

Effect of Soybean Oil on Garlic Volatile Compounds Isolated by Distillation

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Garlic, water, and/or soybean oil were blended and distilled. The volatile isolates were analyzed qualitatively and quantitatively by GC, GC–MS, and HPLC. The contents of 2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin in the volatile isolates were greater if soybean oil was present. The sample without soybean oil contained 1.2% dithiins in the total volatiles, but this increased to 15.6–54.6% if soybean oil was present, although the total volatile compounds decreased at the same time.

Keywords: Garlic; soybean oil; volatiles; distillation; allicin; dithiins

INTRODUCTION

Garlic (*Allium sativum* L.) has historically been used as a food and medicine because of the valuable volatile sulfur compounds it produces. It has been reported that intact garlic cloves contain a flavor precursor, alliin (S-allylcysteine *S*-oxide), and an enzyme, alliinase. When the tissue of garlic is disrupted, alliinase converts alliin into allicin (diallyl thiosulfinate), which is the pungent principle in raw garlic (Stoll and Seebeck, 1951). Allicin is very unstable and can decompose or rearrange to form such sulfide compounds as vinyl dithiins, or ajoenes (Block et al., 1986; Yu and Wu, 1989; Lawson, 1992; Block, 1992).

In addition to its flavoring properties, garlic has been reported to offer beneficial medicinal effects. Yu and Ho (1994) have summarized the recent research results. Allicin, allylmethyl disulfide, and diallyl trisulfide were reported to be beneficial for inhibiting platelet aggregation, while diallyl trisulfide, allyl methyl trisulfide, and diallyl disulfide were competitive inhibitors of soybean lipoxygenase. Lipoxygenase is responsible for the oxidation of lipids in biological systems, and considerable biological damage can be attributed to oxidized membrane lipids. The manifestation of biological damage from oxidized lipids includes promotion of tumors, carcinogenesis/mutagenesis, and cell lysis of membranes. 2-Vinyl-4*H*-1,3-dithiin, a decomposition product of allicin, was found to have antithrombotic activity, and a compatible polymer consisting of 2-vinyl-4*H*-1,3-dithiin copolymerized with *N*-vinylpyrrolidone was reported to exhibit antithrombotic and antibiotic properties. Ajoene from garlic was also reported to be a potent antithrombotic agent.

Although allicin and its transformed products both have medical activity, if we could preserve allicin, it would seem to be beneficial, because of its pungency. In particular, garlic essential oil is usually obtained by distilling a garlic homogenate and is used to produce garlic capsules on the market for health purposes. Therefore, the stability of allicin is important not only for medical use but also for cooking flavor.

This study reports the effect of soybean oil on the allicin content of garlic distillates.

MATERIALS AND METHODS

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

Sample Preparation. Garlic volatiles were isolated according to the following two procedures.

(1) *Steam Distillation and Extraction (SDE).* Garlic (50 g) and distilled water (300 g), with or without soybean oil (50 g), were mixed in a blender for 2 min and then extracted for 2 h in a Likens–Nickerson apparatus (Yu and Wu, 1989). Glass-distilled pentane and diethyl ether (1:1) was used as the extracting solvent, and propyl butyrate was added as an internal standard. The volatile extracts were dried with anhydrous Na₂SO₄, concentrated to a minimum volume by distillation, and further concentrated under a stream of nitrogen gas to a final volume of around 0.2 mL.

(2) *Adsorption.* Garlic (20 g) and a distilled water/soybean oil mixture (the total volume was 250 mL, with soybean oil of 0, 25, 50, 82.5, or 125 mL) were mixed for 2 min in a blender. Each homogenate was put into a 500 mL distillation flask and was distilled using a heating mantle for 1 h. The outlet of the distillation flask was connected to glass column (8.0 cm × 15 mm i.d.) containing 3.0 g of Tenax TA, and each distillate was gradually passed through the column. After distillation, the volatiles adsorbed to Tenax TA were desorbed with 40 mL of ether. After drying on Na₂SO₄ and distilling off ether, the concentrates were treated as aroma concentrate.

Gas Chromatographic Analysis. A quantitative analysis of the volatiles from the garlic samples was conducted by gas chromatography with Shimadzu GC-7A apparatus equipped with a flame ionization detector (FID). A 50 m × 0.25 mm fused silica WCOT capillary column coated with a stationary polar liquid (CP-Wax 52 CB, Chrompak) was used. 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 of the volatile components were calculated with *n*-paraffins (C₆–C₂₆) as references.

Identification of the Volatile Compounds. Identification of the volatile compounds in the aroma isolates was mostly based on gas chromatography–mass spectrometry (GC–MS). The structural assignment of the volatile compounds was accomplished by comparing their mass spectral data, with those from published literature or for authentic compounds and/or their retention time indices with those of authentic compounds.

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

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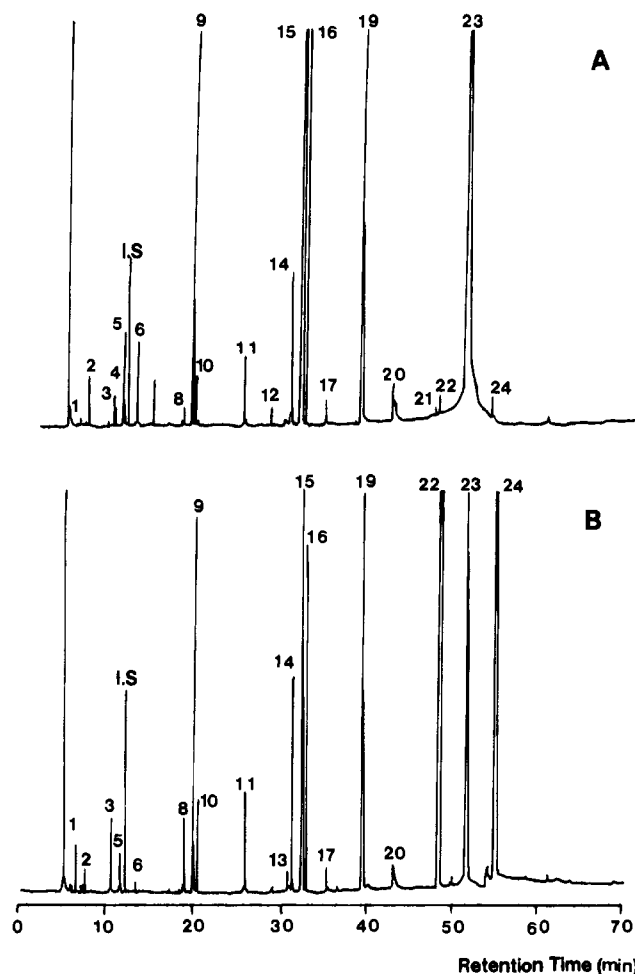


Figure 1. Capillary gas chromatograms of the volatile compounds of garlic in garlic homogenates distilled by SDE without soybean oil (A) and with soybean oil (B).

High-Performance Liquid Chromatographic Analysis. A Shimadzu LC-9A HPLC system was used. A PEGASIL ODS (Senshu Pak.) column, 4.6 mm i.d., with a length of 250 mm was used for separation. The eluant was acetonitrile/water/methanol (50:41:9). Flow rate was 1 mL/min. Detection was based on UV adsorption at 210 nm.

For quantitative analysis, the retention times of standard alliin synthesized according to the method of Iberl et al. (1990a) and dithiins purified by preparative gas chromatography were established. Diethyl disulfide was used as the internal standard.

RESULTS AND DISCUSSION

Figure 1 shows the capillary gas chromatographic separation of the volatile compounds from garlic samples obtained by method 1 with or without the presence of soybean oil, while Table 1 shows the volatile compounds identified in the samples. The major differences between the two samples are the amounts of dithiins, peaks 22 and 24. It has been found that alk(en)yl thiosulfonates could be transformed to 2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin during gas chromatographic analysis by a high temperature at the injection port (Brodnitz et al., 1971; Block, 1985; Yu and Wu, 1989; Iberl et al., 1990b; Lawson, 1992). The presence of small peaks of dithiins in Figure 1A indicates that alliin was decomposed under the steam-distilling conditions and did not appear as a volatile constituent. On the other hand, in the presence of soybean oil, alliin was dissolved in the oil and protected from the decom-

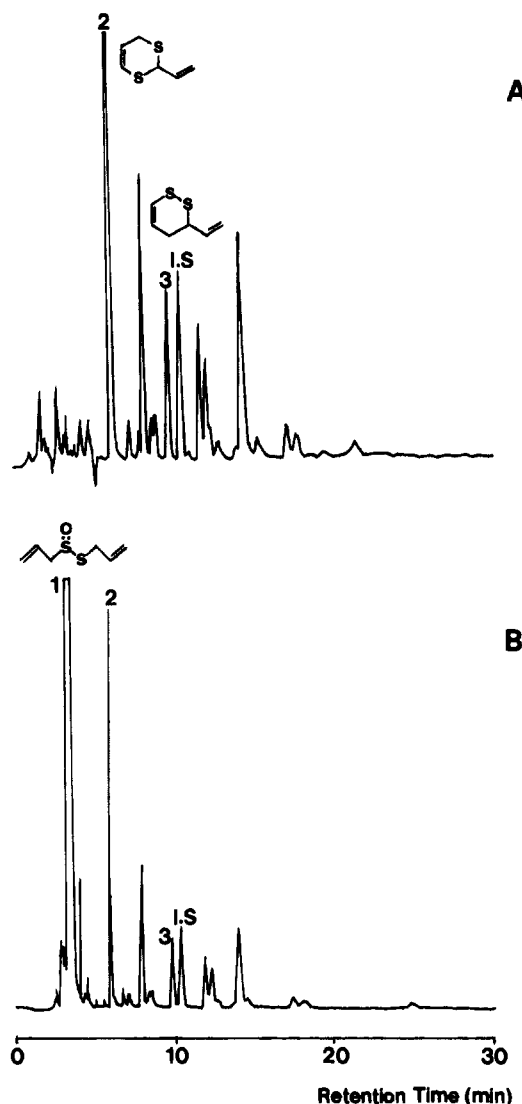


Figure 2. HPLC chromatograms of the distillates of garlic without soybean oil (A) and with soybean oil (B).

position that occurred in an aqueous solution. The large peaks 22 and 24 on Figure 1B indicate that relatively large amounts of alliin were steam-distilled. As shown in Table 1, the sample without soybean oil had much less dithiin content but much greater diallyl disulfide (peak 15) and diallyl trisulfide (peak 23) contents, the other compounds not being significantly different from those obtained with soybean oil. This means that alliin was converted to diallyl trisulfide and diallyl disulfide in the process. Mechanisms for the decomposition or rearrangement of alliin have been proposed by many researchers (Block et al., 1986; Yu and Wu, 1989; Lawson, 1992; Block, 1992), a very complicated series of reactions usually being suggested. However, in this study, we found that diallyl trisulfide and diallyl disulfide were significantly increased with the decreasing alliin. The mechanism for this reaction needs further study.

In regard to the role of vegetable oil in garlic flavor, Yu et al. (1993) have reported the volatile compound contents of deep-oil-fried, microwave-heated, and oven-baked garlic slices. Among these data, it can be seen that the samples treated with soybean oil contained a much higher content of dithiins than those in the absence of an oil. The dithiin content of distilled crushed garlic cloves also was much lower, as reported

Table 1. Volatile Compounds Identified in Garlic Homogenates Distilled without Soybean Oil (A) and with Soybean Oil (B)

peak no.	compound	Rt	KI	yield (mg/100 g of raw garlic)	
				A	B
1	2-propene-1-thiol	6.22	887	0.06	0.28
2	methyl allyl sulfide	7.30	960	0.37	0.12
3	dimethyl sulfide	10.25	1080	0.27	0.60
4	2-vinylcyclopropanal	10.39	1084	0.51	tr ^a
5	2-propen-1-ol	11.28	1109	1.00	0.28
6	diallyl sulfide	13.00	1151	1.04	0.12
8	(Z)-1-propenyl methyl disulfide	18.91	1267	0.25	0.94
9	methyl allyl disulfide	19.91	1283	8.16	4.96
10	(E)-1-propenyl methyl disulfide	20.34	1290	0.81	1.14
11	dimethyl trisulfide	25.93	1380	1.08	1.36
12	propyl allyl disulfide	29.02	1428	0.03	tr
13	3-methyl thiopropanal	30.82	1450	tr	0.50
14	(Z)-1-propenyl allyl disulfide	31.37	1464	3.07	3.34
15	diallyl disulfide	32.46	1480	48.56	13.58
16	(E)-1-propenyl allyl disulfide	32.80	1485	7.67	5.24
17	1,2-dithiacyclopent-3-ene	33.15	1490	0.54	0.40
19	methyl allyl trisulfide	39.48	1588	12.82	7.72
20	allyl thiopropanal	43.13	1646	1.62	0.80
21	propyl allyl trisulfide	47.97	1723	0.53	tr
22	3-vinyl-4H-1,2-dithiin	48.46	1731	0.77	26.46
23	diallyl trisulfide	51.65	1784	71.67	11.96
24	2-vinyl-4H-1,2-dithiin	54.99	1842	0.42	38.34
	total			161.25	118.14

^a Trace (<0.01 mg).**Table 2. Effect on Garlic Volatile Compounds of Different Amounts of Soybean Oil in Garlic Homogenates**

peak no.	compound	Rt	KI	yield (mg/100 g of raw garlic)				
				A ^a	B ^a	C ^a	D ^a	E ^a
2	methyl allyl sulfide	7.65	960	0.02	0.07	0.66	0.15	0.05
3	dimethyl disulfide	10.69	1078	0.05	0.14	0.20	0.06	0.14
5	2-propen-1-ol	11.73	1107	0.11	0.15	0.14	0.05	0.07
6	diallyl sulfide	13.49	1149	0.29	0.18	0.05	0.02	0.03
7	2,4-dimethylfuran	19.19	1261	0.11	0.08	0.11	0.36	0.10
8	(Z)-1-propenyl methyl disulfide	19.48	1266	0.36	0.42	0.97	1.24	0.82
9	methyl allyl disulfide	20.48	1282	4.67	4.07	2.64	2.37	1.59
10	(E)-1-propenyl methyl disulfide	20.92	1289	0.79	0.80	1.14	1.65	0.95
11	dimethyl trisulfide	26.51	1380	0.60	0.65	0.66	1.53	0.60
12	propyl allyl disulfide	26.57	1428	0.25	0.11	0.09	0.08	0.05
13	3-methyl thiopropanal	31.33	1455	0.21	0.23	0.20	1.18	0.30
14	(Z)-1-propenyl allyl disulfide	31.93	1464	5.38	3.33	2.20	4.22	1.60
15	diallyl disulfide	33.02	1480	36.10	18.42	7.99	5.97	3.57
16	(E)-1-propenyl allyl disulfide	33.36	1485	14.48	8.39	3.75	5.28	2.17
18	methyl propyl trisulfide	35.79	1523	0.42	0.31	0.25	0.78	0.21
19	methyl allyl trisulfide	39.99	1587	7.41	5.15	3.28	5.76	2.13
20	allyl thiopropanal	43.58	1644	1.23	0.68	0.48	0.49	0.66
22	3-vinyl-4H-1,2-dithiin	48.95	1731	0.84	5.32	8.79	10.07	8.26
23	diallyl trisulfide	52.13	1784	48.99	16.67	3.47	5.73	1.49
24	2-vinyl-4H-1,3-dithiin	55.51	1842	0.66	5.67	14.67	12.70	11.64
	total			122.97	70.84	51.14	59.69	36.43

^a Water/soybean oil (v/v): A (250/0), B (225/25), C (200/50), D (167.5/82.5), E (125/125).

by Yu and Wu (1989) and other researchers. Therefore, soybean oil has the function of improving the stability of allicin.

To clarify the effect of soybean oil quantitatively, we cooked garlic with different ratios of the water and oil mixture. In this case, the organic volatiles in the distillate were adsorbed directly to the porous polymer (method 2), which was simpler and more suited for repeating the distillation under the same conditions. Table 2 compares the volatile compounds found in the samples without and with different amounts of soybean oil. Again, we found that soybean oil had the function of improving the stability of allicin during the process. With increasing amounts of soybean oil tested, the content of dithiins also increased. The dithiin contents reached even more than 50% of the total volatiles for

the sample with the highest amount of soybean oil, while the sample without soybean oil gave a value of only 1.2%. However, the total content of volatile compounds decreased in the presence of soybean oil, probably due to soybean oil reducing the volatility of these compounds. It is postulated that there was a large portion of volatile compounds remaining in the oil layer and that the garlic flavor was present in the oil.

The presence of allicin in the distillates was proved by HPLC analysis. Figure 2 shows the HPLC chromatograms of the garlic volatile compounds obtained by method 2 with or without the presence of soybean oil. As shown in Figure 2, the sample without soybean oil had no allicin peak, but the sample with soybean oil had allicin as the main constituent, of which content was 13.2 mg/100 g of garlic. Considering the amount of

dithiins, the content of allicin calculated from HPLC analysis is approximately the same as the amount of allicin calculated from GC analysis. Thus, the increasing dithiin content on the gas chromatogram was primarily the result of decomposition of allicin.

Two methods of distillation were used in this study. The first method involved simultaneous distillation and solvent extraction, most of the volatile compounds being distilled out in a short time, although the solvent was returned to provide multiple extractions. The second method involved distillation and trapping, most of the volatile compounds again being distilled out in a short time. While both methods had similar abilities to extract the volatiles, the differing heating time and apparatus resulted in somewhat different yields. The yield by the former method was 161 mg/100 g of garlic, while the latter method gave a yield of 123 mg/100 g of garlic.

There are two possible ways for soybean oil to protect allicin during processing. One is by physical means, the soybean oil and aqueous garlic homogenate being an emulsion in the experiments, with the soybean oil spread on the surface of the garlic particles to protect them (and allicin) from reacting with molecules such as oxygen in the air and the other constituents of garlic. The other is by chemical means, because soybean oil contains unsaturated fatty acids and tends to be autoxidized by heating (Wu et al., 1986; Wu and Liou, 1990; Wu and Chen, 1992). Soybean oil may thus eliminate some compounds or factors that react with allicin, although this postulation needs subsequent evidence.

Summing up our results, allicin was protected by soybean oil during heat processing. Although the total essential oil was less with soybean oil, this result can explain the maintenance of garlic flavor in oil, especially that used in ethnic cooking.

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