

Meat-like Flavor Generated from Thermal Interactions of Glucose and Alliin or Deoxyalliin

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Alliin and deoxyalliin, two important nonvolatile flavor precursors of garlic, were reacted separately with glucose in an aqueous solution at pH 7.5 in a closed sample cylinder at 180 °C for 1 h. The volatile compounds generated were isolated by using a modified Likens-Nickerson distillation-solvent extraction apparatus and analyzed by GC and GC-MS. The isolate from the interaction of alliin and glucose possessed a very good roasted meaty character; the isolate from the interaction of deoxyalliin and glucose possessed a slightly roasted meaty flavor with garlic character. Several pyrazines were identified from both alliin-glucose and deoxyalliin-glucose model systems. Thiazoles, especially 2-acetylthiazole, were found to be the predominant volatile interaction products of alliin and glucose, whereas pyrazines, especially 2,5-dimethyl-, methyl-, and trimethylpyrazine, were found to be the predominant volatile interaction products of deoxyalliin and glucose. Some thiazoles together with some pyrazines, thiophenes, ketones, furans, and cyclic sulfur-containing compounds were thought to contribute to the roasted meat-like flavor in the model systems.

INTRODUCTION

γ -Glutamylalk(en)ylcysteine dipeptides and alk(en)ylcysteine sulfoxides were found to be two important groups of nonvolatile flavor precursors of garlic in intact garlic cloves. During cold storage or the sprouting of the garlic cloves, γ -glutamylalk(en)ylcysteine dipeptides can be transformed to their respective alk(en)ylcysteines by the action of γ -glutamyl transpeptidase. By the action of hydrogen peroxidase, γ -glutamylalk(en)ylcysteines can then be oxidized to their respective γ -glutamylalk(en)ylcysteine sulfoxides. During the physical breakdown of the garlic cells, γ -glutamylcysteine sulfoxides can be transformed to alk(en)yl thiosulfates, the primary flavor compounds of garlic, by the action of alliinase through the dehydration process, accompanied by the formation of ammonia and pyruvic acid (Fenwick and Hanley, 1985; Carson, 1987; Iberl et al., 1990; Lawson et al., 1991a,b; Block et al., 1992a,b; Block, 1992).

Three γ -glutamylalk(en)ylcysteines, i.e., γ -glutamylallyl-, γ -glutamyl-(*E*)-1-propenyl-, and γ -glutamylmethylcysteine, have been found in intact garlic cloves, the previous two compounds being the predominant compounds. Three alk(en)ylcysteine sulfoxides, i.e., *S*-allyl-, (*E*)-1-propenyl-, and methylcysteine sulfoxide, have also been found in intact garlic cloves, with the first being the predominant compound. After being heated in boiling water, γ -glutamylallylcysteine was converted to *S*-allylcysteine (deoxyalliin); γ -glutamyl-(*E*)-1-propenylcysteine was converted to (*E*)-1- and (*Z*)-1-propenylcysteines; and alliin was completely lost in 8 h to unknown compounds (Lawson et al., 1991b; Block, 1992; Block et al., 1993).

Most research work on garlic flavor has been focused on the enzymatic generation of flavor compounds from the nonvolatile flavor precursors of garlic and the stability of these flavor compounds. In the study of fried garlic flavor, we (Yu et al., 1993), however, found that the flavor

precursors of garlic were probably important contributors to the fried garlic character. In a model system study, we (Yu et al., 1994a,b) also observed that the synthesized alliin and deoxyalliin could be self-degraded and generate volatile flavor compounds. It is, therefore, interesting to know whether the flavor precursors of garlic, which are derivatives of amino acids, especially cysteine, can participate in Maillard-type flavor formation. Alliin, the predominant amino acid derivative of garlic, and deoxyalliin, one of the thermal degradation products of γ -glutamylallylcysteine, the predominant glutamyl dipeptides (Lawson et al., 1991a,b; Ueda et al., 1991), were synthesized and then reacted with glucose to study the potential contribution of garlic's nonvolatile flavor precursors to the formation of Maillard-type (fried or baked garlic, and roasted meaty) flavor compounds.

EXPERIMENTAL PROCEDURES

Synthesis and Purification of Deoxyalliin and Alliin. Deoxyalliin and alliin were synthesized according to the procedures of Iberl et al. (1990) with a slight modification as shown in our previous papers (Yu et al., 1994a,b).

Thermal Interaction of Glucose and Alliin or Deoxyalliin. Synthesized alliin (0.005 mole) or deoxyalliin (0.005 mole) was mixed with 0.005 mol of α -D-glucose (Aldrich, 96% purity) in 100 mL of distilled water. The solution was adjusted to pH 7.5 using 2 N NaOH and then was added to a 0.3-L Hoke SS-DOT sample cylinder (Hoke Inc., Clifton, NJ) and sealed. This cylinder was then heated at 180 °C in a GC oven for 1 h. After being cooled to room temperature, an interaction mass of glucose and alliin or deoxyalliin was obtained.

Isolation of the Volatile Compounds. The total reaction mass was simultaneously distilled and extracted into diethyl ether using a Likens-Nickerson (L-N) apparatus (Romer and Renner, 1974) for 2 h. After distillation, 5 mL of heptadecane stock solution (0.0770 g in 200 mL of diethyl ether) was added to the isolate as the internal standard. After being dried over anhydrous sodium sulfate and filtered, the distillate was concentrated to about 5 mL using a Kuderna-Danish apparatus fitted with a Vigreux distillation column and then slowly concentrated further under a stream of nitrogen in small sample vial to a final volume of 0.2 mL.

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Table 1. Final pH, Final Appearance, and Flavor Description of Glucose plus Alliin, Glucose plus Deoxyalliin, and Glucose Model Reaction Systems

model system	final pH	final appearance	flavor description
glucose + alliin	4.6	dark brown	roasted meaty, popcorn undertone
glucose + deoxyalliin	5.4	brown	pungent garlic with roasted meaty
glucose	3.8	slightly brown	slightly burned, caramellic

Gas Chromatographic Analysis and Quantification of Volatile Compounds. A Varian 3400 gas chromatograph equipped with a fused silica capillary column (60 m × 0.25 mm i.d.; 1- μ m thickness, DB-1, J&W Inc.) and a flame ionization detector was used to analyze the volatile compounds. The operating conditions were as follows: injector temperature, 270 °C; detector temperature, 300 °C; helium carrier flow rate, 1 mL/min; temperature program, 40 °C (5 min), 2 °C/min, 260 °C (60 min). A split ratio of 50:1 was used. The amount of volatile compounds was estimated by computing the GC area against that of the internal standard; the same amount of volatile compounds was assumed to have the same peak area. The response factor of all volatile compounds to the FID was assumed to be one. The quantification data of the volatile compounds were based on only one replicate.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. The concentrated isolate was analyzed by GC-MS using a Hewlett-Packard 5840A gas chromatograph coupled to a Hewlett-Packard 5985B mass spectrometer equipped with a direct split interface and the same column as used for the gas chromatography. The GC operating conditions were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 250 °C.

Identification of the Volatile Compounds. Identification of the volatile compounds in the isolate was mostly based on GC-MS, and information from the GC retention index (I_r) used a C₅-C₂₅ mixture as a reference standard. The structural assignment of volatile compounds was accomplished by comparing the mass spectral data with those of authentic compounds available from the Browser-Wiley computer library, the NBS computer library, or previously published literature (Yu et al., 1989, 1993, 1994b). The retention indices were used for the confirmation of structural assignments.

RESULTS AND DISCUSSION

The final pH, final appearance, and flavor description of the thermal reaction products of glucose and alliin and glucose and deoxyalliin as well as the thermal decomposition products of glucose are listed in Table 1. The color development in the reaction systems clearly shows that the degradation or the interactions of reactant(s) did proceed drastically. After the thermal treatment, the final pH of all systems dropped significantly, especially in the glucose only system. The isolate from the glucose and alliin model system possessed a very good roasted meaty character. On the other hand, the isolate from the glucose and deoxyalliin model system had a slightly roasted meaty flavor with pungent garlic character.

The gas chromatographic profiles of the volatile compounds generated from the model reaction systems are shown in Figure 1. Identification and quantification of the volatile compounds generated from the model systems of glucose and alliin as well as glucose and deoxyalliin are made in Tables 2 and 3, respectively. 2-Furfural (peak 1 in Figure 1C) and phenylacetaldehyde (peak 2 in Figure 1C) were the only two volatile compounds identified from the heating of glucose alone. The amounts of 2-furfural and phenylacetaldehyde detected were 25.1 and 13.7 mg/mol of glucose, respectively.

Volatile Compounds Generated in the Glucose and Alliin Model System. As shown in Table 2, some volatile

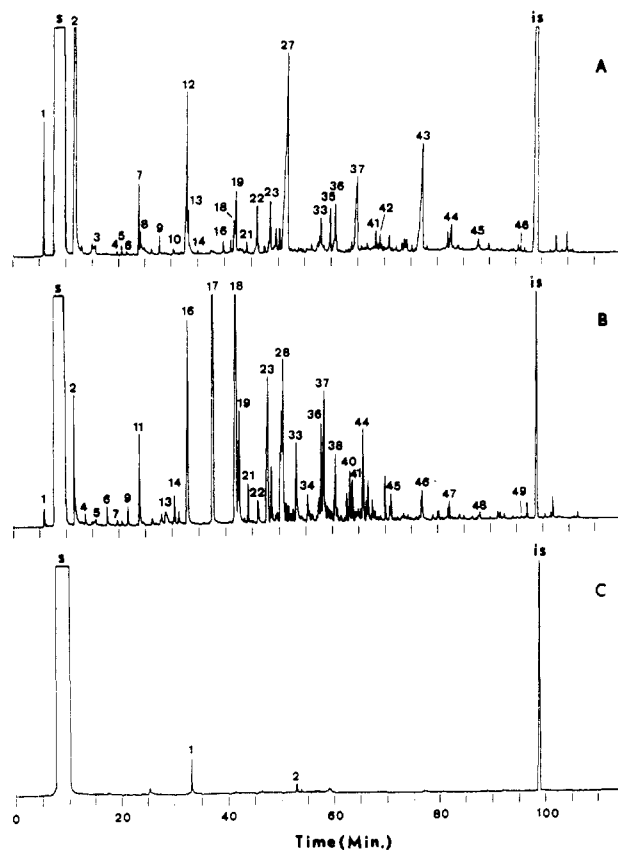


Figure 1. Gas chromatograms of volatile compounds isolated from (A) glucose plus alliin, (B) glucose plus deoxyalliin, and (C) glucose model systems.

compounds identified from the thermal interactions of glucose and alliin were derived from the thermal degradation of alliin, and others were generated from the interactions of alliin and glucose.

Allyl alcohol was the predominant volatile compound found in the thermally degraded solution of alliin. The formation of allyl alcohol from alliin could be explained by the [2,3]-sigmatropic rearrangement of alliin followed by the reduction process (Yu et al., 1994a,b). In the proposed mechanisms, cysteine was also generated. Ammonia, hydrogen sulfide, and acetaldehyde could be derived either from the degradation of cysteine or directly from alliin. The formation mechanisms of most of those compounds degraded from alliin have also been reported (Yu et al., 1994a,b).

As shown in Table 2, nine volatile compounds were thought to be the degradation products of glucose. Among these compounds, only 2-furfural and phenylacetaldehyde were found in the glucose only system. Since none of these nine compounds were found from the thermal degradation of alliin (Yu et al., 1994a,b), alliin was clearly having an effect on the degradation of glucose, which is not too surprising since it may simply take the role of an amino acid in a Maillard reaction. The major interaction products of glucose and alliin were pyrazines and thiazoles. Among them, methylpyrazine, ethylpyrazine, and 2-acetylthiazole were the predominant compounds.

Volatile Compounds Generated in the Glucose and Deoxyalliin Model System. As shown in Table 3, volatile compounds identified from the thermal interactions of glucose and deoxyalliin can be separated into three groups: those generated from the decomposition of deoxyalliin, those generated from the thermal degradation of glucose, and those generated from the interactions of glucose and deoxyalliin.

Table 2. Volatile Compounds Identified from the Thermal Reactions of Alliin and Glucose

peak no. ^a	compound	M _r	RI ^b	mg/mol of alliin
Compounds Generated from Thermal Degradation of Alliin				
1	acetaldehyde	44	<500	16.5
2	allyl alcohol	58	550	340.7
3	acetic acid	60	611	4.3
7	thiazole	85	709	13.3
11	2-methylthiazole	99	791	0.7
16	dipropyl sulfide	118	872	2.8
21	3-methylthiacyclopentane	102	916	3.3
23	2-formylthiophene	112	963	18.3
24	3-formylthiophene	112	974	11.6
29	2-methyl-1,3-dithiane	134	1019	1.4
39	3,6-dimethyl-1,4-dithiane	148	1162	1.2
40	4-methyl-1,2-dithiepane	148	1178	1.5
42	1,2,3-trithiacyclohexane	138	1216	6.0
44	1,2,3,4-tetrathiepane	170	1371	10.6
45	4,6-dimethyl-1,2,5-trithiepane	180	1442	2.8
46	4-ethyl-6-methyl-1,2,3,5-tetrathiane	198	1560	1.1
Compounds Generated from Thermal Degradation of Glucose Catalyzed by Alliin				
4	3,4-dihydropyran	84	664	0.4
5	2,3-pentanedione	100	673	1.2
6	acetoin	88	684	0.8
10	3-hydroxy-3-methyl-2-butanone	102	778	1.9
13	2-furfural	96	804	7.0
14	2-furfuryl alcohol	98	823	0.5
17	2-acetylfuran	110	885	3.3
22	5-methyl-2-furfural	110	937	12.8
28	phenylacetaldehyde	120	1011	1.9
Compounds Generated from Thermal Interactions of Alliin and Glucose				
8	pyrazine	80	712	4.1
9	S-methyl thioacetate	90	752	3.3
12	methylpyrazine	94	800	43.8
15	2,4-dimethylthiazole	113	860	<0.1
18	2,5-dimethylpyrazine	108	891	8.8
19	ethylpyrazine	108	894	15.7
20	2,3-dimethylpyrazine	108	898	1.2
25	2-ethyl-5-methylpyrazine	122	980	7.0
26	2-ethyl-3-methylpyrazine	122	990	2.4
27	2-acetylthiazole	127	995	140.3
30	4-methyl-5-ethylthiazole	127	1023	1.4
31	thienyl alcohol	114	1047	1.0
32	2,3,4-trimethylthiophene	126	1060	4.1
33	2-acetylthiophene	126	1066	13.4
34	2-ethyl-3,5-dimethylpyrazine	136	1072	2.2
35	2-methyl-4-propylthiazole	141	1085	11.8
36	4-methyl-2-propylthiazole	141	1095	18.8
37	benzothiothiophene	134	1145	20.8
38	thieno[3,4-b]thiophene	140	1155	1.8
41	2-acetyl-3-methylthiophene	140	1186	6.0
43	unknown [119 (100), 41 (16), 45 (16), 75 (14), 121 (13), 127 (3), 148 (3), 138 (2)] ^c		1296	69.1
total				842.9

^a Peak number refers to that shown in Figure 1A. ^b Calculated Kovats retention indices. ^c Mass spectrum data.

Diallyl sulfide, mercaptomethylcyclopentane, diallyl disulfide, and (allylthio)acetic acid were found to be the predominant volatile compounds in the degraded solution of deoxyalliin. Allyl mercaptan was proposed to be generated from deoxyalliin through the hydrolysis process or free-radical rearrangement. Diallyl sulfide and diallyl disulfide could then be formed from allyl mercaptan (Yu et al., 1994b). (Allylthio)acetic acid was also thought to be generated from the thermal deamination and decarboxylation of deoxyalliin followed by oxidation (Yu et al., 1994b). Deamination/decarboxylation of deoxyalliin would give allylthioacetaldehyde, which further oxidized to (allylthio)acetic acid. Alternatively, deoxyalliin could

Table 3. Volatile Compounds Identified from the Thermal Reactions of Deoxyalliin and Glucose

peak no. ^a	compound	M _r	RI ^b	mg/mol of deoxyalliin
Compounds Generated from Thermal Degradation of Deoxyalliin				
1	acetaldehyde	44	<500	18.1
2	allyl alcohol	58	543	117.9
4	allyl mercaptan	74	580	8.9
5	acetic acid	60	600	15.2
10	thiazole	85	703	1.6
13	1-mercapto-2-propanol	92	765	29.0
17	diallyl sulfide	114	852	685.6
19	propyl allyl sulfide	116	899	170.1
21	3-(methylthio)cyclopentane	102	918	36.7
23	mercaptomethylcyclopentane	116	958	265.9
24	2-formylthiophene	112	965	55.0
29	2-acetylthiazole	127	992	15.2
31	4-methyl-1,3-dithiacyclopentane	120	1005	12.4
32	(allylthio)acetic acid	132	1013	121.6
33	2-methyl-1,3-dithiane	134	1024	2.2
34	(allylthio)propanol	132	1038	30.0
37	diallyl disulfide	146	1072	203.7
38	2-methyl-1,3-dithiane	132	1095	64.7
40	3-methyl-1,4-dithiacycloheptane	148	1128	51.9
41	3,6-dimethyl-1,4-dithiacyclohexane	148	1134	33.7
42	methyl-1,2,3-trithiacyclopentane	138	1142	9.1
44	3,6-dimethyl-1,4-dithiacyclohexane	148	1157	24.0
45	5-hydroxy-1,2-dithiacyclooctane	164	1221	24.0
47	1,2,3,4-tetrathiepane	170	1361	13.7
48	3,6-dimethyl-1,2,5-trithiepane	180	1445	6.2
49	4-ethyl-6-methyl-1,2,3,5-tetrathiane	198	1559	1.8
Compounds Generated from Thermal Degradation of Glucose Catalyzed by Deoxyalliin				
3	diacetyl	86	568	2.9
6	acetol	74	640	11.5
7	3,4-dihydropyran	84	663	6.6
8	2,3-pentanedione	100	674	4.9
9	acetoin	88	686	17.2
14	3-hydroxy-3-methyl-2-butanone	102	779	31.3
15	sec-butyl ether	130	786	10.5
22	5-methyl-2-furfural	110	938	30.7
25	4,5-dimethyl-4-hexen-3-one	126	972	5.6
Compounds Generated from Thermal Interactions of Deoxyalliin and Glucose				
11	pyrazine	80	710	82.3
12	S-methyl thioacetate	90	755	13.9
16	methylpyrazine	94	802	246.3
18	2,5-dimethylpyrazine	108	894	612.6
20	vinylpyrazine	106	909	3.2
26	2-ethyl-5-methylpyrazine	122	981	85.4
27	2-ethyl-3-methylpyrazine	122	984	116.4
28	trimethylpyrazine	122	987	201.4
30	5-methyl-2-vinylpyrazine	120	1001	5.7
35	2-formyl-5-methylthiophene	126	1062	19.5
36	3-ethyl-2,5-dimethylpyrazine	136	1066	83.1
39	4-methyl-3-thiacyclohexanone	130	1098	11.6
43	2-methyl-3,5-diethylpyrazine	150	1153	13.3
46	unknown (mass spectrum identical to that of peak 43, Table 2)		1296	21.6
total				3655.7

^a Peak number refers to that shown in Figure 1B. ^b Calculated Kovats retention indices.

undergo deamination first to give an alpha-keto acid, which then could undergo decarbonylation to form (allylthio)acetic acid. In addition, ammonia, hydrogen sulfide, and acetaldehyde could also form from the degradation of deoxyalliin. Most of the volatile compounds which are listed in Table 3 as the degradation products of deoxyalliin were derived from the interactions of allyl mercaptan, ammonia, aldehydes, and hydrogen sulfide. Their formation mechanisms have also been described (Yu et al., 1994b).

As shown in Table 3, nine volatile compounds were thought to be the degradation products of glucose. Again,

Table 4. Comparison of the Amounts of Some Important Volatile Compounds Generated from Glucose plus Alliin and Glucose plus Deoxyalliin Model Reaction Systems

compound	concn (mg/mol of alliin or deoxyalliin)	
	glucose + alliin	glucose + deoxyalliin
thiazole	186.3	16.8
pyrazines	85.2	1449.7
allylthio-containing compounds	0.0	1219.9
thiophenes	76.0	74.5

Table 5. Comparison of the Amounts of Volatile Compounds Generated from Glucose plus Alliin and Glucose plus Deoxyalliin Model Reaction Systems

compound	concn (mg/mol of alliin or deoxyalliin)	
	glucose + alliin	glucose + deoxyalliin
compounds derived from alliin or deoxyalliin	436.1	2018.2
compounds derived from glucose	29.8	121.1
compounds derived from the interactions of glucose and alliin or deoxyalliin	377.0	1516.3
total	842.9	3655.7

among these compounds, only 2-furfural and phenylacetaldehyde were found in the glucose only system. Since none of these nine compounds were found from the thermal degradation of deoxyalliin (Yu et al., 1994b), deoxyalliin was also clearly having an effect on the degradation of glucose.

The interaction products of glucose and deoxyalliin shown in Table 3 were mainly pyrazine compounds. Among the pyrazines, 2,5-dimethyl-, methyl-, trimethyl-, and 2-ethyl-3-methylpyrazine were found to be the predominant compounds.

Comparison of Volatile Compounds Generated from Glucose and Alliin and Glucose and Deoxyalliin Model Systems. The comparison of the amounts of some important volatile compounds generated from the model systems is shown in Table 4. The comparison of the amounts of those compounds degraded from alliin or deoxyalliin, those degraded from glucose, and those generated from the interactions of glucose and alliin or deoxyalliin in the model systems are shown in Table 5. The major differences between these two systems were that the formation of thiazoles was favorable in the glucose and alliin system, and the glucose and deoxyalliin system favored the formation of allylthio-containing compounds.

The abundance of thiazoles, especially 2-acetylthiazole, in the glucose and alliin system was thought to be the reason that the roasted meaty character in the glucose and alliin model system is stronger than that in glucose and deoxyalliin system. 2-Acetylthiazole was reported to possess roasty odor (Gasser and Grosch, 1988, 1990), nutty, cereal, and popcorn odor (Pitte and Hruza, 1974), and meaty flavor (Aldrich, 1993). 2-Acetylthiazole has been identified from thermal degradation of alliin (Yu et al., 1994a,b), ribose-cysteine/cystine, and cysteine-pyruvaldehyde model systems (Vernin and Vernin, 1982).

On the other hand, the abundance of allylthio-containing volatile compounds and the lack of thiazole compounds in the glucose and deoxyalliin system may contribute to the pungent garlic odor of the reaction products. Some of the allylthio-containing volatile compounds, especially diallyl sulfide and diallyl disulfide, have been identified

in the volatile flavor of garlic products and were believed to be responsible for the characteristic heated garlic flavor of thermally treated garlic products (Lawson et al., 1991c; Yu et al., 1989; Block et al., 1988).

As shown in Table 4, more pyrazine compounds were generated in the glucose and deoxyalliin system than in the glucose and alliin system. Pyrazines have been widely distributed in thermally processed foods, such as roasted beef, roasted peanut, and roasted barley. The Maillard reaction, which involves the interactions of reducing carbonyl compounds and amino-containing compounds, has been known to be the major mechanism for the formation of pyrazines (Maga, 1982).

Compounds That Probably Contribute to the Meaty Flavor. Among the volatile compounds, thiazole, 2-acetylthiazole, 2,4-dimethylthiazole, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 5-methyl-2-furfural, 2-formylthiophene, 2-acetylthiophene, 2-methyl-1,3-dithiane, diacetyl, acetoin, and 2,3-pentanedione have been reported to be important volatile compounds which could contribute to meaty flavor (MacLeod, 1986; May, 1991; Cerny and Grosch, 1992). No single one of these compounds may be considered to be the character impact compound for the roasted meaty flavor generated in the model systems; however, the combination of these compounds and probably other compounds identified may lead to the good roasted meaty flavor sensation, especially in the glucose and alliin model reaction system.

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