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ARTICLE

Investigation of the Aroma-Active Compounds Formed in the Maillard Reaction between Glutathione and Reducing Sugars

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Aroma-active compounds formed during the thermal reaction between glutathione (GSH) and reducing sugars were analyzed by gas chromatography−mass spectrometry (GC-MS) and GC−olfactometry (GC-O) with aroma extract dilution analysis (AEDA). Application of AEDA to glutathione Maillard reaction products (GSH MRPs) led to the identification of 19 aroma-active compounds in the thermal reaction of glutathione with glucose or fructose. In addition, the carbohydrate module labeling (CAMOLA) approach was also employed to elucidate the formation pathways for selected target sulfur aroma compounds, such as 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde, which have not been reported previously. The intact carbon skeleton of glucose via 3-deoxyhexosone is incorporated into 5-methylthiophene-2-carbaldehyde with the hydrogen sulfide of GSH. On the other hand, the formation of 3-methylthiophene-2-carbaldehyde may occur via the recombination of a C-4 sugar fragment and mercaptoacetaldehyde.

KEYWORDS: Glutathione; aroma-active compounds; 5-methylthiophene-2-carbaldehyde; 3-methylthiophene-2-carbaldehyde; carbohydrate module labeling

INTRODUCTION

Glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH), which is a tripeptide that contains an unusual peptide linkage between the cysteine amine group and the glutamate carboxyl group, is a major source of intracellular low molecular weight amine and thiol groups in most living cells, from microorganisms to humans (*1*). The primary biological function of GSH is acting as a nonenzymatic reducing agent that helps to keep the cysteine thiol side chain in a reduced state (*2*). GSH also acts as an antioxidant, protecting cells from toxins such as free radicals and peroxides, and as a result of its reducing and nucleophilic properties, it prevents cellular oxidative damage in most cells by maintaining their redox potential (*3*).

In food systems, GSH plays an important role in the formation of diverse sulfur-containing volatile compounds during the heating processing via the release of hydrogen sulfide. Zheng et al. (*4*) reported that hydrogen sulfide is released from the cysteine residue in GSH and participates in the generation of sulfur-containing aroma compounds during thermal reactions. It is therefore a key precursor for diverse meaty-type flavor components, such as thiols, thiophenes, thiazoles, and polysulfides, which contain sulfur atom(s), through the Maillard reaction and Strecker degradation (*4*−*6*). Sulfur-containing aroma compounds have been found in vegetables, roasted coffee, roasted seeds, wheat bread, cooked meats, and many different thermally processed foods (*5*−*10*). In general, sulfur-containing aroma compounds possess low odor threshold values and characteristic odor notes and significantly contribute to the overall cooked-meat aroma notes of diverse foods (*6*). In addition, both GSH and its Maillard reaction products (MRPs) were recently shown to have a taste-enhancing ("kokumi") property (*11*).

The potent flavors of thermally processed foods are caused mainly by the formation of Maillard-type reaction products. Because the Maillard reaction can induce a complex reaction cascade that generates diverse flavors, isotopically labeled compounds are very useful for clarifying the reaction pathways and intermediates therein that lead to the formation of certain target molecules. The carbohydrate module labeling (CAMOLA) technique was developed to evaluate the number and relative importance of different pathways that lead to a certain target molecule. In particular, this technique uses a combination of $^{13}C_6$-labeled and unlabeled glucose in a 1:1 ratio to explain the extent of fragmentation of the sugar skeleton and the formation of key transient intermediates involved in the formation of flavor molecules (*12*, *13*). If these transient intermediates combine, a statistically ruled isotopomer of the respective product is formed from these modules. This approach has been used to clarify formation pathways and gain insight into the fragmentation of precursors of MRPs (*12*−*16*).

Although the volatile components have been characterized in various model systems containing GSH [e.g., the interaction with inosine 5′-monophosphate (*5*), furaneol (*4*), or 2,4-decadienal (*16*)] and thermal reactions performed under different pH conditions (*6*), aroma-active compounds and their formation pathways in GSH MRPs have not been determined yet. Therefore, the first objective of this study was to determine the aroma-active compounds in GSH MRPs using aroma extract dilution analysis (AEDA). Another objective was to elucidate the formation pathways for some of the sulfur-containing aroma-active compounds, such as 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde, which are formed from glucose and GSH during

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the thermal reaction. The CAMOLA approach was employed to clarify the formation pathways and evaluate their relative importance.

MATERIALS AND METHODS

Chemicals. L-GSH, D-glucose ([$^{12}C_6$]-D-glucose), D-fructose, *n*-alkane standards (C_8−C_{22}), and sodium sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Dichloromethane of HPLC grade was obtained from Fisher Scientific (Seoul, South Korea). All authentic standard compounds used in this study were obtained from Sigma-Aldrich. For the CAMOLA study, [U-$^{13}C_6$]-D-glucose was obtained from ISOTEC (Milwaukee, WI), and [1-^{13}C]-D-glucose, [2-^{13}C]-D-glucose, and [3-^{13}C]-D-glucose were obtained from Cambridge Isotope Laboratories (Andover, MA).

Model Maillard Reaction Systems. GSH (0.01 M) and either D-glucose or D-fructose (0.01 M) were dissolved in 100 mL of HPLC-grade water (Fisher Scientific). The reaction mixtures were adjusted to pH 7.5 and then sealed in a 200 mL stainless steel cylinder reactor. The cylinder was heated in a 160 °C drying oven for 2 h. After the thermal reaction, the cylinder was cooled in cold water before the cap was opened.

Extraction of Volatile Maillard Reaction Products. After the reaction mixture was cooled, the volatile components were extracted using a simultaneous steam distillation and solvent extraction (SDE) method with a Likens−Nickerson (L-N) apparatus with 50 mL of dichloromethane. After the sample started boiling, SDE was run continuously for 2 h. The extract was dehydrated using anhydrous sodium sulfate and filtered on Advantec 110 mm filter paper (Toyo Roshi Kaisha, Tokyo, Japan) before being concentrated to a final volume of 0.1 mL (for gas chromatography−mass spectrometry analysis) or 0.3 mL (for gas chromatography−olfactometry analysis) using a gentle stream of nitrogen gas.

Analysis by Gas Chromatography−Mass Spectrometry (GC-MS). The volatile extracts from MRPs were analyzed by GC-MS, using a gas chromatograph and mass selective detector (6890N and 5975, respectively; Agilent Technologies, Palo Alto, CA) equipped with a DB-5 ms column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness; J&W Scientific, Folsom, CA). Helium was run as a carrier gas at a constant column flow rate of 0.8 mL/min. A 1 μL aliquot of the MRP extract was injected into the GC column using the splitless injection mode. The oven temperature was initially held at 40 °C for 4 min, then raised to 200 °C at a rate of 2 °C/min, and held there for 10 min. The temperatures of the injector and detector transfer line were 200 and 250 °C, respectively. The mass detector was operated in electron impact mode with ionization energy of 70 eV, a scanning range of 33−550 amu, and a scan rate of 1.4 scans/s.

Aroma Extract Dilution Analysis. The aroma-active compounds of the MRPs were analyzed by GC-O with AEDA. GC-O was conducted using a GC (CP-3350, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port (Alltech Associates, Deerfield, IL) with a DB-5 column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness; J&W Scientific). Effluent from the end of the GC column was split equally 1:1 between the FID and the sniffing port. After the 1 μL aliquot was injected into the GC column, the GC oven temperature was held at 40 °C for 4 min and then increased to 200 °C at 5 °C/min and held there for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively.

The odor descriptions and flavor dilution (FD) factors of each odorant were determined by AEDA (*17*). The original extracts (final volume = 0.3 mL) were diluted stepwise with dichloromethane [each dilution was 1:1 (v/v)], and each dilution was analyzed until no odor was perceivable at the sniffing port. The FD factors were defined as the highest dilution at which a compound could be perceived. Two trained panelists determined the odor descriptions. Their maximum value was then provided as the FD factor of that compound.

Identification of Aroma-Active Compounds. Aroma-active compounds were positively identified by comparing their mass spectral data, linear retention indices (RIs), and aroma properties perceived at the sniffing port with those of authentic compounds. The RI of each compound was calculated using *n*-alkanes C_8−C_{22} as external references (*18*). Otherwise, tentative identification was based on matching RIs and mass spectra of unknowns with those in the Wiley mass spectral database or comparing the RIs and aroma properties of unknowns to those of authentic standards.

Carbohydrate Module Labeling (CAMOLA) Experiment. Equimolar amounts of 0.01 M fully labeled [$^{13}C_6$]-D-glucose and 0.01 M unlabeled D-glucose ([$^{12}C_6$]-D-glucose) were reacted with 0.01 M GSH in a drying oven at 160 °C for 2 h. The reaction mixture was then extracted using the SDE method as described above. The extracts were dehydrated over anhydrous sodium sulfate, concentrated under a gentle stream of nitrogen gas, and then subjected to GC-MS analysis.

Calculation of Isotopomer Proportions. The isotopomer ratios were calculated using the relative signal intensities of the analyzed ions in the mass spectrum of the respective compound. The values of the calculated isotopomer proportions for aroma-active compounds were corrected by subtracting the naturally occurring percentages of ^{13}C (1.1%), ^{33}S (0.76%), and ^{34}S (4.20%). The loss of hydrogen observed with the molecular ion signal was determined in the labeled molecular ions by the ratio $[M^+ - 1]/[M^+]$. Additional data processing was required for 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde; calculation of the isotopomer ratio was based on the $[M^+ - 1]$ ion signal instead of $[M^+]$ because the former was more intense than the molecular ion signal. After correction, any isotopomer percentages below 1% were taken to be 0%.

RESULTS AND DISCUSSION

Aroma-Active Compounds in Glutathione Maillard Reaction Products from Interactions with Different Reducing Sugars. **Table 1** lists the aroma-active compounds identified in GSH MRPs according to the different sugars, their aroma properties, RI values, and FD factors for DB-5 columns. Totals of 17 and 18 aroma-active compounds were identified using AEDA in GSH−glucose (GSH-GLU) and GSH−fructose (GSH-FRU) MRPs, respectively. Some of them (compounds **1**−**11**, **13**, **14**, **16**, and **18**−**21**) were positively identified by comparing their mass spectra, RI values, and aroma properties with those of authentic standards. Although four compounds (**12**, **15**, **17**, and **22**) had a characteristic odor note in GSH MRPs, they could not be positively identified. The total ion chromatograms and flavor dilution chromatograms of aroma-active compounds in glutathione Maillard reaction products are shown in **Figure 1**.

2,5-Dimethylthiophene (**7**, metallic and sulfurous) and 5-methylfuran-2-carbaldehyde (**10**, almond and caramel-like), which had the highest FD factors, were detected in the GSH-GLU MRPs. 2,5-Dimethylthiophene (**7**) has been identified in cooked beef, chicken, and pork liver (*19*, *20*). 5-Methylfuran-2-carbaldehyde, which originated from sugar degradation, has been reported to be one of the major volatile components of the reaction mixture of GSH and glucose (*16*).

2-Methylfuran-3-thiol (**6**, cooked meaty), 2,5-dimethylthiophene (**7**, metallic and sulfurous), 1-furan-2-ylethanone (2-acetylfuran; **8**, balsamic), and 5-methylfuran-2-carbaldehyde (**10**, almond and caramel-like) appeared to have the highest FD factors in the GSH-FRU MRP. 2-Methylfuran-3-thiol, which has a meaty odor note, is reportedly a key aroma contributor to several thermally processed foods, such as cooked beef and roasted coffee, having a low odor threshold value of 0.0025 ng/L in air (*21*). Moreover, it has been identified as being a high aroma value compound in chicken and beef broths and enzyme-hydrolyzed soybean protein (*22*). 1-Furan-2-ylethanone is an important balsamic and cinnamic odorant in natural or processed foods. It has been detected in fruits, flowers, wine, and soy sauce (*23*, *24*). Some studies of the Maillard model system have also determined 1-furan-2-ylethanone to be an important reaction product, and Wang and Ho (*25*) proposed that it was generated from 1,4-dideoxyosone via cyclization and recombination.

2-Methylthiophene (**1**, sulfurous), which was described as a mildly sulfurous and green odorant, had relatively high FD factors of 16 and 64 in the GSH-GLU and GSH-FRU MRPs, respectively. It has previously identified as one of the aroma-active

Table 1. Aroma-Active Compounds Identified in the Thermal Reaction of Glutathione with Glucose or Fructose

compd no.	RI[a]	aroma-active compound	aroma property[b]	FD factor[c] GSH-GLU[d]	FD factor[c] GSH-FRU[e]	ID[g]
1	<800	2-methylthiophene	sulfurous	16	64	MS/RI/odor
2	<800	1,3-thiazole	green, nutty	4	4	MS/RI/odor
3	820	2-methylpyrazine	boiled soybeans and popcorn-like	2	8	MS/RI/odor
4	823	furan-2-carbaldehyde (furfural)	almond-like	32	32	MS/RI/odor
5	856	2-ethylthiophene	sulfurous	4	16	MS/RI/odor
6	860	2-methylfuran-3-thiol (2-methyl-3-furanthiol)	cooked meaty	64	128	MS/RI/odor
7	862	2,5-dimethylthiophene	metallic, sulfurous	128	128	MS/RI/odor
8	905	1-furan-2-ylethanone (2-acetylfuran)	balsamic	16	128	MS/RI/odor
9	948	thiolan-3-one (4,5-dihydro-3(2*H*)-thiophenone)	roasted, meaty	16	32	MS/RI/odor
10	955	5-methylfuran-2-carbaldehyde(5-methyl-2-furfural)	almond-like, caramel-like	128	128	MS/RI/odor
11	965	thiophene-2-thiol	burnt caramel, roasted coffee	8	16	MS/RI/odor
12	1031	unknown	onion-like, metallic	32	128	odor
13	1048	2-methylthiophene-3-thiol	medicinal	16	64	MS/RI/odor
14	1061	1-(1*H*-pyrrol-2-yl)ethanone (2-acetylpyrrole)	nutty, bread-like	8	2	MS/RI/odor
15	1072	unknown	mushroom and soy sauce-like	4	16	odor
16	1081	1-thiophen-2-ylethanone (2-acetylthiophene)	sulfurous	4	8	MS/RI/odor
17	1102	unknown	cabbage-like	2	0[f]	odor
18	1105	2-thiophen-2-ylethanol	sulfurous	0	16	MS/RI/odor
19	1108	5-methylthiophene-2-carbaldehyde (2-formyl-5-methylthiophene)	sulfurous	8	4	MS/RI/odor
20	1109	3-methylthiophene-2-carbaldehyde (2-formyl-3-methylthiophene)	medicinal	4	4	MS/RI/odor
21	1217	4-hydroxy-5-methylfuran-3-one (HMF)	sweet	4	4	MS/RI/odor
22	1535	unknown	soy sauce-like	4	2	odor

[a] RIs were determined using *n*-paraffins C_7−C_{22} as external references. [b] Aroma properties were perceived at the sniffing port. [c] FD factors by two panelists. [d] Glutathion−glucose Maillard reaction product. [e] Glutathion−fructose Maillard reaction product. [f] Not detected. [g] Aroma-active compounds were identified on the basis of the following criteria: MS/RI/odor, mass spectrum, RI, and aroma properties were consistent with those of authentic standards; odor, odor was perceived only at sniffing port by two panelists.

compounds in cooked meat and extruded wheat flour with added cysteine (*26*). 2-Methylthiophene is formed from the reaction of carbohydrate with hydrogen sulfide or amino acids such as cysteine (*26*). Furan-2-carbaldehyde (furfural; **4**, almond-like) exhibited a relatively high FD factor in both the GSH-GLU (FD factor = 32) and GSH-FRU (FD factor = 32) MRPs. Furan-2-carbaldehyde, with a caramel, sweet, and fruity odor note, was an important contributor in heated hydrolyzed vegetable protein and heated aqueous soy extracts (*27*).

1,3-Thiazole (**2**, green and nutty), 2-methylpyrazine (**3**, boiled soybean and popcorn-like), 1-(1*H*-pyrrol-2-yl)ethanone (2-acetylpyrrole; **14**, nutty and bread-like), 1-thiophen-2-ylethanone (2-acetylthiophene; **16**, sulfurous), 5-methylthiophene-2-carbaldehyde (**19**, sulfurous), 3-methylthiophene-2-carbaldehyde (**20**, medicinal), and 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF; **21**, sweet) had relatively low FD factors of 8 or below in both of the GSH MRPs. 4-Hydroxy-5-methylfuran-3-one, with a caramel-like, burnt, and roasted chicory root-like odor note, is one of the Amadori products formed during the Maillard reaction (*7*). 3-Methylthiophene-2-carbaldehyde was identified in chicken, and 5-methylthiophene-2-carbaldehyde, which has an earthy-roast odor note, was reportedly found in cooked beef (*28*).

Ho and co-workers (*4*−*6*, *17*) investigated the model reaction between GSH and glucose. The qualitative and quantitative analyses by GC-MS of the volatile compounds generated from the reaction demonstrated the presence of carbonyls, furans, pyrans, and sulfur- and nitrogen-containing heterocyclic compounds. The major classes of compounds were furans, thiophenes, and polysulfides. The dominant GSH MRPs with the highest yields were the furans. On the other hand, our study identified thiophenes and thiols as major components in GSH MRPs that contributed significantly to the aroma property. They could be the most important aroma-active compounds due to their characteristic odor notes and low odor thresholds. These results indicate that the cooked meat-like and sulfurous odor of both GSH-MRPs might be related to sulfur-containing compounds such as 2-methylthiophene, 2-ethylthiophene, 2-methylfuran-3-thiol (2-methyl-3-furanthiol), 2,5-dimethylthiophene, thiolan-3-one [4,5-dihydro-3(2*H*)-thiophenone], thiophene-2-thiol, 2-methylthiophene-3-thiol, 1-thiophen-2-ylethanone (2-acetylthiophene), 5-methylthiophene-2-carbaldehyde, and 3-methylthiophene-2-carbaldehyde. In addition, 2-methylfuran-3-thiol, 2,5-dimethylthiophene, and 5-methylfuran-2-carbaldehyde could contribute to the distinctive odor notes of GSH-GLU MRP, whereas 2-methylthiophene, 2-methylfuran-3-thiol, 2,5-dimethylthiophene, 1-furan-2-ylethanone, 5-methylthiophene-2-carbaldehyde, an unknown (**12**, onion-like and metallic), and 2-methylthiophene-3-thiol were determined as key aroma-active compounds in GSH-FRU MRP. 2-Thiophene-2-ylethanol (**18**, sulfurous) was not detected in GSH-GLU MRP, despite its high FD factor in GSH-FRU MRP.

Elucidation of Aroma-Active Compounds Formation. The AEDA results indicated that the 17 aroma-active compounds identified in the GSH-GLU MRP listed in **Table 1** include diverse sulfur-containing compounds. The chemical structures of these compounds are shown in **Figure 2**. The major aroma-active compounds formed in the GSH Maillard reaction were thiophene and its derivatives, the abundance of which may be due to the hydrogen sulfide easily released, which is provided by the cysteine residue in GSH. It was reported that the release of hydrogen sulfide from GSH is much faster than that of ammonia (*4*, *6*). Therefore, thiophene derivatives dominated the major sulfur aroma components, whereas thiazole derivatives, which need additional nitrogen for their formation, were almost undetectable in GSH MRPs. Also, Ho et al. (*33*) investigated the Maillard volatile generation of cysteine-containing peptides, GSH, γ-glu-cys, cys-gly, and gly-cys, with glucose and found that GSH generated larger amounts of thiophenes than of thiazoles.

The mass spectra of the aroma-active compounds identified from the thermal reaction of [$^{12}C_6$]-glucose/[$^{13}C_6$]-glucose and GSH were analyzed on the basis of the mass-to-charge ratios

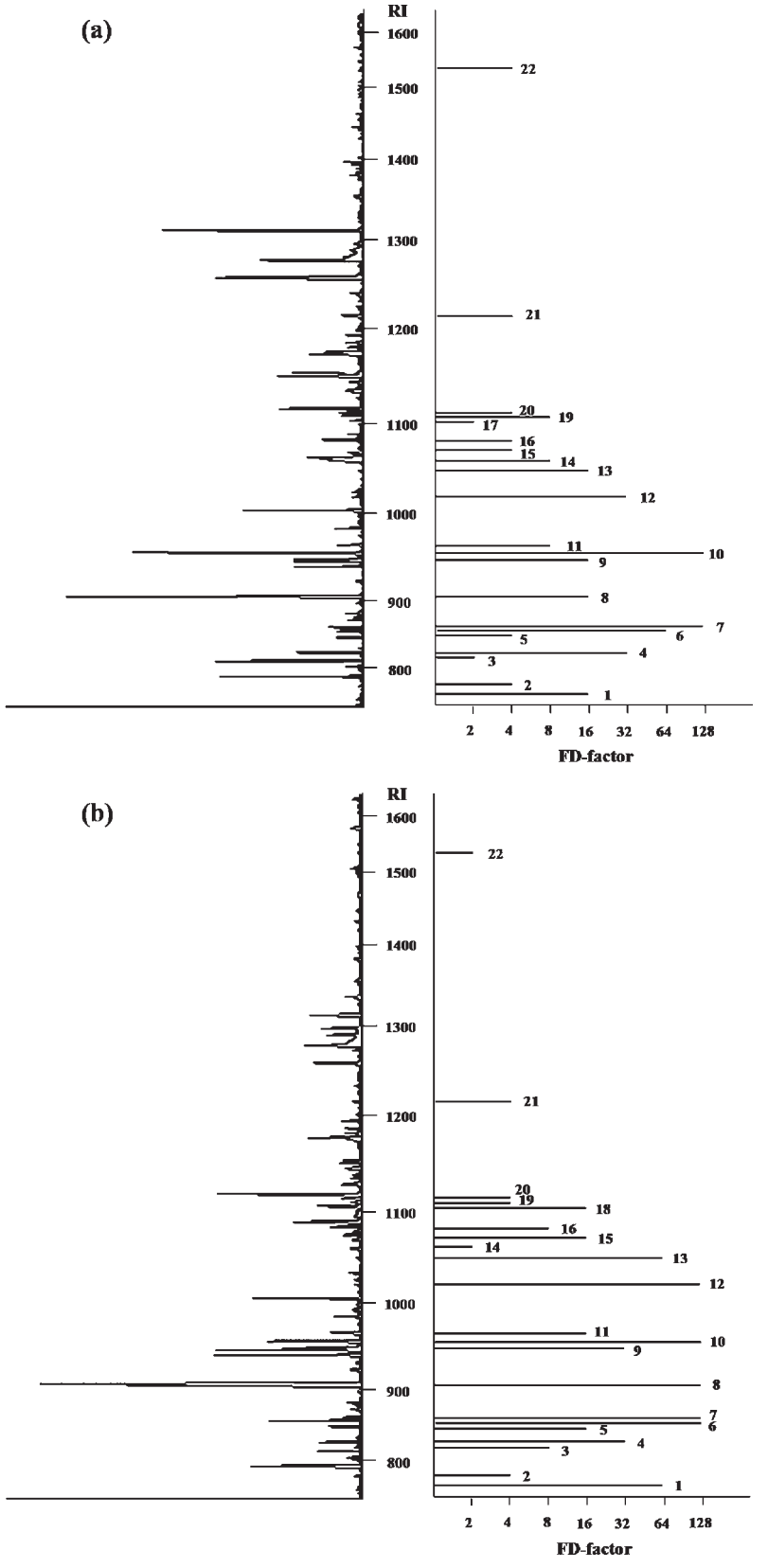

Figure 1. GC-MS total ion chromatograms and flavor dilution chromatograms of aroma-active compounds in glutathione Maillard reaction products: (**a**) glutathione−glucose Maillard reaction product; (**b**) glutathione−fructose Maillard reaction product. Compound numbers corresponding to peak numbers are represented in **Table 1**.

(m/z) of the molecular ions of the isotopomers, which exhibited signals with mass differences of up to $M^+ + 6$ as compared to the spectra obtained from GSH-unlabeled glucose MRP. **Table 2** lists the identified aroma-active compounds and the proportion of their ^{13}C-labeled isotope molecules. The isotopomers indicated that the molecules comprise either unlabeled carbons, fully ^{13}C-labeled carbons, or a mixture of labeled and unlabeled carbon fragments. In the cases of 2-methylthiophene (**1**), furan-2-carbaldehyde (**4**),

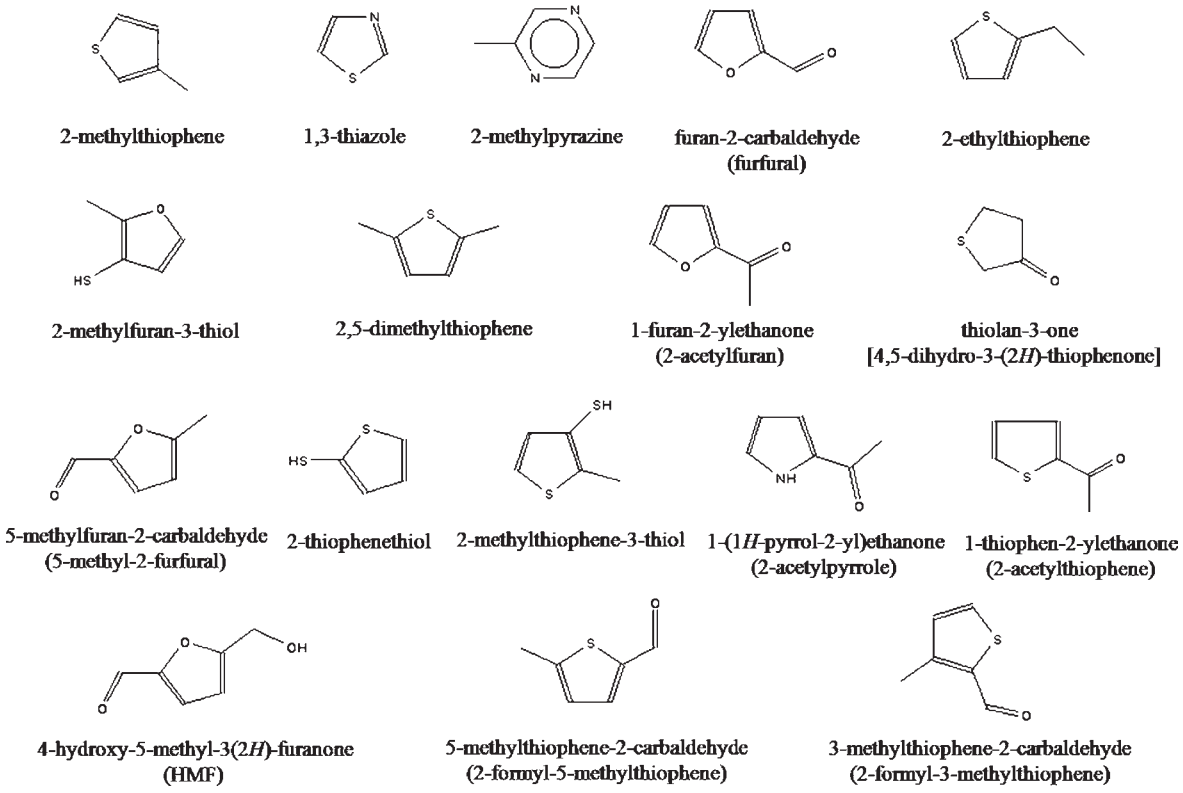

Figure 2. Aroma-active compounds formed from the thermal reaction of glutathione and glucose.

Table 2. Proportion of Isotopomers of Aroma-Active Compounds Formed from the Reaction of Glutathione with [$^{12}C_6$]-Glucose and [$^{13}C_6$]-Glucose

			proportion of labeled carbon atoms in the molecule[c] (%)						
compd no.[a]	aroma-active compound[b]	*m/z* (M^+)	0[d]	1	2	3	4	5	6
1	2-methylthiophene	98	35	6	15	12	1	31	
2	1,3-thiazole	85	52	34	9	5			
3	2-methylpyrazine	94	24	2	23	24	2	25	
4	furan-2-carbaldehyde (furfural)	96	42	2	1	1	1	53	
5	2-ethylthiophene	112	47	4	2	0	0	10	37
6	2-methylfuran-3-thiol (2-methyl-3-furanthiol)	114	32	4	12	6	12	34	
7	2,5-dimethylthiophene	112	46	6	3	1	3	0	41
8	1-furan-2-ylethanone (2-acetylfuran)	110	45	3	0	0	1	2	49
9	thiolan-3-one (4,5-dihydro-3(2*H*)-thiophenone)	102	46	3	3	4	44		
10	5-methylfuran-2-carbaldehyde (5-methyl-2-furfural)	110	46	3	0	0	1	0	50
11	thiophene-2-thiol	116	57	5	12	3	23		
13	2-methylthiophene-3-thiol	130	33	5	12	29	6	15	
14	1-(1*H*-pyrrol-2-yl)ethanone (2-acetylpyrrole)	109	45	3	0	0	0	2	50
16	1-thiophen-2-ylethanone (2-acetylthiophene)	126	47	3	3	3	3	1	40
19	5-methylthiophene-2-carbaldehyde (2-formyl-5-methylthiophene)	126	42	7	4	0	6	0	41
20	3-methylthiophene-2-carbaldehyde (2-formyl-3-methylthiophene)	126	45	5	4	0	41	4	1
21	4-hydroxy-5-methylfuran-3-one (HMF)	126	48	0	0	4	3	10	35

[a] Compd no. represented in **Table 1**. [b] Aroma-active compounds were identified in the thermal reaction of glutathione and glucose by comparing the mass spectra and retention indices with those of authentic compounds. [c] Values were corrected by subtracting the naturally occurring percentages of ^{13}C (1.10%), ^{33}S (0.76%), and ^{34}S (4.20%) in $M^+ + 1$ and $M^+ + 2$. The loss of hydrogen observed with the molecular ion in EI-MS was also corrected in the labeled molecular ions by the ratio $(M^+ - 1)/M^+$. [d] Number of ^{13}C atoms in the molecule.

2,5-dimethylthiophene (**7**), 5-methylfuran-2-carbaldehyde (**10**), 5-methylthiophene-2-carbaldehyde (**19**), and 3-methylthiophene-2-carbaldehyde (**20**), the values for the labeled $[M^+ - 1]$ ion were corrected by the ratio $[M^+]/([M^+ - 1]$ due to the significant loss of hydrogen.

As indicated in **Table 2**, 5-methylthiophene-2-carbaldehyde (**19**) had the isotopomers of $[M^+]$ (*m/z* 126) and $[M^+ + 6]$ (*m/z* 132). Other mixed isotopomers at *m/z* 127−131 were almost undetectable, indicating that the unlabeled and the 6-fold-labeled molecules were present at approximately 1:1. That is, the carbon skeleton of glucose chain remained intact for the formation of 5-methylthiophene-2-carbaldehyde. Also, 2,5-dimethylthiophene (**7**), 1-furan-2-ylethanone (2-acetylfuran) (**8**), 5-methylfuran-2-carbaldehyde (**10**), 1-(1*H*-pyrrol-2-yl)ethanone (2-acetylpyrrole) (**14**), 1-thiophen-2-ylethanone (2-acetylthiophene) (**16**), and 5-methylthiophene-2-carbaldehyde (**19**) showed isotopomeric distribution patterns similar to either only unlabeled or fully ^{13}C-labeled compounds at approximately 1:1. These data suggest that these compounds were formed from the intact carbon skeleton of a C-6 glucose chain without the recombination of

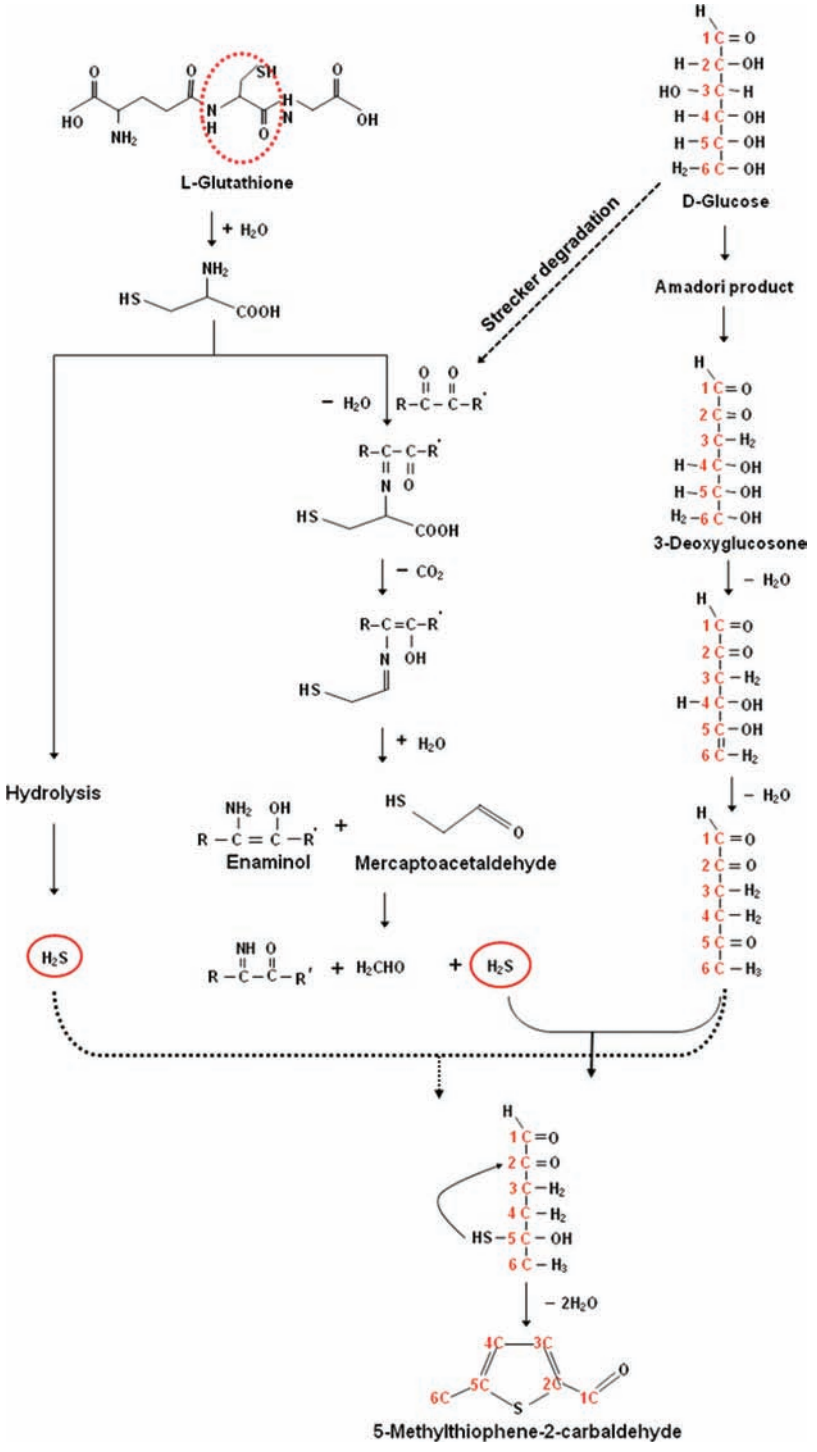

Figure 3. Possible formation pathway for 5-methylthiophene-2-carbaldehyde from the thermal reaction of glutathione and glucose.

glucose fragments. Wang and Ho (*25*) established that the reaction reactivity and mechanism pathway for 1-furan-2-ylethanone (2-acetylfuran) formation differed between ribose and glucose and proposed that it could not be generated from ribose in the presence of cysteine, whereas formaldehyde could form 1-furan-2-ylethanone by the reaction of ribose and glycine.

Furan-2-carbaldehyde (**4**) was formed from the carbon skeleton of glucose. Unlabeled isotopomer and isotopomer with complete 5-fold labeling were found. This means that furan-2-carbaldehyde was formed from glucose through the loss of one carbon atom. These data support the formation of furan-2-carbaldehyde from ribose and cysteine, as postulated by Cerny and Davidek (*15*). Other model experiments with hydrogen sulfide and furan-2-carbaldehyde, which was formed in the Maillard reaction from pentose via 3-deoxyosone, showed that this intermediate is important for the formation of 2-furfurylthiol (*29*).

The isotopomeric distribution patterns of thiolan-3-one [4,5-dihydro-3(2*H*)-thiophenone, **9**] were similar to those of furan-2-carbaldehyde, indicating that unlabeled isotopomer and isotopomer with complete 4-fold labeling appeared at 1:1. This suggests that thiolan-3-one was formed from glucose by the loss of two carbon atoms, but the exact number of carbon units lost from glucose was not confirmed.

Unlabeled ([M^+], m/z 126) and 4-fold-labeled ([$M^+ + 4$], m/z130) isotopomers for 3-methylthiophene-2-carbaldehyde (**20**)

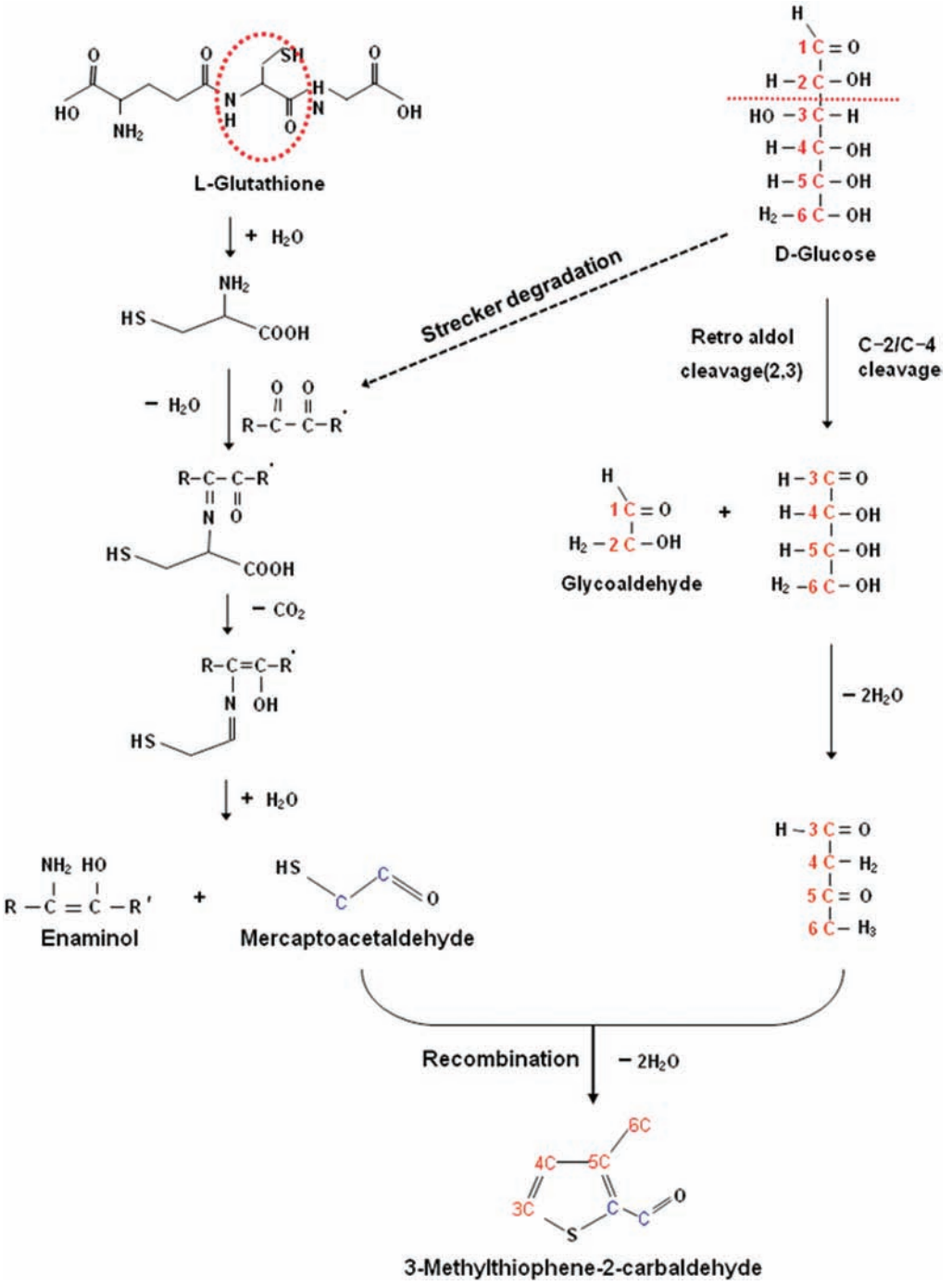

Figure 4. Possible formation pathway for 3-methylthiophene-2-carbaldehyde from the thermal reaction of glutathione and glucose.

were found at approximately 1:1, whereas other labeled isotopomers (m/z 127, 128, 129, 131, and 132) were practically undetectable, which means that the C-4 fragment was split from the glucose carbon skeleton in the formation pathway for 3-methylthiophene-2-carbaldehyde. In the carbon skeleton of 3-methylthiophene-2-carbaldehyde, C-4 was derived from glucose and the C-2 fragment came partly from GSH. For 1,3-thiazole, a molecular ion was identified that was 52% unlabeled ([M^+], m/z 85) and 34% singly labeled ([$M^+ + 1$], m/z 86), suggesting that it was formed from cysteamine derived from cysteine and formaldehyde from glucose to produce thiazole by oxidation of the former, as proposed by Shibamoto (*29*).

The CAMOLA approach was employed to clarify the formation pathways for selected target sulfur aroma compounds, such as 5-methylthiophene-2-carbaldehyde (**19**) and 3-methylthiophene-2-carbaldehyde (**20**). Two methylthiophene-2-carbaldehydes were present as isomers with almost identical mass spectra but somewhat different retention times. These compounds showed different isotopomeric distribution patterns, suggesting that their formations follow different pathways. **Figure 3** illustrates a possible formation pathway for 5-methylthiophene-2-carbaldehyde produced from GSH-GLU MRP. The first step is the formation of hydrogen sulfide, which can be derived from the hydrolysis of cysteine or from the Strecker degradation of cysteine in the presence of dicarbonyl compounds (*30*). The second step involves the intact carbon skeleton of C-6 glucose via 3-deoxyhexosone, which is produced from Amadori intermediates in the Maillard reaction. This intermediate (3-deoxyhexosone) may eliminate water and then react with hydrogen sulfide. Cyclization and elimination of water could

finally yield 5-methylthiophene-2-carbaldehyde. 5-Methylthiophene-2-carbaldehyde has been commonly found in mixtures of cysteine and different sugars (*31*). A previous study found that 5-methylthiophene-2-carbaldehyde was formed from the interaction between C-5 sugar and mercaptoacetaldehyde or by the thermal degradation of C-6 sugars followed by a reaction with hydrogen sulfide in different model systems (*32*).

On the basis of the isotopomeric distribution results, a possible formation pathway for 3-methylthiophene-2-carbaldehyde (**20**) in GSH-GLU MRP is shown in **Figure 4**. The first step forms a glucose C-4 fragment by retro-aldol cleavage. In the next step, Strecker degradation of GSH generates mercaptoacetaldehyde, which is a well-known Strecker degradation product of cysteine (*31*). Strecker degradation or thermal decomposition of cysteine at high temperatures can generate mercaptoacetaldehyde, acetaldehyde, cysteineamine, ethane-1,2-diol, hydrogen sulfide, and ammonia, all of which readily react with sugar degradation products and with each other to form diverse heterocyclic compounds (*32*). One of the intermediates, mercaptoacetaldehyde, reacts with the glucose C-4 fragment. Finally, 3-methylthiophene-2-carbaldehyde can be produced via recombination and cyclization of mercaptoacetaldehyde and glucose C-4 fragment.

[1-^{13}C]-Glucose, [2-^{13}C]-glucose, and [3-^{13}C]-glucose were used in the thermal reaction with GSH to determine how the intact glucose chain is arranged and which carbon position is split off from the intact glucose chain during the formation of 5-methylthiophene-2-carbaldehyde (**19**) and 3-methylthiophene-2-carbaldehyde (**20**), respectively (data not shown). The MS data of 3-methylthiophene-2-carbaldehyde, formed in the reaction between GSH and either [1-^{13}C]-glucose or [2-^{13}C]-glucose, revealed most of the molecular ions were totally unlabeled, indicating that neither the C-1 nor C-2 atom of glucose is involved in the formation of 3-methylthiophene-2-carbaldehyde. A molecular ion that was singly labeled ([$M^+ + 1$], m/z 127) by 83% was found in the reaction between GSH and [3-^{13}C]-glucose. This suggests the molecular structure [3-^{13}C]-3-methylthiophene-2-carbaldehyde, with the labeling position at C-3, which is in agreement with the formation pathway proposed from glucose via the glucose C-4 fragment, generated by cleavage between the C-2 and C-3 atoms of glucose. The MS data clearly show the labeling position of the ^{13}C atom in the molecules generated from labeled glucose ([1-^{13}C]-, [2-^{13}C]-, and [3-^{13}C]-glucose), and GSH. Therefore, the labeling positions of [^{13}C]-glucose were assigned to the carbon skeletons of 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde (**Figures 3** and **4**).

The use of labeled and unlabeled precursors in the thermal reaction was proposed to gain insight into the fragmentation of precursors for the formation of sulfur-containing aroma compounds. The CAMOLA experiments demonstrated that formation of 5-methylthiophene-2-carbaldehyde (**19**) and 3-methylthiophene-2-carbaldehyde (**20**) occurs via the recombination of fragments that may originate from both GSH and glucose. The possible formation pathways for 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde were identified through the recombination of key transient intermediates. The intact carbon skeleton of glucose via 3-deoxyhexosone is incorporated into 5-methylthiophene-2-carbaldehyde, with hydrogen sulfide released from GSH. The formation of 3-methylthiophene-2-carbaldehyde could be explained via the recombination of the C-4 glucose fragment and mercaptoacetaldehyde.

ABBREVIATIONS USED

GSH, glutathione; GSH MRP, glutathione Maillard reaction product; GC-MS, gas chromatography−mass spectrometry; GC-O, gas chromatography−olfactometry; AEDA, aroma extract dilution analysis; FD, flavor dilution; RIs, retention indices; CAMOLA, carbohydrate module labeling.

LITERATURE CITED

(1) Gonzalez, C.; Sanz-Alyayate, M. T.; Agapito; Obeso, A. Effects of reducing agents on glutathione metabolism and the function of carotid body chemoreceptor cells. *Biol. Chem.* **2004**, *385*, 265–274.

(2) Anderson, M.; Meister, A. Dynamic state of glutathione in blood plasma. *J. Biol. Chem.* **1983**, *255*, 9530–9533.

(3) Kenneth, D. T. Redox in redux: emergent roles for glutathione S-transferase P (GSTP) in regulation of cell signaling and S-glutathionylation. *Biochem. Pharmacol.* **2007**, *73*, 1257–1269.

(4) Zheng, Y.; Brown, S.; Walter, O. L.; Mussinan, C.; Ho, C. T. Formation of sulfur-containing compounds from reactions of furaneol and cysteine, glutathione, hydrogen sulfide, and alanine/hydrogen sulfide. *J. Agric. Food Chem.* **1997**, *45*, 894–896.

(5) Zhang, Y.; Ho, C. T. Formation of meat-like aroma compounds from thermal reaction of inosine 5-monophosphate with cysteine and glutathione. *J. Agric. Food Chem.* **1991**, *39*, 1145–1148.

(6) Tai, C. Y.; Ho, C. T. Influence of glutathione oxidation and pH on thermal formation of Maillard-type volatile compounds. *J. Agric. Food Chem.* **1998**, *46*, 2260–2265.

(7) Tressl, R.; Kersten, E.; Nittka, C.; Rewicki. Formation of sulfur-containing flavor compounds from [^{13}C]-labeled sugars, cysteine, and methionine. In *Sulfur Compounds in Foods*; Mussinan, C. J., Keelan, M. E., Eds.; ACS Symposium Series 564; American Chemical Society: Washington, DC, 1993; pp 285−301.

(8) Whitfield, F. B.; Mottram, D. S. Investigation of the reaction between 4-hydroxy-5-methyl-3(2*H*)-furanone and cysteine or hydrogen sulfide at pH 4.5. *J. Agric. Food Chem.* **1999**, *47*, 1626–1634.

(9) Elmore, J. S.; Mottram, D. S.; Enser, M.; Wood, J. D. Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *J. Agric. Food Chem.* **1999**, *47*, 1619–1625.

(10) Block, E.; Naganathan, S.; Putman, D.; Zhao, S. H. Allium chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-heated) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418–2430.

(11) Dunkel, A.; Köster, J.; Hofmann, T. Molecular and sensory characterization of γ-glutamyl peptides as key contributors to the kokumi taste of edible beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **2007**, *55*, 6712–6719.

(12) Schieberle, P. The carbon module labeling (CAMOLA) technique: a useful tool for identifying transient intermediates in the formation of Maillard-type target molecules. *Ann. N.Y. Acad. Sci.* **2005**, *1043*, 236–248.

(13) Schieberle, P.; Bareth, A.; Fischer, R.; Hofmann, T. The carbohydrate module labeling-techniques. A useful tool to clarify formation pathways of key aroma and taste compounds formed in Maillard-type reactions. *Book of Abstracts*, 10th Weurman Flavour Research Symposium, Beaune, France, June 24−28; INRA: Dijon, France, 2002; Abstract O25.

(14) Frank, O.; Hofmann, T. Reinvestigation of the chemical structure of bitter-tasting quinizolate and homoquinizolate and their Maillard-type formation pathways using suitable ^{13}C-labeling experiments. *J. Agric. Food Chem.* **2002**, *50*, 6027–6036.

(15) Cerny, C.; Davidek, T. Formation of aroma compounds from ribose and cysteine during the Maillard reaction. *J. Agric. Food Chem.* **2003**, *51*, 2714–2721.

(16) Zhang, Y.; Ho, C. T. Volatile compounds formed from thermal interaction of 2,4-decadienal with cysteine and glutathione. *J. Agric. Food Chem.* **1989**, *37*, 1016–1020.

(17) Grosch, W. Detection of potent odorants in foods by aroma extraction dilution analysis. *Trends Food Sci.* **1993**, *4*, 68–73.

(18) van den Dool, H.; Kratz, P. D. A generalisation of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

(19) Persson, T.; von Sydow, E. Aroma of canned beef: gas chromatographic and mass spectrometric analysis of the volatiles. *J. Food Sci.* **1973**, *38*, 377–385.
(20) Mussinan, C. J.; Walradt, J. P. Volatile constituents of pressure cooked pork liver. *J. Agric. Food Chem.* **1974**, *22*, 827–831.
(21) Hofmann, T.; Schieberle, P. Chemical interactions between odor-active thiols and melanoidins involved in the aroma staling of coffee beverages. *J. Agric. Food Chem.* **2002**, *50*, 319–326.
(22) Baek, H. H.; Kim, C. J.; Ahn, B. H.; Nam, H. S.; Cadwallader, K. R. Aroma extract dilution analysis of a beeflike process flavor from extruded enzyme-hydrolyzed soybean protein. *J. Agric. Food Chem.* **2001**, *49*, 790–793.
(23) Dreher, J. G.; Rouseff, R. L.; Naim, M. GC−Olfactometry characterization of aroma volatiles from the thermal degradation of thiamin in model orange juice. *J. Agric. Food Chem.* **2003**, *51*, 3097–3102.
(24) Lee, S. M.; Seo, B. C.; Kim, Y. S. Volatile compounds in fermented and acid-hydrolyzed soy sauces. *J. Food Sci.* **2006**, *71*, 146–156.
(25) Wang, Y.; Ho, C. T. Comparison of 2-acetylfuran formation between ribose and glucose in the Maillard reaction. *J. Agric. Food Chem.* **2008**, *56*, 11997–12001.
(26) Majcher, M. A.; Jelen, H. H. Effect of cysteine and cystine addition on sensory profile and potent odorants of extruded potato snacks. *J. Agric. Food Chem.* **2007**, *55*, 5754–5760.
(27) Coleman, W. M.; White, J. L.; Perfetti, T. A. Investigation of a unique commonality from a wide range of natural materials as viewed from the Maillard reaction perspective. *J. Sci. Food Agric.* **1996**, *70*, 405–412.
(28) Wilson, R. A.; Mussinan, C. J.; Katz, I.; Sanderson, A. Isolation and identification of some sulfur chemicals present in pressure-cooked beef. *J. Agric. Food Chem.* **1973**, *21*, 873–876.
(29) Shibamoto, T. Formation of sulfur and nitrogen-containing compounds from the reaction of furfural with hydrogen sulfide and ammonia. *J. Agric. Food Chem.* **1977**, *25*, 206–208.
(30) Mottram, D. S.; Whitfield, F. B. Maillard−lipid interaction in non-aqueous systems: volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *J. Agric. Food Chem.* **1995**, *43*, 1302–1306.
(31) Mottram, D. S.; Nobrega, I. C. C. Formation of sulfur aroma compounds in reaction mixtures containing cysteine and three different forms of ribose. *J. Agric. Food Chem.* **2002**, *50*, 4080–4086.
(32) Güntert, M.; Brüning, J.; Emberger, R.; Kőpsel, M.; Kuhn, W.; Thomas, T.; Werkhoff, P. Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. *J. Agric. Food Chem.* **1990**, *38*, 2027–2041.
(33) Ho, C. T.; Lu, C.-Y.; Wang, Y.; Raghavan, S.; Payne, R. Maillard flavor chemistry of cysteine and cysteine-containing peptides. In *Recent Highlights in Flavor Chemistry and Biology*; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 2008; pp 91−96.

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