Off-Line Supercritical Fluid Extraction of Thiosulfinates from Garlic and Onion

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Supercritical fluid (SF) extracts of fresh garlic (*Allium sativum*) and fresh onion (*Allium cepa*) were analyzed by liquid chromatography, gas chromatography (GC), and mass spectrometry (MS). Allicin (2-propene-1-sulfinothioic acid *S*-2-propenyl ester), the major thiosulfinate found in fresh garlic, was readily extracted by using supercritical CO_2 (SC-CO_2). Under SF extraction conditions using a solvent trap, the yield of allicin from a water homogenate of fresh garlic was 98.2%; however, when a solid-phase trap (i.e., condensation on glass beads) was used, the yield of allicin was 124.6% relative to yields obtained with methylene chloride extraction. An increase in the quantity of thermal decomposition products with respect to allicin was evident when the garlic was extracted at temperatures greater than 36 °C. The identity of allicin in the garlic SF extracts was confirmed by thermospray MS. The SF extraction of yellow onion was ca. 69% as efficient as the extraction with diethyl ether, as determined by GC–MS.

INTRODUCTION

The sulfur compounds found in processed garlic and onion are dependent on the conditions under which such products are manufactured. The characteristic flavors of fresh garlic (*Allium sativum*), onion (*Allium cepa*), and other *Allium* spp. are associated with thiosulfinates, RS(O)SR, and related compounds, formed enzymatically from odorless amino acid precursors when the plants are cut or crushed (Block, 1992). Allicin (2-propene-1-sulfinothioic acid *S*-propenyl ester), derived from the nonvolatile alliin [(+)-*S*-allyl-L-cysteine *S*-oxide], is the major thiosulfinate formed when garlic is crushed and is responsible for much of the bioactivity attributed to garlic extracts (Block, 1992). Harsher processing conditions convert the *Allium* thiosulfinates into sulfinyl disulfides (such as ajoene from allicin) or into the saturated and unsaturated polysulfides found in steam-distilled oils (Block, 1992).

Allicin and related thiosulfinates from onion and other *Allium* spp. have been separated by liquid chromatography (LC) under both normal (Si-LC) and reversed-phase (C_{18}-LC) conditions (Block et al., 1992a; Lawson et al., 1991a,b). Although aqueous *Allium* homogenates can be analyzed directly by C_{18}-LC, (*E*,*Z*)-thiosulfinate isomers are better separated by Si-LC. The addition of methanol to the aqueous homogenate precipitates carbohydrates and proteins in the *Allium* extract. If the aqueous *Allium* homogenate is extracted with methylene chloride, concentrated, and reconstituted as an aqueous solution, the γ-glutamyl peptides and adenosine are removed and the reversed-phase liquid chromatograms are simplified (Lawson et al., 1991a). The methylene chloride or ether extract of the aqueous homogenate can also be directly analyzed by Si-LC or, in the case of onion, by gas chromatography–mass spectrometry (GC–MS) using on-column injection along with cryogenic injector/oven conditions (Block et al., 1992b). LC or low-temperature GC analytical conditions are desirable to minimize decomposition of the thermally unstable thiosulfinates (Block, 1993).

Supercritical fluid (SF) technologies are of interest for the extraction and analysis of natural products because of increasing awareness of the safety hazards related to the use and disposal of conventional organic solvents. Supercritical CO_2 (SC-CO_2) is of particular interest to the food industry. CO_2 is a highly desirable SF because of its low cost, low critical temperature, ease of regeneration, and minimal solvent disposal requirements. Because of the inertness of CO_2, SC-CO_2 extraction provides an accurate representation of the taste, color, and odor of naturally occurring materials (Katauskas and Goldner, 1991). Among the compounds related to food products that have been extracted or chromatographed by using SC-CO_2 are natural products (e.g., flavors/spices, alkaloids, and mycotoxins) (Bicchi et al., 1991; Calvey et al., 1990; Huston and Ji, 1991; Ondarza and Sanchez, 1990; Roach et al., 1989), herbicides/pesticides (France et al., 1991; Hopper et al., 1991; King, 1989), carbohydrates (Calvey et al., 1989; Reinhold et al., 1988), and lipids (Calvey et al., 1991; King et al., 1989; Wehling et al., 1992).

Garlic oil in another vegetable oil has been analyzed by supercritical fluid chromatography (SFC) as a means of demonstrating the specificity and sensitivity of the sulfur chemiluminescence detector (Chang and Taylor, 1990). Decomposition of garlic compounds such as allicin was shown to occur after a 3-h CO_2 extraction (Wagner and Breu, 1989). Garlic products were extracted by using SC-CO_2, and the extracts were analyzed by GC with atomic emission detection (Miles and Quimby, 1990). Because no standards were employed, it is difficult to determine whether the sulfur compounds detected were initially present in the extract or formed during the analysis. GC–MS showed that an SC-CO_2 extract of onion contained 28 sulfur-containing compounds including diallyl thiosulfinate (or its isomer, di-1-propenyl thiosulfinate), propyl methanethiosulfonate, dithiin derivatives, diallyl disulfide, and diallyl trisulfide along with 13 other compounds also found in steam-distilled onion oil (Sinha et al., 1992). None of these compounds was detected by LC, cryogenic GC–MS, or nuclear magnetic resonance (NMR) spectroscopy methods in methylene chloride extracts of several varieties of onion (Block et al., 1992a,b), nor is there any evidence in the literature for the presence of allylic compounds in

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onion (Block, 1992). In addition, no standards were used by Sinha et al. (1992) to verify assignments. Because the GC–MS conditions employed by Sinha et al. included GC injector temperatures of 280 °C, high enough to cause immediate rearrangement of di-1-propenyl thiosulfinate (1-propenyl 1-propenethiosulfinate) or decomposition of the very labile allicin (Block, 1992), it is unlikely that the claims of Sinha et al. are correct. Methylene chloride extracts of fresh garlic homogenates have been analyzed by SFC–MS as a means of demonstrating the ability of SFC to analyze for thermally unstable thiosulfinates containing the allyl moiety (Calvey et al., 1994).

In this paper we report the SC-CO_2 extraction of thiosulfinates, the primary flavor components of garlic and onion, and compare the efficiency of the SC-CO_2 extraction procedure with that achieved using methylene chloride or diethyl ether extraction. Thiosulfinate identity was confirmed by thermospray liquid chromatography–mass spectrometry (LC–MS) and GC–MS methods, while quantitation of the thiosulfinates was achieved by Si-LC (Block et al., 1992a) and GC–MS (Block et al., 1992b). Using LC methodology, we did not find allicin in our SC-CO_2 extract of onion as was recently reported by Sinha et al. (1992). In addition, we found no evidence of methyl 3,4-dimethyl-2-thienyl disulfide or its isomers as reported by Sinha et al. (1992).

EXPERIMENTAL PROCEDURES

Materials. Fresh garlic and onion were obtained from local retail markets. LC solvents (LC grade) were obtained from J. T. Baker. SFC grade CO_2 was obtained from Air Products (Allentown, PA). The chromatographic sand was obtained from Sigma (St. Louis, MO). Hydromatrix (diatomaceous earth) was obtained from Varian (Palo Alto, CA).

LC Analysis. LC analyses were performed by using a Waters Model 600E pump equipped with a Rheodyne Model 7010 manual injector (20-μL loop) and a Hewlett-Packard Model 1040A photodiode array detection system. *System 1* (*C_{18}-LC*) consisted of chromatographic separation on a C_{18} Microsorb cartridge (250 × 4.6 mm i.d.) with a guard cartridge (30 × 4.6 mm i.d.) (Rainin, Woburn, MA). Mobile phases were methanol–water (50:50 v/v) for the allicin determination and methanol–water (80:20 v/v) for the sulfides at a flow rate of 0.8 mL/min. *System 2* (*Si-LC*) consisted of chromatographic separation on a Zorbax silica column (250 × 4.6 mm i.d.) (DuPont Chromatography Products, Wilmington, DE). The linear gradient was 2-propanol–hexane (2:98), which was held for 6 min, then increased by 0.8% 2-propanol/min to 2-propanol–hexane (10:90), and held for 17 min. The flow rate was 1.8 mL/min. *System 3* (*Si-LC*) used the same column as in system 2. The linear gradient was 2-propanol–hexane (2:98) for 10 min, increased by 1.1% 2-propanol/min to 2-propanol–hexane (20:80), and held isocratically for 5 min. The flow rate was 1.6 mL/min.

LC analyses for the preliminary experiments were performed by using an isocratic pump (Kratos Analytical, Ramsey, NJ) equipped with a 5-μL loop manual injector (Valco Instruments, Houston, TX) and employing a variable UV wavelength detector (LDC Analytical, Riviera Beach, FL) set at 254 nm. System 1 was used for the chromatographic separation.

LC–MS Analysis. LC–MS data were collected by using a Vestec LC–MS Model 201A system (Vestec, Inc., Houston, TX) under thermospray conditions with discharge on and no buffer in the mobile phase (vaporizer temperature, 126 °C; block temperature, 300 °C; lens temperature, 125 °C); or by using a Finnigan triple-stage quadrupole MS Model TSQ46 (Finnigan MAT, San Jose, CA) equipped with an Incos data system, a thermospray interface, and a Spectra System P4000 (Spectra-Physics, San Jose, CA). The TSQ46 thermospray conditions were filament on, with 0.1 M ammonium acetate buffer added to the mobile phase (vaporizer temperature, 105 °C; block temperature, 220 °C; quadrupole temperature, 85 °C). Chromatographic separation was achieved with a 5-μm C_{18} Ultrasphere column (250 × 4.6 mm i.d.) (Alltech Associates, Deerfield, IL). Mobile phases were methanol–water (50:50 v/v) for allicin determination and methanol–water (80:20 v/v) for the sulfides. The flow rate was 0.8 or 1.0 mL/min, and 25–75 μL of the extracted solution was injected for analysis.

SC-CO_2 Extraction of Fresh Garlic. In preliminary studies approximately 2–3 g of peeled, fresh garlic was homogenized in 5 mL of water at ambient temperature by using a Tissumizer (Tekmar, Cincinnati, OH). The solution was allowed to stand at room temperature for 10 min to ensure complete enzymatic conversion to the thiosulfinate. The resulting garlic pulp was mixed with chromatographic sand to absorb the liquid and placed into a 100 × 9.4 mm i.d. extraction vessel (Keystone Scientific, Inc., Bellefonte, PA). Approximately 4 mL of the liquid was transferred to the cell. The supercritical fluid extraction (SFE) system was a modified sample preparation accessory (SPA) (Milton Roy, Riviera Beach, FL). Pressure was maintained by using a 50 μm i.d., 23–25 cm linear fused silica restrictor. The garlic was extracted under the various conditions described below. The effluent was trapped in a test tube containing 3 mL of methanol–water (50:50 v/v) or methylene chloride, both held at 0 °C. The aqueous methanol fractions were filtered before LC analysis and stored at −30 °C. The methylene chloride fractions were centrifuged before filtration to break the emulsion caused by the coextraction of water and were stored at −30 °C.

In quantitative studies, the SPA system was further modified to permit the measurement of the amount of CO_2 used in the extractions. A flow meter (Brooks Instrument, Hatfield, PA) and a totalizer (Kessler-Ellis Products Co., Atlantic Highlands, NJ) were connected to the SPA system via a manifold. The manifold was developed to permit three liquid traps in series. The trapping vessels were 16 × 150 mm test tubes. Whole cloves of garlic, ranging from 1.4 to 3.5 g, were homogenized in water at a ratio of 1 g of garlic to 10 mL of water at ambient temperature. The solution was allowed to stand at room temperature for 20 min. Approximately 1.8 g of diatomaceous earth was mixed with 2.5 mL of garlic pulp (0.25 g of garlic extracted). These quantities produced a free-flowing mixture. The addition of more liquid resulted in a mixture that was difficult to add to the extraction vessel. Excess moisture also caused swelling in the extraction cell and reduced the CO_2 flow. The garlic was extracted at 35 °C and 3500 psi for approximately 25–35 min. The extraction was stopped when approximately 25 000 mL of gaseous CO_2 was indicated on the flow totalizer (700–1000 mL/min). The effluent was trapped in 7–10 mL of methanol at approximately 10 °C. The methanol fractions were evaporated to minimal volume by using a rotary evaporator and were redissolved in 2 mL of methylene chloride. Benzyl alcohol (2 μL) was added as an internal standard. Quantitation was by Si-LC as described above. Percent recoveries were determined by comparing the SF extract with the methylene chloride extract.

Additional quantitative data were obtained by using a Prep-Master and AccuTrap (Suprex, Inc., Pittsburgh, PA). The garlic homogenates were prepared as described above. The homogenate (3 mL) was mixed with diatomaceous earth, and the mixture was added to a 10-mL extraction vessel. The garlic was extracted at 35 °C and 240 atm at a flow rate of approximately 3 mL/min after an initial 5-min static period. A total of 40 mL of liquid CO_2 was used for each extraction. The effluent was trapped on glass beads at 0 °C and desorbed by using 1.5 mL of methylene chloride at 20 °C. Benzyl alcohol (3 μL) was added as an internal standard. Quantitation was achieved as described above.

Methylene Chloride Extraction of Fresh Garlic. Peeled garlic was homogenized in water at a ratio of 1 g of garlic to 10 mL of water. The solution was allowed to stand at room temperature for 10–20 min. The garlic pulp was filtered, and the filtrate was then extracted three times with an equivalent volume of methylene chloride. The combined methylene chloride fractions were rapidly evaporated to minimal volume on a rotary evaporator (30 °C). The residue was redissolved in methanol–water (50:50 v/v) or methylene chloride for the appropriate LC analysis. Benzyl alcohol was added as an internal standard for quantitative analyses. The extracts were stored at −30 °C.

SC-CO_2 Extraction of Fresh Onion. Whole onion bulbs or slices were juiced by using an Oster juice extractor (Milwaukee, WI). The solution was allowed to stand at room temperature for

20 min. Approximately 7.5 g of diatomaceous earth was mixed with 10 mL of onion juice (from 15–20 g of onion). These quantities produced a free-flowing mixture. The onion juice was extracted at 35 °C and 3500 psi for approximately 40 min. The effluent was collected in 7 mL of methanol. The methanol fractions were evaporated to minimal volume by using a rotary evaporator and were redissolved in 1 mL of methylene chloride. System 3 described above was used for LC analysis.

GC–MS Analysis. Mass spectra were collected by using a Hewlett-Packard 5898 mass spectrometer (MS Engine) equipped with a Hewlett-Packard 5890 II gas chromatograph with programmable on-column injector and cryogenic cooling (CO_2). An HP/Apollo 400 series computer employing standard Hewlett-Packard HP UX Chemstation software was used for data processing. GC separations were accomplished on an HP-1 (cross-linked methyl silicone gum) column (30 m × 0.53 mm i.d.) with 99.995% helium as the carrier gas. The GC temperature program was as follows: from 0 to 200 °C at 5 °C/min, injector under oven tracking control, transfer line at 100 °C, and column head pressure of 5 psi. The MS source and quadrupole temperatures were maintained at 200 and 100 °C, respectively. Juice (94 mL) obtained from a yellow onion (134 g) was subjected either to SFE (10 mL of juice used) as described above or to liquid–liquid extraction (84 mL of juice) with diethyl ether. The methanolic solution from the SFE was concentrated to a very small volume by using a gentle stream of air. With a 1-μL syringe, 0.3 μL of benzyl alcohol–methanol (10:90 v/v) was added to the SF concentrate, and the solution was analyzed by GC–MS using the response factors previously determined under identical MS operating conditions for standards of each component under either total ion or selected ion monitoring conditions. The remaining 84 mL of juice was filtered through a small pad of Celite, and the filtrate was diluted with 50 mL of saturated sodium chloride solution. The diluted filtrate was extracted with three 200-mL portions of diethyl ether. The combined ether extracts were dried ($MgSO_4$) and filtered, and the filtrate was carefully concentrated to ca. 5 mL on a rotary evaporator with a 20 °C water bath. The volume of the concentrate was further reduced by using a gentle stream of air. This solution was treated with 3 μL of benzyl alcohol–methanol (10:90 v/v). GC–MS analysis was conducted as described above for the SF extract.

RESULTS AND DISCUSSION

In preliminary studies, SFEs were carried out at ambient temperature (25 °C), 36, 45, and 55 °C, and 3500 psi. Analyses of these extracts by C_{18}-LC with methanol–water (50:50 v/v) as the chromatographic solvent resulted in similar chromatograms for the methylene chloride extract (Figure 1A) and the 25 and 36 °C SF extracts. Figure 1B is a chromatogram of the SF extract at 36 °C. At 45 and 55 °C there was a significant increase in early-eluting components, which is illustrated by Figure 1C. When these extracts were analyzed with methanol–water (80:20 v/v) as the chromatographic solvent, additional late-eluting compounds were found in all of the extracts. The 55 °C extract contained the greatest quantity of these components, based on area integration. Retention times of the major late-eluting constituents did not correspond to those of diallyl mono-, di-, or trisulfides. Si-LC (Figure 2) also readily demonstrated the difference between the 36 and 55 °C extracts; the 36 °C extract was again similar to the methylene chloride extract.

The presence of allicin in the extracts was verified by comparing the C_{18}-LC retention times obtained for an extract with that of an allicin standard. LC–MS thermospray data obtained with the Vestec system were used to confirm the presence of allicin (MH^+ ion, m/z 163) in the 25 and 55 °C extracts. For the 55 °C extract under discharge conditions with methanol–water (80:20 v/v) as

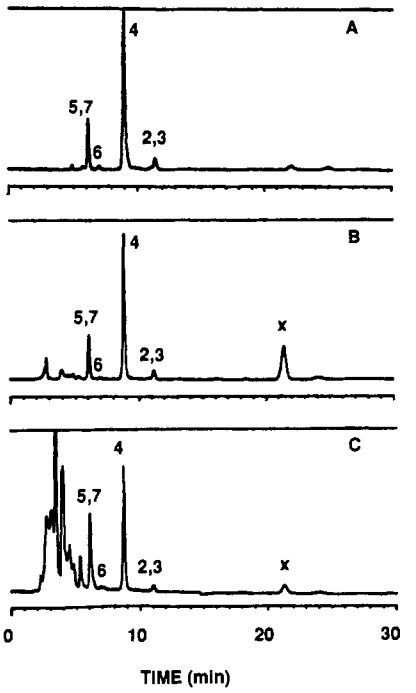

Figure 1. C_{18}-LC chromatograms of fresh garlic extracts. See text for chromatographic conditions (system 1). (A) Methylene chloride extract; (B) 36 °C SF extract; (C) 55 °C SF extract. See Table 1 for peak assignments.

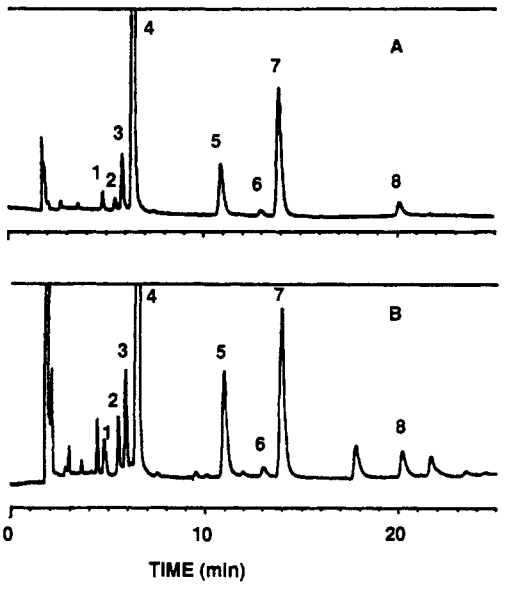

Figure 2. Si-LC chromatograms of fresh garlic extracts. See text for chromatographic conditions (system 2). (A) 36 °C SF extract; (B) 55 °C SF extract. See Table 1 for peak assignments.

the chromatographic solvent, the mass spectrum of the major component that eluted after allicin had a base peak corresponding to the ion at m/z 145. It is likely that dithiins (m/z 144) were formed by thermal decomposition of allicin, as in the case of GC analysis of fresh garlic extracts (Brodnitz et al., 1971; Saito et al., 1989). LC–MS thermospray data obtained with the Finnigan system were used to confirm the presence of allicin in the 35 °C extracts. The C_{18}-LC reconstructed-ion chromatograms obtained for an allicin standard and a garlic extract are shown in Figure 3. The thermospray mass spectra corresponding to the chromatographic peaks labeled in Figure 3B are shown in Figure 4. The spectrum (Figure 4C) corresponding to the chromatographic peak of allicin matches that of an authentic allicin standard. In the allicin

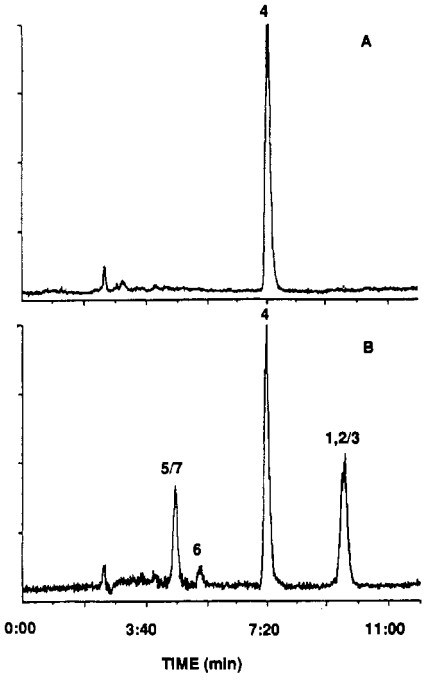

Figure 3. Reconstructed-ion chromatograms for (A) allicin standard and (B) SF extract of garlic. See Table 1 for peak assignments.

spectrum, the base peak is the ion at m/z 163, and the corresponding ammonium adduct ion is at m/z 180. The ion at m/z 121 results from the loss of C_3H_5 from the molecular ion. This assignment was verified by LC–MS/MS data (Matusik et al., 1993), which indicated that the ions at m/z 131 and 145 were not associated with the protonated molecular ion or the ammonium adduct ion. These ions could be attributed to thermal decomposition in the ion source.

In a 4-h extraction of fresh garlic at 3500 psi and 36 °C, fractions were collected every 30 min. Of the quantity of thiosulfinates extracted, 60–70% was obtained in the first hour of the experiment. No decomposition products, such as those obtained when the extractions were conducted at 55 °C, were formed during the 4-h extraction. Preliminary data showed that at 1500 psi and 36 °C only 40–50% of the total thiosulfinates found was obtained in 45 min. An additional 20% was found with each additional increment of 1000 psi in a 45-min period. These data indicated that the choice of 3500 psi at 36 °C permitted sufficient extraction without undue formation of decomposition products. All subsequent studies were geared to improve extraction efficiency at 3500 psi and 35–36 °C.

In preliminary studies, semiquantitative data from the Si-LC analysis showed that SFE at 36 °C yielded approximately 10% of the allicin that was obtained from a methylene chloride extraction. By reducing the amount of garlic homogenized to 0.5 g, so that a 1:10 ratio of garlic to water was used, and by increasing the volume of methylene chloride in the solute recovery trap to 5 mL, the yield increased to approximately 30%. To improve the yield further, whole garlic cloves were homogenized and only 2.5 mL of liquid was added to the extraction cell with diatomaceous earth as the matrix. The trapping solvent was changed to methanol. These changes increased the yield of allicin to 98.2% [percent coefficient of variation (% CV) = 22.1, n = 7]. When the extracted material was trapped by using a solid-phase trap (i.e., condensed on glass beads at 0 °C), a yield of 124.5% (% CV = 14.6, n = 7) was achieved for allicin. This increase in percent yield could have resulted from a reduction in sample manipulation. Tables 1 and 2 compare the total thiosulfinate concentrations of SF extracts to that of a methylene chloride extract of garlic cloves. The mole percent of allicin was higher in the SF extract than in the methylene chloride extract. Total thiosulfinate yields relative to yields obtained with methylene chloride extraction were >90% for the solvent trap system and >119% for the solid-phase trap system.

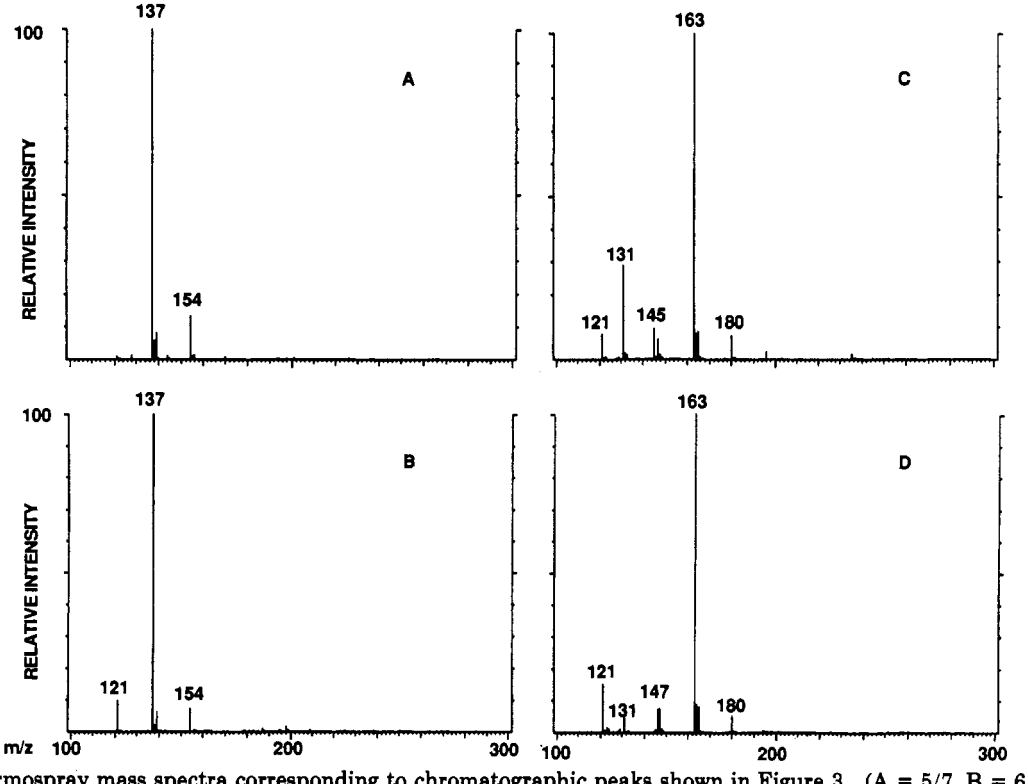

Figure 4. Thermospray mass spectra corresponding to chromatographic peaks shown in Figure 3. (A = 5/7, B = 6, C = 4, and D = 1, 2/3.) See Table 1 for peak assignments.

Table 1. Comparative Concentrations of Thiosulfinates in SC-CO_2 (Solvent Trap System) and Organic Solvent Extracts of Garlic

		SC-CO_2[b]		CH_2Cl_2	
compd no.[a]	compd	μmol/g[c]	mol %	μmol/g[c]	mol %
1	AllSS(O)Propenyl-(*E*)	0.08	0.6	0.12	0.9
2, 3	AllS(O)SPropenyl-(*Z*,*E*)	0.32	2.6	0.33	2.4
4	AllS(O)SAll	9.77	79.0	9.95	73.1
5	AllS(O)SMe	0.60	4.9	0.81	5.9
6	MeS(O)SPropenyl-(*Z*,*E*)	*d*	*d*	*d*	*d*
7	AllSS(O)Me	1.44	11.6	2.15	15.8
8	MeS(O)SMe	0.16	1.3	0.26	1.9
	total thiosulfinates	12.37		13.62	

[a] 1, (*E*)-1-propenesulfinothioic acid *S*-2-propenyl ester; 2, 2-propene-1-sulfinothioic acid *S*-(*Z*)-1-propenyl ester; 3, 2-propene-1-sulfinothioic acid *S*-(*E*)-1-propenyl ester; 4, 2-propene-1-sulfinothioic acid *S*-2-propenyl ester (allicin); 5, 2-propene-1-sulfinothioic acid *S*-methyl ester; 6, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; 7, methanesulfinothioic acid *S*-2-propenyl ester; 8, methanesulfinothioic acid *S*-methyl ester. [b] Data from modified SPA system with solvent trap. [c] Given as micromoles per gram of fresh garlic; used response factors from Block et al. (1992a). [d] Trace amounts present.

Table 2. Comparative Concentrations of Thiosulfinates in SC-CO_2 (Solid-Phase Trap System) and Organic Solvent Extracts of Garlic

		SC-CO_2[b]		CH_2Cl_2	
compd no.[a]	compd	μmol/g[c]	mol %	μmol/g[c]	mol %
1	AllSS(O)Propenyl-(*E*)	0.21	1.2	0.16	1.0
2, 3	AllS(O)SPropenyl-(*Z*,*E*)	0.45	2.5	0.44	2.9
4	AllS(O)SAll	13.18	72.2	10.58	69.3
5	AllS(O)SMe	1.13	6.3	0.96	6.3
6	MeS(O)SPropenyl-(*Z*,*E*)	*d*	*d*	*d*	*d*
7	AllSS(O)Me	2.95	16.2	2.73	17.9
8	MeS(O)SMe	0.34	1.9	0.40	2.6
	total thiosulfinates	18.26		15.27	

[a] 1, (*E*)-1-propenesulfinothioic acid *S*-2-propenyl ester; 2, 2-propene-1-sulfinothioic acid *S*-(*Z*)-1-propenyl ester; 3, 2-propene-1-sulfinothioic acid *S*-(*E*)-1-propenyl ester; 4, 2-propene-1-sulfinothioic acid *S*-2-propenyl ester (allicin); 5, 2-propene-1-sulfinothioic acid *S*-methyl ester; 6, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; 7, methanesulfinothioic acid *S*-2-propenyl ester; 8, methanesulfinothioic acid *S*-methyl ester. [b] Data from PrepMaster system with solid-phase trap. [c] Given as micromoles per gram of fresh garlic; used response factors from Block et al. (1992a). [d] Trace amounts present.

Several commercially available garlic products were extracted by using SC-CO_2 under the conditions described above (SPA system, 36 °C, 3500 psi, 45 min). An SF extract of garlic tablets purchased at a local health food store and reconstituted with water produced a chromatogram that was quite similar to that of fresh garlic. As with fresh garlic, the major thiosulfinates obtained in the extraction were allyl methyl isomers, methyl propenyl isomers, allicin, and allyl propenyl isomers.

In addition, we have extracted yellow onion under similar conditions (35 °C, 3500 psi), using the solvent trap, and analyzed the resulting extracts by LC/UV (Figure 5), LC–MS, and GC–MS (Figure 6). Although there was a 100-fold decrease in the concentration of the thiosulfinates, instrument constraints permitted only a 4-fold increase in the amount of onion extracted through the use of a 32-mL extraction vessel. The larger vessel permitted extraction of 10 mL of onion juice, which represented 15–20 g of whole fresh onion.

The SF extract of 10 mL of juice from a yellow onion was analyzed by GC–MS under previously described conditions (Block et al., 1992b) by using a wide-bore open tubular column (0.53 mm i.d.) with cryogenic (0 °C) on-column injection and initial column temperature conditions, slow column heating (5 °C/min), and a GC–MS

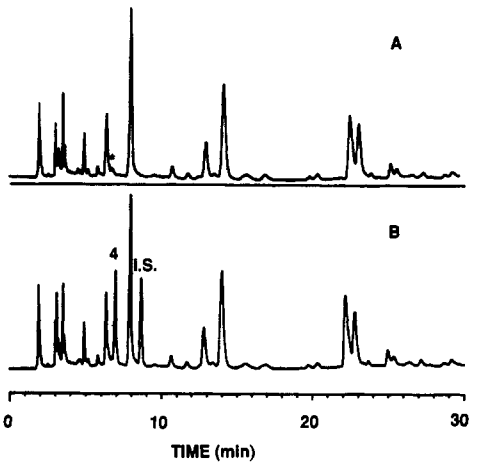

Figure 5. Si-LC chromatograms of fresh onion extracts. See text for chromatographic conditions (system 3). (A) SF extract of yellow onion; (B) SF extract of yellow onion spiked with allicin (4) and benzyl alcohol (IS, internal standard).

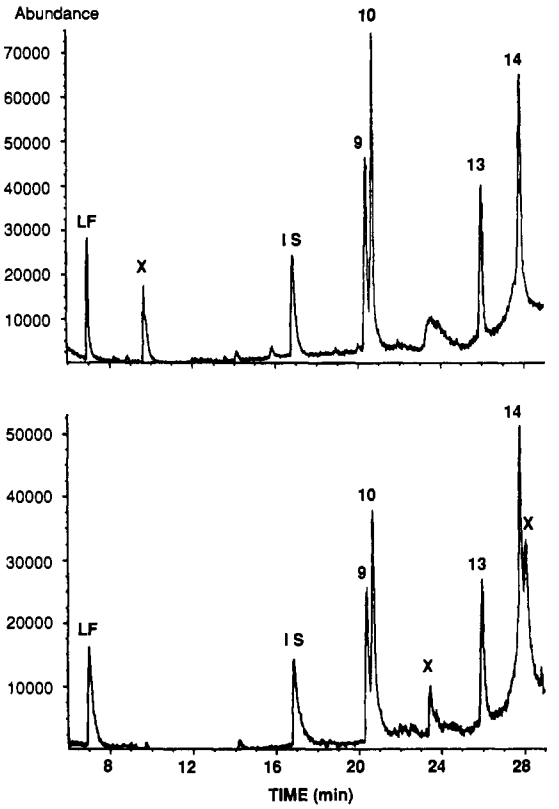

Figure 6. GC–MS total ion chromatograms of (A) diethyl ether extract of onion juice and (B) SC-CO_2 extract of onion juice. The peaks are numbered as indicated in Table 2; LF is propanethial *S*-oxide, IS is the benzyl alcohol internal standard, and X indicates a substance not yet identified.

transfer temperature of 100 °C. Parallel analysis was performed on the diethyl ether extract of another portion of the same juice. The profiles of both extracts (Figure 6), which were previously reported (Block et al., 1992b), show a mixture of thiosulfinates, zwiebelanes, and the lachrymatory factor, propanethial *S*-oxide, and are quite different from that reported by Sinha et al. (1992), who employed much higher injection port temperatures. The amounts of these latter flavorants were ca. 69% as abundant in the SF extract as in the diethyl ether extract (Table 3). In addition, we found no evidence of methyl 3,4-dimethyl-2-thienyl disulfide or its isomers in a comparison with authentic synthetic standards, using GC–MS methodology (Block and Thiruvazhi, 1993). This

Table 3. Comparative Concentrations of Thiosulfinates and Related Compounds in SC-CO_2 and Diethyl Ether Extracts of Yellow Onion

		SC-CO_2		diethyl ether	
compd no.[a]	compd[b]	nmol/g[c]	mol %	nmol/g[c]	mol %
9	MeS(O)SPropenyl-(*E,Z*)	28.1	22.7	41.3	22.9
10	MeSS(O)Propenyl-(*E*)	48.0	38.7	77.0	42.7
11	MeS(O)SPr	0.2	0.2	1.6	0.9
12	MeSS(O)Pr	0.1	0.1	0.8	0.4
13a	*cis*-zwiebelane	19.9	16.1	21.8	12.1
13b	PrS(O)SPropenyl-(*E,Z*)	3.0	2.4	8.0	4.4
14	*trans*-zwiebelanes	24.9	20.1	29.7	16.5
	total thiosulfinates[b]	124.2[d]		180.2[e]	

[a] 9, methanesulfinothioic acid *S*-(*E,Z*)-1-propenyl ester; 10, (*E*)-1-propenesulfinothioic acid *S*-methyl ester; 11, methanesulfinothioic acid *S*-1-propyl ester; 12, 1-propanesulfinothioic acid *S*-methyl ester; 13a, (±)-(1α,2α,3β,4α,5α)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide; 13b, propanesulfinothioic acid *S*-(*E,Z*)-1-propenyl ester (GC peaks for compounds 13a and 13b overlap; analysis requires selective ion methods); 14, (1α,2β,3β,4α,5β)-2,3-dimethyl-5,6-dithiabicyclo-[2.1.1]hexane 5-oxide. [b] We did not search for propanesulfinothioic acid *S*-1-propyl eser [PrS(O)SPr], which was expected to be present in small amounts and requires different column conditions for optimum analysis. [c] Given as nanomoles per gram of fresh onion. [d] Also found: propanethial *S*-oxide at 23 nmol/g. [e] Also found: propanethial *S*-oxide at 7 nmol/g.

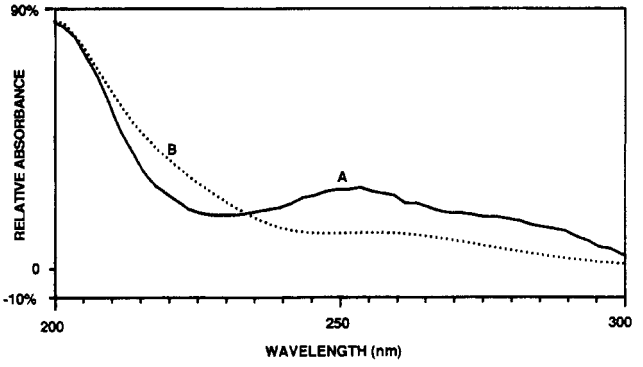

Figure 7. UV spectra of (A) allicin standard and (B) compound(s) eluting before allicin in the SF extract of onion juice.

compound was reported to account for approximately 55% of the total peak area in the GC–MS analysis reported by Sinha et al. (1992).

Because our cryogenic GC–MS conditions did not permit analysis for allicin, we also subjected an SF extract of onion to Si-LC (Figure 5A), under conditions described under Experimental Procedures. The extract was then spiked with a standard solution of allicin containing the internal standard benzyl alcohol. The chromatogram of the spiked onion extract (Figure 5B) indicates that allicin was not present in the original extract. UV spectra (Figure 7) of allicin and the compound eluting before allicin (indicated by an asterisk in Figure 5A) are dissimilar. LC–MS also showed that although a chromatographic component eluting near allicin had an MH^+ ion of m/z 163, its spectrum and retention time did not match those of the allicin standard. LC–MS/MS data further confirmed that this compound was not allicin because of spectral differences (Matusik et al., 1993).

CONCLUSION

SFE of natural products provides an effective alternative to traditional organic extractions. We have demonstrated that the constituents found in an SF extract of an aqueous homogenate of fresh garlic or the expressed juice of a yellow onion are the same constituents found in methylene chloride or diethyl ether extracts of these plants. The yields are >90% in the case of garlic and ca. 69% in the case of onion with respect to organic solvent extractions.

ABBREVIATIONS USED

C_{18}-LC, liquid chromatography with octadecyl bonded phase; GC–MS, coupled gas chromatography–mass spectrometry; LC, liquid chromatography; LC–MS, coupled liquid chromatography–mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance; SC, supercritical; SF, supercritical fluid; SFC, supercritical fluid chromatography; SFE, supercritical fluid extraction; Si-LC, liquid chromatography with silica gel.

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