Volatile Products Formed from L-Cysteine and Dihydroxyacetone Thermally Treated in Different Solvents

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Equimolecular amounts of L-cysteine and dihydroxyacetone were heated at 110°C for 3 hr in different solvent systems such as deionized water, glycerine, or triglyceride. The resulting mixtures were vacuum steam-distilled and each distillate was extracted with ethyl ether. The volatiles in the ether extracts were analyzed by gas chromatography and gas chromatography-mass spectrometry. Differences in the quality and quantity of volatiles formed in the systems were observed. Pyrazines, thiazoles, thiophenes, and some other sulfur-containing compounds were identified in the volatiles. Dimethylpyrazines were formed as major volatiles in the glycerine and triglyceride systems but were minor in the water system. 2-Acetylthiazole in the triglyceride system and 2-acetylthiophene in the glycerine system were secondary abundant products. In the water system, 1-mercapto-2-propanone was found as a major volatile compound, and thiophenes as the next dominants.

The Maillard reaction is significant in the formation of flavors from various heated foods. In the flavor industry, the Maillard reaction is used to produce simulated flavors of thermally treated foods, or 'processed flavors.' Numerous studies have been attempted to identify the precursors of flavors produced by thermal treatment of foods such as meat,^{1,2)} peanuts,^{3,4)} and cacao⁵⁾; the flavor precursors were basically composed of amino acids and sugars. To form cooked meat flavors, it has been shown that sulfur-containing amino acids are important precursors.^{6,7)} Studies on the model reaction systems between sulfur-containing amino acids and sugar have indicated that many interesting compounds, especially sulfur- and nitrogen-containing heterocyclics, found in the volatiles of heat-processed food, can be identified as the reaction products in these systems.^{8–12)} However, these model experiments have been done in a single solvent such as an aqueous solution, rather low boiling organic solvent, triglyceride, or in the dry state, under various heating conditions. In making 'processed flavors,' many kinds of solvent such as water,

sugar alcohols, oils and fats, or their mixtures were used for various applications. It has been observed in some cases that the quality of flavor formed has been changed by the difference of solvent system. The water content of heating systems effects the Maillard reaction.¹³⁾ Hartman *et al.*¹⁴⁾ investigated the effects of water activity on the major volatiles produced from a meat flavor model system containing monosodium L-glutamate, L-ascorbic acid, thiamine hydrochloride, and cystine, and reported qualitative and quantitative differences in volatiles produced. Arnoldi *et al.*¹⁵⁾ reported that the rate of the Strecker reaction of leucine and valine with fructose was higher in cocoa butter-water than in water. Many patents concerning the production of synthetic meat flavor claim cysteine as one of the most important ingredients.²⁾ Dihydroxyacetone is a fission product of sugar and has high reactivity in the browning reaction.¹⁶⁾ Dihydroxyacetone has appeared in a patent to produce roasted meat flavor.¹⁷⁾ Thus, it is the purpose of this study to compare the volatile compounds produced by the reaction of L-cysteine with dihydroxyacetone in dif-

ferent solvent systems; water, glycerine, or triglyceride. Glycerine was selected for its thermostability and the negligible reactivity of sugar alcohols in the Maillard reaction.^{18,19)} As triglyceride, a saturated medium chain triglyceride was used for its high stability to heat and oxidation.

Materials and Methods

Preparation of reaction mixtures. One-tenth mol of L-cysteine and 0.1 mol of dihydroxyacetone were dispersed in 500 g of deionized water, glycerine, or triglyceride. As triglyceride, O.D.O. was used. O.D.O. is a medium chain triglyceride (fatty acid composition: octanoic acid (75%) and decanoic acid (25%)) manufactured by Nisshin Oil Mills Ltd., Tokyo, Japan. These model systems were heated at 110°C for 3 hr in a glass autoclave with stirring.

Isolation of volatiles. Five hundred grams of the reaction product was mixed with 2000 g of deionized water and then steam-distilled under reduced pressure (30 mmHg/40°C). The distillate was condensed with a cold water (3°C) and two cold traps (dry ice-acetone and liquid nitrogen). The distillate (about 1000 ml) from each reaction product was extracted three times with 100 ml of ethyl ether after saturation with sodium chloride. The ether extracts were dried over anhydrous sodium sulfate and concentrated to about 0.1 g under atmospheric pressure. The concentrate was analyzed and measured by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

GC and GC-MS. Gas chromatographic analyses were done on a Shimadzu GC-9A with a flame ionization detector (FID), a flame thermionic detector (FTD), and a flame photometric detector (FPD), fitted with a 0.25 mm i.d. × 60 m fused silica capillary column coated with PEG 20M. The column oven temperature was programmed from 70 to 220°C at 3°C/min. The nitrogen carrier gas flow was 1 ml/min. Peak areas were integrated using a Shimadzu Model Chromatopac 1AX integrator connected to the gas chromatograph. A Hitachi Model M-80B mass spectrometer was used for mass spectral identification of the gas chromatographic components under the following conditions: column, fused silica capillary column (0.25 mm × 60 m) coated with PEG 20M; oven temperature, programmed from 60 to 210°C at 2°C/min; carrier gas, helium; ionizing voltage, 70 eV; accelerating voltage, 3000 V; ion source temperature, 190°C.

Results and Discussion

Sensory evaluation of the reaction mixtures

Table I. FLAVOR QUALITIES OF REACTION MIXTURES FROM L-CYSTEINE-DIHYDROXYACETONE SYSTEMS

System	Flavor description
Triglyceride	Roasted rice, sulfury
Glycerine	Roasted, burnt rice, somewhat roasted meat
Water	Roasted rice (weak), sweet sulfury roasted, somewhat roasted chicken

is presented in Table I. The characters of flavors of the three reaction mixtures somewhat differed. The sensory results indicated the triglyceride and glycerine systems gave more roasted notes and the water system gave a more sulfury note. Therefore, it would be expected that there would be some differences in reaction products. The GC profiles of the volatiles in the reaction mixtures are shown in Fig. 1. The peaks identified are numbered and the peaks not identified are no numbered. Table II lists the volatile components identified along with the peak identification numbers and quantitative data. The volatile components were identified by the comparison of GC retention indices and mass spectra of unknowns with those of authentic samples. The components corresponding to peaks 56 and 64 identified in the triglyceride system were octanoic and decanoic acids, respectively. These two components which could be considered as the degradation products of O.D.O. were excluded from calculating the peak area ratio. Upon examination of Table II, qualitative and quantitative differences in volatile products identified existed among the reaction systems.

Ten pyrazines were detected in the volatiles from the reaction mixtures. These pyrazines have been identified in many model systems of sugar and amino-compounds.²⁰⁾ Pyrazines were detected as major volatile products in the triglyceride and glycerine systems (total area: 60% in the triglyceride system; 70% in the glycerine system). 2,6-Dimethylpyrazine was the largest constituent of the total pyrazines, and the next largest one was 2,5-dimethylpyrazine. The distributions of pyrazines iden-

Fig. 1. Gas Chromatograms of Volatiles Formed in L-Cysteine-Dihydroxyacetone Model Systems.

tified in the triglyceride and glycerine systems were very close to each other. A marked difference was observed in the total percentages of pyrazines formed in the water system, where only 2,5- and 2,6-dimethylpyrazines were detected as minor components (total area: 5%). Shibamoto and Bernhard¹⁹⁾ reported that the largest amount of pyrazine formed in dihydroxyacetone-ammonia system was 2-methylpyrazine and 2,6-dimethylpyrazine was the next largest, and 2,5-dimethyl-, 2,3,5-trimethyl-, unsubstituted-, and 2,3-dimethyl-pyrazine followed in decreasing or-

der. However, this might be due to differences of nitrogen sources and reaction conditions. The presence of larger alkyl- and cyclopentapyrazines and 2-methylquinoxaline in the triglyceride and glycerine systems suggests that the aldol condensation between dihydroxyacetone and its dehydration products and further addition of alkyl group to pyrazine skeleton intermediates occur.

Thiazole compounds were abundant products in the triglyceride and glycerine systems, but were more varied in the glycerine system. In the water system, those were not abundant

Table II. VOLATILE COMPONENTS IDENTIFIED FROM L-CYSTEINE-DIHYDROXYACETONE MODEL SYSTEMS

Compound	Triglyceride system		Glycerine system		Water system	
	Peak No.	Area %	Peak No.	Area %	Peak No.	Area %
Pyrazines						
2,5-Dimethylpyrazine	23	6.9	11	10.4	6	1.5
2,6-Dimethylpyrazine	24	48.5	12	54.8	7	3.7
2,3,5-Trimethylpyrazine	28	1.3	15	2.1		
2-Ethyl-3,5-dimethylpyrazine	32	0.7	17	0.7		
2-Ethyl-3,6-dimethylpyrazine	31	2.4	16	1.2		
2-Ethyl-3,5,6-trimethylpyrazine	35	0.3	18	0.1		
2-Methyl-6,7-dihydro-5 <i>H</i> -cyclopentapyrazine			31	T ^a		
2,5-Dimethyl-6,7-dihydro-5 <i>H</i> -cyclopentapyrazine	41	0.2	27	0.1		
3,5-Dimethyl-6,7-dihydro-5 <i>H</i> -cyclopentapyrazine	44	0.5	32	0.3		
2-Methylquinoxaline			46	0.2		
Thiazoles						
Thiazole	19	0.1	5	T		
4-Methylthiazole			6	0.2		
2,4-Dimethylthiazole	20	0.5	7	0.5		
2,5-Dimethylthiazole			10	3.0		
2-Ethylthiazole	22	0.5	9	0.1	5	0.3
2-Acetylthiazole	39	6.9	25	1.3	15	0.7
2-Acetyl-4-methylthiazole			33	0.7	19	1.2
Thiophenes						
Thiophene-2-carboxaldehyde	43	1.1	30	0.4	17	5.0
5-Methylthiophene-2-carboxaldehyde					22	0.5
2-Acetylthiophene	47	0.7	36	4.1	21	6.4
2-Acetyl-5-methylthiophene			41	T		
2-Acetyl-4,5-dimethylthiophene	49	1.2	40	0.2		
2-Acetyl-3-ethylthiophene	46	0.2	35	0.5		
Tetrahydrothiophen-3-one	37	0.2	21	0.1	12	0.2
2-Methyltetrahydrothiophen-3-one	36	2.6	20	0.9	11	1.6
2-Thiophenethiol			22	0.4		
Other sulfur-containing compounds						
1-Mercapto-2-propanone	25	5.2	13	1.6	8	31.4
3-Mercapto-2-butanone			8	T		
1,2-Ethane dithiol	24	T				
2,5-Dimethyl-2,5-epoxy-1,4-dithiane					16	1.3
3-Methyl-1,2,4-trithiane			41	T		
1,2,3-Trithia-5-cycloheptene (tentative)					42	1.2
Miscellaneous						
Ethanol	5	0.2	3	T	2	0.7
Acetaldehyde	2	T	2	T		
2-Methyl-2-pentenal	13	0.2	4	0.3	4	0.1
2-Pentanone	4	0.3				
2-Heptanone	6	0.5				
2-Methylcyclopentanone	16	0.7				
Cyclohexanone			8	T		
Ethyl acetate	3	0.2				
Methyl octanoate	26	0.6				
γ -Octalactone	52	3.0				
δ -Octalactone	53	1.1				
Octanoic acid	56	L ^b				
Decanoic acid	64	L				

^a T=trace, less than 0.1%.^b L=large, this was excluded from calculating peak area %.

nor varied. The predominant thiazole was 2-acetylthiazole in the triglyceride system, 2,5-dimethylthiazole in the glycerine system, and 2-acetyl-4-methylthiazole in the water system. 2-Acetylthiazole has been identified in the systems of cysteine-ribose,^{8,12)} rhamnose-H₂S-NH₃,²¹⁾ and glucose-cysteamine.²²⁾ Mulders⁸⁾ proposed a possible formation route of 2-acetylthiazole from pyruvaldehyde and cysteine. Dihydroxyacetone is known to be dehydrated to form pyruvaldehyde.²³⁾ It is not readily evident why the predominant thiazole differs in each system.

Ten thiophenes were identified in this study. Eight of them were identified in the glycerine system and 2-acetylthiophene was the major one. In the water system, thiophenes were not varied but the secondary dominating volatiles. 2-Acetylthiophene and thiophene-2-carboxaldehyde were abundant in the water system. On the other hand, the formation of thiophenes was not predominating in the triglyceride system. Mulders⁸⁾ proposed that acylthiophene formed through a Michael addition of the thiol group of mercaptoacetaldehyde, which was a degradation product of cysteine to the α,β -unsaturated carbonyl system. In this system, dihydroxyacetone and also acetaldehyde from cysteine might act as precursors for forming α,β -unsaturated carbonyl.

Dihydroxyacetone and cysteine dispersed in O.D.O. during reaction, but they dissolved in glycerine with the rise of temperature. Therefore, some differences observed between the volatiles produced in the triglyceride and glycerine systems might be due to the fact that dihydroxyacetone and cysteine reacted in a dispersed state in triglyceride and in a dissolved state in glycerine.

1-Mercapto-2-propanone was identified in the three systems examined. This compound was the largest volatile constituent in the water system but not abundant in the other systems. The odor of this compound from the GC trap was described as solvent-, sulfur-, and rubber-like. 1-Mercapto-2-propanone has been identified in the volatiles of heated canned pork meat²⁴⁾ and the reaction mixture of cysteine or

cystine with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone.^{25,26)} 1-Mercapto-2-propanone was prepared by the method of Hromatka and Engel.²⁷⁾ The obtained crystal was shown to be a mixture of 1-mercapto-2-propanone (monomer) and its dimer (2,5-dimethyl-2,5-dihydroxy-1,4-dithiane) by comparing its ¹H-NMR with that of reference 28. When the ether solution of this crystal was injected into the GC under the same conditions used in this study, only one peak appeared. The mass spectrum of this compound showed major peaks at *m/z* 90 (24), 47 (22), 45 (10), 43 (100), 42 (7), 27 (6), and 15 (10) corresponding to the monomer. It has been reported that the monomer and the dimer also exist in equilibrium solution and the monomer is the most stable species.²⁸⁾ It could be thought that 1-mercapto-2-propanone mainly exists as the monomer form in our reaction mixtures.

2,5-Dimethyl-2,5-epoxy-1,4-dithiane, which was identified only in the water system, had not been reported in natural foods nor in a model system. This compound was synthesized from the mixture of 1-mercapto-2-propanone and its dimer by refluxing in benzene with *p*-toluenesulfonic acid. The IR and MS spectra of 2,5-dimethyl-2,5-epoxy-1,4-dithiane are shown in Fig. 2. The ¹H-NMR spectrum (CDCl₃) showed the following; 1.9 ppm (6H, s), 3.4 ppm (2H, d, *J*=9.2 Hz), 3.6 ppm (2H, d, *J*=9.2). The odor of this compound was described as sulfurous and like a putrid radish. The formation mechanism of 1-mercapto-2-propanone and 2,5-dimethyl-2,5-epoxy-1,4-dithiane is postulated as shown in Fig. 3. Dihydroxyacetone can be converted to glyceraldehyde through an enediol intermediate. Glyceraldehyde reacts with hydrogen sulfide which can be formed by the degradation of cysteine to form glyceraldehyde dithioacetal. This dithioacetal can be converted to 2-oxo-thiopropenal by further dehydration *via* methyl ketone homologue. When 2 molecules of 2-oxo-thiopropenal are condensed together, 2,5-dimethyl-2,5-dihydroxy-1,4-dithiane can be generated. 1-Mercapto-2-propanone can be formed through dimer-monomer equilib-

Fig. 2. IR and Mass Spectra of 2,5-Dimethyl-2,5-epoxy-1,4-dithiane.

rium.²⁸⁾ 2,5-Dimethyl-2,5-epoxy-1,4-dithiane can be formed by intramolecular dehydration of 2,5-dimethyl-2,5-dihydroxy-1,4-dithiane.

1,2,3-Trithia-5-cycloheptene, identified ten-

tatively in the water system, has been identified in the thermal degradation products of cysteine at pH 7.1.²⁹⁾ The GC detected by FPD indicates that unknown peaks appearing from

Fig. 3. Postulated Mechanism of the Formation of 1-Mercapto-2-propanone (I) and 2,5-Dimethyl-2,5-epoxy-1,4-dithiane (II).

43.5 min to 50.5 min of retention time on the gas chromatogram of volatiles from the water system were sulfur-containing compounds.

In summary, it has been shown that the solvent of reaction mixture has large effects on the quality and quantity of volatile compounds produced in an amino-carbonyl model system. The reaction in water favors the formation of sulfur-containing products indicating that cysteine prefers degrading to sulfur-containing fragments and releasing hydrogen sulfide in water system. In the triglyceride and glycerine systems, dehydration-type reactions as producing pyrazines and thiazoles are much favored. These differences affect the flavor qualities of model systems and likely those of food systems.

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