

For the qualitative separation of the extracted pigments according to their polarity, a gradient elution system was developed to allow paprika oleoresin pigment separation without saponification. Table 1 shows the gradient elution program using acetone (A) and a mixture of 1 part methanol and 1 part water (B) as eluents. The flow rate was 0.8 mL/min.

Table 2. Quality and Percentage of Recovery of Paprika Oleoresin Using Traditional Isolation Techniques[a]

	oleoresin yield (%)	pigment yield (%)	pigment concn (g/kg)
	Soxhlet Extraction		
acetone	15.6	100.0[b]	26.3
hexane	9.4	87.9	38.6
	Continuous Extraction		
acetone		85.2	34.5
hexane		84.5	34.1
	Maceration		
acetone		94.3	38.2
hexane		91.2	37.0

[a] Values are means of at least two determinations. [b] Reference set as 100%.

For quantitative analysis, the methodology described by Mínguez-Mosquera and Hornero-Méndez (1993) was followed. The oleoresin was saponified, and the pigments were separated using the gradient of acetone and water described by the authors. Pigments were identified by comparison of retention times and spectra of the peaks obtained with those given by the authors for paprika and paprika oleoresins. Quantification was carried out by addition of internal standard (IS) following the procedure described by the authors, calculating response factors for each pigment.

Sample Preparation. The extracted oleoresin was dissolved in 100 mL of acetone. A 1-mL aliquot was placed in a decantation funnel. After addition of 0.25 mL of IS solution with a concentration between 30 and 70 μg/mL IS in acetone, the solvent was evaporated and the residue redissolved in 5 mL of ethyl ether. The pigment solution was saponified by addition of 5 mL of 15% methanolic potassium hydroxide solution. After 1 h at room temperature, 10 mL of ethyl ether and 20 mL of 10% sodium chloride solution were added. The ethyl ether layer containing the saponified pigments was washed repeatedly with water and finally filtered through a layer of anhydrous sodium sulfate. The solvent was evaporated, and the pigments were redissolved in 0.1 mL of acetone. This solution was filtered through a 0.45 μm filter (Millipore, Bedford, MA), and 25 μL was injected into the HPLC system.

RESULTS AND DISCUSSION

The pigment concentration in the oleoresin depends mainly on two parameters, the quality of the fruit and

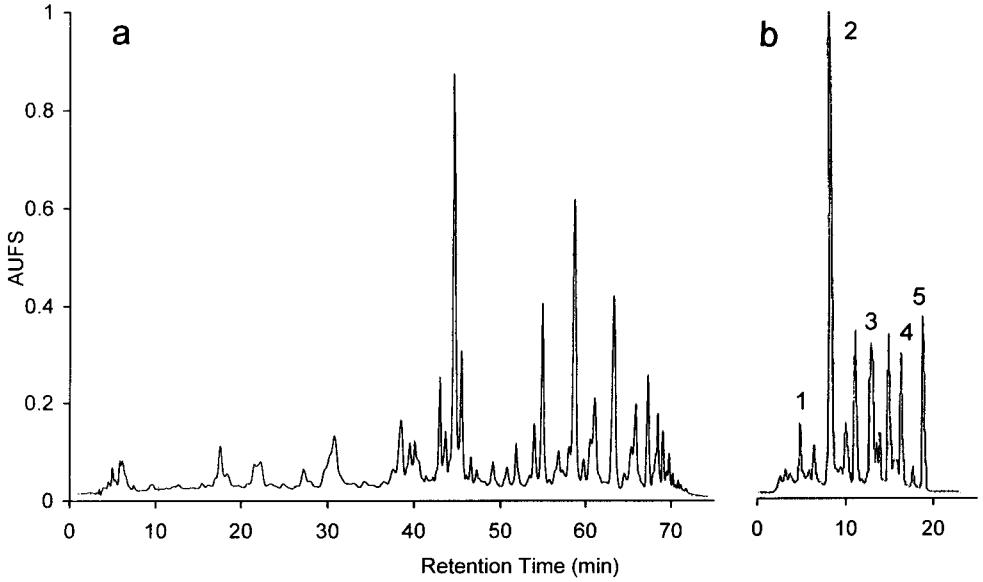

Figure 2. Chromatograms of paprika oleoresin extracted with acetone (a) before saponification and (b) after saponification. Peak identification: 1, capsorubin; 2, capsanthin; 3, zeaxanthin; 4, β-cryptoxanthin; 5, β-carotene.

Table 3. Paprika Oleoresin Extraction by Supercritical CO_2[a]

pressure (psi)	extraction vol (L) 50	100	200	cosolvent (50 L extraction vol) ethanol (1%)	acetone (1%)
	Oleoresin Yield (% of Extracted Paprika)				
2000	10.3	10.0		10.6	
3000	10.8	10.0		10.6	
4000	10.3	10.6	8.9	10.9	
5000	9.3	11.3	10.9	10.9	
6000	9.9	11.6		10.1	9.9
7000	10.0	12.3		9.9	11.9
	Pigment Yield (% of Total Pigment)[b]				
2000	36.0	37.0		34.6	
3000	39.8	47.5		45.1	
4000	47.5	53.9	64.4	49.1	
5000	49.9	55.7	77.8	53.8	
6000	50.1	58.6		56.7	55.7
7000	50.9	62.4		60.4	59.6
	Pigment Concentration in Oleoresin (g/kg)				
2000	13.1	15.0		13.5	
3000	15.7	19.3		17.6	
4000	16.4	23.0	29.8	18.6	
5000	22.5	18.9	29.7	20.4	
6000	18.5	21.1		23.3	23.3
7000	20.4	19.4		25.0	20.7

[a] Values are means of at least two determinations. [b] 100% = Soxhlet extraction with acetone.

the extraction technique employed. With respect to the fruit, the organic solvent will extract all of the lipophilic compounds, which are the pigments and the oil from the pepper pericarp and seeds. The oil is present in much higher quantity than the pigments. In addition, the pigments are located in cellular structures that are more difficult to access for the solvent. The oil is easily extracted at the early stage of the process and subsequently becomes richer in pigments.

Extraction Selectivity. All modifications in the SCF extraction conditions influence the polarity of the extraction fluid, resulting in the extraction of pigment fractions with specific polarity ranges. In fresh red pepper and consequently in paprika, there are a variety of esterified pigments, and with the exception of β-carotene, all of them occur as mono- and diesters. The esterification does not modify the chromatic properties of the pigment but changes completely the chromatographic behavior, giving a range of polarities for the same pigment from a C_{40} dihydroxylic compound (e.g., capsanthin or capsorubin) to C_{76} diesters (e.g., dioctadecanoate of capsanthin or capsorubin). Therefore, the same pigment can appear as several peaks depending on the degree of esterification and structure of the fatty acid. The complex mixture of free pigments and their mono- and diesterified forms is illustrated in Figure 2a.

To evaluate the selectivity of the extraction procedures, it is necessary to employ a chromatographic system to separate all pigments present in the extract in order of polarity. It is not the purpose of this study to extensively identify the complete mixture of pigments and to establish their esterification structures. The identity of the major carotenoid pigments in red pepper and paprika (oleoresin) is well established, and HPLC methods for their quantification are available in the literature (Mínguez-Mosquera and Hornero-Méndez, 1993; Deli et al., 1996; Weissenberg et al., 1997). These methods use saponification as a step previous to quantification in order to eliminate all the esterified forms of the same pigment.

Our HPLC system allowed the separation of more than 73 different peaks (Figure 2a), of which more than 30 peaks had areas greater than 1% of the total area, and their sum comprised more than 82% of the total area. In peaks with an area of more than 1.5%, the peak purity was higher than 95%; in peaks with an area between 0.8 and 1.5%, the peak purity was between 80 and 90%. These findings are indicative of the complexity of the pigment composition found in paprika oleoresin, consisting of a diverse mixture of xanthophyll esters.

The oleoresin obtained by Soxhlet extraction with acetone produced the greatest yield of pigments. It was

Table 4. Fractionated Two-Stage SCF-CO_2 Extraction of Paprika Oleoresin[a]

pressure 1	pressure 2	oleoresin yield (% of extracted paprika)					pigment yield (% of total pigment)[b]					pigment concn in oleoresin (g/kg)				
2000		9.0	8.0	9.2	9.4	10.5	14.1	22.9	18.7	14.8	20.2	6.4	11.8	8.4	6.5	10.7
	3000	2.5					18.6					31.1				
	4000		2.8					17.9					26.9			
	5000			2.3					23.1					42.1		
	6000				1.7					26.0					62.9	
	7000					1.7					27.9					66.7
	total	11.5	10.8	11.5	11.1	12.2	32.7	40.9	41.9	40.8	48.2					
3000			7.9	8.1	8.5	7.8		31.4	40.6	41.7	34.7		16.4	20.7	20.4	18.4
	4000		2.9					14.1					20.6			
	5000			2.8					29.5					44.3		
	6000				2.7					20.4					31.2	
	7000					2.5					25.9					42.6
	total		10.8	10.9	11.2	10.3		45.6	70.1	62.1	60.6					
4000				9.2	9.4	9.2			42.0	34.2	41.3			18.9	15.1	18.6
	5000			2.3					21.6					39.2		
	6000				2.4					14.7					24.8	
	7000					3.6					23.9					27.7
	total			11.5	11.8	12.8			63.7	48.9	65.3					
5000					9.0	9.2				49.6	49.0				22.7	22.0
	6000				4.4					18.7					17.8	
	7000					3.8					19.3					21.2
	total				13.4	13.0				68.3	68.3					
6000						9.2					53.7					24.3
	7000					4.4					22.6					21.4
	total					13.6					76.3					

[a] Values are means of at least two determinations. [b] 100% = Soxhlet extraction with acetone.

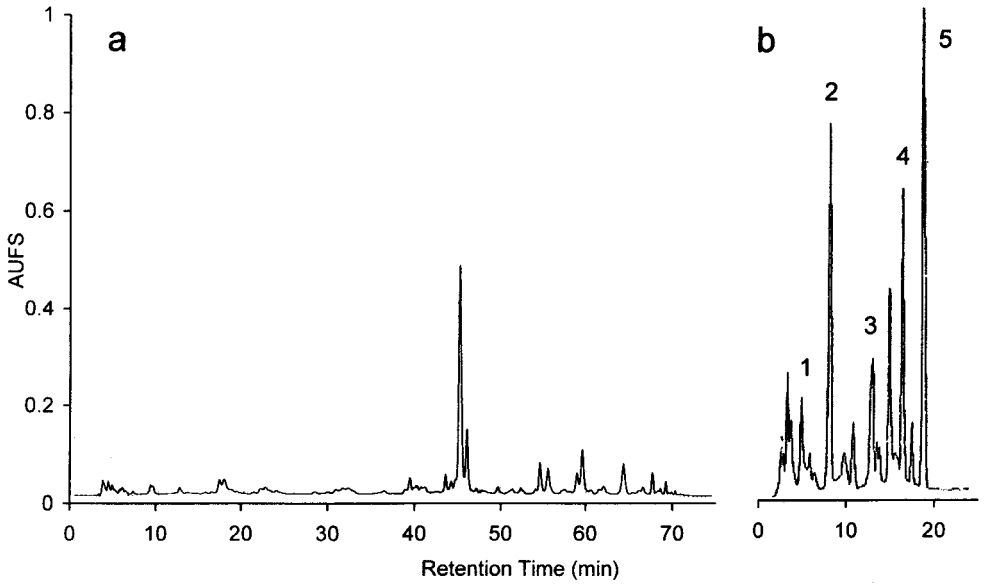

Figure 3. Chromatograms of paprika oleoresin extracted by stages with SCF-CO_2 at 2000 psi (a) before saponification and (b) after saponification. For peak identification, refer to Figure 2.

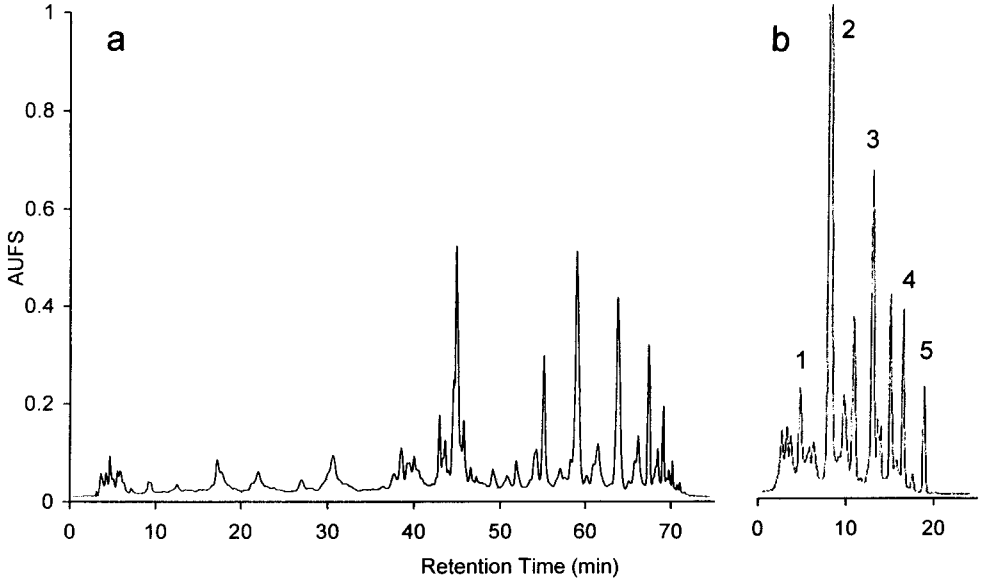

Figure 4. Chromatograms of paprika oleoresin extracted by stages with SCF-CO_2 at 6000 psi (a) before saponification and (b) after saponification. For peak identification, refer to Figure 2.

chosen as the reference and assigned a value of 100% pigment yield. For comparison, Table 2 also shows the results of paprika oleoresin extraction using other extraction procedures with organic solvents. The lower pigment concentration in the oleoresin obtained by Soxhlet extraction with acetone compared to oleoresins produced by traditional methods is due to the extraction of other lipophilic compounds that act as oleoresin diluents (Table 2). All other oleoresins extracted by SCF-CO_2 or organic solvents were compared qualitatively and quantitatively to this reference.

The chromatogram of this reference oleoresin shows a pigment polarity profile with three different parts (Figure 2a). From 0 to 40 min, the more polar compounds, mainly nonesterified xanthophylls, are eluted. In paprika these compounds are naturally found in low concentrations and consequently in the oleoresin. Between 40 and 50 min, the hydrocarbon carotenes are eluted. After 50 min, the chromatogram shows the remaining esterified forms of the xanthophylls with monoesters eluting before the diesters.

The pigments were identified and quantified after saponification according to Mínguez-Mosquera and Hornero-Méndez (1994). Figure 2b illustrates eight pigment components of which five were identified. After hydrolysis of the esters, the pigments can be analyzed and quantified by HPLC in less than 20 min.

Continuous Extraction with SCF-CO_2. Table 3 lists the pigment yields as percentages and the pigment concentrations of the oleoresins at various pressures and extraction volumes. At a constant extraction volume, increasing the pressure increased the pigment concentration. Similarly, at a constant pressure, increasing the extraction volume caused an increase in the pigment yield and concentration. In all cases, the weight of the recovered oleoresin was approximately 10% of the initial weight of the paprika. This similarity in weight occurs because the lipophilic components of the oleoresin are

easily extracted with any of the tested conditions, and varying the extraction volume or pressure only modifies the composition and quantity of pigments solubilized in the oleoresins.

Inclusion of a Cosolvent. The results of the experiments with the cosolvents acetone and hexane are shown in Table 3. At identical conditions, the presence of cosolvent at a concentration of 1% (w/w) of SCF-CO_2 produced higher pigment yields than extractions without cosolvent. This improvement in pigment yield was more pronounced at higher pressures. The percentage of cosolvent used could be considered very low, considering that concentrations of 5−10% cosolvent have been reported elsewhere (McHugh and Krukonis, 1986; Krukonis, 1988; Spanos et al., 1993). It is expected that increasing the cosolvent proportion will increase the pigment yield. Nevertheless, this low concentration was chosen because it already requires five times more organic solvent than sample.

The results showed a considerable variability at identical extraction conditions. The two-headed pump employed caused pressure fluctuations due to the periodic compression and relaxation of each head. At low pressures, where the fluid is more similar to a gas than to a liquid, these pressure fluctuations do not have too much effect. But at high pressures, where the fluid has a compressibility closer to that of a liquid, a compression stroke increases the pressure by 200 psi, while the relaxation period produces a decrease in the pressure by 200 psi. This cycle of varying pressure also affects the flow rate.

Discontinuous Extraction with SCF-CO_2. Table 4 lists the results for paprika extractions that were performed in two consecutive extraction steps at different pressures. The initial stage of the discontinuous extractions efficiently extracted the lipid content from the paprika. Some pigments were extracted during the initial stage as well. The pigment content of the first-step oleoresins was primarily β-carotene. The amount of recovered pigment was dependent on the initial extraction pressure. In general, the oleoresins recovered during the first extraction had low pigment concentrations, since the amount of extracted pigment was low relative to the amount of extracted lipids. Therefore, the oleoresins obtained during the first extraction are classified as β-carotene-rich oils. Because the first extraction successfully removed most of the paprika lipids, the second stage isolated mainly carotenoid pigments, with only small amounts of remaining lipids. The oleoresin produced had a high pigment concentration, with a significant amount of red pigments. The data in Table 4 are consistent for oleoresin yield after the first stage extraction. Variability was observed in pigment yield and pigment concentration due to fluctuation in the flow rate, differences in sample compaction, and extraction time to reach the required 50 L volume of CO_2. In addition, the variation in the first step influences the second-step extraction.

The fact that pigment yield and concentration are pressure dependent is illustrated in Figures 3 and 4. Figures 3a and 4a show the chromatograms of the oleoresins obtained at 2000 and 6000 psi, respectively. These chromatograms can be compared qualitatively. When peaks at similar retention times in these two chromatograms are compared, the peaks in the 6000 psi chromatogram are larger, in accordance with the higher solvating power of SCF-CO_2 at higher pressures. In addition, the chromatogram of the oleoresin extracted at 6000 psi shows many peaks that are not present in the 2000 psi chromatogram. This is due to a change in the selectivity in the extraction fluid. The selectivity varies with extraction pressure since the pressure will determine the density of the fluid, which will then affect the polarity of the fluid. Figures 3b and 4b show these oleoresins after saponification. These chromatograms can be compared quantitatively. The pigment concentrations and their red:yellow ratios are summarized in Table 5.

Table 5. Pigment Concentrations in Extracted Paprika Oleoresins

	concentration (g/kg)		
	acetone extraction	SCF-CO_2 2000 psi	SCF-CO_2 6000 psi
capsorubin	3.29	0.71	5.32
capsanthin	23.30	2.15	39.10
zeaxanthin	9.01	1.31	14.96
β-cryptoxanthin	9.20	2.78	8.78
β-carotene	4.92	2.01	3.02
total	56.36	9.47	76.36
red/yellow ratio	1.30	0.55	1.85

The best conditions for the discontinuous SCF-CO_2 extraction were an initial extraction pressure at 2000 psi, followed by a second extraction at 6000 or 7000 psi. At these conditions, the oil is almost completely extracted in the first stage, while the second stage produces the highest obtainable amount of pigments. The oleoresin obtained during the high-pressure step has a pigment yield of approximately 30% (Table 4). Although this yield seems quite low, it is compensated by a remarkably high concentration of pigments, almost twice the pigment concentration obtained using traditional organic solvent extraction methods. In addition, this oleoresin may be commercially advantageous due to its high red:yellow pigment ratio.

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Received for review February 5, 1999. Revised manuscript received July 2, 1999. Accepted July 7, 1999. Fundación Ramón Areces (Spain) is thanked for the financial support of M.J.-G. as postdoctoral researcher in the Department of Food Science at North Carolina State University.

JF9900985