

# Volatile Compounds in Stir-Fried Garlic

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Stir-fried garlic was extracted by supercritical CO<sub>2</sub> at 200 kg/cm<sup>2</sup> and 40 °C. The extract contained only ~1% of the original frying oil. The volatile compounds of the extract were then analyzed by the purge and trap method and by the simultaneous distillation and extraction method. Dimethyl sulfide, allyl alcohol, diallyl sulfide, methyl allyl disulfide, and diallyl disulfide were the major volatile compounds found by headspace gas analysis. On the other hand, the major volatile compounds of stir-fried garlic isolated by simultaneous distillation and extraction were diallyl disulfide, diallyl trisulfide, and dithiins. The advantages and disadvantages of these flavor isolation methods are discussed and compared with published data. The lower thermal effect of the headspace gas analysis resulted in no propyl sulfides and heterocyclic compounds being found and a higher yield of low-boiling-point compounds. The total percentage of vinyl dithiins of stir-fried garlic was >30% as isolated by the simultaneous distillation and extraction method; however, vinyl dithiins were still not significantly recovered, even when the purge time was prolonged to 35 min.

**Keywords:** *Stir-fried garlic; supercritical CO<sub>2</sub> extraction; purge and trap method; low thermal effect; simultaneous distillation and extraction; low-boiling-point compounds; high-boiling-point compounds*

## INTRODUCTION

To analyze the volatile compounds in foods, the necessary first step is isolation and concentration of the isolates (Teranishi et al., 1971). This first step is needed to separate the minor volatile constituents from the major nonvolatile constituents, which would cause problems in the subsequent identification work if not separated. Simultaneous distillation and extraction (Yu and Wu, 1989) and headspace analysis (Tokimoto and Kobayashi, 1992) for the unstable sulfur-containing compounds in garlic and onion are commonly used for flavor isolation work among the various extraction methods. All of the methods currently used create some problems: (1) a high-temperature treatment may cause artifacts; (2) a low recovery of high-boiling-point compounds results from the headspace analysis; (3) a loss of the original individual component ratios results because of the differing volatility of compounds; (4) a loss of low-boiling-point compounds occurs in the step of concentrating or desorbing the flavor compounds.

Supercritical fluids are currently receiving attention for use in extraction processes because they offer a number of advantages. Generally, they have low viscosity and high diffusivity, resulting in more rapid extraction compared with other solvents. Another advantage of supercritical fluids is their ability to control the solubility of the solute by regulating the temperature and/or pressure. Carbon dioxide is safe for human beings and its critical temperature is 31.3 °C, allowing heat-labile substances to be processed without thermal denaturation or decomposition (Stahl et al., 1987; King and Bott, 1993).

Both volatile compounds and vegetable oils are soluble in common organic solvents, and they are difficult to separate in the subsequent step without harming flavor compounds. Although these two kinds of compound can

also be extracted by a supercritical fluid, separation is possible by temperature or pressure control. It is known (Fridrich et al., 1982) that volatile compounds are generally soluble in CO<sub>2</sub> under a relatively low pressure and that soybean oil is soluble under a relatively higher pressure; therefore, volatile compounds and soybean oil could be separated by controlling the conditions for supercritical CO<sub>2</sub> extraction.

A very common technique in Chinese cooking is stir-frying. By this method, a small amount of vegetable oil is put into a wok and heated to >120 °C. Garlic (*Allium sativum* L.), shallots, or other seasonings are universally applied in stir-frying to produce aroma and flavor, and other food materials are then added to stir-fry. The volatile components, of raw, dried, boiled, deep-oil-fried, microwave-heated, and oven-baked garlic have been well studied. Among these studies, the contribution of alk(en)yl thiosulfonates and their transformation products to the flavor sensation of garlic products were also reported (Stoll and Seebach et al., 1971; Yu and Wu, 1989; Yu et al., 1989, 1993; Lawson and Hughes, 1992; Block, 1992; Ohsumi et al., 1993). However, the volatile compounds of stir-fried garlic have never been reported. In this study, the volatile components in stir-fried garlic were first formed at 125 °C, and the resulting flavored vegetable oil and fried garlic were extracted by supercritical CO<sub>2</sub> at 40 °C. The extracted volatile compounds were further isolated by purge and trap injector system, and by the simultaneous distillation and extraction method, and were finally analyzed by gas chromatography and mass spectrometry.

## EXPERIMENTAL PROCEDURES

**Materials.** Garlic cloves were purchased from a local market in Japan. The vegetable oil used was soybean oil produced in Japan.

**Sample Preparation.** Using a slicer, peeled garlic cloves were cut into slices of ~1-mm thickness. A 35–40-cm diameter Chinese wok was used for stir-frying. The wok was first dried by heating before 100 g of soybean oil was added and heated

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to 125 °C. Garlic slices (333 g) were then added and stir-fried for ~5 min until the desired garlic aroma was achieved. No cover was used. The stir-fried garlic slices were then spread on a cold plate and allowed to cool. This procedure was repeated until enough samples were obtained.

**Supercritical CO<sub>2</sub> Extraction.** The garlic slices (1400 g) that had been stir-fried with oil were put into a 5-L extractor (Akico Company, Japan) and extracted at a pressure of 200 kg/cm<sup>2</sup> at 40 °C for 2 h, using a CO<sub>2</sub> flow rate of 1 m<sup>3</sup>/h and a separator pressure of 40 kg/cm<sup>2</sup> at 30 °C. The yield of the extract was 33.5 g, which contained ~3.0 g of soybean oil as estimated from the residue by the simultaneous distillation and extraction (SDE) method.

**Collection of Volatiles.** The stir-fried garlic volatiles isolated by supercritical CO<sub>2</sub> extraction were collected by the following two methods for sample injection to a gas chromatograph.

**Purge and Trap Injection (PTI).** The liquid (≤0.02 g) obtained from the separator of the supercritical CO<sub>2</sub> extraction apparatus was transferred to the sample flask (25 mL) of a purge and trap injector. A gas chromatographic analysis was carried out according to the headspace gas analysis method with a cryogenic trap (Chrompack, The Netherlands). The sample flask was immersed in a water bath at 40 °C, while the cryogenic trap was maintained at -130 °C by the flow of liquid nitrogen. The cooled trap was heated to 200 °C for 5 min to purge the volatile compounds into the gas chromatograph. The purge times were 2, 5, 15, 25, and 35 min at a helium flow rate of 1.0 mL/min. The purge and trap injector was mounted on a Hitachi G-3000 gas chromatograph with a fused silica column which was coated with a semipolar stationary liquid (DB-5, 60 m × 0.25 mm, J&W Scientific), and a flame ionization detector. The GC oven temperature was held at 40 °C for 10 min and then programmed to increase from 40 to 200 °C at a rate of 4 °C/min. The injector and detector temperatures were 150 and 250 °C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min. The gas chromatography (GC) retention times and Kovats indices were calculated by a Shimadzu C-R 3A Chromatopac data processor.

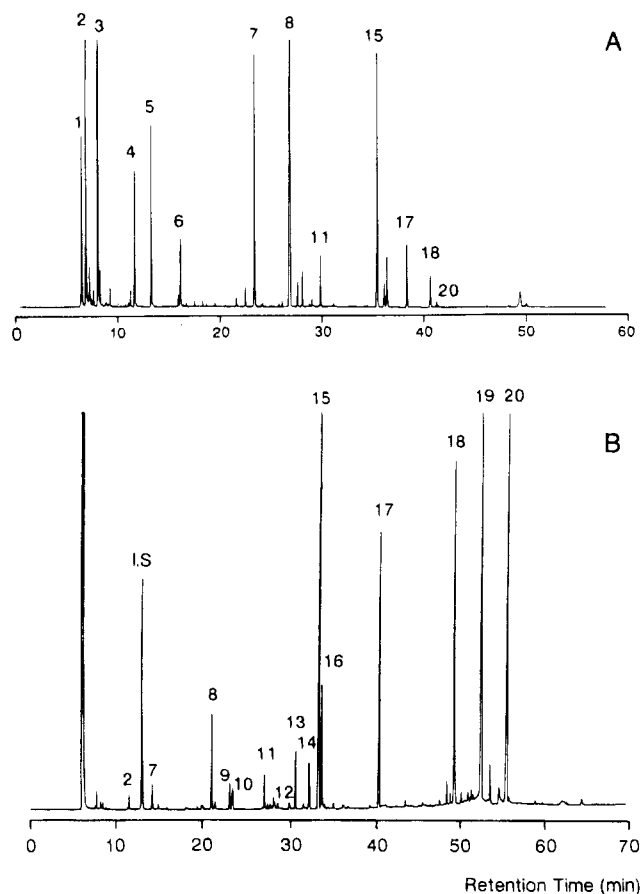
**Simultaneous Distillation and Extraction (SDE).** The liquid (3 g) obtained from the separator of the supercritical CO<sub>2</sub> extraction apparatus and water (200 mL) were distilled and extracted for 2 h in a Likens-Nikerson apparatus. Redistilled pentane and diethyl ether (1:1) were used as extracting solvents, and propyl butylate was added as an internal standard. The volatile extracts were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent was distilled off at 39 °C, and the solvent-free extract was finally concentrated to a final volume of ~0.2 mL by blowing with nitrogen gas.

Volatiles from the garlic samples were quantitatively analyzed by GC with a Shimadzu GC-7A apparatus that was equipped with a flame ionization detector and a 50 m × 0.25 mm fused silica column coated with a polar stationary liquid (CP-Wax 52 CB, Chrompack). The operating conditions were as follows: injector and detector temperature, 200 °C; nitrogen carrier gas flow rate, 1.0 mL/min; oven temperature program, 60 °C (4 min), 2 °C/min, 200 °C (30 min). Linear retention indices for the volatile components were calculated with *n*-paraffins (C<sub>6</sub>–C<sub>26</sub>) as references.

Identification of the volatile compounds in the aroma concentrate was primarily based on GC–mass spectrometry (GC–MS) by comparing their mass spectral data with those from published literature for authentic compounds and by comparing their retention indices with those of authentic compounds.

**Gas Chromatography–Mass Spectrometry (GC–MS).** The GC–MS data were recorded with a Jeol JMS-DX 300 mass spectrometer combined with a Hewlett-Packard 5790 A series gas chromatograph under the same conditions as those used for the GC analysis, except that helium was used as the carrier gas for the SDE method. The ionization voltage was 70 eV.

**High-Performance Liquid Chromatographic Analysis.** Determinations were carried out on a Shimadzu LC-9A HPLC system. A PEGASIL ODS (Senshu Pak) column (4.6 mm i.d., length of 250 mm) was used for separation. The eluant used



**Figure 1.** Capillary gas chromatograms of the volatile compounds in a supercritical CO<sub>2</sub> extract of stir-fried garlic isolated by (A) PTI (purge time, 15 min) and (B) SDE.

for the separation of allicin and allicin transformation products was acetonitrile:water:methanol (50:41:9). The flow rate was 1.0 mL/min, and the detection wavelength was 210 nm.

Quantitative analysis was performed as described in a previous paper (Kim et al., 1995).

## RESULTS AND DISCUSSION

Supercritical CO<sub>2</sub> extraction at 40 °C was used to concentrate the volatiles of stir-fried garlic dissolved in soybean oil. The extract obtained was analyzed by PTI and SDE to examine whether the method of supercritical CO<sub>2</sub> extraction was suitable to collect an attractive cooked flavor from stir-fried garlic. Figure 1 shows the capillary gas chromatographic separation of the volatile compounds of stir-fried garlic obtained by PTI (Figure 1A) and SDE (Figure 1B). The identified volatile constituents corresponding to the peaks in Figure 1 are shown in Table 1. Dimethyl sulfide, allyl alcohol, diallyl sulfide, methyl allyl disulfide, and diallyl disulfide were the major volatile compounds isolated by PTI. On the other hand, diallyl disulfide, dithiins, and diallyl trisulfide were the major volatile compounds isolated by SDE. The volatile compounds isolated by SDE were not found in significant amounts by the PTI method because of the disadvantage of headspace gas analysis for finding the peaks of relatively high-boiling-point compounds such as allicin. In addition, whereas the stir fried garlic contained 420 g of soybean oil in each batch for supercritical extraction, the extract contained <1% of the original fried oil. The low yield of allicin by the PTI method on the gas chromatogram (appearing as dithiins) might be explained by (i) allicin being dissolved in the extracted soybean oil and therefore not being

**Table 1. Volatile Compounds Identified in Stir-Fried Garlic**

peak no.	compound	PTI (A)			SDE (B)			MW
		Rt <sup>a</sup>	KI <sup>b</sup>	% comp	Rt	KI	% comp	
1	acetaldehyde	6.56	— <sup>c</sup>	5.59	—	—	—	44
2	dimethyl sulfide	6.98	—	19.16	11.06	1080	0.27	62
3	allyl alcohol	8.17	682	18.68	—	—	—	58
4	allyl mercaptan	11.77	871	4.12	—	—	—	74
5	methyl allyl sulfide	13.4	922	5.32	—	—	—	88
6	dimethyl disulfide	16.24	970	2.32	—	—	—	94
7	diallyl sulfide	23.47	1085	8.78	13.76	1149	0.80	114
8	methyl allyl disulfide	27.04	1149	19.84	20.77	1281	3.40	120
9	2,5-dimethylpyrazine	—	—	—	22.93	1317	0.93	108
10	2,6-dimethylpyrazine	—	—	—	23.29	1323	1.07	108
11	dimethyl trisulfide	30.1	1203	1.94	26.89	1379	1.26	126
12	allyl propyl disulfide	—	—	—	29.98	1426	0.27	148
13	3-ethyl-2,5-dimethylpyrazine	—	—	—	30.68	1437	2.00	136
14	(Z)-1-propenyl allyl disulfide	—	—	—	32.31	1462	1.80	146
15	diallyl disulfide	35.68	1320	10.41	33.37	1478	17.71	146
16	(E)-1-propenyl allyl disulfide	—	—	—	33.71	1483	4.33	146
17	methyl allyl trisulfide	38.58	1386	2.50	40.40	1585	9.79	152
18	3-vinyl-4H-1,2-dithiin	40.9	1442	1.34	49.30	1727	13.58	144
19	diallyl trisulfide	—	—	—	52.57	1782	25.17	178
20	2-vinyl-4H-1,3-dithiin	41.9	1467	trace <sup>d</sup>	55.73	1836	17.64	144

<sup>a</sup> Rt, retention time (min). <sup>b</sup> KI, Kovats Index. <sup>c</sup> —, Not determined. <sup>d</sup> Trace (<0.01 mg).

**Table 2. Changes in Volatile Compound Composition of Stir-Fried Garlic as a Function of Purge Time (PTI Method)**

peak no.	compound	% comp				
		2 min	5 min	15 min	25 min	35 min
1	acetaldehyde	20.94	9.32	5.39	5.39	7.53
2	dimethyl sulfide	31.47	36.26	19.16	25.04	21.06
3	allyl alcohol	5.20	17.20	18.68	18.09	16.67
4	allyl mercaptan	0.13	9.32	4.12	1.57	2.90
5	methyl allyl sulfide	39.01	9.32	5.32	3.63	4.49
6	dimethyl disulfide	0.78	2.42	2.32	1.49	1.51
7	diallyl sulfide	1.04	5.25	8.78	4.60	4.73
8	methyl allyl disulfide	1.43	9.12	19.84	15.88	16.25
11	dimethyl trisulfide	— <sup>a</sup>	1.04	1.94	1.25	1.76
15	diallyl disulfide	—	0.76	10.41	14.60	17.62
17	methyl allyl trisulfide	—	—	2.50	4.01	3.00
18	3-vinyl-4H-1,2-dithiin	—	—	1.34	3.62	2.17
20	2-vinyl-4H-1,3-dithiin	—	—	trace <sup>b</sup>	0.82	0.32

<sup>a</sup> —, Not determined. <sup>b</sup> Trace (<0.01 mg).

detected as the flavor component in an analysis with a short purge time and (ii) allicin being dissolved in large amount of soybean oil so that the efficiency of CO<sub>2</sub> extraction was low.

One of the advantages of supercritical CO<sub>2</sub> extraction was the low moisture content in the sample used with the purge and cryogenic trap method. The primary problem with a cryogenic trap is that it will also trap water, which is the most abundant volatile in all foods. The sample for this study was a CO<sub>2</sub> extract with no significant amount of water. The common purge time limit was ~2–15 min, because moisture in the sample would condense and form enough ice to block the path in the cryogenic trap after 15 min. The very low moisture content in the sample for this study enabled the purge time to be prolonged. The stir-fried garlic volatiles found with purge times of 2, 5, 15, 25, and 35 min are shown in Table 2. With the short-time analysis, low-boiling-point compounds could be detected in much larger amounts than the higher-boiling-point compounds. As the purge time was prolonged, medium- and high-boiling compounds increased up to ~50% of the total volatiles. Dithiins could be detected on the gas chromatogram with a purge time of >15 min, indicating that allicin was present in the CO<sub>2</sub>-extracted volatile fraction and that a moderate purge time was needed

**Table 3. Volatile Compounds in Stir-Fried Garlic Determined by the SDE Method: Comparison of Two Studies**

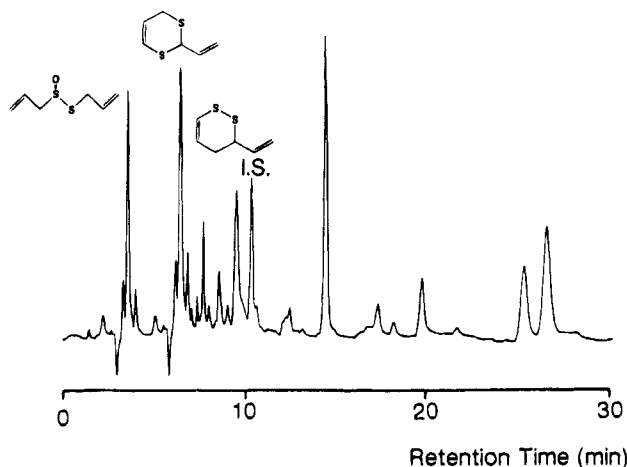
compound	yield, mg/100 g of raw garlic (% comp)		
	raw garlic (Kim et al., 1995)		
	distilled without soybean oil	distilled with soybean oil	stir-fried garlic (this study)
2-propene-1-thiol	0.06 (0.04)	0.28 (0.24)	—
methyl allyl sulfide	0.37 (0.23)	0.12 (0.10)	—
dimethyl sulfide	0.27 (0.17)	0.60 (0.51)	0.04 (0.27)
allyl alcohol	1.00 (0.62)	0.28 (0.24)	—
diallyl sulfide	1.04 (0.64)	0.12 (0.10)	0.12 (0.80)
methyl allyl disulfide	8.16 (5.06)	4.96 (4.20)	0.51 (3.40)
dimethyl trisulfide	1.08 (0.67)	1.36 (1.15)	0.19 (1.26)
propyl allyl disulfide	0.03 (0.02)	— <sup>a</sup>	0.04 (0.27)
diallyl disulfide	48.56 (30.11)	13.58 (11.49)	2.66 (17.71)
methyl allyl trisulfide	12.82 (7.95)	7.72 (6.53)	1.47 (9.79)
3-vinyl-4H-1,2-dithiin	0.77 (0.48)	26.46 (22.40)	2.04 (13.58)
diallyl trisulfide	71.67 (44.45)	11.96 (10.12)	3.78 (25.71)
2-vinyl-4H-1,3-dithiin	0.42 (0.26)	38.34 (32.45)	2.65 (17.64)
total	161.25	118.14	15.02

<sup>a</sup> —, Not determined.

for detection of allicin. The yield of high-boiling-point compounds was somewhat improved with prolonged purge times, which were possible because of the low moisture content.

The other advantage of supercritical extraction was the almost complete exclusion of fat and oil present as food ingredients. In our study, however, 1% or less of soybean oil contaminated the extractable volatile fraction. Therefore, the headspace volatile analysis of this extract would be different from the actual composition of the extracted volatiles.

Therefore, in the next step for the quantitative analysis of the CO<sub>2</sub>-extracted volatiles, we applied the SDE method to the extract and compared the garlic volatiles obtained from the different states of the garlic by the same extraction method. The garlic volatiles from raw garlic isolated by SDE were reported by Kim et al. (1995). The data obtained by SDE in this study are compared with those from the Kim et al. in Table 3. The yield of volatiles from stir-fried garlic (15.0 mg/100 g of raw garlic) was the lowest among the samples; this low yield resulted from the CO<sub>2</sub> extraction of stir-



**Figure 2.** HPLC chromatogram of the supercritical CO<sub>2</sub> extract of stir-fried garlic.

fried garlic before using the SDE method, as discussed in an earlier section of this report.

Although the CO<sub>2</sub> extract of stir-fried garlic had a typical garlic odor, low-boiling-point compounds are not apparent on the GC data shown in Table 3. The major volatile compounds of raw garlic distilled without soybean oil were diallyl trisulfide (44.5%) and diallyl disulfide (30.1%), whereas raw garlic distilled with soybean oil and stir-fried garlic showed two dithiins (54.9% and 31.2%, respectively) as the highest content compounds. This result means that samples treated with soybean oil contained significant amounts of allicin, which was previously found by SDE as dithiins on the gas chromatogram (Kim et al., 1995). It is noteworthy that the high content of allicin in stir-fried garlic originated from its cooking conditions; that is, the presence of oil to dissolve allicin, a moderate cooking temperature, and a short cooking time to avoid the heat decomposition of allicin. Supercritical CO<sub>2</sub> extraction is thus effective for proving the allicin content in cooked garlic.

The presence of allicin in the supercritical CO<sub>2</sub> extracts of stir-fried garlic was verified by HPLC analysis (Figure 2). The content of allicin in the supercritical CO<sub>2</sub> extracts of stir-fried garlic was 20.7 mg/100 g of raw garlic. Calvey et al. (1994) reported that supercritical CO<sub>2</sub> extraction of raw garlic affords 214 mg of allicin per 100 g of raw garlic. Comparing the data of allicin content obtained in this study with that from Calvey et al. (1994), after the stir-frying of garlic, shows that most of allicin has been transformed to dithiins and other sulfide compounds. However, the percent of yield of total compounds from the HPLC analysis in this study showed that supercritical CO<sub>2</sub> extracts of stir-fried garlic yielded ~22% of allicin and 12% of dithiins. Although small amounts of dithiins formed during the process of stir-frying, allicin still survives stir-frying.

Recent studies on garlic flavor have shown that only the methyl, allyl, and 1-propenyl groups, but not the propyl group, were found in the flavor precursors [e.g.,  $\gamma$ -glutamylalk(en)yl cysteine and alk(en)yl cysteine S-oxides] and in the primary flavor compounds [e.g., alk(en)yl thiosulfinates] of garlic (Lawson et al., 1991a-c; Lawson and Hughes, 1992; Lawson, 1992; Block et al., 1992a,b). Yu et al. (1993) postulated that these propyl sulfides should be formed by the reduction of S-compounds during the extreme thermal treatments of frying and baking garlic. In our study, no propyl sulfide was

found by the PTI method, as shown in Table 1; on the other hand, by the SDE method, one propyl sulfide was isolated with a very low yield. In a raw garlic extract, there are two propyl sulfides. These differences can be explained by the short cooking time for stir-fried garlic and the use of soybean oil to protect allicin from decomposition.

Our results indicate that the particular cooking conditions and the use of soybean oil affected the formation of the better flavor of stir-fried garlic.

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Received for review August 30, 1994. Revised manuscript received April 26, 1995. Accepted August 15, 1995.\*

JF940494H

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\* Abstract published in *Advance ACS Abstracts*, October 15, 1995.