Influence of Glutathione Oxidation and pH on Thermal Formation of Maillard-Type Volatile Compounds

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The Maillard-type volatile compounds obtained from the reaction of glutathione and glucose were mainly furans, carbonyl compounds, and sulfur-containing compounds including thiophenes, thiazoles, and cyclic polysulfides. Both pH 8.0 and 6.0 were favorable conditions for sulfur-containing compound formation, whereas acidic conditions were favorable for furan and derivative formation. The reaction between glutathionesulfonic acid, an oxidized form of glutathione, and glucose primarily produced furans, carbonyl compounds, pyrazines, and pyrroles. Furans dominated the products obtained from the reactions at pH 6.0 and 8.0. Pyrazines increased as the reaction pH increased. The disappearance of sulfur-containing compounds in the products of glutathionesulfonic acid and glucose reaction systems indicated glutathionesulfonic acid could not provide hydrogen sulfide for the reaction.

Keywords: *Glutathione; glutathionesulfonic acid; sulfur-containing; oxidation; pH*

INTRODUCTION

Sulfur-containing compounds are known to serve as fundamental elements for many flavors. Sulfur-containing compounds usually possess low odor thresholds, thus contributing to the overall sensory characteristics of certain foods such as meat. In a meat system, the sulfur may come from cysteine, glutathione, proteins, and thiamine. During cooking, interactions occur among the sulfur sources and many other reactants in meat and, as a consequence, sulfur-containing compounds are produced.

Glutathione (γ-Glu-Cys-Gly), a cysteine-containing tripeptide, occurs intracellularly in bacteria, plants, and mammals and serves many biological functions (Merister and Anderson, 1983). Zheng and Ho (1994) reported that glutathione required less activation energy than cysteine to release hydrogen sulfide. With respect to flavor formation, glutathione is an efficient hydrogen sulfide provider for the sulfur-containing aroma compounds, and its contribution to the overall cooked meat aromas is significant.

In food systems, oxidizing environments are unavoidable. These environments are created due to the presence of intentionally added food additives, which are potential oxidizing agents and naturally generate oxidizing agents. In addition, some processing procedures and storage also promote oxidation in foods. Accordingly, food flavor precursors that are exposed to these conditions are likely to also be influenced.

Glutathione is susceptible to oxidizing environments. In addition to oxidizing agents, pH contributes to the oxidation of glutathione as well. Finley et al. (1981) reported that an increase in pH facilitates the conversion of glutathione monomers into dimers. They also indicated that glutathione can be oxidized to glutathionesulfinic acid and glutathionesulfonic acid in the presence of oxidizing agents. Glutathionesulfonic acid and glutathionesulfinic acid are the oxidized forms of glutathione in which the cysteine residue is oxidized to cysteinesulfinic acid and cysteic acid, respectively.

As our previous study has shown, sulfur compounds were the major products in the cysteine/glucose reaction system, whereas pyrazines and furans dominated the oxidized cysteine/glucose reaction system. Cysteine oxidation did significantly change the profile of the Maillard-type volatiles (Tai and Ho, 1997). The current study will focus on another sulfur source, glutathione, and discuss the impact of oxidation on the Maillard-type compound formation in a glutathione/glucose reaction system. In addition, this study will discuss the pH factor on the volatile compound formation as well.

EXPERIMENTAL PROCEDURES

Thermal Reactions. Glutathione or glutathionesulfonic acid (0.0012 mol) (Sigma, St. Louis, MO) with α-D-glucose (Sigma) was dissolved in 50 mL of distilled water. The solution was adjusted to pH, 3.0, 6.0, or 8.0 and was sealed in a 120 mL cylinder reactor. The cylinder was heated in a 160 °C oven for 2 h. After reaction, the cylinder was immersed in an ice−water bath to bring the temperature down and terminate the reaction.

Volatile Isolation. The reaction mixture was mixed with 0.25 mL of internal standard (tridecane, 1000 ppm) and extracted using methylene chloride (50 mL × 4 times). The extract was dehydrated by anhydrous sodium sulfate (Sigma) and filtered. The filtrate was concentrated to 5 mL by a Kuderna-Danish concentrator. Finally, the extract was concentrated to 1 mL under a nitrogen flow.

Gas Chromatographic Analysis. The volatile compounds isolated from the thermal reaction systems were analyzed by a Varian 3400 gas chromatograph (GC) equipped with a fused silica capillary column (60 m × 0.32 mm i.d.; 1 μm film thickness, DB-1, J&W Scientific Inc.) and a flame ionization detector. For each sample, 1 μL was injected into the GC with a split ratio of 25:1. The GC was run with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier flow rate of 1 mL/min. The column temper-

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ature was programmed from 40° to 280 °C with a 2 °C/min increasing rate.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. The concentrated isolates from different reaction systems were analyzed by GC/MS, using a Hewlett-Packard 5790A GC coupled to a Hewlett-Packard 5970A MS. Mass spectra were obtained by electron ionization at 70 eV.

Identification of the Volatile Compounds. The identification of the volatile compounds was based on GC retention indices and GC/MS analysis. The compound from the isolate was identified by comparing the mass spectral data to those of authentic compounds, available in the Wiley 138 computer library or the previous publications, and comparing the retention index to those of authentic compounds or the literature data.

RESULTS AND DISCUSSION

Odor Descriptions. The odor generated from the glutathione/glucose systems at pH 3.0, 6.0, and 8.0 was described by two expert flavorists as a rice cracker-like and sulfur flavor. As the pH increased, the burnt or dark-roasted note increased. Glutathionesulfonic acid and glucose generated rancid candy and old paper notes at pH 3.0; coffee candy, sweet, roasty, and smoky notes at pH 6.0; and cocoa, chocolate, dry prune, peanut butter, smoky, and dark-roasty notes at pH 8.0.

Glutathione and Glucose Reaction System. The reaction of glutathione with glucose was conducted at pH 3.0, 6.0, and 8.0. Total of 37, 47, and 51 compounds were identified in the reaction systems of pH 3.0, 6.0, and 8.0, respectively. The compounds identified in this reaction were carbonyls, furans, pyrans, and sulfur- and nitrogen-containing heterocyclic compounds (Table 1).

Furans are mainly produced through dehydration, fragmentation, and cyclization of sugars (Nursten, 1980). They make a large contribution to the overall sensory properties of foods. As summarized in Table 1, pH 3.0 seems to be a more favorable reaction condition for the formation of furans since the total amount of furans generated at pH 3.0 was higher than that at pH 6.0 and 8.0. 5-Methyl-2-furfural and acetylfuran were the two most dominant compounds at pH 3.0.

2-Acetylfuran is a major compound obtained at all three pH values. It is a degradation product of glucose and is possibly formed through cyclization and dehydration of 1-deoxyhexosone. 5-(Hydroxymethyl)-2-furfural, which is mainly generated via the cyclization of 3-deoxyhexosone, was not detected at pH 6.0 and 8.0, although it was present in a significant amount at pH 3.0. This result may indicate that 3-deoxyhexosone is more likely to be produced at a lower pH.

The higher yield of furan derivatives at a low pH, on the other hand, reduced the amount of carbonyls formed. This may simply be due to the cyclization reaction, which stabilizes the deoxyglucosones and prevents their further fragmentation to small carbonyls. Furthermore, the higher concentration of hydroxyl ion at higher pH values may facilitate fragmentation.

The thiol-containing furan derivative, 2-furfurylthiol, possesses potent roasty and coffee-like aroma (Hofmann and Schieberle, 1995, 1997; Münch et al., 1997). 2-Furfurylthiol is a character impact compound in roasted coffee (Tressl and Silwar, 1981) and has been identified in cooked beef (Farmer and Patterson, 1991), wheat bread (Baltes and Song, 1994), and popcorn (Schieberle, 1991). 2-Furfurylthiol can be simply converted from 2-furfuryl alcohol by substituting the hydroxyl group with a thiol group. Another possible pathway uses 2-furfural and hydrogen sulfide as the precursors (Münch et al., 1997). 2-Furfurylthiol has a low flavor threshold (Hofmann and Schieberle, 1995), which makes it an important flavor contributor in food. The formation of 2-furfurylthiol was slightly more favorable at pH 6.0 and 8.0 than at pH 3.0.

Another thiol-containing furan derivative, 5-methyl-2-furfurylthiol, has a sensory character similar to that of 2-furfurylthiol. Hofmann and Schieberle (1997) have proposed the formation pathway of 5-methyl-2-furfurylthiol via the reaction of 5-methyl-2-furfural and hydrogen sulfide. 5-Methyl-2-furfurylthiol was an important compound obtained from the reaction of rhamnose with cysteine and was a more potent odorant than 2-furfurylthiol (Hofmann and Schieberle, 1997). 5-Methyl-2-furfural, the potential precursor of 5-methyl-2-furfurylthiol, was obtained at its highest yield (94.7 mg/mol) at pH 3.0. The formation of this compound was dramatically reduced at pH 6.0 and 8.0. The amount of 5-methyl-2-furfural, however, did not increase the amount of 5-methyl-2-furfurylthiol at pH 3.0. The yield of 5-methyl-2-furfurylthiol tended to reduce as the pH decreased. Meanwhile, the higher pH values provided more suitable conditions for producing other sulfur-containing compounds. These phenomena did not necessarily indicate a shortage of hydrogen sulfide at lower pH values because Zheng and Ho (1994) have reported that the activation energy for the release of hydrogen sulfide from glutathione follows the sequence pH 9.0 (17.4 kcal/mol) < pH 3.0 (18.5 kcal/mol) < pH 7.0 (22.1 kcal/mol) < pH 5.0 (30.9 kcal/mol). The tendency of the hydrogen sulfide to be set free from glutathione is likely in the order of pH 3.0 > pH 8.0 > pH 6.0. A possible explanation for the much higher yields of the sulfur-containing compounds at higher pH values may be due to the fact that a sufficient amount of hydrogen sulfide is provided in all reaction systems where hydrogen sulfide concentration is not a determining factor.

Furaneol, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, is known for its sweet and fruity attributes. In this study, Furaneol was identified in moderate amounts in all three reaction systems. Furaneol was the thermal reaction product when glucose, fructose, and rhamnose were used as the reactants (Fagerson, 1969; Johnson et al., 1969; Hofmann and Schieberle, 1997).

Thiophene derivatives were the most dominant products at pH 6.0 and 8.0 but not at pH 3.0, possibly due to a deficiency in building elements such as glucose-derived intermediates at pH 3.0. As indicated earlier, the higher yield of furans attenuated the production of other glucose-derived intermediates such as carbonyls. The total yield of thiophenes at pH 3.0 was 93.1 mg/mol, which was relatively less than those of its counterparts at higher pH values. Under pH 6.0 and 8.0, we obtained 311.6 and 326.8 mg/mol, respectively. These yields revealed the significance of thiophenes in a glutathione and glucose reaction system. 2,5-Dimethyl-4-hydroxy-3(2*H*)-thiophenone (thiofuraneol), the analogue of Furaneol, dominated at all three pH values. 2,5-Dimethyl-4-hydroxy-3(2*H*)-thiophenone was described as roasted onion-like in both aroma and taste (Shu et al. 1985). In another study, it was described as roasty and caramel-like (Münch et al., 1997; Hofmann and Schieberle, 1997). The sensory characters described in the latter report are somewhat similar to those of its analogue, Furaneol. Furthermore, the odor thresholds

Table 1. Volatiles (Milligrams per Mole) Obtained from the Reaction of Glutathione and Glucose

compound	RI (DB-1)	pH 8.0	pH 6.0	pH 3.0
carbonyls				
2,3-butanedione	625	3.0	2.2	0.9
2-butanone	631	61.3	36.0	7.3
1-hydroxy-2-propanone	668	31.1	25.8	21.6
5-hydroxy-2-pentanone	687	2.3		
2-pentanone	693	4.8	3.6	
3-pentanone	701	2.7	2.4	
acetoin	705	16.6	13.0	4.8
1-hydroxy-2-butanone	748	5.7	5.3	
3-hexanone	771	1.8		
2-mercapto-3-butanone	789	7.2	8.1	3.3
2,4-hexanedione	862	19.6	24.8	3.1
2,5-hexanedione	888			14.8
3-methyl-3-penten-2-one	902			1.8
2-methyl-1,3-cyclohexanedione	1006	19.6	22.4	12.5
cyclotene	1006	19.0	4.3	10.7
total carbonyls		194.7	147.9	80.8
furans				
2,5-dimethylfuran	715	11.5	11.5	3.6
dihydro-2-methyl-3(2*H*)-furanone	781	3.9	3.7	1.4
2-furfuryl alcohol	835	30.8	32.3	5.1
2-acetylfuran	884	29.2	44.9	43.4
2-furfurylthiol	888	11.9	14.7	9.1
2,5-dimethyl-3(2*H*)-furanone	917	3.1		
5-methyl-2-furfural	934	9.5	17.7	94.7
5-methyl-2-furfurylthiol	993	47.8	47.3	27.8
1-(2-furyl)-1,2-propanedione	1023	3.5	4.3	
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	1046	10.9	14.9	9.5
5-hexyldihydro-2(3*H*)-furanone	1144	8.8	7.9	21.0
2-furancarbothioic acid, *S*-methyl ester	1182			5.7
5-(hydroxymethyl)-2-furfural	1196			30.5
5-acetyl-2-(2-furfuryl)furan	1596	7.6	3.7	
total furans		178.5	202.9	242.3
thiophenes				
thiophene	685	4.1	0.4	6.2
2-methylthiophene	767	11.2	12.4	9.5
3-ethylthiophene	854	2.5		
2,5-dimethylthiophene	859	2.0	2.3	2.5
tetrahydrothiophene-3-one	914	7.6	6.6	3.0
2-thiophenethiol	953	1.6	3.5	8.7
2-methyltetrahydrothiophen-3-one	958	13.9	23.5	12.2
2-thiophenecarboxaldehyde	960			5.6
2-acetylthiophene	1067	8.9	8.9	5.1
2-formyl-5-methylthiophene	1095		6.0	
2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone	1158	259.3	240.4	40.3
5-methyl-2-thienylmethanethiol	1183	2.6	3.6	
3-thiophenecarboxylic acid	1199	9.2		
5-methyl-2-thiophenecarboxylic acid	1255	3.9	4.0	
total thiophenes		326.8	311.6	93.1
thiazoles				
thiazole	726	6.0	5.9	4.7
3-methylisothiazole	796	2.0	2.4	
2,4,5-trimethylthiazole	981	3.6	3.4	
total thiazoles		11.6	11.7	4.7
polysulfides				
3,5-dimethyl-1,2,4-trithiolane	1111	5.5	5.2	
3,6-dimethyl-1,2,4,5-tetrathiane	1401	0.2	2.7	
4,7-dimethyl-1,2,3,5,6-pentathiepane	1667	2.4	1.1	
total cyclic polysulfides		8.1	9.0	
pyrans				
3,4-dihydro-6-methyl-2*H*-pyran	769	4.8	12.4	
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	1124	6.2	16.9	12.3
3,5-dihydroxy-2-methyl-4*H*-pyran-4-one	1178	6.0	7.0	10.0
total pyrans		17.0	30.3	10.5
N-containing heterocyclics				
pyridine	738	2.0	2.6	
methylpyrazine	803	3.2	2.1	
2-acetylpyrrole	1043	28.2	25.6	10.5
total N-containing heterocyclics		33.4	30.3	10.5
others				
acetic acid	650	1.3	7.6	5.3
ethanethioic acid, *S*-methyl ester	758	10.6	12.5	3.9
3-mercaptopropionic acid	973			26.8

of these two analogues were identical, at the value of 1 ng/L in air (Blank and Schieberle, 1993). 2,5-Dimethyl-4-hydroxy-3(2*H*)-thiophenone was also a key product in the model reactions between cystine/Furaneol (Shu et al. 1985), cysteine/Furaneol (Shu et al., 1986), glutathione/Furaneol, sodium sulfide/Furaneol, and alanine/sodium sulfide/Furaneol (Zheng et al., 1997). We also obtained 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone in cysteine/glucose in our previous study (Tai and Ho, 1997). However, we did not identify this compound until after the study was published, and it was therefore not included in the results. The postulated formation pathway was reported by Shu et al. (1985) using Furaneol and hydrogen sulfide as the precursors. The amount of Furaneol obtained in our current study, however, is much less than the amount of 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone obtained at the two higher pH values. 5-Methyl-2-thienylmethanethiol is a possible derivative of 5-methyl-2-furfurylthiol or 5-methyl-2-furfural. 5-Methyl-2-thienylmethanethiol was identified only at pH 6.0 and 8.0 but not at pH 3.0, despite the fact that there were sufficient amounts of 5-methyl-2-furfurylthiol, its potential precursor, across all three pH values. 5-Methyl-2-thienylmethanethiol is a potent odorant due to its low odor threshold (Hofmann and Schieberle, 1997). The unsubstituted thiophene, 2-methylthiophene, 3-ethylthiophene, and 2,5-dimethylthiophene identified in these three reaction systems have been identified in cooked beef, chicken, and pork liver as well (Persson et al., 1973; Nonaka et al., 1967; Janny and Hale, 1974; Mussinan and Walradt, 1974). The sensory property of thiophene was described as sickly and pungent and 2-methylthiophene as green and sweet (Maga, 1975).

Cyclic polysulfides were produced only at pH 6.0 and 8.0 in minor concentrations and were not identified at pH 3.0. The building blocks for cyclic polysulfides are acetaldehyde and hydrogen sulfide, which can be formed through the Strecker degradation of cysteine (Kobayashi and Fujimaki, 1965). 3,5-Dimethyl-1,2,4-trithiolane was originally isolated from boiled beef (Chang et al., 1968). This compound was also produced in a cysteine and glucose reaction system (Tai and Ho, 1997); it was one of the most abundant products. Both 3,6-dimethyl-1,2,4,5-tetrathiane and 4,7-dimethyl-1,2,3,5,6-pentathiepane were identified in the cysteine and glucose reaction model as well. 3,6-Dimethyl-1,2,4,5-tetrathiane is an essential component of boiled mutton flavor (Nixon et al., 1979).

The identified thiazole derivatives include unsubstituted thiazole, 2,4,5-trimethylthiazole, and 3-methylisothiazole. Thiazole derivatives play a substantial role in the characteristic flavor formation of various foods. Nevertheless, they were not the predominant products in both glutathione/glucose and cysteine/glucose (Tai and Ho, 1997) reaction systems. The deficiency of thiazole derivatives is due to the shortage of ammonia, which is provided by glutathione. The release of ammonia is much slower than that of hydrogen sulfide from glutathione. As a result of the competition, thiophene derivatives dominated the products and thiazole derivatives were suppressed in this reaction.

Pyrazines, which are usually identified in the reaction of sugars with amino acids, were barely generated in the reaction systems of pH 6.0 and 8.0. This result is similar to our earlier study of the cysteine and glucose reaction system, by which neither pyrazine nor its derivative was produced (Tai and Ho, 1997; Hofmann and Schieberle, 1997). At pH 3.0, formation of pyrazines was totally blocked even with the high level of precursors such as 1-hydroxy-2-propanone and acetoin existing in this reaction system. High-pH environments are conducive to producing nitrogen-containing heterocyclic compounds, suggesting that the protonized amino group at pH 3.0 is not available to form nitrogen-containing compounds. In addition, freeing the amino group from glutathione is less likely to occur at the lower pH than at the higher pH values. The shortage of ammonia inhibited the interaction between ammonia and α-hydroxyketones, minimizing the formation of pyrazines and other nitrogen-containing compounds. 2-Acetylpyrrole dominated the category of nitrogen-containing compounds at these three pH values. It was generated through the cyclization of aminocarbonyl compounds, which are produced under the Strecker degradation (Nagodawithana, 1995). Another possible formation route is through the participation of 2-acetylfuran and the α-amino group of glutathione or the hydrolyzed constituent amino acids of glutathione.

Fragmentation of sugar is facilitated by the alkaline environments (Feather and Harris, 1973) in which the hydroxyl ions effectively catalyze the transformation of glucose. At pH 3.0, the carbonyls obviously were much less than those obtained at the higher pH values. However, the reason that a greater amount of carbonyls was obtained at pH 6.0 than at pH 8.0 was probably because more carbonyls were further reacted and formed other compounds at pH 8.0. As a consequence, there were fewer carbonyls at pH 8.0. 1-Hydroxy-2-propanone, which is a typical degraded product from glucose, was the most prevalent product in this category. It was also an important product in the cysteine and glucose reaction system. Similar to the result of the cysteine and glucose reaction, only a relatively minor amount of the pyrazine derivatives was identified at pH 6.0 and 8.0, regardless of the rich supply of key precursors such as 1-hydroxy-2-propanone, 2,3-butanedione, acetoin, and 1-hydroxy-2-butanone. 3-Mercapto-2-butanone was described as sulfury and rotten (Hofmann and Schieberle, 1997). Despite its negative sensory character, 3-mercapto-2-butanone is likely to be a potential precursor for thiophenes and thiazoles.

2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one was identified at all three pH values in a moderate amount. It was derived from 1-deoxyhexosone, a degraded compound of glucose, through cyclization and dehydration. 3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one was also derived from 1-deoxyhexosone.

Glutathionesulfonic Acid and Glucose Reaction System. In this reaction series using glutathionesulfonic acid and glucose as the reactants, totals of 31, 31, and 38 compounds were identified at pH 3.0, 6.0, and 8.0 (Table 2). The furans were predominantly produced at pH 3.0 and 6.0. This group occupied ~80% of the identified compounds at both pH values. Among all furans, 5-(hydroxymethyl)-2-furfural was the most abundant. It constituted 87, 84, and 60% of the total furans at pH 3.0, 6.0, and 8.0, respectively. In the reaction system of glutathione and glucose, 5-(hydroxymethyl)-2-furfural was produced only at pH 3.0 and was not significant when compared to the glutathionesulfonic acid and glucose reaction system. The reason that formation of 3-deoxyhexosone, the precursor of 5-(hydroxymethyl)-2-furfural, was such a favored

Table 2. Volatiles (Milligrams per Mole) Obtained from the Reaction of Glutathionesulfonic Acid and Glucose

compound	RI (DB-1)	pH 8.0	pH 6.0	pH 3.0
carbonyls				
3-buten-2-one	621	6.3	11.7	3.9
2-butanone	631	5.1	13.8	14.9
1-hydroxy-2-propanone	668	165.1	58.3	8.9
2-pentanone	680	6.7	10.6	13.9
3-methyl-3-buten-2-one	693	22.0		2.0
acetoin	705	31.7	13.9	2.6
4-methyl-2-pentanone	732	2.4	3.7	6.8
1-hydroxy-2-butanone	748	1.0	2.8	
4-methyl-3-penten-2-one	780	3.4	7.9	11.4
5-methyl-5-hexen-2-one	792			2.2
2-cyclopenten-1-one	799			2.8
4-hydroxy-4-methyl-2-pentanone	814		4.4	10.0
1-(acetyloxy)-2-propanone	826	4.5		
cyclopent-2-ene-1,4-dione	846	1.1		
cyclohexanone	900			1.5
3,4-dimethyl-3-penten-2-one	914	0.7		
2-methylcyclopentanone	970	2.8		
4-propyl-1,3-cyclohexandione	981			5.9
cyclotene	1006	7.7	10.1	5.5
total carbonyls		260.5	137.2	92.3
furans				
2-methylfuran	737	2.6		5.2
dihydro-2-methyl-3(2*H*)-furanone	781	3.7	1.6	
2-furfural	803	42.7	140.0	98.2
2-furfuryl alcohol	835	23.0	52.3	
2(5*H*)-furanone	863	0.7	12.6	7.9
2-acetylfuran	884	2.3	2.5	4.6
2-(1,1-dimethylethyl)-4-methylfuran	915			16.5
5-methyl-2-furfural	934	5.6	1.3	3.0
5-ethyl-2-furfural	1032	18.6	9.8	
1-(2-furyl)-2-hydroxyethanone	1056	7.2	9.6	4.1
5-(hydroxymethyl)-2-furfural	1195	159.4	1244.9	895.9
total furans		265.8	1474.6	1035.4
pyrans				
3,6-dihydro-2*H*-pyran-2-one	883			4.1
3-hydroxy-2-methyl-4*H*-pyran-4-one	1085		5.1	5.1
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	1124		46.5	14.5
3,5-hydroxy-2-methyl-4*H*-pyran-4-one	1178	2.1	6.0	5.2
total pyrans		2.1	57.6	28.9
pyrazines				
pyrazine	722	7.0	1.3	
methylpyrazine	803	16.2	5.0	
2,5-dimethylpyrazine	886	64.5	13.4	
ethylpyrazine	889	1.5		
2,3-dimethylpyrazine	893	2.5		
trimethylpyrazine	980	13.1	6.0	
total pyrazines		104.8	25.7	
pyrroles				
1*H*-pyrrole-2-carboxaldehyde	964	2.1	7.9	
2,5-pyrrolidinedione	1084	15.6	80.4	71.3
5-methyl-1*H*-pyrrole-2-carboxaldehyde	1099		3.7	3.7
total pyrroles		17.7	92.0	75.0
others				
methylbutenol	640	40.6		1.3
acetic acid	650	7.8		
2-methyl-2-butanol	658	10.5	15.2	10.2
3-penten-2-ol	694	39.8	2.2	4.3
ethyl acetate	766	1.4		

trend was not clear. It is possible that it may due to the lack of hydrogen sulfide. Just as the hydroxy ion facilitates glucose fragmentation, hydrogen sulfide could also act as a catalyst. These similar results were also observed in cysteine/glucose versus oxidized cysteine/glucose reaction systems (Tai and Ho, 1997), where 5-(hydroxymethyl)-2-furfural was generated only in the oxidized cysteine and glucose system but not in the reduced cysteine and glucose systems. The yield of 5-(hydroxymethyl)-2-furfural was dramatically reduced at pH 8.0. This outcome was possibly due to the polymerization of 5-(hydroxymethyl)-2-furfural to dark pigments.

The pH value is one of the determining factors of the Maillard reaction. As Mottram and Madruga (1994) have indicated, a change in pH has a significant influence on the production of certain groups of volatiles. For instance, the formation of nitrogen-containing volatiles favors alkaline conditions. Pyrazines were not found in the reaction of pH 3.0 of our study; this result

was in accord with what had been indicated by Mottram and Madruga (1994)—that pyrazines were produced only at pH >5.5. Pyrazines were produced and increased as the pH increased in our study. In fact, pyrazines were the most dominant product of oxidized cysteine and glucose reaction systems (Tai and Ho, 1997). However, the generation of pyrazines in the glutathionesulfonic acid and glucose reaction system was much less as compared to the oxidized cysteine and glucose reaction systems. The glutathionesulfonic acid and glucose reaction system was not able to provide the ammonia for the formation of pyrazines, even though there was enough of the required carbonyls. Methylpyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine were the leading products of this class. Their building blocks, 1-hydroxy-2-propanone or acetoin, were the richest carbonyls at pH 6.0 and 8.0.

The reason that the reaction of glutathionesulfonic acid and glucose could not produce sulfur-containing compounds is the lack of hydrogen sulfide which was supplied by glutathione. The oxidation of the thiol group of glutathione to the sulfate group of glutathionesulfonic acid did restrict the reactivity.

Conclusions. The influence of oxidation of glutathione on the Maillard-type flavor formation is significant. The oxidized glutathione, glutathionesulfonic acid, no longer produced the sulfur-containing compounds while furan and pyrazine derivatives dramatically increased. Furan derivatives dominated the reaction product in quantity at pH 3.0 and 6.0. The influence of pH on the volatile formation is obvious in both reduced and oxidized glutathione reaction systems. Thiophene derivatives, polysulfides, nitrogen-containing heterocyclic compounds, and carbonyls were favorable products in the reactions at higher pH values. The acidic environments were the suitable conditions for the formation of furan derivatives.

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