

Supercritical Carbon Dioxide Extraction of Scotch Bonnet (*Capsicum annuum*) and Quantification of Capsaicin and Dihydrocapsaicin

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Capsaicin and dihydrocapsaicin were extracted using supercritical CO₂ and organic solvents from *Capsicum annuum* var. Scotch Bonnet. Capsaicin and dihydrocapsaicin were quantified by HPLC and characterized by spectral methods. The supercritical CO₂ extract afforded 3.2 and 0.58% capsaicin and dihydrocapsaicin, while the combined organic extract yielded 0.5 and 0.09%, respectively, per gram of dry weight of Scotch Bonnet pepper.

INTRODUCTION

The genus *Capsicum* (family Solanaceae) comprises over 200 varieties ranging from the very hot jalapeno to heatless bell peppers (Andrews, 1984). Their fruits vary widely in size, shape, flavor, and sensory heat. *C. annuum* is the most widely cultivated pepper in the world, and practically all of the commercially available fresh, processed, dried, and frozen peppers belong to this species. *Capsicum* fruits are used throughout the world because of their unique flavor and pungency and also have widespread use as a drug (Newman, 1953). The primary pungent principle from *C. annuum* is capsaicin. Capsaicin and dihydrocapsaicin, which are responsible for 90% of the total pungency (Iwai et al., 1979), are the most abundant principles of hot peppers (Kosuge and Furata, 1970). The *C. annuum* var. Scotch Bonnet, with a heat value of 250 000–300 000 Scoville units, is considered to be the most pungent pepper available in the market. It is grown widely in Jamaica, West Indies, for its pungency and flavor and is presently being cultivated in the United States (Garrett et al., 1991).

A variety of methods are available for the identification and quantification of the capsaicinoids in peppers (Bajaj, 1980; Ramos, 1979; Panker and Magar, 1977; Attuquayefio and Buckle, 1987; Hoffman et al., 1983; Mary, 1984; Saria et al., 1981; Weaver and Awde, 1986; Krajewska and Powers, 1987; Todd and Perun, 1977; Masada et al. 1971). However, HPLC is considered to be the most reliable and rapid method.

Supercritical fluid extraction (SFE) has been widely employed for the extraction of a variety of compounds from different matrices such as polymer fractionation, monomer purification, spice extraction, and coffee decaffeination (Eldridge et al., 1986; Friedrich and List, 1982; Hawthron et al., 1988; Johnston and Penninger, 1989; Krukoni and Kurnik, 1985; McHugh and Krukoni, 1986; McNally and Wheeler, 1988; Stahl et al., 1980). Carbon dioxide is probably the most widely used supercritical fluid because of its critical temperature (31.1 °C), which makes it an ideal solvent for extracting thermally labile materials. Also, CO₂ is nontoxic, nonflammable, and environmentally preferred over organic solvents. In this paper we report the extraction of capsaicin and dihydrocapsaicin from Scotch Bonnet pepper by supercritical CO₂ and organic solvents and separation and quantification of capsaicin and dihydrocapsaicin.

MATERIALS AND METHODS

General Experimental Procedures. Vacuum liquid chromatography (VLC) was performed on silica gel (60-Å pore size, 35–75 μm) and preparative TLC on silica gel plates (20 × 20 cm, 2000, or 250 μm). Medium-pressure reversed phase (C₁₈) column chromatography was carried out on an LC-SORB ODS 24–40 μm, 32 mm/500 mm glass column (Chemco Scientific Co. Ltd., Japan) equipped with a low-pressure pump (Model 81-M-2, Chemco), rheodyne injector (Chemco), and UV-vis 200 detector at 280 nm (Sanki Laboratories Inc., Japan). ¹H- and ¹³C-NMR spectra were obtained on a Varian VXR (500 MHz for proton and 125 MHz for carbon; Varian Co., Sunnyvale, CA). Electron impact mass spectra (EIMS) were obtained on a JEOL Model JMAX 505 mass spectrometer at 70 eV (JEOL JMS Co.). Supercritical CO₂ extraction (SFE) of Scotch Bonnet pepper was conducted on a Dionex 703 SFE equipped with 8 × 32 mL extraction cells (Dionex Co., Salt Lake City, UT) and 703-M solvent modifier.

Plant Material. The matured fruits of *C. annuum* var. Scotch Bonnet were collected from pepper plants grown in the Department of Horticulture greenhouse at Michigan State University, East Lansing, MI. Peppers were lyophilized in a Dura-Dry FTS tray lyophilizer (FTS System Inc.) and stored in sealed plastic bags at -20 °C prior to extraction.

HPLC. High performance liquid chromatography (HPLC) was performed on a Radial-Pak C₁₈ cartridge (4 μm, 5 × 10 mm inserted in a RCM 8 × 10 radial compression module; Waters Associates, Milford, MA) at 280 nm (Waters 490 programmable multiwavelength UV detector). The mobile phase was acetonitrile/water (1:1, 0.75 mL/min) under isocratic conditions.

Production of Pure Capsaicin and Dihydrocapsaicin by Medium-Pressure C₁₈ Column Chromatography from Aldrich Capsaicin. The HPLC (Figure 1) and ¹H-NMR spectrum of capsaicin (Aldrich Chemical Co., Milwaukee, MI) indicated that it was a mixture of capsaicin and dihydrocapsaicin. This "capsaicin" (190.4 mg) was dissolved in acetonitrile (1 mL) and injected into the LC-SORB ODS glass column and eluted with acetonitrile/water (1:1) to afford fractions 1 (200 mL), 2 (150 mL), 3 (20 mL), 4 (15 mL), 5 (40 mL), 6 (130 mL), 7 (150 mL), 8 (200 mL), 9 (210 mL), and 10 (250 mL). HPLC analysis indicated that capsaicin was present in fractions 3–5 and dihydrocapsaicin in fraction 8. Removal of solvent *in vacuo* afforded pure capsaicin (29.5 mg) and dihydrocapsaicin (4.9 mg) from fractions 3–5 and 8, respectively.

Supercritical CO₂ Extraction of Scotch Bonnet Pepper. The lyophilized Scotch Bonnet pepper was milled and the powdered tissue (2.4 g) packed in a 32-mL stainless steel extraction cell (1.5 cm × 20 cm). The initial extraction at 50 °C under 400 atm for 30 min was followed by a 90 min extraction at 600 atm. In both cases the extracts were trapped in chloroform and the combined extracts evaporated to dryness under reduced pressure to afford 0.394 g of an oily residue. The extractions were carried out three times at identical conditions, and the extracts obtained were very similar in weight.

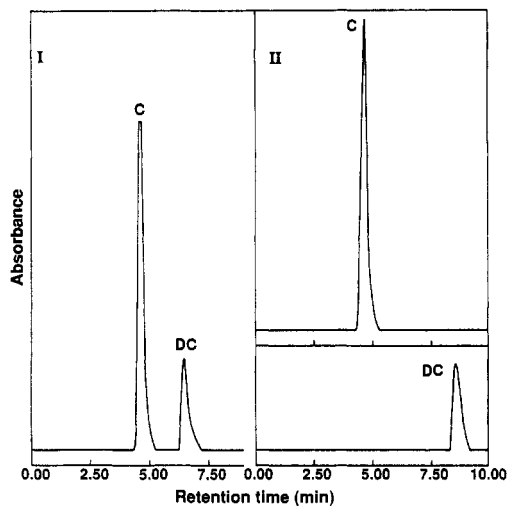


Figure 1. (I) HPLC chromatogram of Aldrich capsaicin: (C) capsaicin; (DC) dihydrocapsaicin. (II) Purified capsaicin (C) and dihydrocapsaicin (DC) after medium-pressure C_{18} column chromatography of the capsaicin–dihydrocapsaicin mixture obtained from Scotch Bonnet pepper from TLC purification.

Isolation of Capsaicin and Dihydrocapsaicin from Scotch Bonnet Pepper. Lyophilized pepper (192 g) was sequentially extracted with hexane (6 L), $CHCl_3$ (2 L), and MeOH (2.5 L). Removal of the solvent *in vacuo* yielded 19.1, 1.5, and 30.7 g of oily extracts, respectively. The MeOH extract did not contain capsaicinoids. Since the $CHCl_3$ extract was relatively small in quantity, it was combined with the hexane extract. A part of this combined extract (10.6 g) was subjected to VLC on a Si gel column (250 g) and afforded the following fractions: fraction 1, hexane (1870 mL); fraction 2, hexane/acetone (4:1, 950 mL); fraction 3, $CHCl_3$ /MeOH (3:1, 500 mL); and fraction 4, MeOH (280 mL). Fraction 2, containing mostly capsaicin and dihydrocapsaicin by TLC, was evaporated to dryness (320 mg) and further purified by preparative TLC using $CHCl_3$ /MeOH (8:1) to yield a mixture of capsaicin and dihydrocapsaicin (R_f 0.56; 192 mg). It was further purified by medium pressure C_{18} column chromatography under isocratic conditions (CH_3CN/H_2O , 1:1). The fractions collected were fraction 1 (150 mL), 2 (70 mL), 3 (10 mL), 4 (30 mL), 5 (20 mL), 6 (40 mL), 7 (10 mL), 8 (20 mL), 9 (80 mL), 10 (150 mL), 11 (60 mL), 12 (120 mL), 133 (50 mL), 14 (150 mL), 15 (120 mL), and 16 (140 mL). Fractions 3–8 and 13–14 afforded pure capsaicin (50.5 mg) and dihydrocapsaicin (21.6 mg), respectively. Similar purification of the supercritical CO_2 extract of Scotch Bonnet (0.394 g) afforded 12.6 and 7 mg of capsaicin and dihydrocapsaicin, respectively.

Standard Curve. Serial dilutions of capsaicin were made to obtain standard solutions containing 400, 200, 100, 80, 40, 20, 10, 8, 4, 2, 1, 0.8, 0.4, 0.2, and 0.1 μ g/mL from a stock solution of pure capsaicin (1.6 mg) in acetonitrile (1 mL). Similarly, solutions of dihydrocapsaicin were also prepared. Standard curves for capsaicin and dihydrocapsaicin were prepared by plotting HPLC peak areas against the concentration for analyses ranging from 400 to 0.1 μ g/mL.

Quantification of Capsaicin and Dihydrocapsaicin in Scotch Bonnet Pepper. The crude extract (10.0 mg) was dissolved in acetonitrile (5 mL) and filtered through the MILLEX-FGS filter (0.2 μ m, Millipore Inc., Bedford, MA). A C_{18} cartridge (1 mL, Waters Chromatographic Division, Milford, MA) was preactivated with 1 mL of CH_3CN . The filtered extract (1 mL) was passed through the cartridge and eluted further with another 1 mL of CH_3CN to afford a total of 2 mL. This solution (50 μ L) was quantified by HPLC for capsaicin and dihydrocapsaicin. The peak areas for capsaicin and dihydrocapsaicin were matched against the respective standard curve to calculate their percentages to the dry weight of Scotch Bonnet.

Capsaicin (1): $C_{18}H_{27}NO_3$; mp, 65–66 °C (uncorrected); 1H NMR ($CDCl_3$) δ 0.94 (6H, d, J = 6.7 Hz, 8'- and 9'- CH_3), 1.47 (6H, m, 2', 3', and 4'- CH_2), 1.97 (1H, m, J = 7 Hz, 10'-CH), 2.19 (2H, t, J = 7.8 Hz, 1'- CH_2), 3.84 (3H, s, OCH_3), 4.34 (2H, d, J = 5.9 Hz, benzylic CH_2), 5.33 (2H, m, 5'- and 6'-vinyl CH), 5.59 (1H,

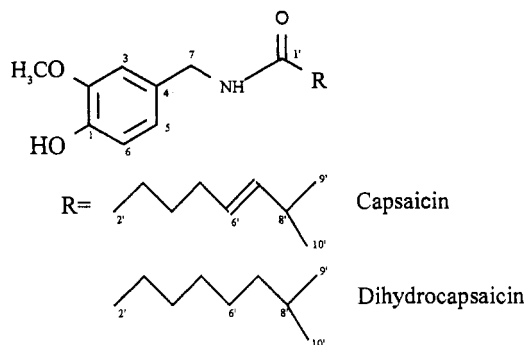


Figure 2. Structures of capsaicin and dihydrocapsaicin.

s, NH), 5.63 (1H, s, phenolic OH), 6.77 (3H, m, 3-, 5-, and 6-aromatic protons); ^{13}C NMR ($CDCl_3$) δ 172.81 (C=O), 146.72, 145.14 (2 \times CO aryl), 55.92 (OMe), 43.53 (CH_2N), 22.63 (2 \times CH_3); EIMS m/z (% intensity) 305 M^+ , $C_{18}H_{27}NO_3$, (16.4), 195 (8.1), 168 (4.5), 152 (12.9), 137 (100).

Dihydrocapsaicin (2): $C_{18}H_{29}NO_3$; 1H NMR ($CDCl_3$) δ 0.84 (6H, d, J = 5.6 Hz, 8'- and 9'- CH_3), 1.37 (10H, m, 2', 3', 4', 5', and 6'- CH_2), 1.58 (1H, m, 7'-CH), 2.18 (2H, t, J = 7.8 Hz, 1'- CH_2), 3.86 (3H, s, OCH_3), 4.35 (2H, d, J = 5.6 Hz, benzylic CH_2), 5.61 (1H, s, NH), 5.63 (1H, s, phenolic OH), 6.80 (3H, m, 3-, 5-, and 6-aromatic protons); ^{13}C NMR ($CDCl_3$) δ 174.6 (C=O), 145.12, 146.68 (2 \times CO aryl), 55.94 (OMe), 43.54 (CH_2N), 22.68 (2 \times CH_3); EIMS m/z (% intensity) 307 M^+ , $C_{18}H_{29}NO_3$ (6.1), 195 (8.1), 168 (4.5), 152 (12.9), 137 (100).

RESULTS AND DISCUSSION

The extracts from Scotch Bonnet pepper using supercritical CO_2 and organic solvents contained a similar ratio of capsaicin and dihydrocapsaicin in the sample of "capsaicin" purchased from Aldrich Chemical Co. The mixture of capsaicin and dihydrocapsaicin obtained from Scotch Bonnet by TLC purification and the commercial sample were purified by C_{18} column chromatography and gave pure capsaicin and dihydrocapsaicin as revealed by HPLC (Figure 1), 1H -NMR, and MS spectra. Capsaicin and dihydrocapsaicin (Figure 2) can be distinguished clearly from their 1H -NMR spectra. The signal at δ 5.3 for two olefinic protons in capsaicin was absent in the 1H -NMR spectra of dihydrocapsaicin. The 1H -NMR spectra of both capsaicin and dihydrocapsaicin from Scotch Bonnet were identical to the capsaicin and dihydrocapsaicin obtained from the purification of the Aldrich sample of capsaicin.

Extraction of the lyophilized Scotch Bonnet using supercritical CO_2 and organic solvents afforded red oily extracts in all cases. The sample of capsaicin purchased from Aldrich contained 67% capsaicin and 33% dihydrocapsaicin, as revealed from its 1H -NMR spectrum. Also, on the basis of the 1H -NMR spectra of the capsaicinoid mixture obtained from Scotch Bonnet, the relative ratio of capsaicin and dihydrocapsaicin in the mixture was 82 and 18%, respectively. Pure capsaicin and dihydrocapsaicin obtained from the purification of the Aldrich capsaicin were used for the quantification of capsaicin and dihydrocapsaicin in Scotch Bonnet.

The supercritical CO_2 extraction of Scotch Bonnet gave 16.4% of extract to the dry weight of the pepper, and the HPLC analysis indicated that it contained 3.2 and 0.5% capsaicin and dihydrocapsaicin, respectively, on a dry weight basis. Organic solvent extraction of Scotch Bonnet pepper afforded a combined total of 26.7% extract and contained only 0.5 and 0.09% capsaicin and dihydrocapsaicin, respectively. This indicated that supercritical CO_2 is a superior solvent to extract capsaicin and dihydrocapsaicin from peppers.

The most pungent pepper, Scotch Bonnet, gave the highest level of capsaicin and dihydrocapsaicin when compared to the organic extracts from seven other varieties of *C. annuum* (data not shown). The higher level of capsaicin indicated that the pungency of Scotch Bonnet pepper is related to the amount of capsaicin present in the pepper and is in agreement with the published results (Mary, 1984; Todd and Perun, 1977). This is the first report of the supercritical fluid CO₂ extraction, purification, and quantification of capsaicin and dihydrocapsaicin from Scotch Bonnet pepper.

LITERATURE CITED

- Andrews, J. *Peppers: the domesticated capsicums*; University of Texas Press: Austin, TX, 1984.
- Attuquayefio, V. K.; Buckle, K. A. Rapid sample preparation method for HPLC analysis of capsaicinoids in *Capsicum* fruits and oleoresin. *J. Agric. Food Chem.* 1987, 35, 777-779.
- Bajaj, K. L. Colorimetric determination of capsaicin in capsicum fruits. *J. Assoc. Off. Anal. Chem.* 1980, 63, 1314-1316.
- Eldridge, A. C.; Friedrich, J. P.; Warner, K.; Kwolek, W. F. Preparation and evaluation of supercritical carbon dioxide defatted soybean flakes. *J. Food Sci.* 1986, 51, 584-587.
- Friedrich, J. P.; List, G. R. Characterization of soybean oil extracted by supercritical carbon dioxide and hexane. *J. Agric. Food Chem.* 1982, 30, 192-198.
- Garrett, L.; Dyremple, B.; Wollo, W. Production of Scotch Bonnet pepper in Missouri: an economic evaluation. *88th ASHS Abstract*, July 19-24, 1991; American Society for Horticultural Science: Alexandria, VA, 1991.
- Hawthron, S. B.; Kreiger, M. S.; Miller, D. J. Analysis of flavor and fragrance compounds using supercritical fluid extraction coupled with gas chromatography. *Anal. Chem.* 1988, 60, 472-477.
- Hoffman, P. G.; Lego, M. C.; Galetto, W. G. Separation and quantitation method of red pepper major heat principles by reverse-phase HPLC. *J. Agric. Food Chem.* 1983, 31, 1326-1330.
- Iwai, K.; Suzuki, T.; Fujiwake, H. Simultaneous microdetermination of capsaicin and its four analogues by HPLC and GC/MS. *J. Chromatogr.* 1979, 172, 303-311.
- Johnston, K. P., Penninger, J. M. L., Eds. *Supercritical fluid science and technology*; ACS Symposium Series 406; American Chemical Society: Washington, DC, 1989.
- Kosuge, S.; Furata, M. Studies on the pungent principle of *Capsicum*. Part XIV: chemical constitution of the pungent principle. *Agric. Biol. Chem.* 1970, 34 (2), 248-256.
- Krajewska, A. M.; Powers, J. J. Gas chromatographic determination of capsaicinoids in green capsicum fruits. *J. Assoc. Off. Anal. Chem.* 1987, 70, 926-928.
- Krukonis, V. J.; Kurnik, R. T. Solubility of the solid aromatic isomers in carbon dioxide. *J. Chem. Eng. Data* 1985, 30, 247-253.
- Mary, C. L. HPLC in the flavor industry. *Food Technol.* 1984, 38, 84-87.
- Masada, Y.; Hashimoto, K.; Inoue, T.; Suzuki, M. Analysis of the pungent principles of capsicum annuum by continued gas chromatography-mass spectrometry. *J. Food Sci.* 1971, 36, 850-860.
- McHugh, M. A.; Krukonis, V. L. *Supercritical fluid extraction: principles and practice*; Butterworth: Stoneham, MA, 1986; pp 23-76.
- McNally, M. E. P.; Wheeler, J. R. Supercritical fluid extraction coupled with supercritical fluid chromatography for the separation of sulfonylurea herbicides and their metabolites from complex matrices. *J. Chromatogr.* 1988, 435, 63-71.
- Newman, A. A. Chemistry of capsaicin—the pungent principle of the *Capsicum* peppers. *Chem. Prod. Chem. News* 1953, 16, 413-418.
- Panker, D. S.; Magar, N. G. New method for the determination of capsaicin by using multiband thin-layer chromatography. *J. Chromatogr.* 1977, 144, 149-152.
- Ramos, P. J. J. Further study of the spectrophotometric determination of capsaicin. *J. Assoc. Off. Chem.* 1979, 62, 1168-1170.
- Saria, A.; Lembeck, F.; Shofitsheh, B. Determination of capsaicin in tissues and separation of capsaicin analogues by HPLC. *J. Chromatogr.* 1981, 208, 41-46.
- Stahl, E.; Schutz, E.; Mangold, H. K. Extraction of seed oil with liquid and supercritical carbon dioxide. *J. Agric. Food Chem.* 1980, 28, 1153-1158.
- Todd, P. H.; Perun, C. Gas-liquid chromatography analysis of capsicum amides. *Food Technol.* 1977, 15, 270-273.
- Weaver, K. M.; Awde, D. B. Rapid HPLC method for the determination of very low capsaicin levels. *J. Chromatogr.* 1986, 367, 438-442.

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