

Extraction of Thiosulfinates from Garlic Using Gas-in-Liquid-Dispersion

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Abstract

Extraction yields of thiosulfinates from garlic were studied by using gas-in-liquid-dispersion (GLD) system to maximize the thiosulfinate extraction. Extracted thiosulfinates were spectrophotometrically quantified using synthesized diallyl thiosulfinate. The conditions for maximum extraction were optimized by response surface methodology. The volatile components extracted by various methods, such as hexane extraction, simultaneous steam distillation and GLD system, were compared by using gas chromatography. The results indicated that the thiosulfinate yield was increased by increasing temperature and nitrogen gas flow rate, while the effects of bubble sizes on thiosulfinate extraction were not significant at the ranges tested. Application of GLD system resulted in extraction of more volatile components than other extraction methods. Therefore, it was suggested that GLD system was one of the efficient extraction methods among the ever introduced ones, for thiosulfinate extraction.

Key words. thiosulfinates, oleoresin, gas-in-liquid-dispersion system, garlic extracts

INTRODUCTION

Garlic (*Allium sativum* L.) is a widely distributed plant in the world and is used not only as a spice and a food, but also as a popular remedy. It is reported that the primary pungent component of crushed garlic is thiosulfinates which is formed enzymatically from an amino acid or its derivatives (1-3). The pharmacologic importances of thiosulfinates have been emphasized because of their antibacterial and antifungal actions (4-7). Thiosulfinates are unstable material degrading easily into volatile sulfur-containing compounds intermediate along with ammonia and pyruvate (8). Jo et al. (9) identified six components, dimethyl sulfide, diallyl sulfide, methyl-1-propenyl disulfide, diallyl disulfide, allyl methyl sulfide and diallyl trisulfide, from garlic essential oil with GC and GC/MS, and found diallyl trisulfide, diallyl disulfide and allyl methyl sulfide to be major volatile components. Recovering these volatile compounds in the form of oleoresin is therefore the key yield-determining factor and the main objective in this study. Bae et al. (10) quantified the oleoresin in garlic extract by measuring the absorbance spectrophotometrically at 420nm and calibrating it with a solution of a defined content of pyruvate, based on the report by Schwimmer et al. (11)

which reported a significant correlation between the amount of enzymatically developed pyruvate present in the juice of comminuted onion and the olfactory threshold concentration of the juice. Jensen et al. (12) elaborated an external standard method utilizing HPLC for the quantitative determination of allicin in garlic. Freeman and Whenham (13) extracted thiopropanal S-oxide from onion with hexane at 0°C and observed the absorbance maximum at 254nm, as compared with that of the synthetic compound as a standard.

Recently, some extraction methods have been studied to be applied to the flavor compound extraction from garlic, such as steam distillation method (14,15), autoclaving method (16) and solvent extractions (10,13) utilizing organic solvents among which, according to findings of Bae et al. (10), methanol showed the most efficient extractability of garlic oleoresin. However, the yield of thiosulfinates was only 19.1% on methanol extraction. Thus, this development work was attempted to utilize gas-in-liquid-dispersion (GLD) system for further improvement of the extraction yield of thiosulfinate. In the GLD system, bubbles at high temperature were dispersed into a continuous liquid phase, thereby generating temperature gradient in air-liquid interface, which causes heat and mass transfer accordingly. Then, the vol-

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atile materials in the continuous liquid phase are evaporated and collected in the cooling tower. This system had been employed in sea water evaporation(17), milk concentration(18) and ethanol recovery from dilute aqueous solution(19). Use of GLD system has several advantages over processes using solvent extraction: Relatively pure volatile materials can be recovered ; the processes can be conducted in a short time and at relatively low temperature ; the initial concentration of the volatiles has little effects on the stripping coefficient and recovery ratio ; and high yields can be achieved(19). Therefore, GLD system was considered appropriate to be applied to recovering the volatile compounds in low content like the thiosulfinates in garlic.

The objectives of this study were to maximize the extraction yield of thiosulfinate using GLD system, to optimize the extraction conditions using response surface methodology and to compare the components of the thiosulfinates extracted by this system with those by various extraction methods.

MATERIALS AND METHODS

Synthesis & purification of diallyl thiosulfinate

Diallyl thiosulfinate was synthesized and purified by modified method of Jansen et al.(12) and Iberl et al.(20) to be used as a standard in determination of total thiosulfinates in garlic extracts. The synthesis is summarized in Fig. 1. Purification of diallyl thiosulfinate was performed on a Sephadex LH-20 column(26mm×580 mm) equilibrated with eluant methanol(60%) in water containing 0.1% formic acid. Synthesized thiosulfinates were dissolved in the eluant to a concentration of 50mg of thiosulfinates/ml of eluant. Three ml of this solution was placed on the column and purified. Finally purified thiosulfinate was stored in a freezer.

Sample preparation

All fresh garlic cloves used in this work were purchased in local market. Peeled garlic cloves were frozen for 24 hr at -60°C , crushed with liquid nitrogen, and then freeze-dried.

Gas-in-liquid-dispersion(GLD) system

A jacket-type GLD system used in this study was specially designed to maintain the constant temperature

using circulation water bath and to condense the stripped volatiles with evaporated solvent in a cooling tower. The schematic diagram for this system is depicted in Fig. 2 and the specification of each part is described in Table 1.

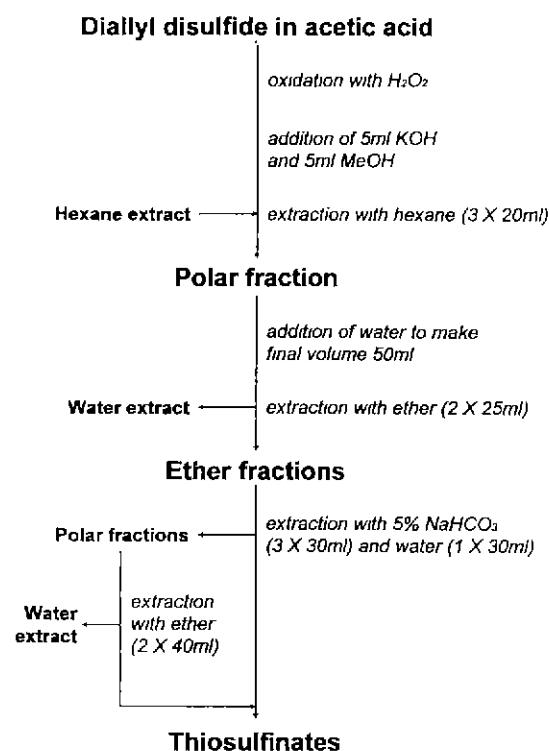


Fig. 1. Schematic diagram for diallyl thiosulfinate synthesis.

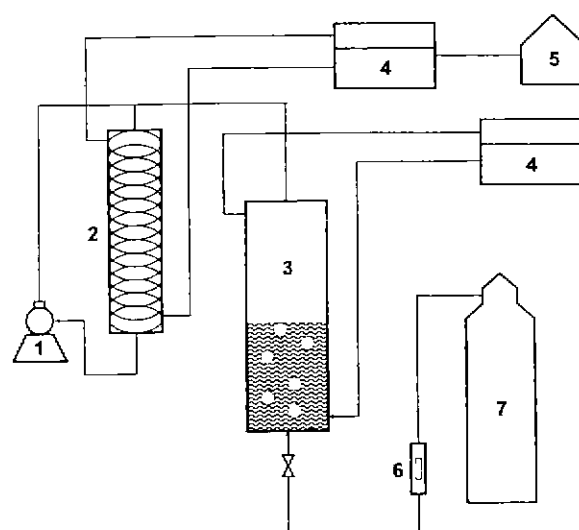


Fig. 2. Schematic diagram of gas-in-liquid-dispersion system for thiosulfate extraction from garlic. 1. Solvent trap 2. Cooling tower 3. Stripping column 4. Circulation water bath(-10°C) 5. Cooling unit 6. Flow meter 7. Nitrogen gas cylinder

Table 1. The specification of each part of gas-in-liquid-dispersion system

Item	Material	Specification
Solvent trap	Pyrex	
Cooling tower	Pyrex	7cm (dia.)×50cm(height) triple layered column
Stripping column	Pyrex	2cm(dia.)×45cm(height)
Distributor	Glass	0.4cm, 0.6cm, 0.8cm(dia.)
Circulation water bath	Stainless steel	
Cooling unit	Stainless steel	Fis system Inc.
Flow meter		
Nitrogen gas cylinder		N ₂ purity : 99.99%

A dispersion of 10g freeze-dried garlic in 100ml distilled water was poured in the stripping column. Nitrogen gas was injected through a single orifice at the bottom of the column to allow the nitrogen gas bubbles rise up through the garlic dispersion. Then, as recommended by Kang et al.(21), 2ml of floating bubble breaker(antifoamer) was added to the dispersion to increase the gas phase hold up and volumetric mass transfer coefficient. While the nitrogen gas bubbles were rising up, heat and mass transfers occurred simultaneously through the surfaces of bubbles. As the bubbles were broken at the interface of gas and liquid phases, the garlic volatiles were condensed in cooling tower which was controlled at -10°C using circulating ethanol(40%) and collected in a trap. Nitrogen was used as a bubble gas in this study to minimize the undesirable changes in thiosulfinates. Extraction was carried out at the N₂ flow rates of 500, 1,000 and 1,500ml/min and temperatures of 25, 50 and 75°C for 2 hrs. The experiments were conducted at relatively low temperature to avoid serious heat-degradation of thiosulfinates. Orifices having 4,000, 6,000 and 8,000µm of diameters were used to produce the bubbles of desired sizes.

Quantification of thiosulfinates

The synthesized diallyl thiosulfinate was serially diluted with hexane to obtain the concentrations from 0 to 0.7µM. The absorbances of the dilution series were then measured with a spectrophotometer(KONTRON Inc., UVIKON 930) at 262nm to prepare a standard curve(13,22). Both 50ml distilled water and 50ml hexane were added to extracted samples. Following each addition, the contents were thoroughly mixed for 2 to 5 min,

Table 2. Coding of independent variable levels for optimization of extraction conditions of thiosulfinates from garlic using GLD system

Coded	Uncoded		
	Flow rate (ml/min)	Diameter of orifice (µm)	Column temp. (°C)
-1	500	4,000	25
0	1,000	6,000	50
1	1,500	8,000	75

and then the mixtures were centrifuged at 3,000rpm for 10 min. Two ml of supernatant of hexane layer were collected and diluted by twenty five times with hexane. The absorbances of each supernatant were measured with a spectrophotometer at 262 nm to calibrate the absorbance into thiosulfinate concentrations in the 20ml thiosulfinate solutions obtained from 1g freeze dried garlic with the standard curve.

Optimization of extraction conditions

After collecting experimental data, response surface methodology(RSM) was employed to optimize the extraction processes(23,24). A central composite design (25) was adopted as summarized in Table 2. It was assumed that one mathematical function is present for the response variable(thiosulfinate content) in terms of three independent processing factors.

To approximate the function, second degree polynomial equations were used:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} X_i X_j + \epsilon$$

where β_0 , β_i , β_{ii} and β_{ij} are constant coefficients and X_i is the coded independent variable, linearly related to independent processing factors.

Statistical Analysis System(26) was used to fit the second order polynomial equation to the experimental data. Differences were considered statistically significant at $p < 0.05$.

Gas chromatographic analysis for the volatile compounds

Gas chromatography was conducted on a G. C.(Star 3400, Varian Co.) equipped with a flame ionization detector. A 30m×0.22mm DB-FFAP column was used. The oven temperature was programmed from 50°C to 230°C at 3°C/min. The injector and detector temper-

atures were 250°C and 270°C, respectively. The carrier gas was nitrogen at a flow rate of 30ml/min. The gas chromatograms of the garlic volatiles extracted by hexane extraction, steam distillation and GLD extraction, were compared. The garlic volatiles were extracted from 10g freeze-dried garlic with hexane for 2 hrs at 25°C according to the solvent extraction method of Oaks et al.(27), and extracted with 100ml water at boiling temperature by using modified Linkens-Nickerson distillation apparatus(28). The gas chromatograms of these extracts were compared with those obtained by GLD method as mentioned previously. The volatiles were collected using diethyl ether and concentrated by the blow-down of nitrogen gas. Two hundreds μ l of diethyl disulfide stock solution(0.451g in 50ml diethyl ether)/one hundred ml of diethyl ether were then added to each extract as an internal standard.

RESULTS AND DISCUSSION

Experimental conditions

For quantitative determination of thiosulfates, a simple and rapid method by spectrophotometry was used. The purity of synthesized thiosulfates was above 99% as determined by HPLC(12). In the GLD system, the heat transfer takes place from gas to aqueous phase through the bubble membranes. Additionally, the vigorous circulations and agitations of the bubbles make the heat transfer more efficient even with small differences in temperatures between gas bubble and aqueous phase. Thus, this study was conducted to apply this efficient system to the thiosulfate extraction from garlic at relatively low temperature to avoid heat degradation of products.

In order to study the effects of N_2 flow rate and temperature, the amounts of thiosulfates extracted from the garlic under some selected conditions were determined and described in Fig. 3. Significant increases in the amounts of extracted thiosulfates by the increasing temperature were observed, mainly due to increased diffusion coefficient and driving force of mass transfer caused by increased differences of temperatures between aqueous solution and N_2 bubbles. This result was in accordance with the report of Lee et al.(19). Cho and Wakao(29) reported that gas flow rate had little influence to the mass transfer of the volatiles because of the coagulation of bubbles in the high flow

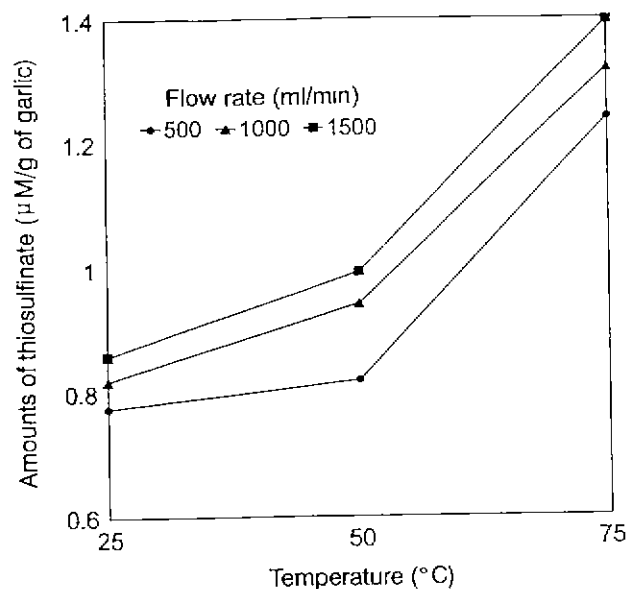


Fig. 3. Effects of temperatures on the thiosulfate extraction by GLD system from 1g of freeze-dried garlic with orifice diameter of 6,000 μ m(based on three determinations).

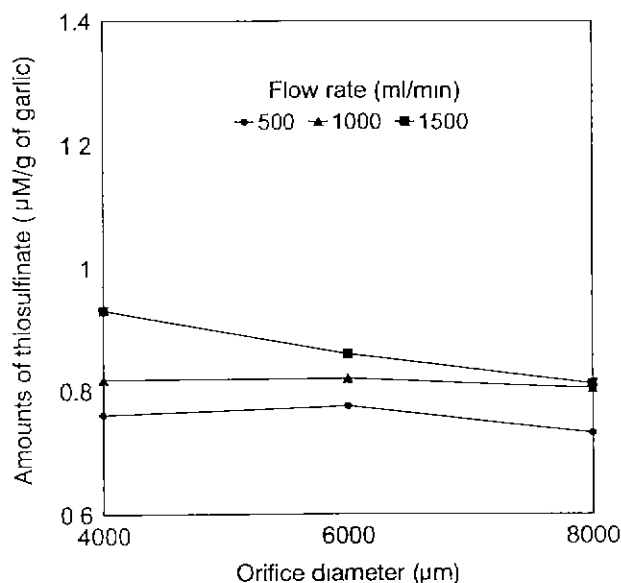


Fig. 4. Effects of bubble sizes on the thiosulfate extraction by GLD system from 1g of freeze-dried garlic at the temperature of 25°C(based on three determinations).

velocities. In this study, however, the increases in N_2 flow rate resulted in steady increases in thiosulfate extraction, because the volumetric mass transfer coefficients increased with an increase in superficial gas velocity(30) and/or N_2 flow rates observed in this study might be low enough to avoid bubble coagulations. In order to study the effects of bubble sizes on thiosul-

finite extraction, the amounts of thiosulfates extracted using orifices of 4,000, 6,000 and 8,000 μm diameters were compared (Fig. 4). Slight decreases in the amounts of extracted thiosulfates by the increasing bubble sizes were observed. Cho and Wakao(29) reported a considerable increase in the volumetric gas and liquid phase mass transfer coefficients by decreasing bubble size and, accordingly, increase in interfacial mass transfer area in aqueous sodium carbonate solution. In this study, however, the effects of bubble sizes on thiosulfate extraction were not so significant as expected.

Statistical analysis

The second order polynomial equation for O. D. values of thiosulfate solutions obtained by GLD system was derived by the central composite design and RSM, and described as follows:

$$Y = 6.265 - 0.000297X_1 - 0.086895X_2 - 0.002285X_3 + 0.00000004922X_1X_1 + 0.000003115X_1X_2 - 0.001365X_2X_2 - 0.000000043125X_1X_3 - 0.00000655X_2X_3 - 0.0000005X_3X_3$$

where: X_1 =orifice diameter(μm); X_2 =temperature($^{\circ}\text{C}$); X_3 =flow rate of N_2 (ml/min); and Y =optical density of thiosulfates (O. D. could be calculated into amount of thiosulfate using standard curve). The analysis of variances for the response variable (Table 3) indicate that the models developed for thiosulfate extractability appeared to be very adequate with satisfactory

Table 3. Analysis of variance in the GLD extraction showing the effect of treatment variables as a linear term, quadratic term and interactions (cross product) on the response variable, amount of thiosulfates extracted from freeze-dried garlic

Regression	D.F.	Sum of squares	R^2	F-ratio	Prob>F
Linear	3	1.021388	0.8869	32.978	0.0028
Quadratic	3	0.078494	0.0682	2.534	0.1953
Cross product	3	0.010490	0.0091	0.339	0.7998
Total regress	9	1.110373	0.9641	11.950	0.0146

Table 4. Pearson correlation coefficient of GLD extraction of thiosulfates from garlic

	Diameter of orifice (μm)	Temperature ($^{\circ}\text{C}$)	Flow rate (ml/min)
O.D. of thiosulfates	- 0.14792	0.90729	0.20450

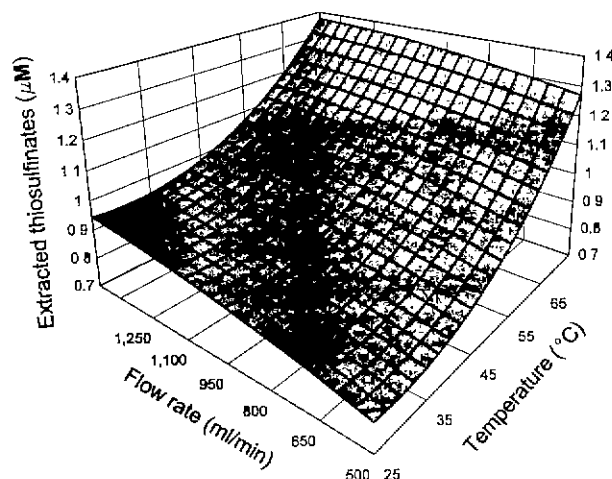


Fig. 5. Response surface of thiosulfate amounts extracted from 1g of freeze-dried garlic using 4,000 μm (dia.) orifice under the conditions of various temperatures and N_2 gas flow rate.

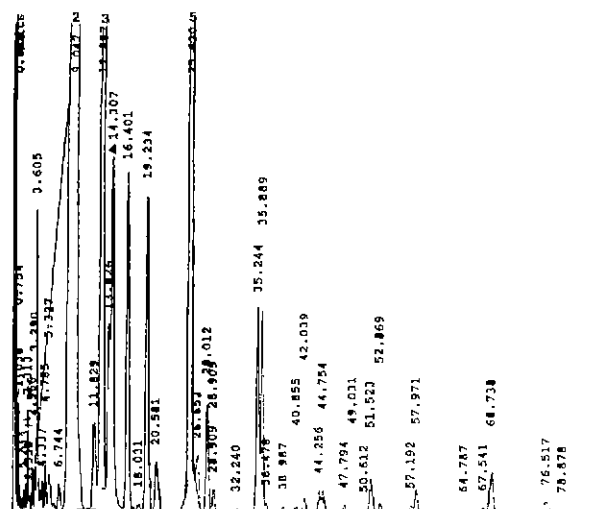


Fig. 6. Gas chromatogram of volatile compounds extracted by hexane extraction (based on three determinations).

1. Allyl sulfide
2. p-cymene
3. Diallyl disulfide
4. Di-n-propyl sulfide
5. Dibutyl disulfide

values of R^2 (0.964). Probability of F-value indicates that each processing variable influences response variable almost independently.

From the statistical analysis (Table 4), the temperature was shown to be the most important factor among the three factors affecting the response variable. Temperature and flow rate showed a positive correlations, while orifice diameter showed a negative correlation. Computer generated response surfaces were obtained using predictive models and are shown in Fig. 5. The maximum extraction was achieved at the highest value

of temperature. Thus, thiosulfinate extraction using GLD system is recommended at the highest temperature in the heat-degradation-avoidable temperature ranges. Under the conditions of 75°C, 1,500ml/min of flow rate and 4,000µm of orifice diameter, the amount of thiosulfinates extracted from 1g of freeze dried garlic in 20ml solution using GLD system (1.453µM/g of freeze-dried garlic) was about eight times higher than that by using methanol extraction(10). Jo et al.(9) reported that thiosulfinates were not susceptible to heat degradation at all at 25°C. Even if thiosulfinate extraction using GLD system was conducted at this temperature(25°C), the amount of extracted thiosulfinates was 0.944(µM/g of freeze-dried garlic), which was approximately five times more than the amount extracted by methanol extraction that is the most efficient extraction methods reported up to now(10).

Profiles of volatile compounds

In this study, the volatile components extracted by various methods were compared. Fig. 6, 7 and 8 showed the gas chromatograms for volatile compounds of garlic extracts isolated by hexane extraction, simultaneous steam distillation and GLD system, respectively. Hexane extraction method extracted 51 components of the volatiles, simultaneous steam distillation extracted 61 and GLD system extracted 63. The most variable components were extracted by GLD system. Among

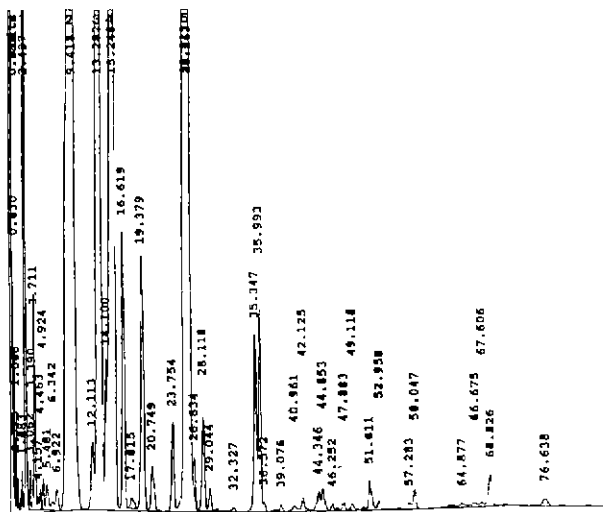


Fig. 7. Gas chromatogram of volatile compounds extracted by steam distillation (based on three determinations).

1. Allyl sulfide
2. p-cymene
3. Diallyl disulfide
4. Di-n-propyl sulfide
5. Dibutyl disulfide

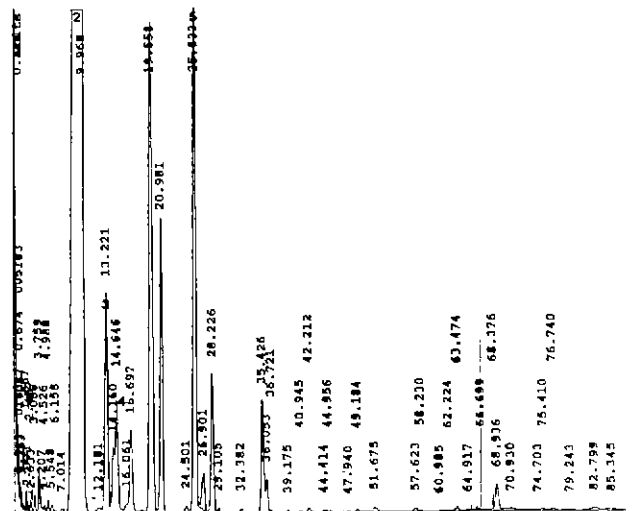


Fig. 8. Gas chromatogram of volatile compounds extracted by gas-in-liquid-dispersion system (based on three determinations).

1. Allyl sulfide
2. p-cymene
3. Diallyl disulfide
4. Di-n-propyl sulfide
5. Dibutyl disulfide

these components, allyl alcohol, p-cymene, allyl sulfide, diallyl disulfide, di-n-propyl disulfide and dibutyl disulfide were identified by comparing retention indices with those of authentic compounds. Allyl alcohol was not found in the gas chromatograms because its retention time was short enough to come out with solvent. All other components except allyl alcohol were identified in the samples.

CONCLUSION

Processes have been successfully developed and optimized to extract thiosulfinates from garlic using GLD system. GLD system was found one of the most efficient methods for thiosulfinate extraction quantitatively. Also, GLD system extracted more volatile components than solvent extraction. However, its practical applications are still suspended, because the reports on the uses of GLD system in food processing are very rare. Therefore, comprehensive studies on the application of GLD system to food processing are required from the physicochemical and toxicological points of view.

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