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EFFECTS OF pH ON THE FORMATION OF FLAVOUR COMPOUNDS OF DISRUPTED GARLIC

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SUMMARY

The effects of pH adjustment during the blending of garlic cloves on the formation of flavour compounds of garlic were studied by high-performance liquid chromatography (HPLC) and gas chromatography (GC). HPLC analysis showed that maximum allicin formation occurred around pH 6.5. By GC analysis it was also found that the two isomeric cyclic compounds 3-vinyl-(4*H*)-1,2-dithiin and 2-vinyl-(4*H*)-1,3-dithiin, which were artifacts formed from allicin during GC, reached their highest levels around pH 6.5, whereas the formation of diallyl trisulphide, diallyl disulphide, methyl allyl disulphide and diallyl sulphide was favoured around pH 9.0.

INTRODUCTION

Garlic (*Allium sativum* Linn.) has been prized for its flavor and pungency for many centuries. Semmler¹ obtained a steam volatile oil from garlic in low yield (0.1–0.2%) and established the importance of diallyl disulphide and diallyl trisulphide in the flavour of garlic distillate. It was also evident at an early stage that the odorous compounds of interest were not present in the plant as such but were formed enzymically when the cellular tissue was disrupted. Cavallito and Bailey² described the isolation of the odoriferous antibacterial substance allicin (diallyl thiosulphinates) by extraction of garlic with ethanol at room temperature. Stoll and Seebeck³ reported that intact garlic cloves contain 0.24% (w/w) of S-allylcysteine S-oxide (alliin), a colourless, odourless solid, and an enzyme, allinase, which converts alliin into allicin. Subsequent research⁴ revealed that the cysteine sulphoxide fraction of garlic consists of 85% of alliin, 2% of S-propylcysteine sulphoxide and 13% of S-methylcysteine sulphoxide. The action of allinase on a mixture of these sulphoxides affords thiosulphinates. In addition to S-substituted cysteine sulphoxides, three (possibly four) γ -L-glutamyl derivatives of S-alk(en)ylcysteine sulphoxides were identified in garlic⁵. These compounds are not cleaved by allinase, they represent only "potentially available" flavor. Consequently, peptidases and transpeptidases which "release" these secondary flavour precursors to primary flavour precursors, that is, thiosulphinates, are important enhancers of the aroma of garlic and its products⁵.

The crude cell-free garlic enzyme solution, of unspecified purity, utilized by Stoll

and Seebeck^{3,6} showed a broad pH optimum of 5–8 and a temperature optimum of 37°C under the conditions used. Using protamine and ammonium sulphate as precipitation agents and following precipitation by fractionation on Sephadex G-200, Mazelis and Crews⁷ obtained a six-fold purification of the enzyme solution and confirmed the observations of Seebeck and Stoll. The purified enzyme possessed a pH optimum of 6.5 when S-methyl-L-cysteine sulphoxide was used as a substrate.

Using gas chromatographic–mass spectrometric (GC–MS) analysis of garlic extracts, Brodnitz *et al.*⁸ revealed the presence of two isomeric cyclic compounds claimed to be 3-vinyl-1,2-dithi-5-ene and 3-vinyl-1,2-dithi-4-ene. These two compounds were postulated to be dehydration products of allicin formed during GC by analogy with the conversion of propyl propanethiosulphinate to 1-propenyl propyl disulphide at 150°C. Brodnitz *et al.* further observed that allicin underwent nearly complete decomposition at 20°C after 20 h. Decomposition of allicin proceeds by several pathways⁹, in one of which it decomposes spontaneously to form two isomeric cyclic compounds, 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin. We also reported that allicin decomposed into these two compounds during GC¹⁰.

Several methods have been reported for the quantitation of thiosulphinates^{11–14}. It might appear that the best method for measuring the flavour and aroma is GC under carefully controlled conditions¹⁵. The individual components can be separated by GC and identified by MS. However, this method is sometimes unsatisfactory, especially when the compound is unstable to heat. For this reason, high-performance liquid chromatography (HPLC) may be a better method for separating the heat-labile compounds, although it also has the disadvantage of poor resolution. In this study, both GC and HPLC were used to determine the effects of pH adjustment on flavour formation in garlic during the blending of garlic cloves.

EXPERIMENTAL

Materials and chemicals

Garlic cloves, of unknown origin, were purchased locally. 2-Vinyl-[4*H*]-1,3-dithiin and 3-vinyl-[4*H*]-1,2-dithiin were synthesized by the method of Bock *et al.*¹⁶ Allicin was synthesized by the method of Block *et al.*¹⁷ using diallyl disulphide as the starting material.

Sample preparation

Peeled garlic cloves (100 g) were blended with 200 ml of distilled water for 5 min in a Waring blender, the pH during blending being adjusted by adding 0.5 *M* sodium hydroxide or 0.5 *M* hydrochloric acid. The homogenate was filtered through a double layer of cheese-cloth to obtain the garlic extract. For HPLC analysis, dimethyl disulphide stock solution (13 ml, 0.5 g in 500 ml of methanol) was added to 7 ml of garlic extract as an internal standard. After filtration through a filter-paper (Toyo No. 2) and a Minipore (Millipore, FG, 0.22 μm), the sample was applied to the HPLC system. For GC analysis, the garlic extract (20 ml) was extracted three times with one volume of diethyl ether. The ether fractions were combined, dried with anhydrous sodium sulphate and then concentrated to a small volume by blowing nitrogen over the surface of the solution in a hood. Dimethyl disulphide stock solution (2 ml, 0.08 g in 100 ml of diethyl ether) was added to the concentrate as an internal standard and 1 μl of

the sample was then analysed by GC. All the GC and HPLC analyses were carried out immediately after the garlic extract had been prepared.

HPLC analyses

A Shimadzu LC-5A HPLC system was used. An ODS column (200 × 4.6 mm I.D.) (Altex) was used for separation with methanol–water (65:35) (methanol of HPLC grade, Merck) as the mobile phase at a flow-rate of 2 ml/min. Detection was based on UV absorption at 254 nm.

Gas chromatography

GC was conducted on a Shimadzu GC-9A instrument equipped with flame ionization detection (FID). A 50 m × 0.22 mm I.D. fused-silica column (Chrompack) coated with CP-Wax 52 CB was used. The oven temperature was programmed from 50 to 200°C at 2°C/min. The injector and detector temperatures were 250°C. The carrier gas was nitrogen at a flow-rate of 0.75 ml/min. The data were recorded on a Shimadzu C-R3A integrator. Values reported are averages of two analyses. The linear retention indices of the volatile components were calculated with C₈–C₂₅ *n*-alkanes¹⁸ (Alltech) references.

Gas chromatography–mass spectrometry

GC–MS was conducted with a Hewlett-Packard 5985B system. The gas chromatograph was fitted with a fused-silica capillary column (bonded CP-Wax 52 CB; 50 m × 0.32 mm I.D.). The oven temperature was programmed from 50 to 200°C at 2°C/min, the injector temperature was 250°C, the carrier gas was helium at a flow-rate of 1.8 ml/min, the ionization voltage was 70 eV and the ion source temperature was 200°C.

RESULTS AND DISCUSSION

Fig. 1 shows the HPLC traces of flavour components of garlic formed at (A) pH 2, (B) pH 6 and (C) pH 10. Seven peaks were detected; however, only peak 5, which had an odour reminiscent of crushed fresh garlic, was collected and was identified as allicin¹⁰. Freeman and McBreen¹⁹ described a spectrophotometric method that measures the absorption maximum of the thiosulphinates at 254 nm, and therefore the HPLC detection in this study was based on UV absorption at 254 nm. The significant differences in HPLC profiles among samples A, B and C in Fig. 1 were postulated to be due to the effect of pH on enzyme activities.

Fig. 2 shows the effect of pH (2.0–10.0 at 1.0-unit intervals) on the formation of allicin as analysed by HPLC. The data were relative to dimethyl disulphide as internal standard. Formation of allicin is favoured around pH 6.5; the optimum pH of allinase is also around 6.5, which is consistent with the results found by Mazelis and Crews⁷.

Fig. 3 shows the gas chromatograms of the volatile components of garlic formed at (A) pH 2, (B) pH 6 and (C) pH 10. Table I shows a comparison of the concentration of volatile constituents in samples of different pH. Identification of the compounds was reported by us previously²⁰. Fig. 4 shows the effect of pH on the formation of 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin, which were confirmed as the major artifacts formed from allicin during the GC^{9,10}. Formation of these two

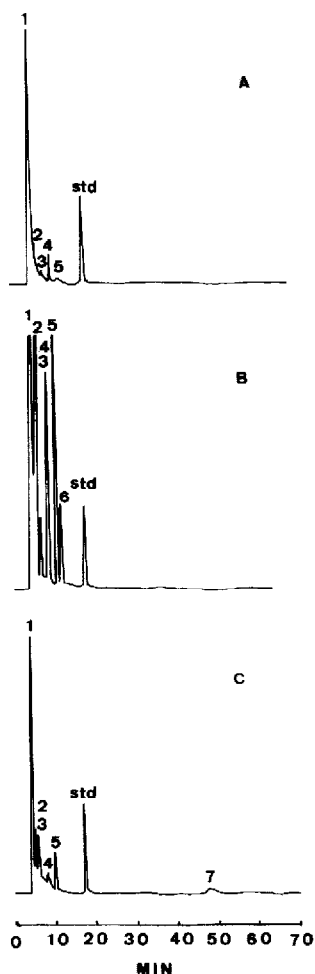


Fig. 1. HPLC separations of flavour compounds of garlic formed at (A) pH 2, (B) pH 6 and (C) pH 10. Peak 5 = allicin.

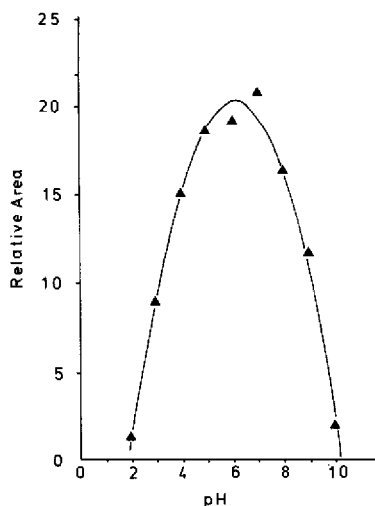


Fig. 2. Relative amounts of allicin formed at different pHs as determined by HPLC. $Y = -23.4788 + 14.473X - 1.18345X^2$ ($r = 0.99$).

compounds is also favoured around pH 6.5, which is consistent with the peak of allicin shown in HPLC analysis. Cavallito and Bailey² found that an aqueous solution of allicin had a pH of approximately 6.5 and, on standing, the acidity slowly increased owing to the formation of small amounts of sulphur dioxide. The antibacterial activity of the solution decreased. Addition of alkalis led to immediate inactivation, with precipitation of allyl disulphide and formation of an alkali sulphite.

Fig. 5 shows the effect of pH on the formation of four additional major compounds, diallyl trisulphide, diallyl disulphide, methyl allyl disulphide and diallyl sulphide. Unlike 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin, the formation

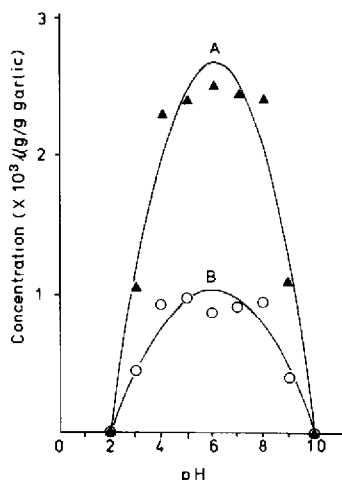
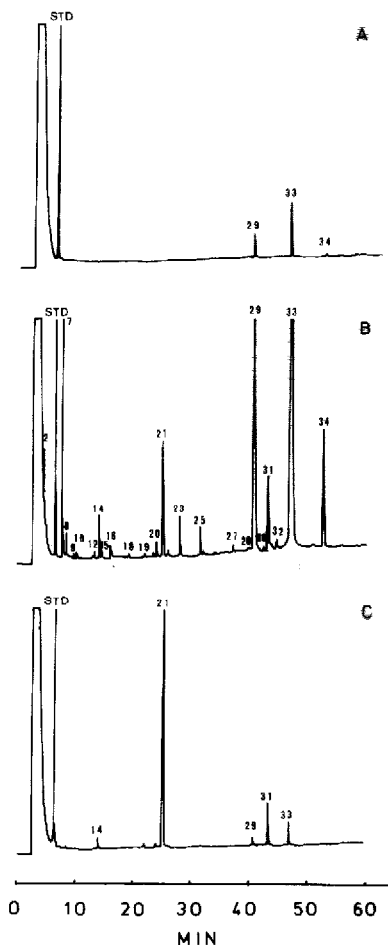


Fig. 3. Capillary gas chromatograms of volatile compounds from garlic formed at (A) pH 2, (B) pH 6 and (C) pH 10.

Fig. 4. Changes in the amount of (A) 2-vinyl-(4H)-1,3-dithiin [$Y = -3275.28 + 1984.01X - 165.049X^2$ ($r = 0.98$)] and (B) 3-vinyl-(4H)-1,2-dithiin [$Y = -1249.96 + 769.544X - 64.3509X^2$ ($r = 0.97$)] formed at different pH values as determined by GC.

of these compounds is favoured around pH 9.0. Schwimmer and Austin²¹ found that γ -glutamyl transpeptidase (GGT) has its optimum activity at pH 9.0. Therefore, it was postulated that these four compounds reach their highest levels around pH 9.0 because alliin readily decomposes into these compounds under alkaline conditions or because the rate of formation of these compounds at pH around 9.0 is greatest as a result of the high activity of GGT. It is possible that GGT in garlic also has an optimum pH at 9.0; at pH around 9.0, GGT readily catalyses the transfer of γ -L-glutamyl-S-allyl sulphoxide to alliin and then alliin is catalysed further by allinase into alliin; under alkaline conditions alliin then readily decomposes into sulphide compounds.

TABLE I
EFFECTS OF pH ON THE FORMATION OF VOLATILE COMPOUNDS OF DISRUPTED GARLIC

Peak No.*	Compound**	I _a *** (CP-Wax 52 CB)	Yield (10 ⁻⁶ g/g of garlic bulb) [§]									
			pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0	
2	Propenylthiol	—	— ^{§§}	2.26	13.40	14.39	11.50	8.93	7.55	6.93	—	
7	2-Propen-1-ol	1125	—	50.84	237.61	219.01	111.18	129.28	85.65	34.88	—	
8	Diallyl sulphide	1148	—	6.86	8.91	10.26	11.53	12.96	13.20	13.31	—	
9	Tetrahydro-2,5-dimethylthiophene	1197	—	2.76	3.42	3.98	3.46	—	—	—	—	
10	C ₆ H ₁₀ S [m/z, 45(100), 42(86), 43(86), 29(86), 55(64), 73(48), 71(28), 64(28)]	1233	—	2.64	3.33	3.81	3.33	—	—	—	—	
12	3-Methyl-2-cyclopentene-1-thione	1261	—	1.01	3.29	4.39	5.07	16.79	—	—	—	
14	Methyl allyl disulphide	1282	—	3.79	27.39	36.05	25.38	23.47	34.09	41.69	6.00	
15	1,3-Dithiane	1296	—	1.03	7.65	7.97	6.13	5.06	5.27	—	—	
16	Aniline	1328	—	—	4.93	13.75	14.19	19.25	13.32	—	—	
18	Dimethyl trisulphide	1380	—	—	3.72	3.97	—	—	—	—	—	
19	Propyl allyl disulphide	1432	—	—	1.99	2.53	—	—	—	—	—	
20	C ₆ H ₁₀ S ₂ [m/z, 73(100), 146(99), 81(79), 41(57), 45(54), 105(45), 39(35), 71(30)]	1471	—	8.37	10.06	11.42	10.33	8.53	7.33	5.74	—	
21	Diallyl disulphide	1490	—	35.19	73.97	91.63	81.19	118.05	243.98	1435.89	561.63	
23	Unknown [m/z, 103(100), 104(64), 45(39), 119(16), 39(15), 69(11), 105(11), 74(8)]	1532	—	15.05	27.15	28.70	25.71	23.14	20.03	10.96	—	

25	Methyl allyl trisulphide	1593	—	2.70	21.17	26.81	22.73	22.49	20.00	20.47	—
27	3,5-Diethyl-1,2,4-trithiolane	1682	—	—	5.31	5.31	4.89	3.34	3.70	—	—
28	Isobutyl isothiocyanate	1753	—	—	5.77	1.94	—	—	—	—	—
29	3-Vinyl-(4 <i>HF</i>)-1,2-dithiin	1761	29.35	454.18	946.48	994.78	879.70	927.47	956.56	429.78	6.20
30	Unknown [<i>m/z</i> , 146(100), 74(73), 73(64), 117(62), 72(55), 71(32), 138(30), 45(23)]	1772	—	4.44	108.32	121.63	102.26	105.84	94.85	41.41	—
31	Diallyl trisulphide	1806	—	25.14	38.50	48.87	63.07	83.12	126.18	176.08	23.04
32	Unknown [<i>m/z</i> , 138(100), 111(92), 109(64), 110(62), 95(60), 123(50), 77(48), 151(36)]	1851	—	2.48	28.44	35.18	35.75	28.64	20.14	10.69	—
33	2-Vinyl(4 <i>HF</i>)-1,3-dithiin	1872	75.28	1058.24	2295.09	2394.87	2501.97	2456.91	2419.54	1097.00	16.27
35	Unknown [<i>m/z</i> , 128(100), 45(37), 99(35), 65(26), 113(21), 110(21), 53(16), 85(15)]	1943	5.41	52.25	134.89	194.87	189.20	151.94	136.67	60.48	—
Total			110.04	1729.23	4010.99	4276.12	4108.57	4145.16	4207.06	3385.31	413.14

* Numbers refer to Fig. 3.

** Numbers in parentheses indicate relative percentage.

*** Calculated Kováts retention indices.

§ Average of two experiments using dimethyl disulphide as internal standard.

§§ Not detected.

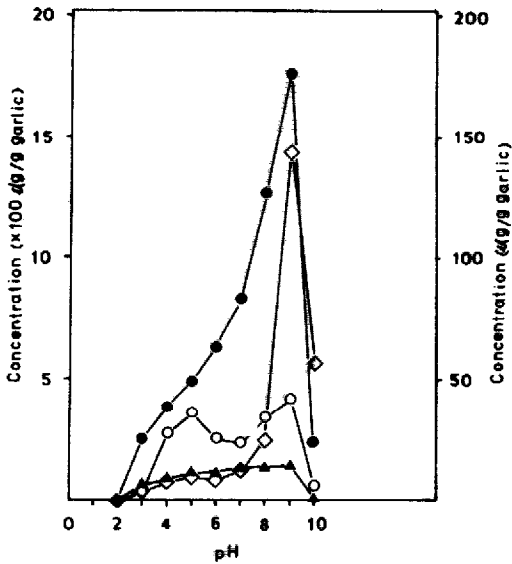


Fig. 5. Volatile compounds of garlic formed at different pH values as determined by GC. \diamond , Diallyl trisulphide; \bullet , diallyl disulphide; \circ , methyl allyl disulphide and \blacktriangle , diallyl sulphide.

CONCLUSION

The results indicate that HPLC is a good method for analysing allicin in garlic and GC is also a good alternative for analysing allicin with the total amount of 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin representing the amount of allicin. Consistent results were obtained when using HPLC and GC to determine the changes in allicin at different pHs. It is also interesting that the formation of diallyl trisulphide, diallyl disulphide, methyl allyl disulphide and diallyl sulphide is favored around pH 9.0.

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