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Formation mechanism of aroma compounds in a glutathione-glucose reaction with fat or oxidized fat

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ABSTRACT

Glutathione and glucose with or without chicken fat/oxidized chicken fat were thermally reacted for generation of stewed meat-like aroma, where 42 sulfur-containing odorants were identified by gas chromatography-mass spectrometry (GC–MS) and gas chromatography–olfactometry (GC-O). The observed effects or interactions on meat flavor formation due to the fats were similar to previous reports of cysteine-reducing sugar reactions. Carbohydrate module labeling approach demonstrated ten alkyl chain compounds were indeed resulted from the lipid degradation-Maillard reaction interactions, whereas the fats had little effect on formation pathways of compounds only derived from the Maillard reaction. Formation pathways of 26 potent aroma compounds were proposed, particularly, involving two benzene derivatives and seven complex thiophenes. Notably, it was found for the first time just 2-ethylthiophene could result from both an intact skeleton of glucose and the lipid degradation product of 2,4-hexadienal, and the carbohydrate modules methylglyoxal and hydroxyacetone could arise from the glutamic acid of GSH.

1. Introduction

Glutathione (γ-Glu-Cys-Gly, GSH), a cysteine-containing tripeptide, can be commonly found intracellularly in bacteria, plants, and mammals and serves many biological functions. GSH prevents cellular damage resulted from electrophilic compounds and chemically reactive intermediates such as free radicals, due to its reducing and nucleophilic properties (Chaudière & Ferrari-Iliou, 1999). Like cysteine, GSH is an important precursor of meaty flavors, which reacts with reducing sugars forming diverse sulfur-containing volatile compounds (El-massry, Farouk, & El-Ghorab, 2003; Lee, Jo, & Kim, 2010; Shedid, 2010; Tai & Ho, 1998; Zhang & Ho, 1991). For example, Lee et al. (2010) identified 12 odor-active sulfur-containing meaty compounds in the thermal reaction of GSH with glucose or fructose.

Meat or meat product is a complexity of free amino acids, peptides, proteins, reducing sugars, and lipids, where the lipids contribute to characteristic flavor of different meat species through thermally oxidization and degradation (Mottram, 1998). As we know, model systems of cysteine-reducing sugars with lipids had been used to study formation mechanism of meat flavor (Elmore, Campo, Enser, & Mottram, 2002; Farmer & Mottram, 1990; Whitfield & Mottram, 1992; Xu et al., 2011; Yang et al., 2015). However, such a model system as GSH-reducing sugar with a lipid was scarcely investigated, though cysteine is usually bound in a peptide.

The carbon module labeling (CAMOLA) approach is a versatile and convenient tool to elucidate formation pathways of flavor compounds and to gain insight into fragmentation of precursors in a model reaction system or food matrix (Cerny & Davidek, 2003; Davidek, Festring, Dufossé, Novotny, & Blank, 2013; Schieberle, 2005; Wang, Yang, & Song, 2012; Xu, et al., 2013). By the CAMOLA technique, pathways to generate 5-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde in glutathione-glucose/fructose systems (Lee et al., 2010), and those to generate the furans from sugars in the presence of different amino acids (van Lancker, Adams, Owczarek-Fendor, de Meulenaer, & de Kimpe, 2011), were clarified. Besides, formation pathway of a compound can be affected by the used reaction conditions. For example, the pathway for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone from glucose under roasting conditions at 160 °C differed from

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that in an aqueous buffer at 140 °C (Schieberle, Fischer, & Hofmann, 2003).

Fat, and oxidized fat prepared by oxidization of fat in control, are commonly utilized to prepare processed-meat flavorings in China (Yang et al., 2015). In this study, volatile flavor compounds formed from the following five model reaction systems, i.e., GSH-Glucose, GSH-Glucose-Chicken fat, GSH-Glucose-Oxidized chicken fat, chicken fat alone, and oxidized chicken fat alone were comparatively investigated by solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC–MS) and gas chromatography–olfactometry (GC-O). Interactions or effects of fat/oxidized fat on flavor formation in the reaction systems with GSH and glucose were explored. Furthermore, formation pathways of the identified potent aroma compounds were elucidated by means of the CAMOLA technique. The work is helpful to gain insight into the formation mechanism of meat flavor and provide guidance for preparation of processed-meat flavorings or processing of meat.

2. Experimental

2.1. Materials and chemicals

Refined and odorless chicken fat, was purchased from Tianjin Mu Yang Oil Factory (Tianjin, China). Oxidized chicken fat, with a peroxide value of 237 meq oxygen/kg and an acid value of 2.00 mg KOH/g, was prepared from the chicken fat according to Yang et al. (2015). Glutathione (99%), [$^{12}C_6$]-D-glucose (98%), [$^{13}C_6$]-D-glucose (99%), and the authentic chemicals (≥95%) used to identify structures of the generated volatile compounds were purchased from *J&K* Chemical Ltd. (Beijing, China). The *n*-alkanes (C_6–C_{27}) for retention indices were purchased from Beijing Chemical Reagents Co. Ltd. (Beijing, China).

2.2. Model reactions

The reaction systems were comprised of (a) GSH-Glucose (Control); (b) GSH-Glucose-Chicken fat; (c) GSH-Glucose-Oxidized Chicken fat; (d) Chicken fat alone (Blank); and (e) Oxidized chicken fat alone (Blank). A 15-ml Synthware glass vial with a screw cap (Beijing Synthware Glass Inc., Beijing, China) was employed. Into 5 mL of sodium phosphate buffer (pH 6.5) were placed GSH (0.3 mmol), glucose (0.3 mmol) and 53.0 mg of chicken fat or the oxidized chicken fat (*ca.* 1% w/w of the total content). On a Parallel Synthesis Poly-block 4 System (Hel Limited Co., England), the reactants were heated at 140 °C while stirring for 5 h. Before the screw cap was opened, the vial was cooled in cold water. Each of the reaction systems was carried out in triplicate.

2.3. Solid phase micro-extraction (SPME)

Volatile compounds in the reaction mixtures were extracted by solid phase microextraction (SPME). A 50/35 μm Carboxen/Polydimethylsiloxane/Divinylbenzene (CAR/PDMS/DVB) fiber (Supelco Inc., Bellefonte, PA) was used. After the sample in a vial was equilibrated at 50 °C for 10 min, the fiber was exposed in the upper space of the vial for 20 min, while the sample was agitated by an electromagnetic stirrer during the adsorption period. After the adsorption, the fiber was directly introduced into a GC injector for the subsequent GC–MS or GC-O analysis.

2.4. Gas chromatography and mass spectrometry (GC–MS)

A 7890B gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA) was used. The chemical separation was performed on a DB-Wax capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, CA). The carrier gas used was helium at 1 mL/min. The initial oven temperature was 40 °C, raised to 70 °C at 5 °C/min; raised to 160 °C at 2 °C/min; and finally raised to 230 °C at 8 °C/min. The injector temperature was 250 °C. Immediately after 0.1 μL of an internal standard (60 ng of 1, 2-dichlorobenzene in 1 μL of methanol) was injected, the fiber was desorbed for 3 min in a splitless mode.

The mass detector was operated at 150 °C in an electron impact mode at 70 eV with no solvent delay. The ion source was set at 230 °C. The transfer line was set at 230 °C. The chromatograms were recorded by full scan detection within the 40–450 mass range.

2.5. Gas chromatograph and olfactometry (GC-O)

An Agilent 7890A gas chromatograph equipped with a FID detector and a DATU 2000 high-resolution olfactometer (DATU Inc. USA.) was used. The carrier gas was nitrogen at 1.0 mL/min. The used capillary column, programmed oven temperatures and desorption of the fiber were as same as those in the aforementioned GC–MS analysis.

Three trained sniffers performed the gas chromatograph and olfactometry (GC-O) analyses. Particularly, for the GSH-Glucose reaction mixtures, aroma extract dilution analysis (AEDA)/GC-O was conducted. The dilutions (1–512) were achieved through a stepwise increase of the injector split ratio (1:2, 1:4, 1:8, … 1:128), and at 128:1 with a contraction of the SPME fiber length exposed in 1/2 and 1/4 of full exposure. The sniffing odor characteristics were recorded, while in the AEDA/GC-O analysis, each odorant was finally assigned a FD factor representing the highest dilution. Retention times of the odor responses were converted into linear retention indices (RI) relative to the series of *n*-alkanes (C_6–C_{27}).

2.6. Identification of volatile flavor compounds

The identification of volatile flavor compounds was based on mass spectra in GC–MS, retention indices (RI) relative to C_6–C_{27} *n*-alkanes in both GC–MS and GC-O analyses, odor characteristics detected in GC-O, and the comparison of the above parameters with those of available chemicals. Regarding the mass spectra analysis, both search of NIST 2015 mass spectra library and subjective interpretation were performed.

2.7. Carbohydrate module labeling (CAMOLA) experiment

The reactions of GSH-Glucose, GSH-Glucose-Chicken fat, and GSH-Glucose-Oxidized Chicken fat were performed as described in the Section 2.2, except that the glucose used was a mixture of equimolar amounts of fully labeled [$^{13}C_6$]-D-glucose (0.15 mmol) and unlabeled [$^{12}C_6$]-D-glucose (0.15 mmol). Volatile compounds in the reaction mixtures were analyzed as described in the Section 2.3 and 2.4, respectively.

Mass spectra of the identified compounds were analyzed on the basis of the mass-to-charge ratios. Proportions of isotopomers of a compound were calculated by normalization of peak areas of the selected ions from M^+ to $M^+ + n$, where M^+ is the molecular ion and n is the number of labeled carbons in an isotopomer. Besides, referring to Cerny and Davidek (2003), the values of the calculated isotopomer proportions were corrected by subtracting the naturally occurring percentages of ^{13}C (1.10%), ^{33}S (0.76%), and ^{34}S (4.20%), and the loss of hydrogen frequently observed with the molecular ion in EI-MS was also corrected in the labeled molecular ions by the ratio $(M^+ - 1)/M^+$.

2.8. Statistical analysis

The results were the averages of three replicates. The figures were plotted with Chemdraw 7.0. In Tables 1 and S1, differences between means were handled by one-way ANOVA with Duncan's multiple range tests using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA). A *p*-level less than 0.05 was defined of significant difference.