Flavor Components in the Maillard Reaction of Different Amino Acids with Fructose in Cocoa Butter–Water. Qualitative and Quantitative Analysis of Pyrazines

Anna Arnoldi,* Claudio Arnoldi, Olga Baldi, and Alberto Griffini

Eight amino acids were reacted with fructose in deodorized cocoa butter–water as a model of the Maillard reaction in cocoa beans. Detailed GC/MS analysis of the volatile compounds obtained is reported with particular reference to similarities and differences in the patterns obtained with the amino acids. A quantitative analysis of pyrazines is described. Among the 22 pyrazines detected, 15 had been already reported in roasted cocoa beans. 2-(3-Methylbutyl)-3,6-dimethylpyrazine was formed in the reaction of leucine, while lysine gave three 2-methoxy-3-alkylpyrazines not reported in cocoa.

Pyrazines are important components of cocoa flavor (Van Praag et al., 1968; Van der Wal et al., 1971; Gill et al., 1984; Carlin et al., 1986). Two recent reviews (Maga, 1982; Fors, 1983) report the literature data on all the pyrazines isolated in foods and, when known, the sensitivity thresholds for taste or smell.

Many theories on the formation of pyrazines during the heating of foods have been proposed. A recent review (Hoskin and Dimick, 1984a) deals with this topic with particular reference to the processing of chocolate. Dawes and Edwards (1966) using model systems with amino acids and fructose identified 2,5-dimethyl- and trimethylpyrazine and concluded that pyrazines come from the condensation of amino acids and sugars. Newell et al. (1967) proposed a mechanism of pyrazine formation involving the production of two aminoacetone molecules that then condense together.

The possible role of ammonia in pyrazine formation was proposed by Van Praag et al. (1968). They reacted fructose with some amino acids or ammonia and found the same pyrazines. They concluded, therefore, that ammonia was an intermediate of the reaction. Because of these results in recent years, the reaction of ammonia with sugars has been studied (Shibamoto and Benhard, 1977).

Koehler et al. (1969) used radioisotopic labeling techniques with the aim of establishing the origin of pyrazine atoms. They concluded that sugars give the carbon atoms and amino acids only nitrogen atoms.

Walradt et al. (1971) deduced that some dihydrocyclopentylpyrazines, characterized by them, are derived probably from the reaction of amino acids with 2-hydroxy-3-methyl-2-cyclopenten-1-one, a sugar degradation product.

Shibamoto and Bernhard (1977) depicted a general scheme for the formation of alkylpyrazines in the reaction of sugars with ammonia, proposing the intermediates that react to give rise to every pyrazine. It must be observed that with these reagents they could not justify the formation of some alkylpyrazines with large substituents (isobutyl, etc.).

More recently with ESR techniques Namiki and Hayashi (1983) succeeded in detecting in the reaction mixture some substituted pyrazine radical cations that retain the amino acid structure. These intermediates then lose CO_2 and the alkyl group to give pyrazines. In this way the authors proposed a reaction pathway in which free ammonia is not formed in the mixture. In a following paper the same authors (Hayashi and Namiki, 1986) detected methyl glyoxal dialkylimine in the reaction of sugars with *n*-butylamine and proposed that similar intermediates give rise to substituted pyrazine radical cations.

The formation of pyrazines and other Maillard products in the reaction of amino acids and sugars has generally been studied in model systems. Water or mixtures of water with alcohols have been used as solvents: methanol (Eichener and Karel, 1972; Lee et al., 1984), ethanol (Ledl, 1982), diethylene glycol (Koehler and Odell, 1970), octanol (Westphal and Cieslik, 1983). The last is the only lipophilic solvent used.

As we are interested in the effect of lipids on the formation of flavor during the roasting of cocoa beans containing up to 55% cocoa butter, in the preceding paper we proposed a model system in which a large amount of cocoa butter was present and we have studied the rate of formation of the Strecker aldehydes from leucine and valine (Arnoldi et al., 1987). We observed that this rate in the presence of cocoa butter was higher than without it and that the aldehydes are also formed without sugars. In this paper we report on a detailed analysis of the flavor components obtained from eight amino acids present in cocoa beans and fructose, which is one of the most abundant sugar in cocoa beans, in the presence of cocoa butter and a quantitation of some of the pyrazines formed. Fructose and the amino acid (alanine, valine, leucine, phenylalanine, threonine, lysine, aspartic acid, glutamic acid) in the presence of a mixture of 96% cocoa butter and 4% water were heated for 3 h at 120 °C with good stirring. Water was added in order to have the cocoa butter to water ratio generally present in cocoa beans. In order to extract the compounds formed from cocoa butter, we chose steam distillation and continuous extraction with dichloromethane. In this way we obtained concentrated solutions suitable for GC/MS analysis.

EXPERIMENTAL SECTION

General Procedure for the Model System. In a 250-mL flask equipped with a reflux condenser, effective magnetic stirrer, and a hydraulic valve, deodorized cocoa butter (23 g obtained from Nestlé) was melted, and then the amino acid (500 mg), fructose (a stoichiometric amount), and water (1 mL) were added. The mixture was stirred at 120 °C for 3 h. Then warm water (100 mL) was added, and the mixture was steam-distilled from the same flask: 100 mL of distillate was collected. The aqueous solution (50 mL) was continuously extracted for 8 h with dichloromethane (200 mL) in an apparatus for the continuous extraction of a liquid with a heavier liquid. The organic layer was dried on anhydrous Na_2SO_4 and the

Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, via Celoria, 2-20133 Milano, Italy (A.A., O.B., A.G.), and Alivar, Corso Vercelli, 28100 Novara, Italy (C.A.).

0021-8561/88/1436-0988$01.50/0

solvent distilled through a 12-cm long Vigreux column at room pressure. The distillation was interrupted when the residue was 1 mL.

Extraction of Deodorized Cocoa Butter. Cocoa butter (24 g) and distilled water (1 mL) were heated for 3 h at 120 °C. Warm water (100 mL) was added, the mixture was steam-distilled, and 100 mL of distillate was collected. The aqueous solution (50 mL) was continuously extracted for 8 h with dichloromethane (200 mL). The organic layer was dried and the solvent distilled through a 12-cm-long Vigreux column. The distillation was interrupted when the residue was 1 mL.

GC/MS Analysis. Qualitative and quantitative analyses of standard mixtures, extracts from model systems, and recovery controls were performed by gas chromatography/mass spectrometry, on a quadrupolar analyzer instrument, Finnigan 4021, equipped with a Super INCOS data system for acquisition and elaboration of data.

A Carbowax 20M wide-bore capillary column was used, Supelcowax-10WB (Supelco Inc.). The column was connected directly to the ion source by the packed column line with a glass-lined microcapillary stainless steel shut-off valve with a zero dead volume instead of a molecular jet separator. This interface allows all the column effluent to reach the ion source. The column was connected to a modified injector for packed columns, by a $^1/_4$ in. × 2 mm (i.d.) Pyrex tube, 140 mm long, with a stainless steel $^1/_4$ in. × $^1/_{16}$ in. Swagelok reducing union. These modifications allowed use of the splitless mode injection in order to resolve some sensitivity problems related to the total ion current mode. The higher capacity of a wide-bore column with respect to traditional capillary columns together with a satisfying separation efficiency gave good results, and in this way we could use two alterne capillary column in the same oven.

Separated compounds were identified by mass spectra. They were compared with literature spectra and, when possible, with the computer library ones. Some peaks were not identified, and therefore they do not appear in tables.

Pyrazines were identified by comparison with commercial standards (Aldrich, purity >97%) and on the basis of characteristic fragmentations (Brophy and Cavill, 1980; Porter, 1985).

Quinoxaline was chosen as internal standard because its structure is similar to pyrazine, its peak was well separated from every other one, and the simplicity of its mass spectra makes easier the detection of superimposed peaks. Either for qualitative or for quantitative analyses the total ion monitoring mode was used (from 33 to 650 μ in 0.95 s).

Analytical Conditions. GC: Supelcowax-10WB column, 30 m × 0.75 mm (i.d.) (bonded phase); film thickness, 1 μm; carrier gas, He at 3 mL/min (at 70 °C); linear rate, 27.3 cm/s; injector, 250 °C; program, 70 °C for 4 min, 5 °C/min to 180 °C, isocratic 180 °C, interface 250 °C; splitless mode. MS: electron energy, 70 eV; emission current, 0.25 mA; electron multiplier, 1500 V; preamplifier sensitivity, 10^{-8} A/V; ionization chamber T, 250 °C, source configuration, EI/DIR; scanning 33–650 μ in 0.95 + 0.05 s.

Analysis of Standard Solutions of Pyrazines. Pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, ethylpyrazine, tetramethylpyrazine, 2-methoxy-3-(1-methylethyl)pyrazine, and 2-methoxy-3-(1-methylpropyl)pyrazine were commercial samples. Sensitivity thresholds (which are about 25–30 mg L^{-1}) and response factors were determined from the analysis of nine solutions containing from 40 to 400 mg L^{-1} of each pyrazine and 116.5 mg L^{-1} of quinoxaline.

RESULTS

The detected compounds were divided in four classes: Table I, aliphatic, aromatic, and a few heterocyclic compounds; Table II, furans and pyrroles; Table III, pyrazines. The relative retention time (t_{RR}) taking quinoxaline as standard and the percent area of each compound are also reported.

In cocoa butter we have detected alkylbenzenes, acetophenone, benzaldehyde, benzenemethanol, and aliphatic compounds, some of which are not reported in tables. The only heterocyclic compound is benzothiazole, while we could not detect furans, pyrroles, and pyrazines.

The compounds of Table I are generally similar in cocoa butter and in model systems. In particular benzothiazole is certainly derived from cocoa butter (Hoskin and Dimick, 1984b). On the contrary, the large amount of benzaldehyde and benzenemethanol formed in the reaction with phenylalanine indicates that these are among the most important decomposition products for this amino acid.

Furans (Table II) were detected in all model systems, often in large amounts. Some of them, 2-acetylfuran, 5-methyl-2-furancarboxyaldehyde, and furfuryl alcohol, were detected often; others, only in some systems. Aspartic and glutamic acids gave the largest number of different furans.

Only four pyrroles (Table II) were detected; in particular 5-methylpyrrole-2-carboxyaldehyde and 2-acetylpyrrole were detected in five model systems, while the other two were only detected with one and two amino acids, respectively.

Pyrazines are generally considered very important components of roasted food flavor, in particular chocolate. We have detected 22 pyrazines with different substituents (Table III). This table reports (in brackets) the percent area of each pyrazine with respect to the total area of pyrazines in every model system. Some pyrazines are aspecific, for example 2,5-dimethylpyrazine, representing about 60% of total pyrazines, and ethylpyrazine; the second largest component (17–37%), except in the cases of lysine and threonine. Generally, higher molecular weight pyrazines are more specific and were detected with only a few amino acids. Glutamic and aspartic acids give the lowest number of pyrazines (two and three, respectively). In the case of leucine 10 different pyrazines were detected; the presence of 2-(3-methylbutyl)-3,6-dimethylpyrazine suggests the possibility that the alkyl chain of the amino acid is preserved intact in this pyrazine. In the case of lysine three 2-methoxy-3-alkylpyrazines were detected. Also threonine gives different pyrazines.

As we had at our disposal authentic samples of eight pyrazines, we determined the response factors with respect to quinoxaline and could perform a quantitation by GC/MS of some pyrazines formed in the model systems. Details of the experimental procedure will be published elsewhere.

Table IV reports the amount of pyrazines obtained in the different model systems.

CONCLUSION

We have reported a detailed comparison of the compounds formed from eight different amino acids in the Maillard reaction in a lipidic medium more similar to cocoa beans and other oleaginous seeds than the traditional aqueous medium. As it concerns the origin of pyrazines, we have shown that some of them are aspecific, i.e. they are formed with every amino acid, and others are specific. Specific pyrazines have long, branched, or oxygenated substituents, and we suppose that the substituents of the amino acids are in some way involved in their formation. Our data are slightly different from those reported in the

Table I. Aromatic, Aliphatic, and Other Heterocyclic Compounds[a]

compound	t_{RR}[b]	Ala	Val	Leu	Phe	Thr	Lys	Asp	Glu	cocoa butter
1,2-dimethylbenzene	0.222			0.41						
isopropylbenzene	0.244	2.14								
1,3-dimethylbenzene	0.267						5.15			27.53
styrene	0.338	3.33	2.15	0.98	0.66			1.59	0.49	
3-hydroxybutan-2-one	0.377							0.36		
cyclohexanone	0.389	0.68	0.67	0.43			2.08	0.39		4.84
hydroxyacetone	0.396		3.42			1.27				
1,4-dimethyl-2-ethylbenzene	0.413									0.79
3,4-dimethyl-1-ethylbenzene	0.435						0.17			
1,2,3,4-tetramethylbenzene	0.442						0.33			0.25
4-methylhexan-2-one	0.458		2.60							
2,5-dimethyl-2-(hydroxymethyl)-1,3-dioxolane	0.460	6.80	2.95		2.60	2.81	2.46		1.31	
5-methylhexan-2-one	0.468									1.43
nonanal	0.485	1.44				1.10			0.62	1.84
(1,1-dimethylpropyl)benzene	0.489						0.77			
2-methylcyclopentanol	0.493	4.96	4.18	1.91	1.71	2.02		2.31	1.85	
2,4-dimethyl-1-ethylbenzene	0.501						0.23			
1,2,4,5-tetramethylbenzene	0.522						1.69			1.95
1,2,3,5-tetramethylbenzene	0.528						2.83			3.77
4-methyl-2,3-dihydro-1*H*-indene	0.571						1.05			
5-methyl-2,3-dihydro-1*H*-indene	0.575									0.71
3-methyl-1-isopropylbenzene	0.591									1.00
(*E*)-(1-methylpropenyl)benzene	0.603									1.94
1-ethyl-2,4,5-trimethylbenzene	0.619									0.23
1,2,3,4-tetrahydronaphthalene	0.627	0.72	0.53	0.41		0.26		0.44	0.61	
benzaldehyde	0.635	0.15	0.56	0.09	24.56			0.20		0.41
3-methylhexan-1-ol	0.654									0.34
2-methylpropanoic acid	0.663		3.96							
4-methylbenzaldehyde	0.736					0.40				
acetophenone	0.765									0.40
1,2-dihydroindazol-3-one	0.834							0.30		
1-phenyl-2-propanone	0.839				0.55					
naphthalene	0.846	0.31					3.39		0.21	2.85
3-phenylpropanal	0.888				0.18					
4-methylpentanoic acid	0.893			1.54						
2-(2-butoxyethoxy)ethanol	0.895	1.19	0.68		0.23	0.80	2.76	0.43	0.66	0.43
(*E*,*E*)-2,4-decadienal	0.909					0.61			0.46	1.0
2-hydroxy-3-methyl-2-cyclopenten-1-one	0.929						1.20			
benzenemethanol	0.970	1.08	0.95	1.22	17.00	1.26		1.28	1.00	0.41
α-ethylidenebenzeneacetaldehyde	1.024				3.43					
benzothiazole	1.041	1.37	1.50	1.06	0.45	1.85	0.50	1.25	1.19	2.90
phenol	1.076				0.67					

[a] Amount expressed of percent area. [b] t_{RR} = relative retention time.

Table II. Furans and Pyrroles[a] Detected in the Model Systems

	t_{RR}[b]	Ala	Val	Leu	Phe	Thr	Lys	Asp	Glu
	Furans								
2-methyl-4,5-dihydro-3(2*H*)-furanone	0.352	1.63							
5-methyl-2(3*H*)-furanone	0.542								0.66
furan-2-carbaldehyde	0.570	1.42					7.70		12.46	16.27
2-acetylfuran	0.615	0.73	5.00	3.17	1.67	9.32	1.25	12.49	9.78
5-methylfuran-2-carbaldehyde	0.687	2.12	2.03	0.29	0.16	11.31	0.92	19.95	36.02
4,5-dimethyl-4,5-dihydro-2(3*H*)-furanone	0.754	0.29	0.22						1.97
furan-2-methanol	0.766	3.64	4.26	7.21		7.10		1.29	0.75
2,5-dimethyl-2,3-dihydrofuran	0.799							0.26	
2(3*H*)-furanone	0.873		0.31	0.16				0.37	
methylfuran-3-carboxylate	1.091							1.31	1.38
total percent area of furans		9.83	11.82	10.83	1.83	35.43	2.17	48.13	66.83
	Pyrroles								
5-methyl-1-(2-methylpropyl)-1*H*-pyrrole-2-carboxaldehyde	0.917		0.36	0.62					
2-acetylpyrrole	1.056		6.62	1.81		1.11	2.62	2.19	
5-methyl-(1*H*)-pyrrole-2-carbaldehyde	1.168			0.49	0.29		6.28	1.16	0.89
4-butylpyrrolidine-2,5-dione	1.175			0.42					
total percent area of pyrroles			6.98	3.34	0.29	1.11	8.90	3.35	0.89

[a] Amount expressed as percent area. [b] t_{RR} = relative retention time.

literature. In fact, Koehler and Odell (1970) and Van Praag et al. (1968) found the same pyrazines when reacting either amino acids or ammonia alone with sugars and therefore concluded that ammonia was an intermediate in the reaction, whereas the pattern we find is more complex and different depending on the amino acid used. It may be supposed that the reason for the discrepancy is the use of packed gas chromatographic columns by Koehler and Odell (1970) and Van Praag et al. (1968), which allowed the detection of only the more abundant, but aspecific, pyrazines, in particular methyl-, dimethyl-, and ethyldimethylpyrazines. In our case, the more efficient

Table III. Pyrazines[a] Detected in the Model Systems

substituent	t_{RR}[b]	Ala	Val	Leu	Phe	Thr	Lys	Asp	Glu
methyl	0.348		1.54 (6.2)	0.82 (3.5)	0.20 (0.5)				
2,5-dimethyl	0.412	17.25 (62.5)	14.06 (56.4)	14.39 (62.2)	10.18 (25.5)	3.90 (20.0)	3.29 (26.8)	13.37 (59.2)	6.66 (62.6)
2,6-dimethyl	0.422					8.11 (43.5)			
ethyl	0.424	9.59 (34.8)	4.43 (17.8)	4.74 (20.5)	4.76 (11.9)		1.98 (16.1)	7.98 (35.4)	3.98 (34.4)
2,3-dimethyl	0.436		1.90 (7.6)		0.42 (1.1)				
2-ethyl-6-methyl	0.482					0.35 (1.9)			
2-ethyl-5-methyl	0.484		1.38 (5.6)	0.91 (3.9)	0.34 (0.9)				
trimethyl	0.499	0.20 (0.7)	0.97 (3.9)	0.48 (2.1)	0.22 (0.6)	1.09 (5.9)			
2-ethyl-3,5-dimethyl	0.543	0.55 (2.0)	0.22 (0.9)					1.22 (5.4)	
2-ethyl-3,6-dimethyl	0.545			0.38 (1.6)	0.14 (0.4)	2.25 (12.0)			
2,6-diethyl	0.562			0.16 (0.7)		1.07 (5.7)			
tetramethyl	0.577			0.54 (2.3)					
2-ethenyl-5-methyl	0.590		0.42 (1.7)	0.27 (1.2)					
2-ethenyl-6-methyl	0.602			0.19 (0.8)					
2,3-diethyl-5-methyl	0.593					0.35 (1.9)			
2-methoxy-3-(1-methylpropyl)	0.598						3.25 (26.4)		
2-methoxy-3-(2-methylpropyl)	0.623						2.43 (19.8)		
2-propyl-3,6-dimethyl	0.638					0.75 (4.0)			
2-methoxy-3-butyl	0.671						1.34 (10.9)		
1,2,3-triazolo[1,5-*a*]	0.759				23.66 (59.2)				
2-(3-methylbutyl)-3,6-dimethyl	0.760			0.25 (1.2)					
(*E*)-5-methyl-2-(1-propenyl)	0.825					0.76 (4.1)			
total percent area of pyrazines		27.59	24.92	23.13	39.92	18.63	12.29	22.57	10.64

[a] Pyrazine amount expressed as percent area; in parentheses percent area with respect to the total percent area of all pyrazines. [b] t_{RR} = relative retention time.

Table IV. Pyrazines Detected in Model Systems (Micrograms)

substituent	Ala	Val	Leu	Phe	Lys	Thr	Asp	Glu
methyl		31.2	20.3	17.0				
2,5-dimethyl	203.9	151.3	125.7	200.8	14.14	20.8	195.7	86.1
2,6-dimethyl						38.4		
ethyl	116.8	56.8	65.9	99.2	11.9		119.5	56.8
2,3-dimethyl		26.9		19.3				
trimethyl		15.3	9.6	9.9		6.9		
tetramethyl			13.6					
2-methoxy-3-(1-methylpropyl)					3.6			

Table V. Comparison of Pyrazines Reported in Roasted Cocoa and Detected in Model Systems

Common Pyrazines[a]	
methyl	2-ethyl-3,5-dimethyl
2,3-dimethyl	2-ethyl-3,6-dimethyl
2,5-dimethyl	tetramethyl
2,6-dimethyl	2-ethenyl-5-methyl
ethyl	2-ethenyl-6-methyl
2-ethyl-6-methyl	2-(3-methylbutyl)-3,6-dimethyl
2-ethyl-5-methyl	2,3-diethyl-5-methyl

Pyrazines Detected Only in Model Systems
- 2,6-diethyl
- 2-propyl-3,6-dimethyl
- (*E*)-5-methyl-2-(1-propenyl)
- 2-methoxy-3-(1-methylpropyl)
- 2-methoxy-3-(2-methylpropyl)
- 2-methoxy-3-butyl
- 1,2,3-triazolo[1,5-*a*]

[a] Van Praag et al., 1968; Van der Wal et al., 1971; Gill et al., 1984; Carlin et al., 1986; Hoskin and Dimick, 1984.

capillary columns also made possible the detection of specific pyrazines.

The efficiency of our model system in cocoa butter in reproducing the Maillard reaction in cocoa beans can be evaluated from Table V, which reports the pyrazines detected in the eight model systems in comparison with the pyrazines reported in chocolate flavor (Van Praag et al., 1968; Van der Wal et al., 1971; Gill et al., 1984; Carlin et al., 1986;, Hoskin and Dimick, 1984a). Until now, more than 80 pyrazines have been detected by extraction of roasted cocoa beans. We have detected 22 pyrazines. In particular we found only one tetraalkylpyrazine, tetramethylpyrazine, while in chocolate flavor eight tetraalkylpyrazine were detected; we could not isolate dihydrocyclopentylpyrazines. However, it is important to note that the authors extracted very large amounts of cocoa beans (in general many kilograms) and that many compounds were detected only in traces.

One of the most dramatic differences was the detection of three methoxypyrazines in lysine reaction but not in chocolate. As lysine is one of the amino acids present in cocoa beans, this fact is worthy of further investigation.

From the results shown, we conclude that the proposed model system in cocoa butter can be a useful tool in the study of the Maillard reaction in cocoa beans.

Registry No. L-Ala, 56-41-7; L-Val, 72-18-4; L-Leu, 61-90-5; L-Phe, 63-91-2; L-Lys, 56-87-1; L-Thr, 72-19-5; L-Asp, 56-84-8; L-Glu, 56-86-0; 2-methyl-4,5-dihydro-3(2*H*)-furanone, 3188-00-9; 5-methyl-2(3*H*)-furanone, 591-12-8; furan-2-carbaldehyde, 98-01-1; 2-acetylfuran, 1192-62-7; 5-methylfuran-2-carbaldehyde, 620-02-0; 4,5-dimethyl-4,5-dihydro-2(3*H*)-furanone, 6971-63-7; furan-2-methanol, 98-00-0; 2,5-dimethyl-2,3-dihydrofuran, 17108-52-0; 2(3*H*)-furanone, 20825-71-2; methylfuran-3-carboxylate, 13129-23-2; 5-methyl-1-(2-methylpropyl)-1*H*-pyrrole-2-carboxaldehyde, 66054-34-0; 2-acetylpyrrole, 1072-83-9; 5-methyl-1*H*-pyrrole-2-carbaldehyde, 1192-79-6; 4-butylpyrrolidine-2,5-dione, 114819-82-8; methylpyrazine, 109-08-0; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; ethylpyrazine, 13925-00-3; 2,3-di-

methylpyrazine, 5910-89-4; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-5-methylpyrazine, 13360-64-0; trimethylpyrazine, 14667-55-1; 2-ethyl-3,5-dimethylpyrazine, 13925-07-0; 2-ethyl-3,6-dimethylpyrazine, 13360-65-1; 2,6-diethylpyrazine, 13067-27-1; tetramethylpyrazine, 1124-11-4; 2-ethenyl-5-methylpyrazine, 13925-08-1; 2-ethenyl-6-methylpyrazine, 13925-09-2; 2,3-diethyl-5-methylpyrazine, 18138-04-0; 2-methoxy-3-(1-methylpropyl)pyrazine, 24168-70-5; 2-methoxy-3-(2-methylpropyl)pyrazine, 24683-00-9; 2-propyl-3,6-dimethylpyrazine, 18433-97-1; 2-methoxy-3-butylpyrazine, 32737-11-4; 1,2,3-triazolo[1,5-*a*]pyrazine, 51392-75-7; 2-(3-methylbutyl)-3,6-dimethylpyrazine, 18433-98-2; (*E*)-5-methyl-2-(1-propenyl)pyrazine, 18217-82-8; D-fructose, 57-48-7.

LITERATURE CITED

Arnoldi, A.; Arnoldi, C.; Baldi, O.; Griffini, A. "Strecker Degradation of Leucine and Valine in a Lipidic Model System". *J. Agric. Food Chem.* **1987**, *35*, 1035–1038.

Brophy, J. J.; Cavill, G. W. K. "Naturally Occurring Pyrazines and Their Mass Spectrometric Characterization". *Heterocycles* **1980**, *14*, 477–504.

Carlin, J. T.; Hsieh, O. A. L.; Sun Hwang, L.; Ho, C. T.; Chang, S. S. "Comparison of Acidic and Basic Volatile Compounds of Cocoa Butters from Roasted and Unroasted Cocoa Beans". *JAOCS, J. Am. Oil Chem. Soc.* **1986**, *63*, 1031–1035.

Dawes, I. W.; Edwards, R. A. "Methyl Substituted Pyrazines as Volatile Reaction Products of Heated Aqueous Aldose, Aminoacid Mixtures". *Chem. Ind. (London)* **1966**, 2203.

Eichener, K.; Karel, M. "The Influence of Water Content and Water Activity on the Sugar–Amino Browning Reaction in Model Systems under Various Conditions". *J. Agric. Food Chem.* **1972**, *20*, 218–233.

Fors, S. "Sensory Properties of Volatile Maillard Reaction Products and Related Compounds". in *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 185–286.

Gill, M. S.; Mac Leod, A. J.; Moreau, M. "Volatile Components of Cocoa with Particular Reference to Glucosinolate Products". *Phytochemistry* **1984**, *23*, 1937–1942.

Hayashi, T.; Namiki, M. "Role of Sugar Fragmentation in the Maillard Reaction". *Amino-carbonyl Reactions in Food and Biological Systems*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Elsevier: Amsterdam, 1986; pp 29–38.

Hoskin, J. C.; Dimick, P. S. "Role of non Enzymatic Browning during the Processing of Chocolate—A Review". *Proc. Biochem.* **1984a**, *19*, 92–104.

Hoskin, J. C.; Dimick, P. S. "Role of Sulphur Compounds in the Development of Chocolate Flavour—A review". *Proc. Biochem.* **1984b**, *19*, 150–156.

Koehler, P. E.; Odell, G. V. "Factors Affecting the Formation of Pyrazine Compounds in Sugar Amine Reaction". *J. Agric. Food Chem.* **1970**, *18*, 895–898.

Koehler, P. E.; Mason, M. E.; Newell, J. A. "Formation of Pyrazine Compounds in Sugar–Aminoacids Model Systems". *J. Agric. Food Chem.* **1969**, *17*, 393–396.

Ledl, F. "Formation of Colored Products in Browning Reactions: Reaction of Dihydroxy Acetone with Furfural". *Z. Lebensm. Unters. Forschung.* **1982**, *175*, 349–352.

Lee, C. M.; Sherr, B.; Koh, Y. N. "Evaluation of Kinetic Parameters for a Glucose–Lysine Maillard Reaction". *J. Agric. Food Chem.* **1984**, *32*, 379–382.

Maga, J. A. "Pyrazine in Flavor". In *Food Flavours. Part A. Introduction*; Morton, I. D., MacLeod, A., J. Eds.; Elsevier: Amsterdam, 1982; pp 283–324.

Namiki, M.; Hayashi, T. "A New mechanism of the Maillard Reaction Involving Sugar Fragmentation and Free Radical Formation". In *The Maillard Reaction In Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; p 21–46.

Newell, J. A.; Mason, M. E.; Matlock, R. S. "Precursors of Typical and Atypical Roasted Peanut Flavor". *J. Agric. Food Chem.* **1967**, *15*, 767–772.

Porter, Q. N. *Mass Spectrometry of Heterocyclic Compounds*, 2nd ed.; Wiley: New York, 1985.

Shibamoto, T.; Bernhard, R. A. "Investigation of Pyrazine Formation Pathways in Sugar–Ammonia Model Systems". *J. Agric. Food Chem.* **1977**, *25*, 609–614.

Van der Wal, B.; Kettenes, D. K.; Stoffelsma, J.; Sipma, G.; Semper, A. T. "New Volatile Components of Roasted Cocoa". *J. Agric. Food Chem.* **1971**, *19*, 276–280.

Van Praag, M.; Stein, H. S.; Tibbetts, M. S. "Stean Volatile Aroma Constituents of Roasted Cocoa Beans". *J. Agric. Food Chem.* **1968**, *16*, 1005–1008.

Walradt, J. P.; Pittet, A. O.; Kinlin, T. E.; Muralidhara, R.; Sanderson, A. "Volatile Components of Roasted Peanuts". *J. Agric. Food Chem.* **1971**, *19*, 972–979.

Westphal, G.; Cieslik, E. "Studies of the Maillard Reaction. 8. Reaction of Glucose with Phenylalanine in 1-Octanol". *Nahrung* **1983**, *27*, 55–62.

Received for review June 15, 1987. Revised manuscript received February 29, 1988. Accepted March 22, 1988.

Comparison of the Volatile Compounds Obtained from Thermal Degradation of Cysteine and Glutathione in Water

Yuangang Zhang, Mingjien Chien, and Chi-Tang Ho*

The thermal degradation of both cysteine and glutathione was studied in a closed model system. Aqueous solutions of each reactant were adjusted to pH 7.5 and heated at 180 °C, representing frying temperature. From the degradation of cysteine, 34 volatile compounds were identified and the major products were 2,4,6-trimethylperhydro-1,3,5-thiadiazine and 2,4,6-trimethylperhydro-1,3,5-dithiazine. Of the 17 compounds identified as products of glutathione degradation, the isomers of 3,5-dimethyl-1,2,4-trithiolane were the major products.

Hydrogen sulfide is an important reactant in the production of meat flavors (MacLeod, 1987). It is liberated from cysteine, cystine, and glutathione (γ-Glu-Cys-Gly) when meat is heated. Glutathione releases hydrogen sulfide rapidly during the early stages of cooking (Mecchi et al., 1964; Ohloff et al., 1985).

The question of whether Maillard reaction products derived from peptides differ from those derived from their constituent amino acids has been raised by Nursten (1987). The effect of pH on the volatile components formed from a dilute aqueous solution of cysteine heated at 160 °C for

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903 (Y.Z., C.-T.H.), and Givaudan Corporation, Clifton, New Jersey 07014 (M.C.).

0021-8561/88/1436-0992$01.50/0