JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Formation of 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone through Methylglyoxal: A Maillard Reaction Intermediate

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The caramel-like aroma compound, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) was quantified and verified by HPLC and GC-MS in the Maillard reaction based on methylglyoxal (MG). The reaction was performed in the 0.5 M phosphate buffer by heating MG with or without either glycine or cysteine at 120 °C for 1 h. MG alone or MG with cysteine could produce increased level of DMHF with pH increased, whereas MG with glycine had contrary trend. Experiments using a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_6$]glucose indicate that in the presence of glycine or cysteine, glucose skeleton kept intact during DMHF formation since a 1:1 mixture of [$^{13}C_6$]DMHF and [$^{12}C_6$]DMHF was formed. Acetylformoin was detected in the glucose with amino acid reaction system as a precursor of DMHF, while in the MG reaction systems, acetylformoin could not be identified. It is suggested different pathways of DMHF formation via MG and glucose.

KEYWORDS: 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone; methylglyoxal; glucose; acetylformoin; carbon module labeling technique

INTRODUCTION

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF, known as Furaneol), with an intense caramel-like aroma, was originally discovered as a key flavor component of strawberry in 1965 (*1*). Till now, DMHF has been found as an important odor-active compound in various natural and processed foods such as pineapple, raspberry, tomato and grape as well as in roasted coffee, bread crust, roasted almond and soy sauce (*2–9*). Because of its widespread occurrence, DMHF became a major reactant in generating other flavor compounds. At low pH, DMHF has been shown to react with cysteine or hydrogen sulfide in generating meat-like aroma compounds (*10, 11*). Some roast aroma compounds such as alkylpyrazines could also be generated through the decomposition of DMHF with phenylalanine (*12*).

The formation pathways of DMHF have been studied in model experiments of thermal degradation of 6-deoxysugars, hexoses and pentoses in the presence or absence of amino acids (*13–15*). Generally, DMHF can be formed through 2,3-enolization of 6-deoxysugars, hexoses and pentoses leading to 1-deoxyosones as intermediates (*16*). Compared to hexoses and pentoses, 6-deoxysugars such as rhamnose are more effective in forming DMHF through 2,3-dioxo-4,5-dihydroxyhexane which is not easily formed from hexoses and cannot be generated from pentoses (*13*). Schieberle in 1992 (*14*), and later Hofmann and Schieberle in 2001 (*17*) showed that DMHF can be formed from hexose via acetylformoin reduction which may proceed either by disproportionation reaction or a Strecker reaction with amino acids. In addition, hexose or pentose can be cleaved into methylglyoxal and 1-hydroxy-2-propanone to generate DMHF, and this reaction has been proved to be a major pathway when glucose is reacted with proline in an aqueous solution (*18*). Only one study has shown the formation of DMHF from pentoses. Blank and Fay (*15*) indicated that elongation of pentoses by Strecker aldehyde of glycine was an alternative pathway of DMHF formation in which acetylformoin was also proposed as an intermediate (*15*).

Methylglyoxal (MG), also known as 2-oxopropanal or pyruvaldehyde, is generated from 3-deoxyosone in Maillard reaction as an intermediate (*19*). Due to its reactivity, MG plays an important role as a precursor of aroma and color compounds especially in the Strecker degradation, a major flavor generation reaction. MG with amino acids undergoes Schiff base formation, decarboxylation and α-aminoketone condensation leading to heterocyclic aroma compounds such as pyrazines, pyrroles and pyridines.

As mentioned above, methylglyoxal can recombine with 1-hydroxy-2-propanone to form DMHF as one possible pathway, but systematic studies on possible pathways of DMHF formation through methylglyoxal, particularly its relationship with different amino acids, have not yet been performed. This study aimed at understanding DMHF formation from methylglyoxal in the presence or absence of two specific amino acids, glycine and cysteine, in phosphate buffer solution. The relationship between DMHF formation pathways from methylglyoxal and glucose

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10.1021/jf8012025 CCC: $40.75
Published on Web 07/02/2008

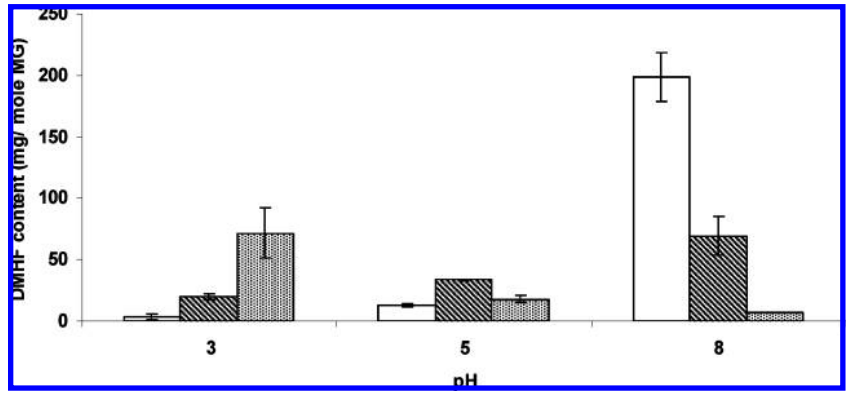

Figure 1. Effect of pH on 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone formation in 1.4 M MG phosphate buffer solution incubated with or without 1.0 M glycine or 1.0 M cysteine: MG only (open bars); MG-cysteine (slashed bars); MG-glycine (dotted bars). Values for DMHF are the means ± standard deviation (SD), each analyzed three independent times. Statistical significance was examined using Student's *t* test comparison between the means. A *p* value of >0.05 was considered statistically significant.

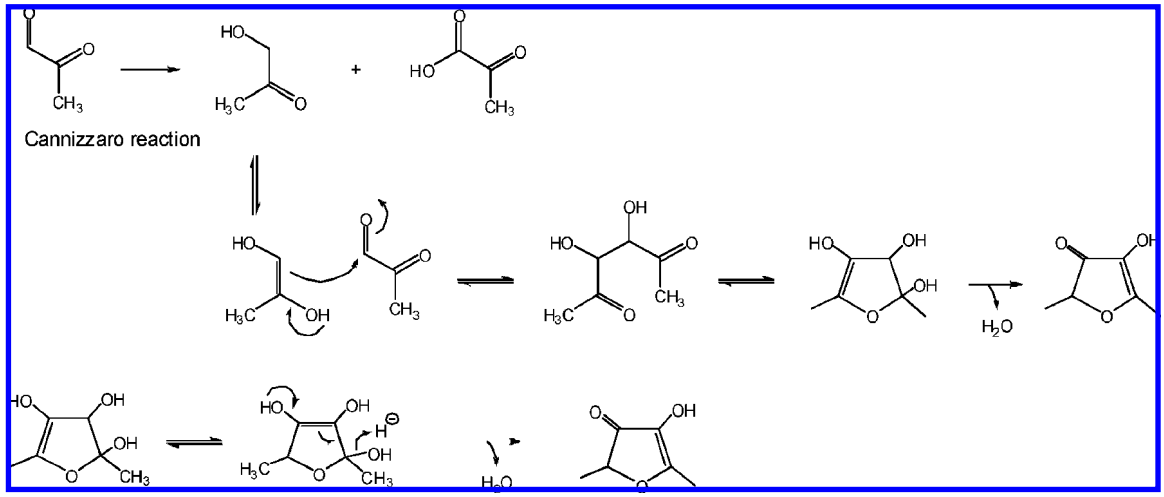

Figure 2. Reaction pathway leading methylglyoxal to 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone via Cannizzaro reaction.

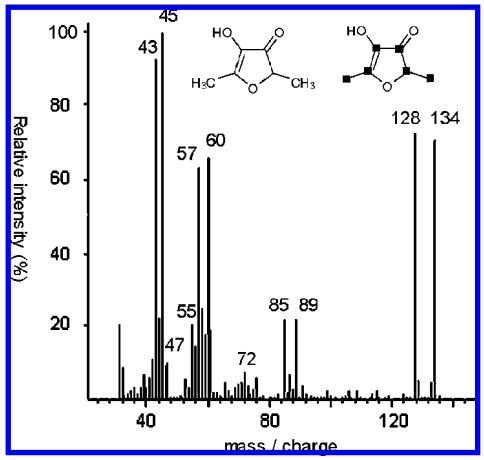

Figure 3. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone from a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_6$]glucose.

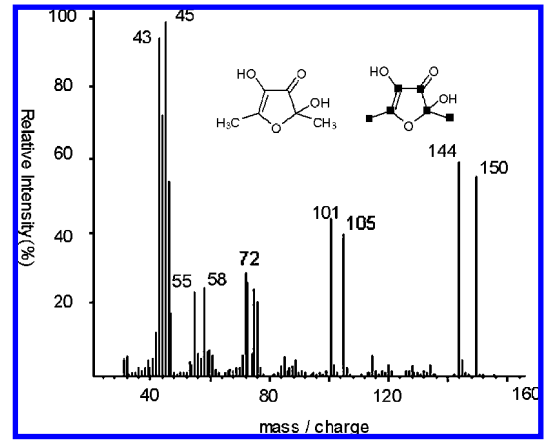

Figure 4. GC-MS spectrum of acetylformoin from a 1:1 mixture of [$^{13}C_6$]-glucose and [$^{12}C_6$]glucose.

was investigated by using the carbon module labeling (CAMOLA) technique (*18*).

MATERIALS AND METHODS

Materials. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone, methylglyoxal (40 wt % in water), L-cysteine, L-glycine, sodium hydroxide and [^{13}C6] or [^{12}C6]D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC grade water, acetonitrile, dichloromethane and methanol were purchased from Fisher Scientific (Springfield, NJ).

Preparation of Model Systems. MG, glucose, cysteine and glycine were dissolved in the phosphate buffer (0.5 M, pH 3.0, 5.0, and 8.0), separately. The pH was adjusted with 1 N sodium hydroxide. The concentrations were 1.4 M, 1.4 and 1 M for MG, glucose and amino acids, respectively. Two model systems were set up. The first one contained an aliquot (1 mL) of MG and phosphate buffer solution. In the second group, an aliquot of MG was mixed with either glycine or cysteine. All these samples were prepared in sealed glass tubes and heated at 120 °C for 60 min. All reacted samples were cooled by an ice bath and centrifuged at 14 × 1000 rpm (16000*g*) for 5 min before HPLC analysis.

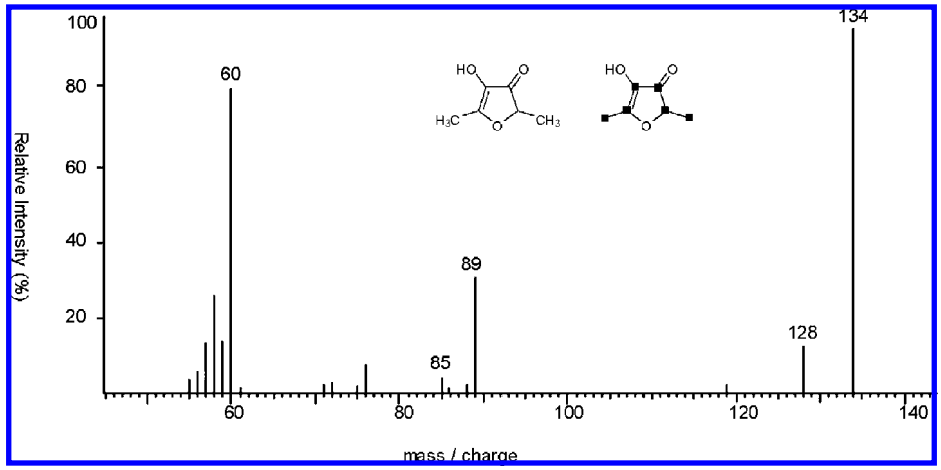

Figure 5. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone from a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_3$]methylglyoxal.

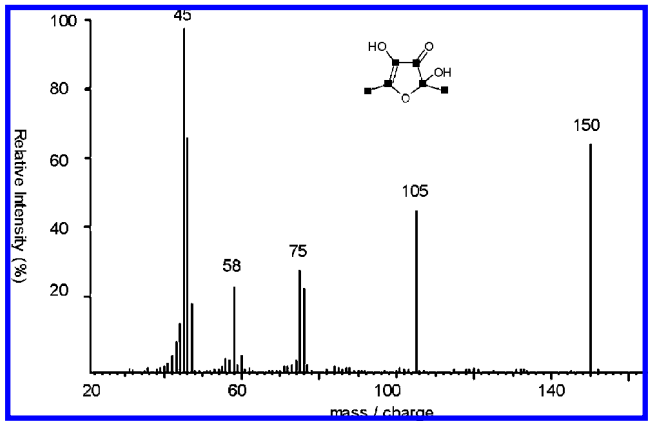

Figure 6. GC-MS spectrum of acetylformoin from a 1:1 mixture of [$^{13}C_6$]-glucose and [$^{12}C_3$]methylglyoxal.

The carbon module labeling (CAMOLA) technique (*19*) was used to verify and evaluate the formation pathways of DMHF from MG and glucose. A 1:1 mixture of 1.4 M [$^{13}C_6$]glucose and 1.4 M [$^{12}C_6$]glucose (containing natural abundant ^{13}C) were reacted with 1 M glycine or cysteine at 120 °C and pH 5 for 60 min, and another similar model, a 1:1 mixture of 1.4 M [$^{13}C_6$]glucose and 1.4 M [$^{12}C_3$]methylglyoxal (containing natural abundant ^{13}C) were reacted with 1 M glycine or cysteine at 120 °C and pH 5 for 60 min. Then, the reaction mixture was extracted three times with 10 mL methylene chloride. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under nitrogen gas for the GC-MS analysis.

Analysis of DMHF by HPLC. The Dionex UltiMate 3000 LC Modules equipped with a pump (model: LPG-3400 pump, Sunnyvale, CA), UV−vis detector (model VWD-3400 detector), and an autosampler (model: WPS-3000 SL) were used. A Luna C18 (Phenomenex, Torrance, CA) column (150 × 4.6 mm i.d., 3 μm particle size) was used for DMHF analysis. The column temperature was maintained at 25 °C in a column oven (Dionex model STH 585). The mobile phase for the HPLC system consisted of HPLC grade water with 0.15% acetic acid (v/v; solvent A), acetonitrile (solvent B) and methanol (solvent C) with a constant flow rate set at 0.5 mL/min. HPLC gradient programs were modified according to the method reported by Haleva-Toledo et al. (*20*) for DMHF analysis as follows: 6% solvent B and 6% solvent C, and they increased together to 15% over 16 min, then decreased to 6% over 4 min. The whole program ran for 20 min. DMHF was detected with a UV wavelength at 290 nm and the injection volumes were 30 μL. The external standard quantification method was applied in this study. Every single peak area for the quantification was laid in the linear range of standard curve.

GC-MS System. The analyses of volatiles were performed with a HP6890 gas chromatograph. The Agilent gas chromatograph (6890 Series) was equipped with a manual (7673 Series Injector) and a mass spectral detector (EI, 70 eV). The column was HP-1701 (14% (cyanopropyl-phenyl) methylpolysiloxane capillary (60 m × 0.25 mm id, film thickness = 0.25 μm). The hydrogen, air, and makeup gas (helium) flow rate was at 30.0, 300.0, and 5.0 mL/min, respectively. The injector was in 1:1 split mode. The 1.0 mL/min constant carrier gas (helium) flow rate was set. The GC oven temperature was programmed as follows: the initial oven temperature 40 °C was set and increased to 280 °C at a rate of 5 °C/min held for 12 min. The total run time was 60 min. The injector temperature was 250 °C and detector temperature was 250 °C.

Statistical Analysis. Data were expressed as means ± standard deviation (SD) and represent three independent analyses. Statistical significance was examined using Student's *t* test comparison between the means. A *p* value of >0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Quantification of DMHF Formation from MG. DMHF as an intense caramel-like aroma compound with low odor threshold has been widely used in the flavor industry. So far, the studies on formation pathways of DMHF have been mainly focused on different hexoses, 6-deoxysugars and pentoses. In current study, the generation of DMHF from MG, an important flavor precursor for processed foods was carried out. Generally, MG and 1-hydroxy-2-propanone, the two major degradation products in Maillard Reaction, could react together to form DMHF via 2,5-dioxo-3,4-dihydroxyhexane (*18*). However, when MG was heated alone at 120 °C, the formation of DMHF was observed (**Figure 1**), and the MG level was significantly increased as pH of the reaction increased. MG, one of the dicarbonyl compounds, may transform into 1-hydroxy-2-propanone and pyruvic acid through the Cannizzaro reaction (**Figure 2**), and subsequently lead to DMHF by reacting MG with 1-hydroxy-2-propanone (*18*). DMHF formation from MG was pH-dependent because the Cannizzaro reaction is a base preferential reaction.

The aroma compounds generated in Maillard reaction depend on the study models such as composition of sugars, amino acids as well as the reaction condition. DMHF was produced at different levels either directly from MG or from MG in the presence of glycine or cysteine. At pH 8, Strecker degradation was the major reaction which consumed most of MG in the presence of amino acids. However, cysteine could be degraded into hydrogen sulfide which can be used as a reducing agent to produce 1-hydroxy-2-propanone from MG. At pH 5, Cannizzaro reaction and Strecker degradation became weaker, and reduction activity of cysteine was the main effect on DMHF formation,

consequently cysteine reacting with MG generated a high level of DMHF. Cysteine may change its role from a reductant to an inhibitor at pH 3. The inhibitory effect of thiol group in cysteine on DMHF formation has been observed particularly at pH 3 (*21–23*). Haleva-Toledo et al. (*20*) showed that cysteine and *N*-acetylcysteine inhibited DMHF formation at pH 3 by a nucleophilic attack of thiol group to the open carbonyl form of DMHF (*20*). It was very interesting to observe that the generation of DMHF from MG and glycine increased as pH decreased. Further studies are required to explain this phenomenon.

Verification of DMHF Formation through MG and Glucose Pathways. Maillard reaction involving thermal degradation of carbohydrates and amines could induce a complex reaction cascade in which aroma, taste and color compounds are generated through cyclization and fragmentation of carbohydrates. DMHF, on one hand, can be formed through cyclization of an intact carbohydrate via acetylformoin as an intermediate. On the other hand, the carbohydrate may be cleaved into fragments such as MG and 1-hydroxy-2-propanone, which may recombine to form DMHF. In order to show DMHF formation mechanisms, the carbon module labeling (CAMOLA) was used. CAMOLA is a powerful technique to elucidate different pathways and evaluate the relative importance of each pathway (*18*). Equal molar of [$^{13}C_6$] labeled and [$^{12}C_6$] unlabeled glucose were mixed in the presence of glycine or cysteine, and the isotopomers of DMHF were analyzed by GC-MS. If the glucose carbon skeleton keeps intact in DMHF formation, equal molar of [$^{13}C_6$] labeled DMHF and [$^{12}C_6$] unlabeled DMHF should be obtained. However, if the fragmentation of glucose occurs before DMHF formation, up to seven isotopomers with different numbers of labeled carbons may be formed. The results demonstrated that five isotopomers [$^{13}C_1$] to [$^{13}C_5$] from glucose fragmentation, were not observed in the presence of glycine and cysteine, and a 1:1 mixture of [$^{13}C_6$]DMHF and [$^{12}C_6$]-DMHF was obtained (**Figure 3**), suggesting no breakdown of glucose during DMHF formation. Generally, fragmentation degree is related to the temperature. When reaction temperature was increased to 165 °C, still no fragmentation of glucose occurred in the DMHF formation (data not shown). Previous studies suggested that acetylformoin was an important precursor which could be reduced to DMHF (*17*). In the current experiments, molecular ion of *m*/*z* 144 representing the [$^{12}C_6$]acetylformoin was present in an equal intensity to *m*/*z* 150 ([$^{13}C_6$]-acetylformoin) indicating that acetylformoin as one of DMHF intermediates was also generated from intact glucose (**Figure 4**). It is therefore concluded that in the presence of glycine or cysteine, DMHF can only be formed through the intact glucose.

As an important intermediate during thermal degradation of glucose, MG itself could generate DMHF with or without amino acids. If a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_3$]MG reacted with glycine or cysteine at 120 °C, a 4:1 mixture of [$^{13}C_6$]DMHF and [$^{12}C_6$]DMHF was obtained. Because some of the MG involved in the Strecker degradation, only 20% of DMHF was formed from MG, and the rest 80% was formed from glucose (**Figure 5**). However, no [$^{12}C_6$]acetylformoin was observed suggesting that acetylformoin was not a precursor during the DMHF formation from MG (**Figure 6**). In all of DMHF and acetylformoin isotopomers, only [$^{13}C_6$] labeled and [$^{12}C_6$] unlabeled, were observed. Glucose kept carbon skeleton intact during DMHF formation even its fragment MG was present, which indicated that MG and cyclization of intact glucose pathways were parallel since the precursors of these two pathways were different.

In conclusion, the results of this study indicate that MG, depending on the pH differently affected DMHF generation in the presence or absence of amino acids. DMHF level increased as pH increased when cysteine reacted with MG, whereas the trend was reversed in the presence of glycine. When glucose reacted with glycine or cysteine, glucose skeleton kept intact in the formed DMHF as well as its precursor acetylformoin. Acetylformoin was not formed in the reaction between MG and either glycine or cysteine.

LITERATURE CITED

(1) Willhalm, B.; Stoll, M.; Thomas, A. F. 2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one. *Chem. Ind. (London)* **1965**, *38*, 1629–1630.

(2) Rodin, J. O.; Himel, C. M.; Silverstein, R. M.; Leeper, R. W.; Gortner, W. A. Volatile flavor and aroma components of pineapple. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. *J. Food Sci.* **1965**, *30*, 280–285.

(3) Pabst, A.; Barron, D.; Etiévant, P.; Schreier, P. Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *J. Agric. Food Chem.* **1991**, *39*, 173–175.

(4) Buttery, R. G.; Takeoka, G. R.; Krammer, G. E.; Ling, L. C. Identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) and 5-methyl-4-hydroxy-3(2*H*)-furanone in fresh and processed tomato. *Lebensm. Wiss. Technol.* **1994**, *27*, 592–594.

(5) Rapp, A.; Knipser, W.; Engel, L.; Ullemeyer, H.; Heimann, W. Off-flavor compounds in the berry and wine aroma of grapevine hybrids. I. The strawberry-like aroma. *Vitis* **1980**, *19*, 13–23.

(6) Tressl, R.; Bahri, D.; Köppler, H.; Jensen, A. Diphenols and caramel compounds in roasted coffees of different varieties. II. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 111–114.

(7) Schieberle, P. Studies on bakers yeast as source of Maillard type bread flavour compounds. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R., Eds.; Birkhäuser; Basel, Switzerland, 1990; pp 187−196.

(8) Tei, Y.; Yamanaishi, T. Flavor components of roasted almond. *Agric. Biol. Chem.* **1974**, *38* (12), 2329–2336.

(9) Steinhaus, P.; Schieberle, P. Characterization of the key aroma compounds in soy sauce using approaches of molecular sensory science. *J. Agric. Food Chem.* **2007**, *55*, 6262–6269.

(10) Shu, C. K.; Ho, C. T. Effect of pH on the volatile formation from the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. *J. Agric. Food Chem.* **1988**, *36*, 801–803.

(11) Zheng, Y.; Brown, S.; Leding, W. O.; Mussinan, C.; Ho, C. T. Formation of sulphur-containing flavour compounds from reactions of furaneol and cysteine, glutathione, hydrogen sulphide, and alanine/hydrogen sulfide. *J. Agric. Food Chem.* **1997**, *45*, 894–897.

(12) Jutta, K. K.; Werner, B. Model reaction on roast aroma formation. VIII. Volatile reaction products from the reaction of phenylalanine with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) by cooking in a laboratory autoclave. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 14–16.

(13) Hofmann, T.; Schieberle, P. Identification of potent aroma compounds in thermally treated mixtures of glucose/cysteine and rhamnose/cysteine using aroma extract dilution techniques. *J. Agric. Food Chem.* **1997**, *45*, 898–906.

(14) Schieberle, P. Studies on the formation of furaneol in heat processed foods. In *Flavor Precursors: Thermal and Enzymatic Conversions*; Teranishi, R., Takeoka, G. R., Güntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 164−175.

(15) Blank, I.; Fay, L. B. Formation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone through Maillard reaction based on pentose sugars. *J. Agric. Food Chem.* **1996**, *44*, 531–536.

(16) Hodge, J. E.; Mills, F. D.; Fisher, B. E. Compounds of browned flavor derived from sugar−amine reactions. *Cereal Sci. Today* **1972**, *17*, 34–40.

(17) Hofmann, T.; Schieberle, P. Acetylformoin−an important progenitor of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 2-acetyltetrahydropyridine during thermal food processing. In *Flavour 2000 Perception Release Evaluation Formation Acceptance Nutrition/ Health*; Proceedings of the 6th Wartburg Aroma Symposium; Eisenach, M., Rothe, Eds.; 2001, 311−322.

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

(19) Namiki, M.; Hayashi, T. Formation of novel free radicals products at the early stage of Maillard reaction. *Prog. Food Nutr. Sci.* **1982**, *5*, 81–91.

(20) Haleva-Toledo, E.; Naim, M.; Zehavi, U.; Rouseff, R. L. Effects of L-cysteine and *N*-acetyl-L-cysteine on 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), 5-(hydroxymethyl)furfural, and 5-methylfurfural formation and browning in buffer solutions containing either rhamnose or glucose and arginine. *J. Agric. Food Chem.* **1999**, *47*, 4140–4145.

(21) Friedman, M.; Molnar-Perl, I. Inhibition of browning by sulfur amino acids. I. Heated amino acid−glucose systems. *J. Agric. Food Chem.* **1990**, *38*, 1642–1647.

(22) Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. II. Fruit juices and protein-containing foods. *J. Agric. Food Chem.* **1990**, *38*, 1648–1651.

(23) Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. III. Apples and potatoes. *J. Agric. Food Chem.* **1990**, *38*, 1652–1656.

Received for review April 16, 2008. Revised manuscript received May 29, 2008. Accepted June 1, 2008.

JF8012025