Elucidating the Role of Low Molecular Weight Peptides as Maillard Reactant Flavor Precursors in Chicken Meat

by

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ABSTRACT

Low molecular weight (MW) peptides (1KDa) may act as flavor precursors in the Maillard reaction and contribute to the overall aroma and flavor formation in meat. The contribution of carnosine, glutathione (GSH), and cystinylglycine (Cys-Gly) to meat flavor formation was examined in the model systems after heating at 180° C in the presence of ribose to mimic the meat roasting conditions and physiological concentrations at pH 6.3. The generated volatile organic compounds (VOCs) were extracted using two different solvent extraction methods and were analyzed by gas chromatography-mass spectrometry. Pyrazines and pyridines dominated the reaction mixtures containing carnosine, while the sulfur-containing VOCs were found in the model systems containing GSH and Cys-Gly. The GSH system generated cyclic polysulfides, which have not been previously reported in the Maillard reaction of peptides. These results suggest an active contribution of low MW peptides to the overall aroma and flavor formation in meat systems.

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LIST OF ABBREVIATIONS

AGEs	Advanced Glycation End-products
DKPs	Diketopiperazines
FD	Flavor Dilution
FFT	2-Furfurylthiol
GC-MS	Gas Chromatography-Mass Spectrometry
G6P	Glucose-6-Phosphate
LRI	Linear Retention Index
MFT	2-Methyl-3-furanthiol
MRPs	Maillard Reaction Products
MW	Molecular Weight
PUFAs	Polyunsaturated Fatty Acids
R5P	Ribose-5-Phosphate
SDE	Simultaneous Steam Distillation and Solvent Extraction
VOCs	Volatile Organic Compounds

Chapter 1. Introduction

Analysis of the volatile composition of heated meat is always a topic of interest in the manufacture of imitation meat flavorings. Maillard reaction is one of the most important chemical reactions contributing to the flavor and color development in cooked foods. The nature and concentrations of reactants, pH, temperature, and water activity are among important factors affecting the Maillard reaction which may be controlled to optimize the desirable sensory characteristics of foodstuffs such as meat. Raw meat has little or no aroma with metallic and blood-like taste (Crocker, 1948), however, when heat is applied, thousands of volatile organic compounds (VOCs) generate and give rise to the desirable sensory characteristics of the meat products. It is noteworthy that not all of these VOCs have the same degree of flavor significance, and only those with low odor thresholds were regarded as essential aroma contributors (Van Ba, Touseef, Jeong, & Hwang, 2012). In post-slaughter muscle, a large number of amino acids and low MW peptides are released during meat aging following the proteolytic activity of enzymes to degrade muscular proteins (Schreurs, 2000). In the presence of other organic constituents, such as carbohydrates, lipids, and vitamins, they comprise a pool of flavor precursors which undergo thermal interactions can develop characteristic flavors of meat foods.

Due to the complexity of food matrices, simplified model systems containing known concentrations of Maillard reactants are generally employed to study the chemistry of meat flavor formation. The mechanisms pertinent to the Maillard reaction between amino acids and reducing sugars are well recognized, however, little is known when low molecular weight (MW) peptides are involved.

Gas Chromatography (GC) is a common tool used to separate organic compounds in a given sample. When coupled with Mass Spectrometry (MS), it is a powerful analytical

technique with numerous applications in various scientific disciplines. Based on retention times eluted in a gas chromatogram as well as mass-to-charge ratios collected in a mass spectrum, determination and identification of the compounds of interest can be accomplished. In flavor chemistry, other complementary techniques, such as Liquid Chromatography-Mass Spectrometry (LC-MS), GC-Tandem Mass Spectrometry (GC-MS/MS), GC-Flame Ionization Detection (GC-FID), GC-Olfactometry (GC-O) and Nuclear Magnetic Resonance (NMR) may also be included in the analytical procedures based on the objectives of a study.

Only a few studies have been dedicated to the Maillard reaction of peptides. Among which none used concentrations of reactants and pH values similar to those expected for meat and meat products. Therefore, the main objective of this study was to investigate the nature and potential contribution of VOCs generated from the Maillard reaction systems containing natural concentrations of selected peptides with a pH value similar to those expected in aging meats (pH 6.30). Carnosine, GSH, and Cys-Gly were chosen as the low MW peptides (< 1 kDa) to act as potential NH_2 donors in thermal reactions with ribose in aqueous solutions.

Generated VOCs from the model systems were analyzed using GC-MS, and their relative flavor significance was examined based on previously reported flavor studies related to cooked meat. The formation of key aroma compounds from Maillard reactions of low MW peptides in this study can provide critical information to assess the potential contribution of these peptides to overall aroma and flavor of cooked meats.

Chapter 2. Literature Review

2.1. Meat Flavor and Associated Key Aroma Compounds

Flavor is an organoleptic function of foods and is of significance in determining the ultimate acceptability and quality of food products (Mottram, 2007). The flavor formation in meat and meat products has been investigated extensively. Thousands of VOCs have been reported in foods, among which, over 1000 VOCs were identified in meat (Mottram, 1998). It is however important to note that not all of the detectable VOCs in a given matrix are active aroma compounds. Aroma compounds or odorants are VOCs that can be perceived by the human olfactory system. It is generally accepted that only odorants with relatively low odor thresholds and present at low concentrations are key aroma constitutes (Mottram, 1998), and hence responsible for the overall sensory quality of foods including cooked meat and meat products.

Raw meat has little or no aroma with metallic and blood-like taste (Crocker, 1948). Upon heating, the non-volatile compounds present in raw meat also called flavor precursors will react to generate a wide range of different classes of VOCs and provide the particular sensory properties of cooked meat. These chemical reactions include the thermal degradation of sugars, lipids, and thiamin, as well as the Maillard reaction between amino compounds and reducing sugars. In addition, the generated non-volatile intermediates and VOCs from any of these chemical reactions may then interact with each other to produce new VOCs. In general, the odorants originated from Maillard reactions and the Strecker degradations (reactions between **α**-dicarbonyl compounds and amino acids) are mainly responsible for the typical meaty flavor, while those derived from lipid degradation give rise to the species-specific flavors (Bodrero, Pearson, & Magee, 1981; Mottram, 2007).

Studies on the flavor formation in meat have been conducted in various animal species, which include poultry (chicken, turkey, and duck) (Gasser & Grosch, 1990; Noleau &

Toulemonde, 1986; Wu & Liou, 1992), beef (Gasser & Grosch, 1988; Kerscher & Grosch, 1997; Leod & Ames, 1986), pork (Mottram, 1985; Xie, Sun, Zheng, & Wang, 2008), goat (Madruga, Elmore, Dodson, & Mottram, 2009), lamb (Roldán, Ruiz, del Pulgar, Pérez-Palacios, & Antequera, 2015), and fish (Guillén & Errecalde, 2002; Methven, Tsoukka, Oruna-Concha, Parker, & Mottram, 2007). Based on their chemical structures, VOCs derived from cooked meat are generally found as aldehydes, ketones, alcohols, esters, hydrocarbons (aliphatic and aromatic), phenols, and heterocyclic compounds with the elements of sulfur, nitrogen, and/or oxygen in the ring structures. It was reported that among the total volatile constitutes isolated from roasted beef, 90% in volume was from lipid, while around 40% of which in the aqueous fraction were considered to be heterocyclic compounds (Bailey, 1983).

2.1.1. Lipid-derived VOCs. In view of lipid-derived VOCs, aldehydes, ketones, alcohols, esters, hydrocarbons, and alkylfurans can all be formed from oxidation of fatty acids in the lipid fractions of meat (Varlet, Prost, & Serot, 2007). The odor thresholds of these VOCs are generally higher than those of the heterocyclic compounds arising from the water-soluble components via the Maillard reaction, hence, their aroma impact is qualitatively less significant (Mottram, 1998). Nevertheless, lipid-derived VOCs play a vital role in the overall perceived sensations of cooked meat. Not only many of them are quantitatively predominant in the volatile profile of cooked meat (Madruga et al., 2009; Noleau & Toulemonde, 1986), but also some of the compounds are responsible for the authentic flavors between meat species.

Due to their chemical reactivity and natural organoleptic properties in foods, lipid-derived aldehydes are critical compounds to flavor chemists. Indeed, they impart the typical fatty and species-authentic aroma notes depending on the fatty acid composition. Chicken meat generally

has a higher content of unsaturated fatty acids than beef or pork. After filtering out the fats and fat solids, the chicken broth was shown to have a distinct flavor quality, while little difference was detected from the broths of beef, pork, or mutton. It was suggested that VOCs generated from the thermal degradation and oxidation of lipids were responsible for such flavor differences (Rothe, Kirova, & Schischkoff, 1981). Interestingly, flavor of the beef broth (with fat filtered out) could be manipulated to be "chicken-like" after vigorous shaking with highly unsaturated vegetable oil (Rothe et al., 1981). On the other hand, the occurrence of a high proportion of branched chain saturated fatty acids in mutton fat is associated with the characteristic mutton flavor (also known as "Soo" in Chinese), which results in its low consumer popularity in many places around the world (Wong, Nixon, & Johnson, 1975).

Aroma attributes of some lipid-oxidation aldehydes, as well as their relative flavor significance in meat were further elucidated in the volatile analysis. Comparison of volatile compounds isolated from boiled beef and chicken broth suggested that carbonyl compounds stemmed from unsaturated fatty acids accounted for the authentic flavor of chicken (Gasser and Grosch, 1988; 1990). In particular, *(E, E)* 2, 4-decadienal was found to be an important flavor contributor whose flavor dilution (FD) factor in the aroma extract dilution analysis (AEDA) was higher than the other aroma constituents. 2-undecenal, an aldehydes isolated from the same volatile fraction, also seemed to be a potent odorant due to its relatively high FD value. Moreover, in a review article of poultry meat flavor, hexanal and 2, 4-decadienal were reported to be two of the most abundant aldehydes in roasted chicken meat (Shi & Ho, 1994). As the odor threshold of 2, 4-decadienal (0.00007mg/kg) was much lower than that of hexanal (0.0045mg/kg) (Van Germert, & Nettenbrijer, 1977), the former aldehyde should be paramount in chicken flavor development (Shi & Ho, 1994). Many of aldehyde compounds identified in the

volatile fractions of cooked chicken were also aroma constituents in duck meat. Such a similarity might be owing to the comparable fatty acid composition between two species in which both meat types contained a high content of polyunsaturated fatty acids (PUFAs) (Chen, Song, Ma, 2009; Liu, Xu, Ouyang, & Zhou, 2006; Liu, Xu, & Zhou, 2007). Other chemical classes, such as alcohols, furans, ketones, and some of the hydrocarbons, can be generated from the same lipid decomposition pathway like aldehydes, and together contribute to the overall

cooked meat flavor.

2.1.2. VOCs from Maillard reactions and the associated Strecker degradation

Aldehydes and ketones are compounds not only formed from thermal oxidation of lipids, but also produced by Maillard reactions. Reductones and dehydroreductones are sugar degradation products derived from the Maillard reaction. The breakdown of these intermediates can give rise to smaller reactive carbonyl compounds via retro-aldo reactions (Varlet et al., 2007). In addition, Strecker degradations of amino compounds in the presence of dicarbonyls can also lead to the formation of corresponding Strecker aldehydes and α-aminoketones. Therefore, the nature of aldehydes and ketones generated can be attributed to the associated chemical reactions. Some important aldehydes formed in the Strecker degradation are 3-methylbutanal (from leucine), 2-methylpropanal (from valine), methional (from methionine), acetaldehyde (from alanine), and phenylacetaldehyde (from phenylalanine) (Mottram, 2007). Due to their aroma characteristics and relatively low odor thresholds, Strecker aldehydes have generated a great deal of interest in flavor research studies. For instance, the odor of 3-methylbutanal was described to be closely related to cheese, malt, and dark chocolate (Beal & Mottram, 1994; Chen, et al., 2009). The relatively high scores in flavor dilution (FD) factors as

well as in odor activity values (OAVs) determined it to be one of the aroma-active compounds in roasted duck meat (Chen et al., 2009). 2-methylpropanal was found to be the smallest odor-active aldehyde in the volatile extract of cooked farmed obscure puffer. Flavor potency of this volatile in fish foods was suggested since it could exhibit nutty, malty, and burnt-like odor notes (Tao, Wu, Zhou, Gu, & Wu, 2014). Additionally, methional was considered as a primary odorant in both boiled beef and chicken broth as its FD values in these foods were relatively high (Gasser & Grosch, 1988 & 1990). Flavor impressions of methional were reported to have potato and meat-like aromas with a low odor threshold of merely 0.2µg/L in water (Buttery, Teranishi, Ling, & Turnba, 1990; Gasser & Grosch, 1988). In terms of sugar-derived carbonyl volatiles, 2, 3-butanedione and 3-hydroxy-2-butanone are two ketones frequently found in cooked meat. With strong buttery and creamy odors, 2, 3-butanedione is an important flavor component in a variety of heated foods, including beef (Rivas-Cañedo, Juez-Ojeda, Nuñez, & Fernández-García, 2011), goat meat (Madruga et al., 2009), duck (Chen et al., 2009), fish (Tao et al., 2014), and crab (Chung, & Cadwallader, 1994). Using model systems, the formation of this compound has been validated from sugar degradation via the Maillard reaction (Yaylayan, & Keyhani, 1999). Following the same formation pathway, 3-hydroxy-2-butanone is another sugar-derived product which contributes to the buttery and sour aromas in the cooked foods (Chen et al., 2009; Roldán et al., 2015). It was found to be one of the quantity dominators in the flavor composite of roasted pork (Xie, et al., 2008). Due to the nature of carbonyl compounds, 2, 3-butanedione and 3-hydroxy-2-butanone are highly reactive and readily participate in subsequent Maillard reactions and/or Strecker degradations. In this process, they react with ammonia (NH_3), hydrogen sulfide (H_2S), or other intermediate derivatives to give rise to a wide range of heterocyclic volatile compounds as the final reaction products (Mottram, 2007; Xie, Huang, & Ho, 1999).

Heterocyclic volatile compounds, such as furans, thiophenes, thiazoles, pyrazines, pyridines, pyrroles, and oxazoles, are significant contributors for the sensory impressions of meat and meat products. Main sources of these compounds are from Maillard reactions and Strecker degradations. As previously mentioned, carbonyl compounds produced from Maillard reaction and lipid degradation can further react with amino compounds or other reactive intermediates to form a myriad of character impact heterocyclic compounds (Mottram, 2007). It has been recognized that Strecker degradations are imperative in this case, as they provide a pathway for the introduction of sulfur and nitrogen atoms into heterocyclic ring structures. Therefore, the nature of amino acids partly determines the ultimate presence of heteroatoms in these volatile compounds (Farmer, 1994; Mottram, 2007).

Furan and furan derivatives are a class of volatile compounds present in all heated foods. They can be the products of solely sugar degradation (caramelization) or of Maillard reactions after a series of rearrangement and dehydration of sugars (Mottram, 2007; Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995). This class of chemical compounds generally imparts caramel-like, fruity, nutty, and sweet odors with their flavor thresholds higher than those of sulfur- and nitrogen-containing heterocyclic volatiles (Mottram, 2007). Although they cannot contribute to the "meaty" flavor in their own right, they are important intermediate precursors of heterocyclic aroma compounds, such as thiophenes, furanthiophenes, and furanthiols, of which odor properties are of prime importance to the overall sensory quality of meat.

Sulfur-containing volatile compounds, both aliphatic and heterocyclic, are essential flavor constituents in meat foods. According to a review of Maarse (1991),

aliphatic sulfur compounds are more related to boiled meat and generally have low odor thresholds. H_2S, dimethyl sulfide, dimethyl disulfide, methanethiol, and methional are some of some of the examples, and they have been reported in the aroma extracts of cooked meat. Odors of these volatiles have been described as sulfurous, cabbage, onion, garlic-like, pungent, and diffusive (Fors, 1983). Sulfur heterocyclic volatile compounds are known for their sensory properties by having savory, meaty, roasted, and boiled aromas, and it is a class of chemical constituents with particular flavor importance owing to their low odor thresholds.

From a standpoint of flavor development, H_2S has been well accepted as a major source of heterocyclic sulfur compounds. Origins of hydrogen sulfide in chicken meat are primary glutathione in muscle non-protein, and cysteine/cystine in muscle protein (Mecchi, Pippen, & Lineweaver, 1964). When meat is subjected to heat, H_2S is released from Strecker degradation or thermal decomposition of cysteine in accompany with the liberation of mercaptoacetaldehyde, acetaldehyde, 1, 2-ethanediol, and NH_3 (Güntert et al., 1990). These products are highly reactive and can interact with each other or with dicarbonyl compounds to form sulfur heterocyclic compounds (Güntert et al., 1990; Lee, Jo, & Kim, 2010).

A large amount of flavor studies on cooked meat and model systems have been focused on the identification of VOCs and the determination of their individual flavor significances. 2-methyl-3-furanthiol (meat-like, sweet), 2-furfurylthiol (roasted), 2, 4, 5-trimethylthiazole (earthy) and methional (cooked potato-like) were found to be the primary odorant compounds in chicken broth (Gasser & Grosch, 1990), while 2-methyl-3-furanthiol and its disulfide bis(2-methyl-3-furyl)disulfide (meat-like), methional, and 2-acetylthiazole (roasted) were the potent sulfur containing volatiles in cooked beef (Gasser & Grosch, 1988). Odor thresholds of these compounds are exceptionally low, and some of which were reported at the concentrations of

0.0025-0.001ng/L in air of 2-methyl-3-furanthiol (Gasser & Grosch, 1990), 0.0045-0.002ng/L in air of 2-furfurylthiol (Gasser & Grosch, 1990), 0.02ng/kg in water of bis(2-methyl-3-furyl)disulfide (Buttery, Haddon, Seifert, & Turnbaugh, 1984), 0.2ug/kg in water of methional (Guadagni, Buttery, & Turnbaugh, 1972), and 10ug/kg in water of 2-acetylthiazole (Schutte & Teranishi, 1974).

Nitrogen-containing heterocyclic compounds are associated with the roasted flavors of cooked meat, and they are frequently reported in meat processed by high-heat thermal treatments, such as grilling (Madruga et al., 2009; Mottram, 1985), roasting (Noleau & Toulemonde, 1986; Wu & Liou, 1992; Xie et al., 2008), pressure-cooking (Farkaš et al., 1997; Mussinan, Wilson, & Katz, 1973), and deep-frying (Tang, Jin, Shen, Ho, & Chang, 1983). This class of VOCs comprises of mainly thiazoles, pyrazines, pyridines, pyrroles, oxazoles, and their derivatives. Pyrazines generally contribute to the pleasant and desirable sensory attributes of meat foods by providing nutty and roasted aromas. Pyrazines identified in roasted pork were described as popcorn-like of 2-methylpyrazine, roasted and popcorn-like of 2, 5-dimethylpyrazine, and nutty and roasted of 3-ethyl-2, 5-dimethylpyrazine (Xie et al., 2008). Pyridines are another class of nitrogen heterocyclic volatiles whose odors were usually reported as green, burnt, and astringent (Fors, 1983; Van Ba, Touseef, Jeong, & Hwang, 2012). Although they have not received much attention due to the lower flavor significance, they still contribute to the overall sensory impression of roasted foods. In particular, some alkylpyridines isolated from the basic fraction of roasted lamb fat are partly responsible for the low acceptance of this kind of meat (Buttery, Ling, Teranishi, & Mon, 1977). Thiazoles, which contain both sulfur and nitrogen in the cyclic structures, are one of the major classes of aroma compounds in meat flavor composition. They afford the characteristic nutty, roasted, meaty, and sulfurous notes of cooked

meat and related products (Fors, 1983). Discrepancy in the odor descriptions of thiazoles between studies can be attributed to the particular concentrations and food matrices in which they occurred. For instance, benzothiazole identified in cooked beef was reported to have burnt and meaty aromas by Machiels, Van Ruth, Posthumus, & Istasse (2003), but described as pyridine-like and metallic by Gasser & Grosch (1988). Odors of this compound in roasted pork also were found to be roasted, nutty, musty, and sweet (Xie et al., 2008), but rubbery and musty in roasted duck (Chen et al., 2009). 2-acetylthiazole is known for its roasted, nutty, and popcorn-like aromas. The odor threshold of this compound was reported at only 3ug/L in water (Marchand, de Revel, & Bertrand, 2000).

From the point of view of chemical property, the odor thresholds and associated organoleptic qualities of heterocyclic volatile compounds are determined by a couple of parameters, such as volatility, polarity, molecular structure (functional groups; number or position of double bonds), and molecular size (such as chain length) (Boelens, & van Gemert, 1986; Teranishi, Buttery, & Guadagni, 1974). It has been demonstrated that furans with a thiol group in the β-position generally elicit strong meaty aroma and taste characteristics (Evers, Heinsohn, Mayers, & Sanderson, 1976). The degree of unsaturation and the position of the methyl group are also of significance for the flavor impact of some of the thiofuran derivatives (Van den Ouweland, Demole, & Enggist, 1989). Van den Ouweland et al. (1989) demonstrated that those with at least one double bond and a methyl group on the ortho position adjacent to the thiol group give a strong meaty flavor. In addition, as the length of side chains increases, the odor thresholds of alkylthiazoles and alkylpyrazines decreases, and the organoleptic qualities of pyrazines become more intensive (Teranishi et al., 1974). In general, mono-, di-, tri-, and tetramethylpyrazines have higher odor threshold values in comparison to those with substitution

by one or more ethyl group(s) (Guadagni, Buttery, & Turnbaugh, 1972). Moreover, the presence of an alkoxyl group appears to have an essential odor threshold-lowering effect on the corresponding pyrazine compounds (Shibamoto, 1986; Teranishi et al., 1974).

2.2. Main Chemical Reactions in Meat Cooking

There is a wide range of flavor sensations that can be perceived in meat. Depending on types of meat and cooking methods employed, flavors of cooked meat can range from being relatively bland and broth-like, to flavors with a strong meaty aroma and distinct roasted odors. Primary chemical reactions contributing to the flavor formation in meat include pyrolysis of amino acids and peptides, carbohydrate caramelization, Maillard reactions of sugars and amino compounds, thermal oxidation and degradation of lipids, as well as thiamin degradation (Mottram, 1991). Thermal decomposition of carbohydrates (caramelization), and amino acids and peptides (pyrolysis) generally require much higher temperatures than the other reactions. These reactions occur only on the surface of meat subjected to high heat treatments, and therefore, play a minor role in flavor formation of meat (Mottram, 1991).

2.2.1. Lipid Degradation. The thermally induced oxidation and degradation of lipids is an important route to introduce aroma volatile compounds during meat cooking. Fatty acids, both saturated and unsaturated, in adipose tissues and intramuscular fat of meat are capable of being oxidized and transformed into respective hydroperoxides intermediates, which can then decompose to give a complex mixture of odorant constituents upon heating (Van Ba et al., 2012). The oxidation of unsaturated fatty acids is initiated by an attack of molecular oxygen on the double bonds of the acids with the abstraction of hydrogen atoms and the formation of lipid radicals. Subsequent self-

rearrangement and further reaction with oxygen of these radicals yield different ranges of hydroperoxides (Mottram, 1991). These intermediates are very active and readily break down down into alkoxy and hydroxyl radicals. Decomposition of the alkoxy radicals (β-scission) generates a vast number of odorant compounds, such as aldehydes, ketones, alcohols, acids, hydrocarbons, lactones, furans, and esters (Varlet et al., 2007). Given the fact that fatty acids with methylene groups ($R{=}CH_2$) adjacent to double bonds are the most susceptible to the oxidative attack, oleic, linoleic, linolenic, and arachidonic acids, which are the main sources of unsaturated fatty acids in meat, are therefore the most vulnerable fatty acids to thermal degradations upon cooking (Baines & Mlotkiewicz, 1984; Mottram, 1991).

Compared to the heterocyclic volatile compounds formed from water-soluble precursors in Maillard reactions, lipid-derived odorants are more important in distinguishing different species flavors. In meat carcasses, intramuscular fat contents (marbling) and membrane lipids are the main sources of lipid-derived volatile compounds (Van Ba et al., 2012). Therefore, lipid contents and degrees of oxidation occurring in these fractions are strongly related to the ultimate sensory quality of the cooked meat. Other considerations of flavor impact from lipid degradation include the interactions with other chemical reactions during cooking, as well as the development of rancidity throughout meat storage. Findings from the previous model studies and cooked meat systems showed that an involvement of lipids can both quantitatively and qualitatively change the volatile compositions of Maillard reaction mixtures, and it was presumably due to the competition for flavor intermediates between the two chemical reactions (Mottram, 1985; Tang et al., 1983; Whitfield, Mottram, Brock, Puckey, & Salter, 1988). Influences of lipids on the overall meat flavor can be in part achieved by inhibiting the formation of some of the Maillard heterocyclic compounds, such as alkylpyrazines, while facilitating the production of many other

long-chain alkyl substituted heterocyclic odorants that are exclusively derived from Maillard-lipid interactions (Mottram & Edwards, 1983; Whitfield et al., 1988). Following the same formation mechanism of desirable flavor compounds, lipid oxidation also leads to off-flavor or rancidity during refrigerated storage which can adversely affect the sensory quality of meat (Brunton, Cronin, & Monahan, 2002; Mottram, 1991).

2.2.2. Maillard Reaction and the Associated Strecker Degradation. Maillard reaction is one of the most important non-enzymatic chemical reactions responsible for the flavor and color (browning) formation in foods (Mottram, 2007). This thermally induced reaction was firstly recognized by Louis-Camille Maillard in his prominent work on the sugar-amino acid reaction in 1912. However, it was 40 years after this discovery that John Edward Hodge proposed a coherent scheme of the reaction in 1953.

According to Hodge's scheme (Hodge, 1953) and subsequent reviews (Mottram, 1991 & 2007; Varlet et al., 2007), reaction mechanisms associated with the Maillard reaction can be divided into three stages. The initial stage involves the condensation of the carbonyl group of a reducing sugar with the amino group of an amino compound, such as an amino acid, peptide, or protein. The product is unstable in the aqueous solution and readily forms a Schiff base by eliminating a molecule of water. Subsequent non-reversible isomerization of the Schiff base gives rise to a more stable *N*-substituted glycosyamine. If an aldose, an aldosyamine is formed and then rearranged to the corresponding Amadori product (1-amino-1-deoxy-2-ketone) by the acid-catalysis (Hodge, 1953). If a ketose, the generation of ketosyamine undergoes rearrangement to give a Heyns product (2-amino-2-deoxaldose) (Mottram, 1991). In the intermediate stage, the reactive Amadori or Heyns rearrangement product spontaneously undergoes

enolization, deamination, fragmentation, and dehydration to form a wide range of reductones and dehydroxyreductones. Specifically, the dehydration of 3-deoxysones resulting from 1, 2-enolization of the Amadori products leads to furfurals from pentoses, or 5-methylfurfurals and 5-hydroxymethylfurfurals from hexoses (Mottram, 1991). Alternatively, 2-acetylfuran and furanones of aldosugars are the dehydration products of 1-deoxysones via the route of 2, 3-enolization (Mottram, 1991; Tressl, Grunewald, Silwar, & Bahri, 1979). What's more, retro-aldolizations of reductones and dehydroxyreductones also lead to a great number of smaller carbonyl compounds, such as diacetyl, acetoin, and hydroxyacetone (Varlet et al., 2007). Many of the aforementioned products are not only flavor constituents, but more importantly, they can readily react with amino acids in Strecker degradations to generate crucial heterocyclic flavor compounds. The final stage of Maillard reactions in Hodge's scheme comprises of aldo condensation, aldehyde-amine polymerization, and formation of nitrogenous heterocyclic compounds, which involve a collection of previously formed breakdown products. In this stage, the condensations of carbonyl compounds with each other, or with amino compounds are necessities for the formation of character impact odorants and brown nitrogenous polymers (melanoidins).

Strecker degradation is generally considered as a concomitant reaction occurring during the Maillard reaction. If the Maillard reaction is viewed as the degradation of reducing sugars catalyzed by the amino acids, Strecker degradation is then, per se, the decomposition of amino acids initiated by the dicarbonyl compounds. Pertinent mechanisms of this reaction have been comprehensively illustrated by Rizzi (2008) and Yaylayan (2003). Based on the review of Rizzi (2008), Strecker degradation of an amino acid begins with the nucleophilic attack of an unprotonated amino group to a carbonyl group with the formation of an unstable hemiaminal

condensation product. The removal of a water from this intermediate yields a Schiff base (α-iminocarbonyl) which can readily undergo decarboxylation to liberate a molecule of carbon dioxide and give azallylic zwitterions. It is by the hydrolysis of these intermediates that the formation of a Strecker aldehyde and an α-amino carbonyl compound can be finally achieved. Although α-dicarbonyl compounds derived from Maillard reactions are usually considered as the primary reagents in the associated Strecker degradations, in fact, any active α-carbonyl compounds can act as Strecker reagents provided they are capable of inducing oxidative deamination of the amino compounds via the formation of Schiff bases (Mottram, 2007; Rizzi, 2008). In this case, the carbonyls required may not be necessarily obtained from a carbohydrate source as Maillard intermediates. Other organic constituents indigenously present in meat, such as lipids, can also involve in the Strecker degradation by contributing α-unsaturated carbonyl compounds to the reaction through thermal oxidative degradation (Rizzi, 2008). Strecker degradations provide a rich source of intermediates for the formation of many classes of heterocyclic compounds that are essential for meat flavor.

Maillard reactions and Strecker degradations have implications other than flavor formation in foods. During food processing and storage, the interactions between reactive carbonyls and amino compounds could lead to the loss of essential amino acids and functional peptides and proteins, which in turn lower the nutritional values of foods. At the biological level, such interactions result in the formation of low molecular weight advanced glycation end products (AGEs) (Van Nguyen, 2006). The physiological impact of AGEs related to ageing and complications in some of the chronic diseases, such as diabetes, atherosclerosis, and neurodegenerative diseases, has been extensively discussed

in multiple studies (Baynes, & Thorpe, 2000; Vlassara & Palace, 2002; Sasaki et al., 1998). Meanwhile, potential mutagenic or carcinogenic Maillard reaction products (MRPs), such as acrylamide, furans, and heterocyclic amines, are of particular concerns regarding thermal-treated foods (Bakhiya & Appel, 2010; Mottram, Wedzicha, & Dodson, 2002; Skog, Johansson, & Jägerstad, 1998; Surh, Liem, Miller, & Tannenbaum, 1994). Nevertheless, antioxidant activities exerted by certain MRPs have received much attention as one of the advantages of Maillard reactions (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2000).

2.3. Flavor Precursors in Raw Meat

Flavor is a perception comprised of mainly taste and aroma (ASTM International, 2009). Taste sensations are triggered when taste compounds bind to the specialized taste buds located in the mouth, stimulating the basic sensations that are perceived as sweet, sour, salty, bitter, and umami. Olfactory perceptions are initiated by a mixture of volatile components transported to the olfactory receptor cells through nares and nasopharynx. The combination stimulates sensory signals which after being processed by the brain can facilitate odor discrimination (Stevenson & Wilson, 2007).

Flavor precursors in raw meat can be categorized into lipids and water-soluble compounds (Mottram, 1998). Natural concentrations of these precursors differ both between animal species and muscle types. Diets, breeds, and sex of the animals, as well as pre-slaughter handling procedures and post-slaughter storage conditions are some of the determinants of the differences (Van Ba et al., 2012). So far, biochemical and physiochemical changes in post-mortem muscles as well as the underlying mechanisms have been substantially reviewed (Bechtel, 1986; Schreurs, 2000). The relative importance of individual precursors for cooked meat flavors was also addressed in the previous studies.

2.3.1. Lipid precursors. Intramuscular triglycerides and structural phospholipids are the main sources of fatty acids in meat muscles. Naturally, the proportion of PUFAs in phospholipids is much higher than that in neutral lipid triglycerides (Fogerty, Whitfield, Svoronos, & Ford, 1990; Mottram, 1998). Specifically, the levels of linoleic acid as well as its synthetic PUFAs, such as arachidonic acid, are much greater in muscle phospholipids as compared to triglycerides (Wood et al., 2008). In view of heat liability, meat phospholipids were thermally susceptible to hydrolysis. Researchers found that there was a remarkable loss of PUFAs and fatty aldehydes in lean meats after cooking, whereas, only a slight release of fatty acids was observed from intramuscular triglycerides (Fogerty et al., 1998). Considering that PUFAs are exceptionally vulnerable to thermal oxidation and degradation, higher levels of PUFAs in raw meat conceivably give rise to a larger amount of lipid-derived VOCs during meat cooking. Elmore, Mottram, Enser, & Wood (1999) reported that a higher level of lipid oxidation products, especially saturated and unsaturated aliphatic aldehydes, was released from the cooked beef containing a greater amount of PUFAs.

The type of muscle fibers is closely related to their fatty acid compositions. For example, raw chicken meat has a higher content of intramuscular PUFAs compared to beef and pork (Rhee, Anderson, & Sams, 1996). Particularly, the quantities of linoleic acid (18:2n-6) and the related longer chain PUFAs in pork muscles were more notable than those in ruminant meats which in turn contained relatively higher levels of linolenic acid (18:3n-3) and its synthesized PUFAs (Enser, Hallett, Hewitt, Fursey, & Wood, 1996). Compared to beef, pork, and chicken, fish flesh has a richer profile of PUFAs, particularly the n-3 PUFAs (Lovell, 1980). When heat is applied, these fatty acids are

readily subjected to autoxidation, leading to a series of decomposition products, which contribute to the authentic "fishy" and "fatty" aromas of cooked fish (Varlet et al., 2007).

In meat, features of flavor profiles from lipids are affected by several factors, which include but are not limited to the variations of fatty acid compositions; the liability of PUFAs to autoxidation; as well as the self-owned flavor properties of the lipid breakdown products.

2.3.2. Water-soluble Flavor Precursors. This class of organic compounds in meat consists of free sugars, sugar phosphates, nucleotide/nucleosides, free amino acids, peptides, and thiamin (Mottram, 1998). Sugar and sugar related components are glycogen-derived products, while proteolytic degradation in post-mortem muscles accounts for the generation of small molecular weight amino acids and peptides. Contributions of water-soluble flavor precursors to the overall sensory quality of meat have been studied in numerous model systems and raw meat with certain amounts of these compounds added prior to cooking. The subsequent results are usually analyzed by both sensory and instrumental assessments.

Nucleotides and related compounds. Inosine 5'-monophosphate (IMP), inosine, and hypoxanthine in meat carcass are all originated from ATP. After the death of an animal, glycogen stored in the muscle is the main energy reservoir, which upon glycogenolysis and glycolysis can break down into ATP. The simultaneous degradation of AMP coming from ATP gives IMP (Tikk et al., 2006). As a result, the concentration of IMP increases at the early stages post-slaughter, but it progressively degrades into inosine, and further ribose and hypoxanthine by the corresponding enzymes during ageing (Aliani, Farmer, Kennedy, Moss, & Gordon, 2013; Tikk et al., 2006; Williamson, Ryland, Suh, & Aliani, 2014).

Previous studies have indicated the importance of IMP in meat quality as both a taste enhancer (Kato & Nishimura, 1987), and a flavor precursor. From a flavor aspect, it can undergo

Maillard reactions with other amino acids to generate desirable VOCs giving off the characteristic cooked meat flavors (Farmer, Hagan, & Paraskevas, 1996). The taste property of IMP is clear and can be explained by its chemical structure as a 5'-nucleotide with a hydroxyl group in the 6-position (Reineccius, 2005). This water-soluble compound holds an umami taste, and in combination with monosodium glutamate (MSG), it gives a synergistic flavor-enhancing effect on foods. According to the previous literature, effects of IMP on the aroma formation in meat seem to be concentration-dependent and species-specific. Farmer, Hagan, & Paraskevas (1999) found that at four times the natural concentration (340mg/100g), IMP added in raw meats resulted in a significant increase in meaty and roasted aromas in both pork and beef. However, when the addition of IMP was two fold the reported concentration (170mg/100g), the increase of meaty aroma was only observed in pork. Aliani & Farmer (2005b) reported that there was no significant effect of IMP on the flavors of cooked chicken with the concentration added at twice (150mg/100g) the natural levels. In addition to sensory analysis, the identification of volatile compounds from raw meat with IMP added provides a complementary evidence of potential flavor impact of this compound on meat studied. In beef treated with 4-fold the natural concentration of IMP, the roasted aroma given off appeared to be intensified and the green odor was suppressed. At the same time, a concomitant change in the quantities of 2-methyl-3-(methyltrithio)furan and hexanal was also detected (Farmer et al., 1996 & 1999). At a higher concentration of 10 times as much as the natural levels added to beef, IMP was shown to be a precursor of a number of dicarbonyl, sulfur-substituted furan, and disulfide compounds in which many of them possess part of the representative beef flavors (Mottram & Madruga, 1994).

Reducing sugars and related phosphorylated sugars. The influence of carbohydrates on the generation of aroma volatiles has been explored in various types of meat at different times post-slaughter. In most cases, the determination of natural concentrations of flavor precursors in fresh meat, as well as their changes during conditioning is critical in regards to evaluating the flavor-generating potentials of these compounds. Ribose, glucose, and their phosphorylated sugars are some of the monosaccharides that have been intensively studied. The natural concentrations of these compounds and their respective flavor contributions vary between meat species and under different storage conditions.

Based on the previous study, the natural concentrations of monosaccharides in pork after 6 days of ageing at 4℃ were followed by the order from greatest to least as glucose (4.0umol/g), glucose-6-phosphate (G6P) (2.6umol/g), ribose (0.3umol/g), and no detection of ribose-5-phosphate (R5P) (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009). Similarly, the quantity of glucose (4.6umol/g) in pork after conditioning for 22 days at 2℃ was found to be greater than the amount of ribose (0.1umol/g) or G6P (2.6umol/g) (Meinert, Andersen, Bredie, Bjergegaard, & Aaslyng, 2007). The variation between two studies was suggested to be caused by the different muscle types and ageing conditions employed in the studies (Meinert et al., 2009). Besides pork, the average concentrations of ribose were significantly lower than glucose and G6P in bison muscle over 21 days chilled storage conditioning at 4℃. Again, no ribose phosphates was detected in this study. Similarly, the much greater level of glucose found in red meat was also observed in poultry muscle. As reported by Aliani & Farmer (2002), the average quantity of glucose was much higher than that of G6P, ribose, and R5P in fresh chicken breast muscle. Other than the inherent differences between meat species, variations in the individual study designs, such as meat cuts, storage temperatures and duration, as well as extraction

methods and analytical approaches are all factors influencing the eventual amounts of carbohydrates detected in post-mortem muscles.

Effects of time post-slaughter on sugar concentrations in meat have been investigated. In chicken meat, the concentration of ribose was low at early stages post-slaughter, but increased remarkably and linearly with time during chilled storage (Aliani et al., 2013). In contrast, R5P firstly increased during early storage stages, but decreased with time after 28h post-slaughter. The amounts of both glucose and G6P declined to some degrees after storage. Similarly, ribose in bison muscle increased substantially with time across meat conditioning, but no significant differences were observed in the case of glucose and G6P at the end of conditioning (Williamson et al., 2014). In general, the post-mortem duration required for ageing and concomitant muscular tenderization is shorter in poultry than in red meat (Dransfield & Sosnicki, 1999; Schreurs, 2000). Variations in the natural concentrations of sugars in meat may reflect the dynamic interchange between sugars and their breakdown products by the action of endogenous enzymes. However, it is difficult to pinpoint the actual contribution of particular pathways to the formation of sugars, as there are generally several of them running parallel during ageing.

Ribose present in post-slaughter muscle is mainly from IMP by dephosphorylation, and there could be more than one potential breakdown pathway leading to the release of ribose (Lee & Newbold, 1963). The generation of R5P can be achieved by the IMP degradation or by the pentose phosphate pathway (Lee & Newbold, 1963). R5P appears to be reactive, and it was shown to be readily converted into G6P and ribose in pork samples (Meinert et al, 2009). Glucose and G6P are well-known glycogen

metabolites. The interchange of these compounds with other sugars and related sugar compounds contribute to the rise or fall in their respective concentrations in muscle post-slaughter.

The content of ribose in meat is relatively low, however, a slight change in its concentration may lead to a significant alteration in the sensory quality of cooked meat. Aliani & Farmer (2005b) reported that ribose added at 2-4 times the natural concentration in chicken meat, which corresponded to the natural variations between commercial sources, resulted in a marked increase of meaty, roasted, and chicken flavors in cooked meat samples. Farmer et al. (1996 & 1999) found that the addition of ribose at twice the natural level (600mg/100g) in pork meat considerably increased both the meaty and roasted aromas after cooking. In comparison to ribose, glucose appears to be more important in the flavor formation of pork foods (Aliani & Farmer, 2002; Meinert et al., 2009). However, no significant effect of glucose on the flavor of cooked chicken was detected even more than 4-fold the natural concentration was added prior to the thermal treatment (Aliani & Farmer, 2005b). Compared to ribose and glucose, their phosphorylated sugars seem to play a minor role in the flavor development of meat (Aliani & Farmer, 2005b; Meinert et al., 2009).

In the Maillard reaction, the higher reactivity of ribose over glucose is known for its propensity to the reaction as a pentose. The less bulky skeleton and a higher proportion of the open-chain form in the solution render the proceedings of a reaction easier in ribose than in glucose (Hayward & Angyal, 1977; Laroque et al., 2008). In model systems, R5P was found to be more reactive than ribose, though its effect on the flavor formation of meat was less significant. In the reaction with cysteine, R5P yielded a

larger quantity of volatile compounds, especially mercaptoketones and their oxidized disulfides, than those from ribose (Mottram & Nobrega, 2002). The plausible mechanism leans toward a non-Maillard type breakdown of R5P. Instead of forming Amadori intermediates, R5P may be directly converted into 4-hydroxy-5-methyl-3(2H) furanone via the dephosphorylation-dehydration reaction. This reactive furanone can readily form VOCs with meaty aromas, such as furanthiols and thiophenes, in the presence of H_2S (Farmer et al., 1999; Mottram & Nobrega, 2002; Van Den Ouweland & Peer, 1975).

Free amino acids and small molecular weight peptides. Irrespective of animal species, protein degradation occurring in the conversion from muscle to meat is a prime factor determining the post-mortem tenderization, as well as the flavor and texture development in cooked meat. The degradation of key myofibrillar and associated proteins is believed to be enzyme-dependent, in other words, it relies on the pH and ambient temperatures for the optimal activity of enzymes in meat during ageing (Niewiarowicz, Pikul, Trojan, & Thomson, 1978; Quali, 1992).

Free amino acids and smaller MW peptides are the major products of protein degradation. They possess multifunctional properties in foods and biological systems. In terms of meat palatability, free amino acids and peptides can serve as taste-active compounds by providing the flavor background of meat products (Dunkel & Hofmann, 2009; Solms, 1969). In Maillard reactions, amino acids are essential flavor precursors in the formation of desirable volatile compounds. Previous studies on meat flavor have been focused on the facet of free amino acids as taste and aroma contributors. Much less attention was paid on the role of peptides, especially those with low MW, as flavor precursors in meat.

Effects of ageing on the generation of proteolytic products are of great interest in meat processing. In general, there is a substantial increase in the concentrations of free amino acids and peptides with time post-slaughter. Based on ante- and post-mortem factors, the degree of increments varies between animal species. Non-protein-nitrogen (NPN), which corresponds to the muscle's non-soluble compounds containing nitrogen (Bruas-Reignier & Brun-Bellut, 1996), is frequently used as an indicator of meat proteolysis. During the post-rigor period, the NPN content increased significantly, but at different rates in chicken (Khan & Berg, 1964), beef (Bruas-Reignier & Brun-Bellut, 1996), goat (Feidt, Brun-Bellut, & Dransfield, 1998), and lamb (Sylvestre, Feidt, & Brun-Bellut, 2001). It suggests that free amino acids and peptides are liberated in this process. Direct measurements of the contents of free amino acid and peptide have been reported, and a general agreement on their significant increases with time post-slaughter during meat ageing is obtained (Feidt, et al., 1998; Williamson, et al., 2014). Variations in the concentrations of amino acids and peptides are common between animal species or meat cuts (Cornet & Bousset, 1999; Niewiarowicz, et al., 1978). These differences, may not only indicate the extent of enzymatic activities working on the muscular proteins during ageing (Moya, Flores, Aristoy, & Toldra, 2001; Nishimura, Rhue, Okitani, & Kato, 1988), but also contribute to the flavor quality of individual cooked meat samples.

During meat storage, the release of peptides from proteins is catalyzed by proteinases, such as cathepsins and calpains, while smaller peptides and free amino acids are liberated by the action of peptideses and aminopeptidases (Feidt et al., 1998; Moya et al., 2001; Nishimura, Rhyu, & Tajima, & Kato, 1996). The increase in the level of smaller sized peptides seems to be a trend with time post-slaughter. Nishimura et al. (1988) reported that in beef, pork, and chicken, the levels of oligopeptides increased after storage, whilst the rises were significant only in

chicken and pork. Similarly, the proteolytic activities in goat meat were also studied. After storage of 25 days at 2℃, the amount of larger peptides (> 4KDa) reduced to 20% of its initial level, while small MW peptides (< 2KDa) elevated by about 50% with storage time. The change in the quantity of intermediate MW peptides (1.9 to 4KDa) was shown to be more pH-dependent (Feidt et al., 1998). Results aforementioned were in accordance with observations obtained from other meat types (Bauchart et al., 2006; Claeys, De Smet, Balcaen, Raes, & Demeyer, 2004; Sylvestre et al., 2001), which all suggest alterations in the peptide levels during meat ageing are closely related to the nature of post-mortem muscles.

Contributions of free amino acids to the development of meat taste and aroma have been elaborated by many researchers. However, few studies have investigated the roles of small peptides, as proteolytic products from meat ageing, in the formation of flavor volatiles induced by the Maillard reaction. Among the peptides isolated from meat, carnosine (β-alanyl-L-histidine), anserine (β-alanyl-N(π)-methylhistidine), and glutathione (γ-L-glutanyl-L-cysteinyglycine) are three endogenous peptides that have gained particular interests due to their multifunctional properties in foods and biological systems.

Carnosine and its methylated derivative anserine are histidine-containing peptides. They are present in an appreciable amount in the skeletal muscles of most of the vertebrate species (Boldyrev & Severin, 1990). These peptides have strong antioxidant and buffering capacities, and thereby provide benefits for meat quality and shelf life maintenance (Kohen, Yamamoto, Cundy, & Ames, 1988). Carnosine appears to be effective in inhibiting lipid oxidation either by inactivating free radicals or by forming complexes with metals to decrease their prooxidant

activities (Decker, Crum, & Calvert, 1992; Quinn, Boldyrev, & Formazuyk, 1992). It was reported that the addition of carnosine together with ascorbic acid and ribose in minced bison meat synergistically increased the aroma intensity and the color acceptability of grilled patties (Aliani, Ryland, Williamson, & Rempel, 2013). The antioxidant activity of carnosine and is attributed to the imidazole moiety of histidine in the peptide. However, methylation of one or two of the nitrogen atoms in the imidazole ring could conserve or impede the antioxidant property as in the case of anserine and other histidine-related compounds like 1-methyl-*L*-histidine (Kohen et al., 1988).

Although carnosine and anserine exhibit slightly sour and astringent tastes in nature (Dunkel & Hofmann, 2009), they were found to be key molecules giving the typical thick-sour and white-meaty characters of double-boiled chicken broth (Dunkel & Hofmann, 2009). On the other hand, Pereira-Lima, Ordoñez, de Fernando, & Cambero (2000) suggested that there was a significant correlation between the levels of carnosine and anserine and the development of characteristic beef broth flavor. In their study, a greater impact was found from an increase of anserine compared to carnosine. Aside from directly contributing to meat flavors, these skeletal peptides can also interact with key flavor compounds to affect the sensory perceptions of muscle foods (Gianelli, Flores, & Toldrá, 2003). Compared to anserine, carnosine has a higher affinity for hexanal, 2-methylbutanal, 3-methylbutanal, and methional, which are aroma compounds generated from lipid degradation or Strecker degradation of amino acids (Gianelli, Flores, & Toldrá, 2003). The selective interactions of these peptides with certain flavor compounds were considered as a result of their chemical structures and subsequent binding potentials (Gianelli, Flores, & Toldrá, 2003; Zhou & Decker, 1999).

The contents of carnosine and anserine vary depending on the types of muscles. In general, glycolytic muscles contain higher concentrations of carnosine than those in oxidative muscles (Cornet & Bousset, 1999; Maikhunthod & Intarapichet, 2005; Peiretti, Medana, Visentin, Dal Bello, & Meineri, 2012). Whereas, the trend for anserine contents in regards to muscle types is vague (Cornet & Bousset, 1999). In individual meat samples, there is a seemingly inverse relationship between the levels of carnosine and anserine (Aliani & Farmer, 2005a; Mora, Sentandreu, & Toldra, 2007; Peiretti et al., 2011). Carnosine predominates in mammalian species, whilst anserine is much more abundant in non-mammalian species (Aliani & Farmer, 2005a; Mora et al., 2007; Peiretti et al., 2011).

Due to the absence of aminoacylhistidine dipeptidase in the skeletal muscles, the levels of carnosine and anserine remained stable over meat storage (Bauchart et al., 2006; Cornet & Bousset, 1999). However, they are lost to a certain extent in meat after cooking. Compared to microwave-cooking and broiling, water-boiling caused the most significant loss of carnosine and anserine in beef and turkey after cooking (Peiretti et al., 2012). In addition, cooking temperature is more important than heating time in determining the depletion of these peptides in cooked meat (Pereira-Lima et al., 2000). Researchers suggested that the greater loss of carnosine and anserine in boiled meat could be attributed to their high solubility and ease of liberation (Peiretti et al., 2012; Pereira-Lima et al., 2000).

To date, research attempting to elucidate the role of carnosine and anserine as flavor precursors in the Maillard reaction is sparse. In a recent study, VOC profiles formed from the reaction mixtures containing an equimolar of ribose, cysteine, with or

without carnosine, heated at 180℃ for 2 hours at pH 5.0 or 8.5 were investigated (Chen & Ho, 2002). Results showed that the addition of carnosine appeared to inhibit the formation of some of the prominent sulfur flavor compounds, such as thiophenes and furanthiols, whilst increasing the yields of other nitrogen-containing heterocyclics, like thiazoles and pyrazines. Under the identical condition, several pyrazine compounds were identified from the reaction between carnosine and ribose, suggesting the role of carnosine as a nitrogenous source in the Maillard reaction (Chen & Ho, 2002).

GSH and cysteine-glycine (Cys-Gly). GSH is an endogenous peptide present virtually in all living cells (Meister, 1974). The sulfhydryl group in the cysteine residue of GSH accounts for its multifaceted physiological functions, including antioxidant defense, metal chelation, detoxification, and cell metabolic regulation (Liu & Eady, 2005; Sen, 1997; Wu, Fang, Yang, Lupton, & Turner, 2004). In contrast to the physiological significance, much less information was acquired for the direct impact of GSH as a non-volatile constituent on cooked meat flavor. With no effect on the basic tastes, GSH elicited a characteristic *kokumi* flavor by enhancing the continuity, mouthfulness, and thickness in model beef extracts (Ueda, Yonemitsu, Tsubuku, Sakaguchi, & Miyajima, 1997). Furthermore, beef soups containing Maillard reaction products (MRPs) of GSH had a stronger beef flavor than the control or soups with MSG added, and they had a comparable sensory quality to the samples with GSH added at a similar concentration (Hong, Jung, Kim, Lee, & Kim, 2010).

In terms of aroma perceptions, GSH is believed to be a crucial precursor of sulfur-containing flavor compounds in meat upon cooking. Sensory characteristics exhibited by this peptide in the thermal degradations with/without sugars have been shown to be highly associated with cooked meat flavors (Lee, Kwon, Kim, & Kim, 2011). In combination with other

constituents, it has been commercially used to produce imitation meat flavors in the food industry (Rhee, 1989). Within the studies focusing on the Maillard reactions of GSH, El-Farouk, & El-Ghorab (2003) found that by refluxing an equal molar ratio of ribose and GSH constituted amino acids for 3 hours at pH 7.1, a pronounced boiled-meat flavor with low intensity of roasted and burnt notes was obtained. Identification of VOCs from the model system showed that sulfur-containing odorants were predominant in the product mixture whilst pyrazines or oxazoles was absent. These observations agree with the fact that the release of H_2S is much faster than NH_3 in the thermal degradation of GSH (Zheng & Ho, 1994). Because of this, it enables H_2S to react faster with carbonyl compounds derived from sugars, leading to a greater yield of heterocyclic compounds containing sulfur than those with nitrogen (Lee et al., 2010).

In order to demonstrate the role of GSH and its related peptides in the production of meat flavors, mechanisms underlying GSH degradation were also investigated. Ueda et al. (1997) reported that the thermal degradation of GSH in aqueous solutions at 98℃ and at pH 5.0 or higher produced glutathione disulfite (GSSG), pyroglutamic acid (PCA) and cyclic Cys-Gly, in which PCA was derived from the glutamic residue of GSH. Ho, Lu, Wang, Raghavan, & Payne (2008) found a more complex degradation mixture from GSH heated at 160℃ for 1hr at pH 7.5. It was proposed that during the Maillard reaction, GSH might split in the position of glutamyl and cysteinyl, giving rise to PCA, cysteine and Cys-Gly dipeptides (Wang, Yang, & Song, 2012). The Cys-Gly dipeptide could later form cyclic (Cys-Gly) [also known as diketopiperazines (DKPs)], while the straight chain Cys-Gly may serve as a Maillard reactant in the subsequent reactions (Ho et al., 2008). Studies of the Maillard volatile generation of GSH and its related peptides showed that volatile products formed from Cys-Gly were qualitatively

comparable to those from GSH (Ho et al., 2008; Wang et al., 2012). When heated with glucose, a greater amount of volatile compounds was generated from Cys-Gly as compared to its reverse sequence Gly-Cys (Ho et al., 2008; Lu, Hao, Payne, & Ho, 2005).

Currently, the biological functions of dipeptide Cys-Gly in living organisms are still a field of much unknown. Early studies on the natural occurrence of Cys-Gly as well as GSH-related metabolisms may provide some indications of the potential physiological importance of this peptide. Based on the γ-glutamyl cycle proposed by Meister (1974), Cys-Gly is endogenously originated from the breakdown of GSH by the action of γ-glutamyl peptidase (EC 2.3.2.2). Within the cycle, the homeostasis of GSH is rigorously regulated by a series of enzyme-catalyzed reactions which involve a dynamic breakdown and synthesis of the related compounds. GSH deficiency caused by either nutritional and diseased-associated factors, or dysfunctions of one or more of the enzymes in the γ-glutamyl cycle may lead to an abnormal content of Cys-Gly in cells (Meister, 1974; Perry & Hansen, 1981). The regular concentration of Cys-Gly in human plasma was reported as 9-11μmol, or approximately one-fifth that of cysteine (Armstrong, 1979; Perry & Hansen, 1981). This peptide was also found in the plasma from bovine, rat and rabbit blood (Armstrong, 1979), and it was isolated from the water-soluble metabolite fraction of chicken extracts (Aliani, unpublished data). Comprehensive investigation of this dipeptide is worthy since it is not only metabolically significant to GSH homeostasis, but also potentially important in the thermal formation of flavor compounds due to the presence of a thiol group.

2.4. Peptides in Maillard Reactions and Associated Flavor Formation Mechanisms

Peptides, as one of the most common nutrient constituents, have been reported in a wide range of food systems, such as meat extracts (Mabrouk, 1976; Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011) and hydrolyzed vegetable proteins (Manley, McCann, & Swaine Jr, 1981). They are compounds of great interest not only because they provide multiple nutritional and biological functions (Udenigwe & Howard, 2013), but also they modify sensory quality of foods by affecting their taste (Dashdorj, Amna, & Hwang, 2015; Wang, Maga, & Bechtel, 1996) and aroma sensations (Ho, Oh, Zhang, & Shu, 1992). Chemical modifications of peptides, as well as their influence on food properties have been comprehensively reviewed by Lanker, Adams, & De Kimpe (2011).

It is well established that the omissions of peptides from Maillard reactions can have a direct effect on the final organoleptic quality of foods which cannot be compensated by the addition of amino acids (de Kok & Rosing, 1994; Mohr, Rohrle, & Severin, 1971). Maillard reactions between free amino acids and sugars have been thoroughly reviewed in previous studies. However, reactions of peptides and sugars with respect to flavor formation are still less understood.

2.4.1. Peptides reactivity in Maillard reactions. The reactivity of peptides in Maillard reactions has been measured based on the nature of peptides at issue, such as chemical structure, chain length, amino acid composition and sequence, and hydrolysis susceptibility of peptide bonds. The majority of studies focusing on the thermal reaction of peptides and carbonyl compounds were performed using model systems to yield relatively well-defined mechanisms (Yang, Wang, & Song, 2012).

In order to explore the effect of peptide chain length on the volatile formation of peptides in Maillard reactions, glycine and its homopolymers were frequently used due to their simple structures (Kim & Lee, 2009). Oh, Shu, & Ho (1991) reported that in the reactions with glucose at 180℃ at pH 4-5 for 2hr, glycine and triglycine generated larger amounts of pyrazines than those from diglycine and tetraglycine. The quantities of furfural and 5-(hydroxymethyl) furfural obtained from diglycine and tetraglycine were otherwise greater than the amounts formed from glycine and triglycine. Reasons for the similarities of the relative abundance of pyrazines of glycine with triglycine, and diglycine with tetraglycine were suggested, in which triglycine could be cleaved into glycine and diglycine via DKPs, whilst tetraglycine was primarily degraded into diglycine in the browning reactions (Oh et al., 1991). Lu et al. (2005) also found that the reactivity of triglycine in the formation of pyrazines was similar to that of glycine but greater than diglycine in the Maillard reaction. Such a difference was possibly caused by the higher availability of free glycine from triglycine due to the lower electron density of the peptide bond. In the case of di- or tetraglycine, the hindrance of peptide-bond cleavage rendered these amino compounds more efficient in catalyzing the formation of sugar derivatives rather than contributing to the backbones of pyrazine compounds in the Maillard reaction (Lu et al., 2005; Izzo & Ho, 1992). By quantifying the non-volatile components formed from the heated reaction mixtures of di-and triglycine with glucose, triglycine was mainly degraded into cyclic Gly-Gly and glycine, while cyclic Gly-Gly and diglycine were the major non-volatile compounds of diglycine/glucose reaction mixture (Lu et al., 2005). These results were in agreement with the observations obtained by Kim & Lee (2009) in their similar model systems.

According to the previous literature, results from the use of different parameters in the evaluation of peptide reactivity and the rates of Maillard reactions were not consistent. de Kok & Rosing (1994) considered that the rate of sugar conversion was a better parameter for these purposes. In their study, the reactivity of peptides was increased from glycine to diglycine, but decreased to triglycine in the reactions with glucose under both buffered and non-buffered conditions. The much higher reactivity of diglycine was possibly caused by the intramolecular protonation of the COOH terminus on the imine nitrogen of the dipeptide-glucose adduct (de Kok & Rosing, 1994). However, decreasing or increasing the chain length to glycine or triglycine could otherwise reduce the reactivity of the peptide, since the proximity of COOH terminus to its imine group of the adduct was inhibited (de Kok & Rosing, 1994).

Amino acid sequence is another important factor influencing the reactivity of peptides. In this case, volatile profiles isolated from the Maillard reactions of peptides and their reverse sequences, or of peptides with the same amino acids at the N- or C-terminus are frequentyl studied. Lu, Payne, Hao, & Ho (2008) investigated the hydrolysis susceptiblity of Gly-Ser and Ser-Gly heated at 160℃ for 1hr at pH 7.5 without sugars. Identification of volatile compounds from the reactions of Gly-Ser, Ser-Gly, and their constituted amino acid mixture Gly + Ser with glucose under the same condition (pH not mentioned) was also conducted. The researchers found that Gly-Ser upon heating was more liable to cleavage compared to its reverse sequence. In the Maillard reactions, Gly-Ser generated more pyrazines than Ser-Gly, whilst the yields of furfural and 5-methylfurfural were greater in the case of Ser-Gly. The higher hydrolysis rate of Gly-Ser was attributed to the amino acid serine at the C-terminal position. The hydroxyl group of

Ser can intramolecularly attack the carbonyl carbon of the peptide bond, which facilitates the breakdown of Gly-Ser. However, the self-catalysis of Ser-Gly was hindered due to the unfavorable four-membered ring transition state (Lu et al., 2008; Yashiro et al., 2003). Since the peptide bond of Gly-Ser is more susceptible to hydrolysis, the higher availability of free amino acids from this peptide could render the Maillard formation of pyrazines easier as in the case of triglycine.

Effects of amino acid composition on the reactivity of peptides also involve the intramolecular catalysis of peptide-sugar adducts or Maillard reaction intermediates. These phenomena have been observed in the dipeptides whose C-terminal amino acids bear an extra carboxylic side chain. de Kok & Rosing (1994) compared the reactivity of dipeptides Gly(Met)-X (X=Gly, Val, Thr, Pro, Phe, Glu, Lys, Asp, and His) in the reactions with glucose at 100℃ at pH 5.6. They found a "Glu effect" on accelerating the rate of reactions when "X" was substituted by glutamate, whereas, such an effect was absent in the Pro-X systems. Due to the fact that proline contains a secondary amine, the positively charged amino group (iminium cation) in the Pro-X/sugar Schiff base intermediate resists acid hydrolysis, and hence, it cannot be deprontonated to yield neutral glycosylamine derivatives (de Kok & Rosing, 1994). However, the presence of a basic group ϵ-NH_2 at the C-terminus appeared to facilitate the intramolecular deprotonation of the intermediate as seen in the Pro-Lys reaction (de Kok & Rosing, 1994; Oh, Hartman, & Ho, 1992).

2.4.2. The VOC formation from peptide/sugar reaction model systems. The role of peptides as flavor precursors in thermal reactions has been investigated to a limited extent. Peptides appear to have complex effects on flavor formation. It was considered that the volatile compounds produced by peptides were qualitatively similar to those from free amino acids in the

Maillard reaction (Van Lancker et al., 2011). Moreover, Maillard reactions of peptides were shown to generate specific volatile compounds which could not be found in the case of free amino acids (Oh, Shu, & Ho, 1992). With regard to volatile products, Strecker aldehydes were also detected in the thermal reactions of dipeptides and sugars. Considering that peptides cannot undergo typical Strecker degradations due to the blocking of amino groups, the identification of Strecker aldehydes may suggest the hydrolysis of peptides to a certain extent during the reactions (Oh, Hartman, & Ho, 1992; Van Lancker, Adams, & De Kimpe, 2012). For peptides with more than two amino acids, it is postulated that they tend to hydrolyze first to dipeptides which preferably react with sugar derivatives to generate flavor compounds instead of further breaking down into amino acids (Oh, Hartman, & Ho, 1992). It agrees with the mechanistic interpretation proposed by Steinberg & Bada (1983) who validated the decomposition of larger peptides in neutral pH region to the corresponding cyclic dipeptides.

Pyrazines are one of the most important classes of volatile compounds formed from Maillard reactions. Recent work on the interactions of peptides and carbonyl compounds has disclosed some of the formation patterns of pyrazines correlated to peptide reactivity. It leads to a conclusion that each peptide may serve as a unique flavor precursor in the overall sensory development of foods (Rizzi, 1989). Recently, Van Lancker, Adams, & De Kimpe (2010) reported that more pyrazines were produced from dipeptides, as compared to the corresponding amino acid mixtures, in the reactions with glucose, methylglyoxal, or glyoxal at 130℃ for 2hr at pH 8.0. The yields of 2, 5(6)-dimethylpyrazine and trimethylpyrazine were particularly high in the case of the dipeptides, whilst the amounts of unsubstituted pyrazine and amino acid specific

pyrazines were greater in the reactions with free amino acids. As Strecker aldehydes are required in the formation of amino acid specific pyrazines, the detection of these pyrazines from the dipeptide systems indicated peptide bond cleavage during the reactions (Van Lancker et al., 2010). Other than the unsubstituted and methyl-substituted pyrazines, ethyl-substituted pyrazines were also Maillard products of peptides (Lu et al., 2005; Van Lancker et al., 2010, 2012). In general, pyrazines with one or more ethyl side chains have lower odor thresholds than those substituted only by the methyl groups (Guadagni et al., 1972). These compounds are more important for roasted meat aromas, and their formations were favored in the Maillard reaction of glycine or triglycine as compared to that of diglycine (Lu et al., 2005). In whey protein isolates, peptides generated upon hydrolysis were found to significantly increase the formation of pyrazines when heated with glucose, whereas free amino acids originally present in hydrolyzed whey protein appeared to play a minor role (Scalone, Cucu, De Kimpe, & De Meulenaer, 2015).

Pyrazinones are peptide-specific Maillard reaction products which cannot be formed from free amino acids. Van Chuyen, Kurata, & Fujimaki (1973) reported that a series of pyrazinone derivatives [2-(3'-alkyl-2'-oxopyrazine-1'-yl) alkanoic acids] were formed from the reactions of various dipeptides with glyoxal at 100℃ for 30min at pH 5.0. Although the dipeptide Gly-Leu has a very bitter taste, its corresponding pyrazinone product [2-(2'-oxopyrazin-1'-yl) isocaproic acid] had an astringent, a little sour and later a mild taste. The production of 2-pyrazinone compounds from dipeptides with reverse sequences (Gly-Leu & Leu-Gly), or from peptides with different chain lengths (diglycine, triglycine, and tetraglycine) was also observed in the reactions with glucose (Oh, Shu, & Ho, 1992). Specifically, pyrazinones generated by the dipeptides Gly-Leu and Leu-Gly were qualitatively similar, but slightly different in quantity (Oh, Shu, & Ho, 1992). The

production of pyrazinones was proposed through the reactions of α-dicarbonyl compounds and dipeptides (Oh, Shu, & Ho, 1992). It seems that the kinds of pyrazinones formed by a peptide in the Maillard reaction are specific to the nature of its amino acid residues (Oh, Shu, & Ho, 1992; Van Chuyen et al., 1973).

In addition to pyrazines and pyrazinones, some of the novel Strecker aldehydes were also reported as the major volatile products in several peptide model systems, such as formaldehyde and isovaleraldehyde from Gly-Leu/glyoxal (Van Chuyen et al., 1973), phenylacetaldehyde from Lys-Phe/glucose (Van Lancker et al., 2010), 2-methylpropanal from Va-Gly/fructose, and 3-methylbutanal from dipeptide Leu-Gly or tripeptides Ala-Leu-Gly with fructose (Rizzi, 1989). More complex condensation products of Strecker aldehydes were also identified. For example, the aldo condensation of 3-methylbutanal from the reaction of Leu-Gly/glucose gives 2-isopropyl-5-methyl-2-hexenal which has a sharp cocoa-like aroma (Hartman, Scheide, & Ho, 1983; Oh, Shu, & Ho, 1992). Although the generation of Strecker aldehydes from Maillard reactions of peptides is much less efficient than that from the corresponding free amino acids (Van Lanker et al., 2010, 2012), these reactive carbonyl compounds are paramount for the Maillard flavor formation of peptides in foods upon thermal treatments.

Chapter 3. Hypothesis and Objective

3.1. Hypothesis

The protein hydrolysis of muscles post-mortem can generate considerable amount of peptides including low molecular peptides that may react as NH_2 donnors in the Maillrad reaction with reducing sugars. Therefore, we hypothesized that:

Model systems containing the expected natural concentrations of ribose and selected low MW peptides (MW < 1KDa) in aging meat may generate important volatile organic compounds (VOCs) with known contribution to the overall aroma and flavor of cooked meat.

3.2. Objective

The main objective of this study was to identify and to quantify VOCs formed from the Maillard reaction model systems containing ribose and selected low MW peptides (carnosine, glutathione, and cysteine-glycine) at their expected natural concentrations in raw meat and at their expected physiological pH (6.30) when heated at 180℃ or 217℃ for 2hr. The selection of peptides was based on their biological significance and flavor-generating potential (e.g. by containing sulfur) in meat as previously mentioned. Ribose was chosen as the sugar for its high reactivity in the Maillard reaction.

Chapter 4. Materials and Methods

4.1. Chemicals

D-(-)-ribose, L-carnosine, L-glutathione reduced, Cys-Gly (TLC), dichloromethane of HPLC grade (≥ 99.8%), internal standard 1, 2-dichlorobenzene (*40023* Supelco, ON, Canada), anhydrous sodium sulfide, and C_8-C_{20} alkane standards (~40mg/L for each in hexane, *04070* Sigma) were all purchased from Sigma-Aldrich Ltd. Sodium phosphate buffer (1M, pH 6.30) was prepared from the stock solutions of sodium phosphate monobasic anhydrous (NaH_2PO_4) and sodium phosphate dibasic anhydrous (Na_2HPO_4) in the laboratory. In-house Mili-Q water was used to dissolve reactants of the model systems.

4.2. Buffer Preparation

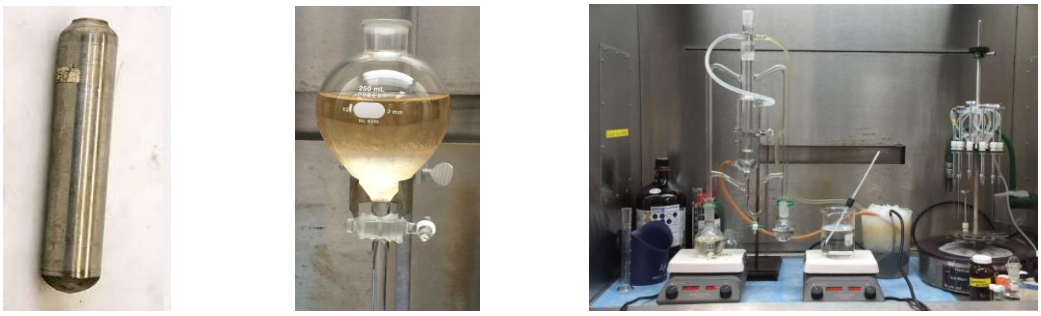
Sodium phosphate buffer (1M, pH 6.30) was prepared as follows. Stock solution A: 12g of sodium phosphate monobasic anhydrous (NaH_2PO_4) was dissolved in 100mL deionized water. Stock solution B: 14.2g of sodium phosphate dibasic anhydrous (Na_2HPO_4) was dissolved in 100mL deionized water. A mixture of 77.5mL of stock solution A and 22.5mL of stock solution B were thoroughly mixed to make 100mL 1M sodium phosphate buffer. The pH value of the buffer was adjusted with NaOH (10M) solution. Due to the strong buffering capacity of carnosine, no buffer was added to the model systems containing this peptide.

4.3. Model Maillard Reaction Systems

Ribose and one of the following peptides, carnosine, glutathione, or Cys-Gly, were dissolved in 110mL or 150mL of deionized water (**Table 1**). Prior to the thermal reactions, pH of the reaction mixtures containing carnosine were adjusted with NaOH

(1M) and/or HCl (1M) solutions, while the reaction mixture(s) of GSH or Cys-Gly were adjusted with sodium phosphate buffer (1M, pH 6.30) to the same pH value of 6.30. The mixture solution was then transferred into a 150mL-Hoke cylinder (Hoke, Inc., SC, USA) and heated in a conventional oven at 180 ℃ or 217 ℃ for 2hrs (**Table 2**). After the reaction, the cylinder was immediately cooled under cold running water before the cap was opened. Model systems with either ribose alone or water under the same experimental conditions were served as controls for peptide model systems. Each model system was performed in triplicate. Volumes and pH values of the mixtures were recorded before and after heating. The VOCs were extracted from reaction mixtures by either a Liquid/Liquid (L/L) extraction method or using a simultaneous steam distillation and solvent extraction (SDE) method (**Table 2**).

Figure 1. The Hoke cylinder and the extraction methods applied in the model systems



Hoke cylinder **L/L Extraction** **SDE**

4.4. Extraction Methods for VOCs

Liquid/Liquid (L/L) extraction. Upon completion, the reaction mixture was spiked with 100 μL of internal standard (1mg 1, 2-dichlorobenzene /1mL in methanol) and extracted with dichloromethane (50 mL × 3 times) using a separatory funnel. The mixture was thoroughly shaken for at least 5 min to achieve complete extraction. When the solvent and water were

clearly separated (held for ~20 min), the solvent layer was collected into a flask and dried with anhydrous sodium sulfide.

Simultaneous steam distillation and solvent extraction (SDE). Volatile compounds formed from the thermal reactions were extracted using a modified Likens-Nickerson apparatus. The reaction mixture was spiked with 1, 2-dichlorobenzene (100 μL) and was transferred into a flat bottom flask (250 mL) and heated to the boiling temperature (500rpm at 380°C). Upon boiling, the solvent flask containing 50mL of dichloromethane was heated in a water bath at 40℃. In general, when steams from sample and solvent reach the cold finger of the apparatus the condensation occurs and water and solvent are separated based on their density and return to their corresponding flasks. At this stage, the equilibrium is achieved and the VOCs extraction starts. SDE extractions were continuously run for 2hr and the final distillates were dried with anhydrous sodium sulfide and kept at 4℃ overnight. Final dried extracts from either the L/L or SDE extraction method were concentrated to a final volume of 0.5-1mL under a gentle stream of nitrogen gas, and stored at -20°C until analysis by GC-MS.

4.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis of volatile compounds was performed on a Varian 450-Gas Chromatography (Agilent Technologies, Walnut Creek, California, USA) equipped with a low speed fused-silica capillary column (5%-Pahenyl)-methylpolysiloxane (30m × 0.25mm i.d. × 0.25um film thickness, DB-5ms, Agilent J &W GC Columns, USA) and a Varian 240-MS/4000 Mass Spectrometer (Agilent Technologies). An aliquot (1 μL) of extract was injected into the GC with a split ratio of 20:1. The column temperature was held at 40℃ for 5 min, then ramped to 100℃ at 2℃/min, followed by 200℃ at 5℃/min,

and finally to 250℃ at 40℃/min and held for 5 min. The total running time was 61.25 min with the filament delay time programmed to 3 min. The GC was operated with an injector temperature of 270℃ and a detector temperature of 200℃. Helium (Ultra high purity 5.0) was used as carrier gas with a constant flow of 1 ml/min. The mass spectrometer was operated in full scan electron impact (EI) mode with an ionizing voltage of 70 eV, and it was scanned from *m/z* 40 to 650.

4.5.1. Identification of VOCs. The collected volatile compounds were analyzed based on the calculated linear retention indices (LRIs) and the mass spectral data retrieved from the GS-MS analysis. Compounds from the isolate was identified by comparing their LRIs and the mass spectra to those of authentic compounds available in the NIST Search v. 2.0 and other authorized sources as reported in the LRI & Odor Database (http://www.odour.org.uk) and the National Institute of Standards and Technologies (NIST) Chemistry WebBook (http://webbook.nist.gov/chemistry), and other previous publications. Personal interpretations were applied when there were more than two reference compounds of which LRIs and mass spectra provided by the NIST database were closely related to the unknown compound, or when only the mass spectral data was available for the reference compound. In these cases, a tentative identification was made.

4.5.2. Calculation of Linear Retention Index (LRI). A standard mixture of n-alkanes (C_8-C_{20}, ~40mg/L each in hexane) was added to the extract isolate at a ratio of 1:9 before GC-MS analysis. These alkanes were used as external standards to determine the LRI of each volatile compound in the sample. The following equation was used to calculate the corresponding LRI (Van den Dool & Kratz, 1963): $LRI(x) = [n + (Rx\text{-}Rc\text{-}1)/(Rc\text{-}Rc\text{-}_1)] \times 100$, where "R" is the retention time of a peak; "x" is the unknown compound; "c-1" refers to the alkane before the

unknown and “c” is the alkane after the unknown; “n” represents the number of carbon atoms of alkane before the unknown.

4.5.3. Semi-quantification of VOCs. The detected volatile compounds were semi-quantified using the base ion peak of the IS. The calculation was done by multiplying the ratio of the base ion peak area of the compound of interest to that of the IS (1, 2-dichlorobenzene, m/z=146) by the amount of IS applied in the extract (100 μg). The quantity of each volatile compound was then converted into mg of volatiles produced from 1mol of the selected peptide (carnosine, GSH, or Cys-Gly).

4.6. Statistical Analysis

Paired-samples *t* test was used to examine the mean pH changes of model solutions with heating (compared to pH 6.30). One-way analysis of variance (ANOVA) was conducted *1)* to evaluate the differences of pH between model systems before and after reactions, as well as the differences of pH changes between model systems after heating; *2)* to evaluate the effect of model systems on the quantities of selected volatile compounds. Tukey’s multiple comparison test was used to determine least squares mean treatment differences when significant ($P \leq 0.05$). All statistical analyses were carried out using SPSS Statistics Software Version 22.0 (IBM, USA).

Chapter 5. Results and Discussion

5.1. Rationales for the Choice of Model Systems

5.1.1. Concentrations of reactants. The choice of concentrations of the reactants used in this study was selected based on their known natural amounts in raw chicken meat. Due to various factors such as storage time and temperature, a high coefficient of variation (CV %) for meat flavor precursors is expected (Aliani & Farmer, 2002; Aliani & Farmer, 2005a). The concentrations used in our study were mainly based on known reported concentrations in the literature and those obtained from our recent results for chicken meat extracts (n=6 individual chickens). In this experiment, chicken breast and thigh samples (water soluble extracts) were analyzed from 4hrs post-slaughter up to 6 days of storage at 4˚C using a liquid chromatography-quadrupole time-of-flight-mass spectrometry (LC-QTOF-MS) approach (Unpublished data).

Model systems of ribose/carnosine (RC), ribose/carnosine, heated at 217℃ **(**RCH), and ribose/GSH (RG) were studied at the early stages of our study where the choice of concentrations used for ribose and peptides were based on the reported natural concentrations in 100g of raw meats. As the study progressed, adjustments were made to ensure a better accuracy. Considering that 150g raw meat (the approximate weight of a chicken breast) is the portion size normally consumed by the population in a given meal (United States Department of Agriculture [USDA], 2015), and muscle meat contains ~75% water (USDA, 2013). Amounts of peptides and the volume of water were re-adjusted in the model systems of ribose/carnosine, adjusted (RCA), ribose/GSH, adjusted (RGA), and ribose/Cys-Gly (RCG) (**Table 1**).

Ribose. Natural concentration of ribose in raw chicken breast was reported as 25mg/100g wet weight (Aliani & Farmer, 2002), which was equivalent to 37.5mg/150g.

Carnosine. Natural concentration of carnosine in the non-protein fraction of raw chicken breast was reported as ~350mg/100g (Aliani & Farmer, 2005a), and it was equivalent to ~530mg/150g.

GSH. The concentration of GSH used in the RG model system was based on the natural concentration of this peptide in chicken meat (per 100g) as previously reported (Jones et al., 1992; Jones, 1995). The natural concentration of GSH in fresh pork (loin, fat trimmed), which was reported as 630nmol/g wet weight (equivalent to 30mg/150g), was chosen to represent the maximum amount of GSH that can be obtained from a portion of raw meat (Wierzbicka, Hagen, & Jones, 1989), and it was applied in the RGA system.

Cys-Gly. The natural concentration of this dipeptide in raw meat has not been previously reported. Therefore, results obtained from LC-QTOF-MS of water soluble compounds in chicken extracts (7.5mg/150g) were used.

5.1.2. Parameters used in the model systems.

pH value. All reaction mixtures were adjusted to a final pH value of 6.30 using NaOH and HCl solutions, or sodium phosphate buffer as shown in **Tables 2 & 3**. It was the mean value of the duplicate pH measurements from 4 individual chicken breast and thigh samples kept at 4℃ for 7hr, 1, 2, 4, and 6 days (Aliani et al, 2016, unpublished data), which was in agreement with the reported values under similar conditions (Aliani, Farmer, Kennedy, Moss, & Gordon, 2013).

Temperature and heating duration. The use of temperature (180℃) and reaction time (2hr) was based on the work of Chen & Ho (2002). With an attempt to investigate the effect of a higher temperature on the formation of nitrogen-containing volatile

compounds, 217℃ (the maximum temperature held by the oven in our laboratory) was applied in the RCH model system.

Extraction methods for VOCs. L/L extraction method was used at the first stage of the study (in the RC & RCH model systems) according to Chen & Ho (2002). After realizing the SDE method was a more frequently cited sample preparation method in the volatile analysis, it was used for the rest of the model systems (RCA, RGA, & RCG).

5.2. Some Changes of the Model Solutions after Heating

Due to the complexity of Maillard reactions, model systems are the preferred tools to investigate the reaction. A model system is ideal to control the reaction conditions and to investigate different steps of the VOC generation. In our current study, model systems containing ribose and the selected low MW peptides (at pH 6.30) were heated at either 180℃ or 217℃ for 2hr. A summary of all model systems used in this Thesis is provided in **Tables 1 & 2**. A summary of the changes obtained for pH, aroma, and color of individual model solutions after the thermal treatment is provided in **Tables 3 & 4**.

pH. The initial pH values obtained from the model systems used in our study ranged between 3.55 and 8.19 for GSH (RGA) and carnosine (RCA), respectively (**Table 3**). Given the fact that pH has a major influence on the nature of VOCs generated from the Maillard reacton (Madruga & Mottram, 1995), it was critical to adjust the pH of all model solutions to an expected physiological pH value in meat. Reaction mixtures containing carnosine were basic with pH values greater than 8.0 (**Table 3**). In contrast, mixtures containing GSH had the lowest pH values. The natural pH of the RCG model solution was close to those of the blank controls. The relatively low pH levels of the GSH reaction mixtures before pH adjustment is due to the

acidic nature of this peptide. In general, the average pH decline of the model systems was significant after heating ($P < 0.001$). Particularly, the pH decreases in the model systems

Table 1. Summary of the model systems containing ribose and a selected peptide used in this Thesis.

	Amount (mg)						
	RC & RCH	***RCA***	***RG***	***RGA***	***RCG***	***R***	***W***
Ribose	25	35	25	35	35	25	--
Carnosine	350	530	10	30			
GSH							
Cys-Gly					7.5		
	Concentration (mol/L)						
	RC & RCH	***RCA***	***RG***	***RGA***	***RCG***	***R***	***W***
Ribose	0.001	0.002	0.001	0.002	0.002	0.001	--
Carnosine	0.01	0.02					
GSH			0.0002	0.0009			
Cys-Gly					0.0004		
Mili-Q water (mL)	150	110	150	110	110	150	150

Abbreviations:
RC: ribose/carnosine; **RCH**: ribose/carnosine at 217℃; **RCA**: ribose/carnosine adjusted; **RG**: ribose/GSH; **RGA**: ribose/GSH adjusted; **RCG**: ribose/Cys-Gly; **R**: ribose only; **W**: water only.

Table 2. Parameters used for the model reactions.

Model Systems	Temperature	Buffer	Extraction method
RC	180℃	--	L/L
RC	217℃	--	L/L
RCA	180℃	--	SDE
RG	180℃	Yes	SDE
RGA	180℃	Yes	SDE
RCG	180℃	Yes	SDE
R	180℃	Yes	SDE
W	180℃	Yes	SDE

Note: sodium phosphate buffer (1M, pH 6.30) was used to adjust the pH of reaction mixtures before heating.

Table 3. Summary of changes in pH for the Maillard reaction mixtures before vs. after thermal reactions.

Model systems	RCA	RC	RCH	RGA	RG	RCG	R	W
Initial pH	8.19[a] (0.02)	8.24[a] (0.02)	8.20[a] (0.03)	3.55[e] (0.03)	3.89[d] (0.01)	5.32[c] (0.01)	5.80[b] (0.20)	5.85[b] (0.10)
Adjusted pH	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30
Final pH	5.92[ab] (0.07)	6.13[a] (0.01)	5.88[b] (0.11)	4.17[c] (0.05)	4.06[c] (0.08)	3.61[d] (0.15)	4.15[c] (0.07)	5.85[b] (0.03)
pH decline[1]	0.38[cd] (0.07)	0.17[d] (0.01)	0.42[c] (0.11)	2.13[b] (0.05)	2.24[b] (0.08)	2.69[a] (0.15)	2.15[b] (0.07)	0.45[c] (0.03)

Notes: each pH value was an average of triplicate measurements.
[1] pH decline: all data was calculated based on the formula (adjusted pH – final pH).
[a, b, c, d]: Mean values (n=3, followed by standard derivations reported in the brackets) in the same line with the same superscript do not differ significantly ($P \geq 0.05$).

of carnosine (RCA, RC, RCH) were significantly less than the systems containing GSH (RGA, RG) or Cys-Gly (RCG) ($P < 0.05$), albeit buffer was applied before heating these peptides. Mean pH reduction in the model system of Cys-Gly was significantly greater than that in the GSH systems ($P < 0.05$). For the controls, pH value of the model system containing only ribose or water was decreased at the same degree as in the case of GSH or carnosine respectively ($P < 0.05$). Due to the presence of an imidazole moiety carnosine has a very strong pH buffering capacity (Crush, 1970) by which the pH decrease during the thermal reactions of carnosine and a reducing sugar can be impeded. Reasons for pH decrease after Maillard reactions of peptides could be partly explained by the fact that the amino termini of peptides are converted in the reactions with sugars, whereas, the carboxyl residues remain intact (de Kok & Rosing, 1994). On the other hand, the liberation of carboxylic acids from sugar degradation during the Maillard reaction may also contribute.

After condensing with an amino residue, the Schiff base of an aldose can undergo the 1, 2- or 2, 3-enolization route to form 3- or 1-deoxyosones (Mottram, 2007). These dicarbonyl

intermediates are very reactive and are susceptible to further decomposition to low MW aldehydes and organic acids. Brands & van Boekel (2001) reported that glucose can be into formic acid and acetic acid through different breakdown pathways. Davidek, Devaud, Robert, & Blank (2006) considered the hydrolytic β-dicarbonyl cleavage as the major pathway in the generation of acetic acid during Maillard reactions. In line with these observations, ribose in our case might also be capable of producing carboxylic acids from the corresponding dicarbonyl intermediates in the thermal reaction with peptides, which eventually increased the acidity of the model solutions.

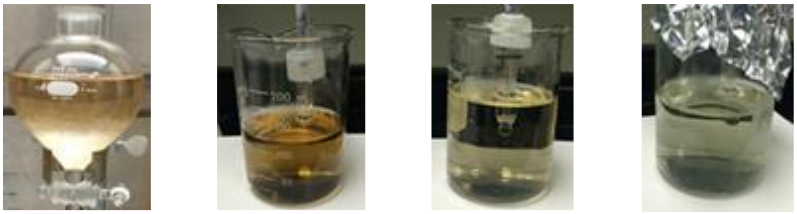
Aroma & Color. The color and aroma development of model solutions after heating were personally evaluated by the researcher. Model solutions containing higher concentrations of reactants (RCA, RGA) showed a higher degree of browning compared to the reaction mixtures with lower concentrations (RC, RCH, & RG). Although the final color of the ribose control could not be clearly differentiated from what was seen before the treatment, slightly fruity and sweet aroma elicited from the solutions manifested the decomposition of ribose to some of the odorant components. The model solutions of RC and RCH possessed a mild sweet and boiled meat aroma, and following an increase of the reactant concentrations, a strong sweet and slightly burnt odor was perceived in the RCA model solution. As expected, the reaction mixtures with GSH or Cys-Gly had a sulfurous note that could not be detected in the carnosine models. In addition, model solutions containing GSH after reactions possessed an aroma closely related that of chicken-broth. The thiol residue of GSH and Cys-Gly could be a source of volatile compounds of which flavor impressions were distinct from those produced by the systems without sulfur in their reactants.

Table 4. Summary of changes in aroma and color for the Maillard reaction mixutres after heating at 180℃ or 217℃ for 2hr.

Model Systems	Aroma	Color
RCA	Strong sweet, and slightly burnt	Yellow dark brown
RC	Mild sweet and boiled meat	Light yellow brown
RCH	Mild sweet and boiled meat	Light yellow brown
RGA	Strong sweet and sulfurous (chicken broth-like)	Yellow
RG	Sweet and sulfurous boiled chicken	Light yellow
RCG	Sweet and sulfurous	Light yellow
R	Slightly fruity and sweet	May have color
W	None detected	No color

Note: aroma & color of the model solutions were personally evaluated by the researcher after heating.

Figure 2. Color of the some of the peptide model solutions after heating.



RC **RCA** **RGA** **RCG**

5.3. General Characteristics of VOCs Formed from the Peptide Model Systems

The major VOCs isolated from model systems containing ribose and the three low molecular weight peptides (carnosine/GSH/Cys-Gly) used are summarized in **Table 6**. These VOCs were extracted during 2hr of reaction at either 180℃ or 217℃. The selected VOCs (n=70) were clustered in nine different chemical groups. The identified VOCs were classified into

hydrocarbons (n=9), aldehydes (n=5), ketones (n=8), phenols (n=4), furans (n=3), an alcohols, as well as sulfur- (n=22) and nitrogen-containing (n=13) heterocyclic compounds. Five other compounds were not classified as any of the previous eight groups.

The identities of forty-four VOCs have been determined by comparing their mass spectra data and LRI values with those of reference compounds available in the NIST library. Eleven identities were assigned to the compounds whose mass spectra were in agreement with the reference compounds in the library, but their LRI measurements were beyond the alkane standard range. Fifteen identities were tentatively suggested by the comparison of their mass spectra (and LRIs) with those of related compounds. Chemical structures of some of the heterocyclic VOCs have been provided in **Appendix I, Table 7**.

Sulfur-containing compounds were identified in the model systems of GSH and Cys-Gly, whereas, the majority of nitrogen-containing volatiles, especially pyrazines and pyridines, were only detected in the carnosine reaction systems. The identified sulfur volatile compounds included thiophenes, mercaptoketones, furanthiols, thiophenethiols, and cyclic polysulfides. Some of these compounds have been previously reported from the Maillard reaction of a reducing sugar with cysteine or GSH (Hofmann & Schieberle, 1995; Madruga & Mottram, 1998; Zhang & Ho, 1991a, b), as well as from the thermal degradation of cysteine and/or GSH alone (Shu, Hagedorn, Mookherjee, & Ho, 1985; Umano et al., 1995). Sulfur-containing volatiles can influence the sensory perception of cooked meat by mainly affecting the meat-like, sulfurous, and roasted notes. Given their low odor thresholds, they are generally accepted as important flavor constitutes in meat foods. With respect to the nitrogen-containing compounds, the overall formation

tendency shown in our carnosine model systems was aligned with what was observed by Chen & Ho (2002), and this albeit the much lower concentration of reactants used in our study. In both studies, the majority of nitrogen-containing volatile products were pyrazines and pyridines, and most of them were alkyl-substituted. The importance of this group of heterocyclic volatiles is generally attributed to their aroma properties of being roasted, nutty, and burnt in cooked foods. Other products, such as 2-acetylpyrido [3, 4-*d*] imidazole, 3-methyl-2, 5-piperazinedione (tentative), and a methyl-substituted pyrimidinone (compound **60, Appendix II, Figure 10**) which appeared to be reactant-specific products, could be of value for further investigation in regard to their formation pathways in the Maillard reaction.

5.3.1. Comparison of GSH and Cys-Gly in the Maillard reaction. There have been several model studies investigating the VOC formation from Maillard reactions of GSH and Cys-Gly. However, none of them has been conducted in reactions related to the natural physiological condition in meat (**Table 5**). Among the total twenty-two reaction products identified in the GSH and Cys-Gly model systems, only three were thiazoles, and no pyrazines was detected. After the thermal degradation of cysteine and GSH at pH 7.5, Zhang, Chien, & Ho (1988) reported that more than 90% of the sulfur-containing volatiles from cysteine contained nitrogen atoms, while only 9% of the heterocyclic compounds from GSH contained both sulfur and nitrogen atoms, and all of them were thiazoles. In addition, the nitrogen-containing cyclic sulfides formed from cysteine degradation were not found in the case of GSH in their study. As proposed by the authors, the formation of a much less number of both sulfur- and nitrogen-containing volatiles from GSH was owing to the fact that GSH evolved H_2S more rapidly than NH_3.

For pyrazines, they are preferably formed at high pH (Meynier & Mottram, 1995). In this study, pH decreases in the model solutions of GSH and Cys-Gly were more prominent than those of carnosine after the reaction, which could further suppress the formation of pyrazines in these systems. El-massry et al. (2003) considered mode of GSH reactivity in the Maillard reaction might inhibit the formation of pyrazines. As both sulfur derivatives and pyrazines require the same dicarbonyl precursors, the preferential formation of sulfur volatiles could disadvantage pyrazine synthesis from this peptide. In addition, the rapid release of H_2S from GSH or Cys-Gly during the Maillard reaction may reduce the reactive dicarbonyl compounds into less reactive α-hydroxycarbonyls. Therefore, Strecker degradations and subsequent pyrazine formation in the cases of these peptides would become less favorable (Ho et al., 2008).

As mentioned in the literature review, GSH in the Maillard reaction could cleave into 5-oxoproline and the dipeptide Cys-Gly which then forms cyclic (Cys-Gly) (Wang et al., 2012). Additionally, Steinberg & Bada (1983) proposed that upon heating peptides would undergo internal aminolysis reaction by which they decomposed to diketopiperazines. The hydrolysis of these cyclic dipeptides could give rise to the corresponding dipeptides and free amino acids. If the same decomposition mechanism can be applied in the Maillard reaction of GSH, the formation of cyclic (Cys-Gly) (which has been observed in the previous literature), as well as the dipeptide Cys-Gly and free amino acid cysteine is plausible. In the presence of sugars or sugar derivatives, products from peptide degradation can serve as Maillard reactants and participate in the formation of sulfur-containing volatile compounds.

5.3.2. Cys-Gly in the Maillard reaction. Cys-Gly gave limited volatile products (n=24) compared to other peptides used. In addition, the generated volatiles were also found in the model systems of GSH. Ho et al. (2008) compared the volatile profile of

Table 5: Comparison of reactant concentraions used in the current RGA model system1 to those in the previous literature.

Literature	Maillard model systems	Reactant concentrations
Zhang & Ho (1991a)	Glucose + GSH pH 7.5	Glucose: 50× higher GSH: 250× higher
Zhang & Ho (1991b)	IMP + GSH pH 2.2	IMP: 25× higher GSH: 55.6× higher
Ho, Oh, Zhang, & Shu (1992)	Glucose + GSH pH 7.5	Eqiumolar of the reactants
Tai & Ho (1998)	Glucose + GSH pH 3.0, 6.0, and 8.0	Glucose: not mentioned GSH: 120× higher
El-massry, Farouk, & El-Ghorab (2003)	Ribose + GSH pH 7.1	Equimolar of the reactants
Ho, Wang, Raghavan, & Payne (2008)	Glucose + GSH pH 5.5	Glucose: 20× higher GSH: 44× higher
Lee, Jo, & Kim (2010)	Fructose/glucose + GSH pH 7.5	Sugar: 5× higher GSH: 11× higher
Lee, Kwon, Kim, & Kim (2011)	Ribose/xylose/glucose + GSH pH 7.0 or 11.0	Sugar: 2.5× higher GSH: 5.6× higher
Wang, Yang, Song (2012)	Xylose + xylose (labeled) + GSH + thiamine pH 4.8	Xylose (unlabeled): 6.7× higher GSH: 3.8× higher

[1]**RGA:** reactant concentrations of this model system are ribose-0.002M, GSH-0.0009M.

Cys-Gly to that of GSH in the reaction with glucose (160℃ for 1hr at pH 5.5). They found that except the absence of pyrazines, classes of aroma compounds were quantitatively and qualitatively comparable between the two reaction systems. Mode of peptide fragmentation is

another subject of interest in regard to the Maillard volatile formation. The thiol side chain of cysteine or cysteine-containing peptides is considered to be reactive and can act as both intermolecular and intramolecular nucleophile in reactions (Freitas, O'Hair & Williams, 1997; Reid, Simpson, & O'Hair, 1998; O'Hair, Reid, & Styles, 1998). Specifically, in the gas phase fragmentation reactions of cysteine-containing peptides (where other amino acid residues were glycine), a release of NH_3 was observed when cysteine was positioned in the N-terminus of peptides, whereas, water was lost in the other cases (O'Hair et al., 1998). When it was applied in the case of Cys-Gly, the thermal degradation of the peptide initiated by the presence of reducing sugars or dicarbonyl compounds might give off NH_3. Based on the previous literature, it is likely that the interactions between H_2S, NH_3, and other reactive intermediate derivatives generated from the Maillard reaction/Strecker degradation of peptides give rise to many important heterocyclic volatile compounds.

5.4. Classes of VOCs Identified in the Peptide Model Systems

5.4.1. Hydrocarbons. Most of the hydrocarbons found in the peptide model systems were aromatic, and they were also present in the control systems. Except styrene (compound **6**) and D-limonene (compound **9**), the yields of these compounds were not significantly different between the reaction systems ($P > 0.5$). Aromatic hydrocarbons are common volatile compounds in meat flavor profiles (**Appendix I, Table 1**), but their origin from the related products is ambiguous. Comparison of the volatile components from cooked duck showed that both cooking methods (boiling/roasting) and retention matrices (meat/fat/gravy) influenced the final amounts of hydrocarbons isolated from the systems (Wu & Liou, 1992). Whereas in *sous-vide* cooked lamb, neither temperature nor time was shown to have an impact on their abundance (Roldán et al., 2015). Flavor

contribution of this chemical family to meat is minor, however, limonene identified in roasted pork was described to have floral, green, and sweet odors (Xie et al., 2008).

5.4.2. Aldehydes & ketones.

Heptanal (compound **20**) was more frequently reported as a lipid degradation product in food systems. Its flavor characteristics were generally demonstrated as nutty, burnt, fatty, and green (**Appendix I, Table 2**). Effect of cooking methods on the presence of this aldehyde was negligible, since it was found in meat exposed to boiling, roasting, or grilling treatments (Mottram, 1985). Identification of this compound in our peptide model systems (**Appendix III, Chart 2**) indicates that sugar degradation in the presence of amino groups could be a potential pathway for its formation. Also, its absence from the carnosine systems may suggest the importance of types of amino compounds as for heptanal formation.

3-Hydroxy-2-butanone (compound **11**), also known as acetoin, is the most prevalent ketone identified in this study (except the control models). Comparison of the quantity between systems showed that it was dominated in the carnosine model systems (**Appendix III, Chart 1**). As an α-hydroxycarbonyl compound, 3-hydroxy-2-butanone is a known sugar degradation product which can contribute to the overall sensory perception of cooked meat (Buttery et al., 1990; Xie et al., 2008). Besides that, it can act as a flavor precursor by providing the carbon backbone for the formation of heterocyclic aroma volatiles (Huang, Fu, & Ho, 1996; Xi et al., 1999). Based on the literature, a high cooking temperature is preferred in the formation of this compound (**Appendix I, Table 2**).

The origin of 3-hydroxy-2-butanone from Maillard reactions via the direct reduction of 2, 3-butanedione has been proposed by Wnorowski & Yaylayan (2000). In general, retro-aldolization and α- and β-dicarbonyl cleavage are major reaction routes for the formation of α-hydroxylcarbonyl and α-dicarbonyl compounds in sugar fragmentation (Novotny, Cejpek, & Velisek, 2007). It is well accepted that sugar decomposition in the Maillard reaction is catalyzed by the alkaline environment where more reducing sugars are in open chain forms and the basic strength of amino acids is retained. It is concluded that at low pH, Amadori intermediates favorably undergo 1, 2-enolization to give 3-deoxyosones which are then transformed into the corresponding furfurals. Whereas, at high pH, Amadori products undergo 2, 3-enolization to produce 1-deoxyosones that can be converted into furanones and/or further degraded into reactive small carbonyl compounds (Chen & Kitts, 2011; Hodge, 1953; Mottram, 2007; Nursten, 1981). The reactions of ribose and selective peptides in the slightly acidic aqueous solutions (pH 6.3) all led to a decrease in pH after heating, and the decline in the model systems of GSH and Cys-Gly was more significant than that of carnosine. The much greater yield of furfural as compared to 3-hydroxy-2-butanone from the current models could be explained by the relatively low pH condition where sugar transformation via the route of 1, 2-enolization was favored. Results from our study also showed some agreement with observations obtained from the similar reaction systems. Tai & Ho (1998) suggested that the higher level of furan derivatives in accompany with the reduced amount of carbonyls from the reactions of glucose and GSH at a low pH could be attributed to the cyclization reaction which stabilized the deoxyglucosones from further decomposing into small carbonyls. In addition, dicarbonyl compounds (like glyoxal and

2, 3-butanedione) that are commonly encountered in the sugar degradation during the Maillard reaction were not found in our case, and it could be possibly owing to their high volatility and/or swift reactions with other compounds to produce secondary products.

Phenylglyoxal or benzaldehyde. The identity of compound **21** was suggested to be either phenylglyoxal or benzaldehyde (**Appendix II, Figure 1a & b**) in the GC-MS analysis. According to the NIST library, both candidates have a close *m/z* distribution pattern, however, the definite identification could not be made due to the lack of LRI reference to phenylglyoxal. In this study, compound **21** was identified in the carnosine model systems and it was one of the dominant compounds in quantity (**Appendix III, Chart 3**). Benzaldehyde is known for its strong sensory property by giving bitter, nutty, almond, and burnt aromas (Tao et al., 2014) in various cooked meat systems (**Appendix I, Table 8**). The formation of benzaldehyde has been observed from the pyrolysis of phenylalanine, or from the Maillard reactions of phenylalanine and its analogous compounds (Adamiec, Rössner, Velíšek, Cejpek, & Šavel, 2001; Chu & Yaylayan, 2008). Varlet et al. (2007) pointed out that the oxidation of toluene or other hydrocarbons, like styrene or methylstyrene, could contribute to the formation of benzaldehyde in a weak part. However, this possibility, if it was involved in our case, should only play a negligible role, since aromatic hydrocarbons identified in the thermal reactions between ribose and carnosine were also observed in other model systems including the controls. Further investigations should be carried out to finalize the identity of compound **21**, and to clarify the pertinent Maillard formation mechanism in which carnosine is one of the starting materials.

5.4.3. Furans. Furfural (**28**), 2-furanmethanol (**29**), and 2-acetylfuran (**30**) were furans identified in the current study. Furans are well-known products formed from the thermal degradation of carbohydrates (caramelization), or from the Maillard reactions involving an

amino compound. Besides their self-own flavor characteristics, they are also building blocks for heterocyclic aroma compounds, such as thiophenes, furanthiols, and pyrroles.

Furfural (compound **28**) possesses sweet, caramel-like, and almond odor properties, and it is formed from 3-deoxyosone with a loss of water (Cerny & Davidek, 2003). Furfural was identified in all model systems except the water blank (**Appendix III, Chart 4**). The higher yields of furfural ($P < 0.001$) in the model systems of RG and RCG have been previously explained by the favorable formation of 1, 2-enaminol at relatively low pH in the section of 3-hydroxy-2-butanone. As reactions progressed, the exhaustion of peptides could in turn lead to furfural accumulation in the reaction mixtures, since furfural can further interact with the amino compound and its Strecker intermediates available in the system. However, it should be addressed that the decision of reactant concentrations in the current study was not made to optimize the volatile compounds but instead it was an attempt to mimic the natural presence of flavor precursors in fresh meat through which to investigate their potential impact in the Maillard-type flavor formation.

Table 6. The list of VOCs generated from the model systems containing ribose and selective peptides.

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
							RCA	RC	RCH	RGA	RG	RCG		
	Hydrocarbons													
1	**3-Methylhexane** (tentative)	C_7H_{16}	3.3	<800	100	MS	1.12 (0.49)	4.93 (1.17)	5.90 (1.83)	27.97 (16.19)	208.76 (232.17)	41.14 (7.63)	2.13 NS	R, W
2	**Methylcyclohexane**	C_7H_{14}	3.9	<800	98	MS	0.07 (0.02)	0.19 (0.06)	ND	2.05 (1.02)	8.19 (8.86)	2.37 (0.00)	2.23 NS	R, W
3	**Toluene**	C_7H_8	5.1	<800	92	MS	0.90 (0.67)	0.45 (0.17)	0.06 (0.00)	4.78 (0.59)	15.35 (18.67)	11.08 (3.63)	2.03 NS	R, W
4	**Ethylbenzene**	C_8H_{10}	8.7	858	106	MS, LRI	0.01 (0.00)	0.30 (0.04)	0.03 (0.03)	0.58 (0.39)	1.33 (1.51)	0.55 (0.36)	1.67 NS	R, W
5	**o/m/p-Xylene**	C_8H_{10}	9.1	867	106	MS, LRI	0.04 (0.00)	0.28 (0.04)	0.02 (0.01)	1.02 (0.00)	3.99 (4.58)	1.50 (0.76)	1.91 NS	R, W
6	**Styrene**	C_8H_8	10.0	890	104	MS, LRI	0.14^{b} (0.02)	0.20^{b} (0.22)	0.54^{b} (0.04)	3.41^{b} (0.59)	10.23^{a} (3.54)	7.91^{a} (1.37)	23.13 ***	R, W
7	3-Propylcyclohexene	C_9H_{16}	12.8	954	124	Tentative	0.10^{a} (0.02)	ND	ND	ND	ND	ND	49.00 ***	
8	**1,2,3/1,3,5/1,2,4-Trimethylbenzene or 1-Ethyl-2/3/4-methylbenzene**	C_9H_{12}	14.4	992	120	MS, LRI	ND	ND	0.05 (0.02)	0.58 (0.39)	1.02 (1.77)	1.11 (1.10)	1.07 NS	R, W
9	**D-Limonene**	$C_{10}H_{16}$	15.9	1028	136	MS, LRI	0.04^{b} (0.00)	0.17^{b} (0.10)	ND	1.02^{ab} (0.00)	3.38^{a} (2.62)	2.37^{ab} (0.00)	5.21 **	R, W
	Alcohol													
10	2-Hexanol	$C_6H_{14}O$	6.7	811	102	MS, LRI	0.13 (0.00)	1.29 (0.93)	0.24 (0.16)	2.73 (0.59)	10.23 (9.87)	5.54 (1.37)	2.75 NS	R, W
	Ketones													
11	**3-hydroxy-2-butanone**	$C_4H_8O_2$	3.6	<800	88	MS	2.06^{c} (1.82)	21.24^{b} (2.13)	45.27^{a} (7.77)	5.46^{c} (1.56)	ND	ND	82.49 ***	

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
12	3-Hydroxy-3-methyl-2-butanone	$C_5H_{10}O_2$	4.1	<800	102	MS	0.10^{c} (0.02)	0.22^{c} (0.04)	ND	2.73^{ab} (0.59)	8.19^{a} (6.39)	4.75^{ab} (0.00)	4.76 *	
13	**2,4-Pentanedione**	$C_5H_8O_2$	5.5	<800	100	Tentative	ND	0.04^{b} (0.07)	ND	10.23^{a} (1.02)	ND	ND	297.92 ***	
14	1,2-Cyclopentanedione	$C_5H_6O_2$	11.5	924	98	Tentative	ND	0.58^{a} (0.28)	0.17^{b} (0.04)	ND	ND	ND	12.18 ***	
15	2,5-Hexanedione	$C_6H_{10}O_2$	11.8	929	114	MS, LRI	ND	0.50^{a} (0.31)	ND	ND	ND	ND	7.90 **	
16	3-Methyl-1,2-cyclopentanedione	$C_6H_8O_2$	14.5	991	112	Tentative	ND	0.32^{a} (0.06)	ND	ND	ND	ND	75.00 ***	
17	4,4-Dimethyl-2-cyclohexene-1-one	$C_8H_{12}O$	15.6	1018	124	MS, LRI	0.06 (0.07)	ND	0.06 (0.01)	ND	ND	ND	3.63 *	
18	Benzophenone	$C_{13}H_{10}O$	36.6	1628	182	MS, LRI	ND	0.09^{a} (0.04)	0.06^{a} (0.00)	ND	ND	ND	20.20 ***	
	Aldehydes													
19	Pentanedial	$C_5H_8O_2$	10.6	903	100	MS, LRI	ND	ND	0.84^{a} (0.06)	ND	ND	ND	507.0 ***	
20	**Heptanal**	$C_7H_{14}O$	10.6	904	114	MS, LRI	ND	ND	ND	0.85 (0.30)	2.56 (3.11)	0.63 (0.14)	1.83 NS	
21	Phenylglyoxal/ Benzaldehyde	C_7H_6O	13.2	962	106	Tentative	21.63^{b} (1.02)	24.32^{ab} (1.48)	26.71^{a} (2.05)	ND	ND	ND	431.70 ***	
22	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	16.5	1041	124	MS, LRI	8.69^{a} (2.72)	3.08^{b} (0.59)	7.66^{a} (2.59)	ND	ND	ND	20.06 ***	
23	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	40.1	1754	234	Tentative	0.01^{b} (0.00)	0.17b (0.07)	0.06^{b} (0.00)	0.44^{b} (0.41)	2.25^{a} (1.42)	0.24^{b} (0.00)	6.05 **	
	Phenols													
24	**Phenol**	C_6H_6O	14.1	982	94	MS, LRI	ND	0.13^{a} (0.00)	0.043^{b} (0.02)	ND	ND	ND	140.80 ***	

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
25	**2/3/4-Methylphenol**	C_7H_8O	17.1	1054	108	MS, LRI	ND	ND	0.26[a] (0.11)	ND	ND	ND	16.00 ***	
26	o/m/p-tert-Butylphenol	$C_{10}H_{14}O$	26.1	1295	150	MS, LRI	0.07 (0.09)	ND	ND	ND	ND	ND	2.08 NS	
27	**2,4-bis(1,1-Dimethylethyl)phenol**	$C_{14}H_{20}O$	33.0	1506	204	MS, LRI	0.01[b] (0.02)	0.24[b] (0.07)	0.06[b] (0.00)	ND	2.25[a] (1.42)	0.55[b] (0.14)	6.74 **	
	Furans													
28	**Furfural**	$C_5H_4O_2$	7.7	834	96	MS, LRI	1806.83[c] (355.8)	1989.12[c] (137.2)	2915.42[c] (508.6)	9578.40[bc] (2118.6)	15199.57[ab] (7355.09)	22056.18[a] (5378.8)	14.21 ***	R
29	**2-Furanmethanol**	C_5H_6O2	8.6	856	98	MS, LRI	ND	0.11[a] (0.04)	ND	ND	ND	ND	25.00 ***	
30	**2-Acetylfuran**	$C_6H_6O_2$	10.9	910	110	MS, LRI	0.44[a] (0.09)	0.37[a] (0.07)	0.19[b] (0.00)	ND	ND	ND	53.26 ***	
	S compounds													
31	**Thiazole**	C_3H_3NS	4.3	<800	85	MS	ND	ND	ND	13.64[a] (2.13)	8.19[ab] (6.39)	12.66[a] (3.63)	13.236 ***	
32	Tetrahydro-2-/3-methylthiophene	$C_5H_{10}S$	4.8	<800	102	MS	ND	ND	ND	0.41[b] (0.10)	ND	2.37[a] (0.00)	1550.31 ***	
33	**2-/3-Methylthiophene**	C_5H_6S	5.2	<800	98	MS	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
34	Mercaptoacetone	C_3H_6OS	5.9	<800	90	MS	ND	ND	ND	31.04[a] (3.13)	ND	ND	295.75 ***	
35	**2-Methyl-3-furanthiol**	C_5H_6OS	9.0	866	114	MS, LRI	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
36	**2-Furfurylthiol**	C_9H8OS	10.8	911	164	MS, LRI	ND	ND	ND	25.24[a] (0.59)	ND	0.79[b] (1.37)	848.55 ***	

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
37	**Dihydro-3(2H)-thiophenone**	C_4H_6OS	12.8	987	102	Tentative	ND	ND	ND	1.71[a] (0.59)	ND	0.95[ab] (1.23)	5.07 **	
38	2-/3-Thiophenethiol	$C_4H_4S_2$	13.6	973	116	MS, LRI	ND	ND	ND	0.58[a] (0.39)	ND	ND	6.72 **	
39	**Dihydro-2-methyl-3(2H)-thiophenone**	C_5H_8OS	14.2	1056	116	Tentative	ND	ND	ND	1.71[a] (0.59)	ND	ND	25.00 ***	
40	**2-/3-Thiophenecarboxaldehyde**	C_5H_4OS	14.8	1002	112	MS, LRI	ND	ND	ND	109.50[a] (14.22)	9.21[c] (5.32)	27.69[b] (4.94)	130.96 ***	
41	**2-Acetylthiazole**	C_5H_5NOS	15.6	1020	127	MS, LRI	ND	ND	ND	7.16[a] (1.02)	2.35[b] (1.24)	1.35[bc] (0.90)	41.37 ***	
42	3-(Methylthio)thiophene	C_5H_6S2	17.0	1056	130	Tentative	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
43	2-Methyl-1,3-dithiane	$C_5H_{10}S_2$	17.9	1077	134	MS, LRI	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
44	**2/3-Thiophenemethanol**	C_5H_6OS	19.2	1107	114	Tentative	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
45	[1,3]-/[1,4]- Dithian-2-one	C_4H_6OS	21.7	1174	134	MS, LRI	ND	ND	ND	1.71[a] (0.59)	ND	ND	25.00 ***	
46	4-Methyl-3H-1,2-dithiole-3-thione	C_4H_4S3	23.1	1211	148	MS, LRI	ND	ND	ND	1.36[a] (0.59)	ND	ND	16.00 ***	
47	**Benzothiazole**	C_7H_5NS	23.7	1227	135	MS, LRI	ND	ND	ND	ND	11.26[a] (9.87)	ND	3.90 *	
48	[1,2,3,4]Tetrathiane	$C_2H_4S_4$	27.5	1338	156	MS, LRI	ND	ND	ND	0.41 (0.00)	ND	ND	NS	
49	Hexathiane	S_6	33.0	1508	192	MS, LRI	0.24 (0.42)	ND	ND	11.26 (5.70)	13.61 (20.15)	ND	1.70 NS	

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
50	**Lenthionine (1,2,3,5,6-pentathiepane)**	$C_2H_4S_5$	37.0	1545	188	MS, LRI	ND	ND	ND	0.79[a] (0.41)	ND	ND	10.80 ***	
51	**Hexathiepane (1,2,3,4,5,6-hexathiepane)**	CH_2S_6	39.3	1629	206	MS, LRI	0.01[b] (0.02)	ND	ND	5.46[a] (3.13)	ND	ND	9.13 ***	
52	Cyclic octaatomic sulfur	S_8	47.4	>2000	256	MS, LRI	0.69[b] (1.07)	ND	ND	56.97[a] (41.45)	10.23[ab] (12.41)	3.16[b] (1.37)	4.84 *	
	N compounds													
53	**Pyrazine**	$C_4H_4N_2$	4.3	<800	80	MS	0.38[a] (0.11)	ND	0.24[b] (0.04)	ND	ND	ND	35.49 ***	
54	**Pyridine**	C_5H5N	4.8	<800	79	MS	0.24[b] (0.02)	0.47[a] (0.04)	0.30[b] (0.07)	ND	ND	ND	95.87 ***	
55	**2/3/4-Methylpyridine**	C_6H_7N	7.0	817	93	MS, LRI	1.72[a] (0.15)	1.18[b] (0.10)	0.39[c] (0.06)	ND	ND	ND	268.01 ***	
56	**2-Methylpyrazine**	$C_5H_6N_2$	7.4	828	94	MS, LRI	0.33[a] (0.11)	0.22[ab] (0.10)	0.09[bc] (0.04)	ND	ND	ND	15.08 ***	
57	**2/3/4-Ethylpyridine**	C_7H_9N	10.6	902	107	MS, LRI	0.09[b] (0.00)	ND	0.22[a] (0.04)	ND	ND	ND	99.85 ***	
58	**2,5/6-Dimethylpyrazine**	$C_6H_8N_2$	11.1	913	108	MS, LRI	ND	ND	1.27[a] (0.13)	ND	ND	ND	267.77 ***	
59	**2,3-/2,4-/3,4-/3,5/3,4-/2,5-/2,6-Dimethylpyridine**	C_7H_9N	11.9	928	107	MS, LRI	0.13[b] (0.04)	ND	0.90[a] (0.00)	ND	ND	ND	1299.00 ***	
60	1-/5-Methyl-2(1H)-pyrimidinone or 1-/2-/6-Methyl-4(1H)-pyrimidinone	$C_5H_6N_2$	14.2	985	110	MS, LRI	ND	0.09[b] (0.04)	0.37[a] (0.04)	ND	ND	ND	138.90 ***	
61	**2-Ethyl-3/5/6-methylpyrazine**	$C_7H_{10}N2$	14.9	1002	122	MS, LRI	ND	ND	0.22[a] (0.07)	ND	ND	ND	25.00 ***	

No.	Compounds	Formula	RT	LRI[1]	MW	Identification	Model Systems Relative concentrations (mg/mol)[2]						F-values (5, 12)	Controls[3] (R/W)
62	1-(3H-imidazol-4yl)-Ethanone	$C_5H_6N_2O$	17.5	1065	110	Tentative	0.74[a] (0.57)	0.71[ab] (0.13)	0.06[bc] (0.00)	ND	ND	ND	7.14 **	
63	3-Methyl-2,5-piperazinedione	$C_5H_8N_2$	19.8	1125	128	Tentative	ND	ND	ND	1.02 (0.00)	ND	ND	NS	
64	1H-Indol-4/5ol	C_8H_7N0	31.8	1466	133	MS, LRI	ND	ND	0.47[a] (0.37)	ND	ND	ND	4.99 *	
65	**2-acetylpyrido[3,4-d]Imidazole**	$C_8H_7N_3$	34.1	1541	161	MS, LRI	ND	0.67[a] (0.07)	0.84[a] (0.23)	ND	ND	ND	46.47 ***	
	Others													
66	2,5/6-Dimethylhydroquinone	$C_8H_{10}O_2$	20.3	1137	138	Tentative	0.30[a] (0.11)	ND	0.24[a] (0.13)	ND	ND	ND	11.39 ***	
67	2,5-/6-Di-tert-butylbenzoquinone	$C_{14}H_{20}O_2$	31.5	1460	220	Tentative	0.004[b] (0.001)	0.11[ab] (0.07)	0.03[b] (0.03)	ND	1.64[a] (1.38)	0.12[ab] (0.12)	3.92 *	R, W
68	N-Acetonylphthalimide	$C_{11}H_9NO_3$	39.5	1731	203	MS, LRI	ND	0.23[a] (0.11)	0.18[a] (0.06)	ND	ND	ND	14.45 ***	
69	Isobutyl phthalate	$C_{16}H_{22}O_4$	42.8	1859	278	Tentative	0.14[b] (0.07)	0.88[b] (0.41)	0.15[b] (0.04)	2.39[b] (0.59)	17.40[a] (4.69)	3.96[b] (1.37)	32.67 ***	R, W
70	Dibutyl phthalate	$C_{16}H_{22}O_4$	45.2	1954	278	MS, LRI	0.57[b] (0.22)	16.90[b] (7.37)	3.12[b] (0.54)	13.64[b] (2.13)	391.94[a] (203.83)	24.52[b] (3.63)	10.45 ***	R, W

Notes: VOCs in bold were previously reported in the cooked meat systems (see Appendix I).

[a, b, c, d]: Mean values (n=3, followed by standard derivations reported in the brackets) in the same line with the same superscript do not differ significantly.

NS: not statistically significant $P \geq 0.05$, * $P \leq 0.05$, **$P \leq 0.01$, *** $P \leq 0.001$.

ND: not detected in the samples.

[1]**LRI:** Linear Retention Index (Calculated for each VOC using a mixture of Alkanes C_8 to C_{20} analyzed by the same GC-MS method).

[2]**Amount of VOCs:** all VOCs were semi-quantified using an internal standard (1, 2-dichlorobenzene, 100 μg) added in the models; each data represented the mean of triplicate measurements.

[3]**Controls (R/W):** VOCs also found in the ribose and/or water control model systems; **R**: ribose control; **W**: water control.

2-Furanmethanol (compound **29**) was only identified in the RC system. However, the prominent abundance of furfural eluted in gas chromatograms might be at the expense of the detection of 2-furamethanol in the other systems (its peak could be possibly masked by that of furfural due to a close retention time). 2-furanmethol was described to have a burnt and mild odor in meat (Chen et al., 2009), and it can be directly converted from furfural in the Maillard reaction (Chen, Chin, & Ho, 2004).

2-Acetylfuran (compound **30**) was described to have sweet and balsamic odor notes (Lee et al., 2010). Cooking methods that require high-heat treatments and low water content tend to favor the formation of this odorant (**Appendix I, Table 4**). In our study, 2-acetylfuran was exclusively identified in the carnosine systems (**Appendix III, Chart 5**). The formation of this compound was considered to be affected by both sugars and amino acids (Wang & Ho, 2008; Wang, Juliani, Simon, & Ho, 2009). In terms of sugars, ribose was found to be more reactive than glucose in the production of 2-acetylfuran (Wang & Ho, 2008). Depending on the types of amino acids, ribose can be either converted into 1-deoxypentosone which reacts with formaldehyde to generate 2-acetylfuran, or self-degraded into formaldehyde to form the furan compound after the reaction with ribose. However, when the amino acid was cysteine, ribose fragmentation and the subsequent formation of 2-acetylfuran were inhibited (Wang & Ho, 2008). Results of our study were in agreement with these observations, which again indicated that the formation of 2-acetylfuran was catalyzed by the Maillard reaction (as it was absent in the ribose control), whereas it could be discouraged in peptides with cysteine at the N-terminus.

5.4.4. Sulfur-containing VOCs. Many sulfur-containing volatile compounds generated from our current model systems are known flavor constituents of cooked meat subjected to a range of thermal treatments. The whole fraction of these compounds is

believed to contribute to the overall meaty and sulfurous flavor impressions of meat foods. Based on the summary of sulfur-containing VOCs related to cooked meat (**Appendix I, Table 5**), the formation of some of these volatiles was more favorable than the others under certain cooking conditions, indicating their relative importance in meat associated with cooking methods.

Mercaptoacetone (compound **34**) (**Appendix II, Figure 3**) was the only sulfur-containing ketone among the identified volatiles, and it was found in the RGA system. Mercaptoacetone has been captured in the flavor profile of pressure-processed pork meat (Uchman & Jennings, 1977). The odor threshold of this odorant was relatively high when compared to that of 2-methyl-3-furanthiol or furfurylthiol, but it is still within the range of ng/L in air (Hofmann & Schieberle, 1995). In Maillard reactions, mercaptoketones were suggested to be formed from the reactions between alkanediones and H_2S (Madruga & Mottram, 1998). Hofmann & Schieberle (1998) showed that the interaction of pyruvaldehyde and H_2S gave rise to mercaptoacetone which in turn acted as a precursor of 2-methyl-3-furanthiol.

Furanthiols and thiophenes. This class of volatiles included two furanthiols, 2-furfurythiol (compound **36**) and 2-methyl-3-furanthiol (compound **35**); six thiophenes, tetrohydro-2/3-methylthiophene (compound **32**), 2/3-methylthiophene (compound **33**), 2/3-thiophenecarboxaldehyde (compound **40**), dihydro-3(2H)-thiophene (compound **37**), dihydro-2-methyl-3(2H)-thiophene (compound **39**), 2/3-thiophenemethanol (compound **44**); and two thiol-substituted thiophenes, 2/3-thiophenethiol (compound **38**) and 3-(methylthio)-thiophene (compound **42**). The compounds of **37, 39, 42,** and **44** were

tentatively identified. In the model system of Cys-Gly, only compound **32, 36, 37**, and **40** were found.

2-methyl-3-furanthiol (MFT) and 2-furfurylthiol (FFT) have been identified as character impact compounds in meat flavor with exceptional low odor thresholds (Gasser & Grosh, 1988 & 1990; Hofmann & Schieberle, 1995). MFT possesses desirable sulfurous, meaty and boiled odor notes while FFT has distinctive roasted and coffee-like aromas. Sensory studies on the Maillard reaction products of ribose and GSH revealed that flavor properties of MFT and FFT were highly related to beef flavor (Lee et al., 2011). The occurrence of MFT and FFT in cooked meat and meat products has been summarized in **Appendix I, Table 5**.

Hofmann & Schieberle (1998) reported that ribose was the most effective carbohydrate precursor for both MFT and FFT within the investigated reducing sugars. They found that in reactions with H_2S, FFT was preferably formed from furfural than from ribose or 3-deoxyribosulose. Whereas in another reactions with H_2S, 4-hydroxy-5-methyl-3(*2H*)-furanone (NF) was more efficient in the production of MFT than ribose. Both 3-deoxyribosulose and NF are pentose degradation intermediates derived from the Maillard reaction through different pathways. The authors also proposed that the formation of FFT was achieved by the direct sulfurylation of furfural with H_2S, while the formation of MFT comprised of several reaction steps. In contrast, Cerny & Davidek (2003) observed that the majority of MFT produced from the reaction of NF, [$^{13}C_5$] ribose, and cysteine stemmed from ribose. However, it should be noted that in their study the two carbonyl sources, NF and ribose, were mixed to react with H_2S for which they might compete in the generation of MFT. Other carbonyl intermediates could also contribute to the formation of MFT depending on the source of reducing sugars and specific parameters applied in the reactions (Cerny & Davidek, 2003; Hofmann & Schieberle, 1998).

Under the studied condition, a non-significant amount of MFT ($P > 0.5$) was identified in the RGA model system, while FFT was detected in both the RGA and RCG systems. In light of the similar chemical structures shared by MFT and FFT, different efficacies in the production of corresponding rearranged Amadori products could be a factor affecting the ultimate distributions of the two odorants. Additionally, the course of MFT formation requires further reaction with a reducing agent (Hofmann & Schieberle, 1998), which might be more energy-consuming than the one-step substitution in the case of FFT. Furthermore, nature of the model systems may affect the retention of MFT, since this compound is unstable and can be easily oxidized or interact with other components present in the systems (Tang, Jiang, Yuan, & Ho, 2013).

Dihydro-3(2H)-thiophenone (compound **37**) ***and dihydro-2-methyl-3(2H)-thiophenone*** (compound **39**). Identification of the two volatile compounds was tentatively established in our study. Compound **37** was observed in both the RGA and RCG systems, whilst compound **39** was only detected in the RGA system. The natural presence of dihydro-3-(2H)-thiophenone and its methyl analogue in meat has been previously reported (Methven et al., 2007; Mottram, 1985; Wilson, Mussinan, Katz, & Sanderson, 1973). Dihydro-3-(2H)-thiophenone was identified as an aroma-active compound in the reaction mixtures of GSH with glucose and fructose. It has been described to have roasted and meaty odors in the GC-O analysis (Lee et al., 2010; Xu et al., 2013). Using carbohydrate module labeling technique (CAMOLA), the origin of dihydro-3-(2H)-thiophenone generated from reactions of GSH and glucose was traced back to the sugar by a loss of two carbon atoms (Lee et al., 2010). This compound was

also found in the thermal degradation of cysteine without carbohydrates (Shu et al., 1985), indicating the potential involvement of other formation mechanisms.

2/3-thiophenecarboxaldehyde (compound **40**) was found in the model systems of GSH and Cys-Gly. It was one of the most dominant compounds amongst the detected sulfur-containing volatiles. Concentrations of this compound in the corresponding model systems are provided in **Appendix III, Chart 7**. Possessing a characteristic sulfurous odor note, 2-and 3-thiophenecarboxaldehyde are a novel aroma constituents of meat flavor, and 2-thiophenecarboxaldehyde was identified as a flavor-active compound in chicken broth (Gasser & Grosch, 1990). The formation of 2-thiophenecarboxaldehyde from an aqueous reaction mixture of furfural, H_2S, and NH_3 under cooking condition has been observed by Shibamoto (1977). He suggested that the exchange of the oxygen atom of the furan ring in furfural by H_2S could lead to the odorant. In Maillard reactions, 2/3-thiophenecarboxaldehyde is a known product of GSH (Ho et al., 1992; Tai & Ho, 1998; Zhang & Ho, 1991a, b), however, it was not found in the model systems of GSH or Cys-Gly established by Ho et al. (2008). The detection of this compound together with other essential sulfur-containing heterocyclic constituents in the current RCG system indicates that the manner of dipeptide Cys-Gly could be similar to that of GSH in the Maillard volatile formation.

2/3-methylthiophene (compound **33**) is a volatile constituent distributed in a wide range of meat systems (**Appendix I, Table 5**). Sensory impressions of this compound were described as sulfurous, green and onion-like (Gasser & Grosch, 1990; Lee et al., 2010; Xu et al., 2013). Under the studied condition, the yield of this compound identified in the RGA system was not significant ($P > 0.05$). In the similar peptide model systems established by Ho et al. (2008), both 2- & 3-methylthiophene were observed in the thermal reactions of GSH and Cys-Gly with

glucose. Also, the total amounts of thiophenes, especially 2-methylthiophene, identified in the two systems were much greater than those formed by the related peptides Gly-Cys and γ-Glu-Cys. Such a variation, as suggested by the authors, were possibly due to the N-terminal position of cysteine in the Cys-Gly dipeptide where the residue can directly access the sugar molecule and undergo Strecker degradation to release H_2S. Cerny & Davidek (2003) demonstrated that 2-methylthiophene can be derived from intact ribose with a sulfur atom being introduced into the ring structure by H_2S. The absence of 2/3-methylthiophene in the thermal degradation of cysteine or GSH alone agrees with the statement, as given by Zhang & Ho (1991a), suggesting that the generation of thiophenes could be catalyzed by the presence of reducing sugars in the Maillard reaction.

Information of the natural presence of ***tetrahydro-2/3-methylthiophene*** (compound **32**, **Appendix II, Figure 2**) in meat is limited, and its formation in the Maillard reactions of cysteine or GSH has not been previously reported. In the current study, this compound was identified in both the RGA and RCG model systems. Interestingly, the yield from Cys-Gly was significantly greater than that from GSH ($P < 0.001$) despite a lower concentration of reactants being employed in the former reaction. At this point, neither flavor properties nor the formation mechanisms of tetrahydro-2/3-methylthiophene has been previously demonstrated. However, the hydrogenation of 2/3-methylthiophene under proper conditions might be a route for the formation of this volatile compound (Zhao, Oyama, & Naeemi, 2009).

The occurrence of ***2/3-thiophenethiol*** (compound **38**) in cooked meat was not common. but it was frequently reported as a volatile product in the model systems of cysteine and GSH (Cerny & Davidek, 2003; El-massry et al., 2003; Lee et al., 2010;

Madruga & Mottram, 1998; Tai & Ho, 1998). In Maillard reactions, the formation of 2/3-thiophenethiol was initiated not only by thermal treatments, but also by mild reaction conditions conditions such as in wine ageing (25℃) (Marchand et al., 2000). 2-thiophenethiol has been described to have a burnt or roasted coffee-like aroma with an odor threshold value of ~0.8μg/L in water (Marchand et al., 2000). Besides the Maillard reaction, 2/3-thiophenethiol can be formed from the thermal degradation of cysteine preferably at low pH (Shu et al., 1985; Tai & Ho, 1988). Chemical mechanisms governing its formation from solely cysteine and from Maillard reactions have been postulated by Shu et al. (1985) and Cerny & Davidek (2003), respectively. In particular, 3-thiophenethiol formed from the reaction of ribose and cysteine was suggested to be originated from the amino acid rather than the sugar (Cerny & Davidek, 2003).

2/3-thiophenemethanol (compound **44**) was tentatively identified in the RGA system. It has been isolated in the flavor composities of pressure-cooked beef (Wilson et al., 1973) and braised chicken (Duan et al., 2015). Based on the collected literature, 2/3-thiophenemethanol is a known product of Maillard reactions of cysteine (Chen & Ho, 2002; Chen, Xing, Chin, & Ho, 2000), whereas its presence in the related systems of GSH is rarely reported. 2-thiophenemethanol is possibly formed by the replacement of an oxygen atom with H_2S on the furan ring of 2-furanmethanol (Chen et al., 2004). Due to the lack of reference of 2/3-thiophenemethanol from GSH, further identity verification of this compound is needed.

Thiazoles. In the current study, thiazole (compound **31**), 2-acetylthiazole (compound **41**) and benzothiazole (compound **47**) are the volatile products found in this class. Thiazole and 2-acetylthiazole were present in the model systems of GSH and Cys-Gly (**Appendix III, Chart 6 & 8**), while benzothiazole was only found in the RG system. Under the studied condition, the amounts of thiazole identified from the model systems of

RGA and RCG were not significantly different from each other ($P > 0.5$). It aligns with the results from the previous reaction mixtures of GSH and Cys-Gly with glucose as reported by Ho et al. (2008).

Thiazoles are important flavor components in meat, and they contribute to the roasted, nutty and meaty aromas in related products. The general route for the Maillard formation of thiazoles is considered to be through the interaction of H_2S, and NH_3 with a mixture of α-dicarbonyl compound and aliphatic aldehydes. The reaction of an aldehyde and NH_3 produces an imine intermediate and the interaction of an α-dicarbonyl compound and H_2S yields an α-mercaptoketone. Further action of the mercaptoketone on the imine produces a thiazolidine compound which after oxidation can give rise to the corresponding thiazole (Huang & Ho, 2001; Mottram, 2007). Mechanistic interpretations of individual formations of thiazole and 2-acetylthiazole have been provided by Lee et al. (2010) and Cerny & Davidek (2003), and Mulders (1973) respectively. Both thiazole and 2-acetylthiazole can also be formed from the thermal degradations of cysteine and GSH without sugars (Zhang et al., 1988). In our study, the presence of thiazoles in the Cys-Gly system indicates that the dipeptide may undergo Maillard reactions/Strecker degradations to give H_2S, NH_3, α-dicarbonyls, and other degradation derivatives which are required for the formation of thiazole compounds. In the case of benzothiazole, its odors between studies varied. It was reported to be burnt- and meat-like in cooked beef (Machiels et al., 2003), but rubbery and metallic in roasted duck (Chen et al., 2009). Besides being a natural meat volatile constituent, this compound was also isolated from the thermal reaction of inosone 5'-monophosphate and cysteine (Madruga & Mottram, 1998).

Benzothiazole is generally regarded as a Maillard reaction product, but the explicit formation mechanism is still unclear at this point.

Cyclic polysulfur compounds. In this class, volatile compounds includes 2-methyl-1, 3-dithiane (compound **43**) (**Appendix II, Figure 4**), 1, 3/4-dithian-2-one (compound **45**) (**Appendix II, Figure 5**), 4-methyl-3H-1,2-dithiole-3-thione (compound **46**) (**Appendix II, Figure 6**), [1, 2, 3, 4]-tetrathiane (compound **48**) (**Appendix II, Figure 7**), hexathiane (S_6) (compound **49**), lenthionine (1, 2, 3, 5, 6-pentathiepane) (compound **50**) (**Appendix II, Figure 8**), hexathiepane (1, 2, 3, 4, 5, 6-hexathiepane) (compound **51**) (**Appendix II, Figure 9**), and cyclic octaatomic sulfur (S_8) (compound **52**). With the exception of S_6 and S_8, all other cyclic polysulfur compounds were only identified in the RGA model. To our best knowledge, none of them has been previously reported in the Maillard reaction of peptides. Only lenthionine and hexathiepane have been isolated in the flavor profiles of meat and related products.

2-methyl-1, 3-dithiane (compound **43**) was described to have an onion and garlic-like taste (Shu, Hagedorn, Mookherjee, & Vock, 1981). Yu, Wu, & Ho (1994) identified [1, 2, 3, 4]-tetrathiane and 2-methyl-1, 3-dithiane from the thermal reactions of alliin and deoxyalliin with glucose. It is noteworthy that certain degrees of roasted meaty flavor were elicited from both model solutions after heating. Whilst neither of the products was considered as character aroma compounds in the model solutions, their combination with other volatile constituents was thought to contribute to the overall meaty flavor of the model solutions (Yu et al., 1994). In contrast to [1, 2, 3, 4]-tetrathiane, the presence of its isomers, such as [1, 2, 5, 6]-tetrathiane and [1, 2, 4, 5]-tetrathiane, was more frequently reported in the model systems of cysteine (Madruga & Mottram, 1998; Mottram &

Nobrega, 2002; Yu & Zhang, 2010). 1, 3/4-dithian-2-one has not been isolated in any food systems, but its isomeric compound 1, 2-dithian-4-one was found in heated salmon extract (Methven et al., 2007). S_8 was a volatile product in the thermal reaction of ascorbic acid and cysteine at pH $\geq$ 6.0 (Yu & Zhang, 2010).

Lenthionine (compound **50**) & *hexathiepane* (compound **51**). Information pertaining to these two compounds is relatively limited, and they have been identified in the flavor extracts of boiled mutton (Nixon, Wong, Johnson, & Birch, 1979) and cooked ham (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2014) respectively. Lenthionine and hexathiepane were firstly isolated from the extracts of dried Shiitake mushroom by Morita & Kobayashi (1966). The mass spectra previously obtained for their structural assignments (Morita & Kobayashi, 1967) further support the identification of both compounds in our RGA model system. Previous studies on the physiochemical properties of lenthionine showed that the solubility of this compound was low in water and in polar solvents, but significantly increased in vegetable oil. This compound was stable in solutions with pH between 2 to 4, but it decomposed rapidly in pH above 5 (Wada, Nakatani, & Morita, 1967). As volatile sulfur compounds, lenthionine and hexathiepane are liable to high temperature in gas chromatographic separation (Chen & Ho, 1986). It was reported that the amounts of both compounds recovered from the headspace aqueous extract of Shiitake mushroom at 70℃ (for 30min) were 3-and 6-fold respectively less than those obtained at 22℃ (Dermiki, Phanphensophon, Mottram, & Methven, 2013). The identification of both lenthionine and hexathiepane in the current study indicates a certain degree of retention capability of our model systems for these sulfur aroma components.

With respect to the flavor contribution to food products, lenthionine possesses a characteristic aroma of Shiitake mushroom which has been used as a taste and flavor enhancer in a number of traditional Asian dishes (Morita & Kobayashi, 1966; Wada et al., 1967). The organoleptic potency of lenthionine could be in part attributed to its low odor threshold values.They have been determined at a concentration between 12.5-25 ppm in vegetable oil, and a much lower level between 0.27-0.53 ppm in distilled water (Wada et al., 1967). Ito, Toyoda, Suzuki, & Iwaida (1978) presumed that lenthionine extracted from dried Shiitake mushroom would gradually decompose to carbon disulfide in aqueous solutions.

When it comes to the origin of cyclic polysulfur compounds, the reaction between aldehydes, H_2S, and NH_3 is considered to be a major pathway (Boelens, van der Linde, de Valois, van Dort, & Takken, 1974; Zhang et al., 1988). Lentinic acid was proposed as the enzymatic precursor of lenthionine in Shiitake mushroom (Yasumoto, Iwami, & Mitsuda, 1971). The chemical structure of this peptide was characterized as a S-substituted cysteine sulfoxide derivative with a glutamic acid at the N-terminus and a methylsulfonyl group at the C-terminus. Similar to GSH, lentinic acid also has an γ-peptide bonding between the carboxyl group of glutamic acid and the amino group of the cysteinyl residue. Besides being enzymatically liberated from food systems, the formation of lenthionine and hexathiepane from the reaction mixture of xylose and thiamin has been previously reported (Hincelin, Ames, Apriyantono, & Elmore, 1992). It was postulated that the interaction between H_2S, and 1, 1-ethanedithiol could form lenthionine. 1, 1-ethanedithiol can be synthesized from the reaction of H_2S and acetaldehyde, both of which are the Strecker degradation products of cysteine (Boelens, van der Linde, de Valois, van Dort, & Takken, 1975). Comparison of the quantity of cyclic polysulfur compounds in the RGA system indicates that the formation of some of these compounds could

be more favorable than the others under a given condition. Taking into account the artificial synthesis of lenthionine and hexathiepane from simple reaction materials (Morita & Kobayashi, 1966; Wada et al., 1967), as well as nature of their precursors in foods, further investigation of the relative formation mechanisms of these compounds would be of high value to afford a better understanding of the role of peptides as Maillard reactants in flavor development.

5.4.5. Nitrogen-containing VOCs.

Pyrazines and pyridines are ubiquitous volatile constituents. The presence of these compounds in foods subjected to high heat treatments is commonly reported. Pyrazines impart roasted and nutty odor notes to foodstuffs. Individual flavor characteristics of 2-methylpyrazine and 2, 5/6-dimethylpyrazine have been previously described in cooked meat (Chen et al., 2009; Xie et al., 2008). In particular, the odor characteristics of 2, 5-dimethylpyrazine considerably resemble the flavor of potato chips, and it has a flavor threshold of 2 ppm in oil and 1 ppm in water (Deck & Chang, 1965; Maga & Sizer, 1973). Compared to pyrazines, pyridines have less pleasant odors, and their impact on (whether enhancing/diminishing) the overall flavor quality of food products is not quite as clear-cut (Ohloff & Flament, 1978; van Boekel, 2006). For the pyrazine and pyridine compounds identified in this study, their occurrence in meat and meat products is summarized in **Appendix I, Table 6**. It is shown from the table that temperatures required for the production of simple pyrazines are relatively broad. However, as the degree of alkyl-substitution increases, the formation of both pyrazines and pyridines becomes temperature-dependent. For instance, pyrazine, 2-methylpyrazine, and 2, 5/6-dimethylpyrazine are simple pyrazines whose formation was not considerably

related to cooking methods, whereas the occurrence of 2-ethyl-3/5/6-methylpyrazine was limited to meat treated with high temperature and generally at low water content.

In this study, both pyrazines and pyridines were exclusively found in the model systems of carnosine, and they were the major nitrogen-containing volatile constituents. Pyrazine (1, 4-diazine) (compound **53**), 2-methylpyrazine (compound **56**), 2, 5/6-dimethylpyrazine (compound **58**), 2-ethyl-3/5/6-methylpyrazine (compound **61**), pyridine (compound **54**), 2/3/4-methylpyridine (compound **55**), 2/3/4-ethylpyridine (compound **57**), and 2, 3/2, 4/2, 5/2, 6/3, 4/3, 5-dimethylpyridine (compound **59**) were successfully identified. Under the current gas chromatographic conditions, a complete separation of certain pyrazines or pyridines cannot be accomplished due to the little difference between their mass spectra. It was decided to include all possible compounds in one eluting peak and consider them as one compound in the following discussion. Results of this study showed that temperature affected the distribution of these nitrogen heterocyclic volatiles. For example, 2-methylpyrazine was observed in all three carnosine systems (**Appendix III, Chart 9**), while 2-ethyl-3/5/6-methylpyrazine (**61**) was only detected in the reaction extract of RCH. In a related ribose/carnosine model system conducted by Chen & Ho (2002), most of the pyrazines and pyridines formed in their study at pH 8.5 were also reaction products of our systems at pH 6.3.

The formation of pyrazines has been studied in a number of model systems involving different complexity of carbonyl and nitrogen sources. Reactions of glucose with H_2S or NH_3, or both revealed that NH_3 could serve as a reactant and a base-catalyst in the formation of pyrazines. Also, the production of nitrogen heterocyclic compounds could suppress the yields of sugar derivatives and sulfur heterocyclic volatiles formed in the reactions (Sakaguchi & Shibamoto, 1978; Shibamoto, Akiyama, Sakaguchi, Enomoto, & Masuda, 1979; Shibamoto &

Russell, 1977). Shibamoto & Bernhard (1975) suggested that there were at least two ways for the incorporation of nitrogen atoms into a pyrazine ring, one from free NH_3, and the other directly from an amino acid or amine nitrogen. It is known that NH_3 can be emanated from the degradation of amino acids, peptides or proteins upon heating. The interactions between amino acids and reducing sugars produce reactive dicarbonyl compounds which can catalyze the Strecker degradation of amino acids to give rise to the intermediate precursors of pyrazines. Little discussion has been undertaken to verify both pathways in the pyrazine synthesis as well as their respective reaction efficiencies. Peptides have gained a great deal of interest as compounds with flavor-generating potency in the Maillard reaction. It is speculated that their roles in the formation of pyrazines have been underestimated (Rizzi, 1989; Scalone et al., 2015; Van Lancker et al., 2010, 2012).

In general, variables affecting the formation of pyrazines include the nature and ratio of reactants, pH value, water activity, reaction temperature, and heating time. In view of pH effects, basic environment facilitates the formation of pyrazines by increasing the reactivity of amino acids towards the carbonyl groups of reducing sugars. However, possibly due to the increased production of melanoidin pigments, such a catalytic effect could be offset when the solution alkalinity reaches a certain limit (Bemis-Young, Huang, & Bernhard, 1993; Koehler & Odell, 1970). Temperature can affect the kind and quantity of pyrazines, and it is suggested that the formation of individual pyrazine compounds may require different activation energies (Huang, Bruechert, & Ho, 1989). Moreover, depending on the severity of thermal treatments, the major route governing the formation of pyrazines could be varied (Koehler & Odell, 1970).

Interactions between α-aminocarbonyl derivatives are considered as the general route for pyrazine formation (Rizz, 1972). For pyrazines with complex branched chains or higher molecular weights, the involvement of various aldehydes derived from Strecker degradations of amino acids seems to be important for their formation (Flament, 1981; Rizz, 1972). Shibamoto & Bernhard (1977) suggested that the combination of different α-aminocarbonyl fragments accounted for the generation of individual alkylpyrazines. Knowing that α-aminocarbonyls are structural-relative products of α-dicarbonyls derived from the Strecker degradation, factors that affect the formation of these dicarbonyl precursors could in turn influence the generation of pyrazines.

So far, evidence of peptides as flavor precursors in Maillard reactions is far from conclusive. Van Lancker et al. (2010, 2011, 2012) have contributed some of the notable work in this field. They suggested that a different mechanism should account for the formation of α-aminoketones from dipeptides since they cannot undergo the typical Strecker degradation due to the presence of peptide bonds.

In the investigation of volatile profiles from Maillard reactions of glucose with free amino acids, dipeptides, or tripeptides, researchers found that the total amounts of pyrazines, especially 2, 5(6)-dimethylpyrazines and trimethylpyrazine, produced by the dipeptides were much greater than those given by the corresponding free amino acid mixtures or tripeptides (Van Lancker et al., 2010 & 2012). The higher reactivity of dipeptides could be attributed to the catalysis of dipeptide-sugar Amadori rearrangement adducts by which accelerating the overall rate of the reaction (de Kok & Rosing, 1994; Van Lancker et al., 2012).

The reaction mechanism governing the Maillard formation of pyrazines from dipeptides has been proposed by Van Lancker et al. (2010). They hypothesized that the reaction was

initiated by the nucleophilic attack of the α-amino group of a dipeptide to the carbonyl group of a dicarbonyl compound. It gave rise to an α-ketoimine product containing both imine (-C=NR) and amide (-$CONH_2$) functional groups. Followed by enolization and subsequent hydrolysis, the rearranged intermediate (4-hydroxy-2-azadiene) then cleaved into an α-aminoketone and a complex α-dicarbonly compound. By means of this, materials required for the formation of pyrazines are generated, and the nitrogen atom originated from the dipeptide can be incorporated into the rings of pyrazines through α-aminoketones.

As mentioned in the literature review, the release of Strecker aldehydes from Maillard reactions of peptides indicated a certain degree of peptide bond hydrolysis (Rizzi, 1989; Van Lancker et al., 2010). In this case, the free amino acids released from peptides could undergo Strecker degradations to form pyrazine compounds. However, the actual behavior of peptides in the Maillard reaction could be much more complex, since volatile profiles given by the dipeptides and their corresponding free amino acids were strongly different in the Maillard reaction (Van Lancker et al., 2010).

To review our study, dipeptides of carnosine (β-alanine-L-histidine) and Cys-Gly may undergo the similar reaction steps to produce pyrazine compounds. 2, 5-dimethylpyazine was identified in the reaction of glucose and Cys-Gly (pH 5.5, 160℃, 1hr) (Ho et al., 2008), however, no pyrazine was detected in our model system of ribose and Cys-Gly (pH 6.3, 180℃, 2hr). Variations between the studies are more likely due to the nature of model systems with respect to the manner of pyrazine synthesis. In the case of carnosine, effects of its amino acid composition on the conformation of the corresponding dipeptide-sugar adducts and thereafter the pyrazine formation are

unknown at this point. Under certain conditions, histidine in peptides may accelerate the rate of Maillard reactions, possibly by facilitating the formation of rearranged Amadori intermediates. Mori, Bai, Ueno, & Manning (1989) reported that the presence of histidine, as a positively charged amino acid at the second position of the investigated dipeptides (Val/Ala-His) and tripeptides (Gly-His-Gly), significantly increased the reactivity of peptides to the site of glyceraldehyde. Despite the rate of reactions may not be necessarily correlated with the efficacy in pyrazine formation, it is still of value to consider the related mechanisms from an overall Maillard reaction perspective.

Besides histidine, the reaction scheme depicted by Van Lancker et al. (2010) has rationalized the potential importance of β-alanine as for carnosine in the pyrazine synthesis. Based on their scheme, β-alanine, the N-terminal amino acid of carnosine, can directly interact with the dicarbonyl compound to initiate the productin of α-aminoketones. However, β-position of the amino group may render the behaviors of this amino acid and its related peptides differ from those of alanine and the derived peptides in the Maillard reaction. So far, volatile compounds from Maillard reactions of β-alanine have not been reported. Lien & Nawar (1974a, b) found that modes of the thermal decomposition of alanine and β-alanine were significantly different from those of valine, leucine, and isoleucine. Also, β-alanine appeared to decompose at lower temperatures as compared to alanine (Lien & Nawar, 1974 a, b). Inclusion of Maillard reactions of β-alanine in the future study may help to validate the reaction mechanisms hypothesized by the previous researchers. In short, data from this study shows that carnosine, of which the concentration employed is close to what is naturally present in fresh meat, still has the capability to produce flavor compounds by acting as a nitrogen donor in the Maillard

reaction. Nevertheless, further work is needed to elucidate the reaction mechanisms of carnosine, or other low MW peptides, in the Maillard formation of aroma compounds, as well as to determine the degree of flavor contribution of these peptides to the overall sensory impression of meat foods.

5.4.6. Reactant-specific volatile products.

3-methyl-2, 5-piperazinedione (compound **63**) (**Appendix II, Figure 11**). Compound **63** was only present in the RGA reaction mixture, and it was tentatively identified as 3-methyl-2, 5-piperazinedione in the GC-MS analysis. 3-methyl-2, 5-piperazinedione is considered as a DKP product of the dipeptide Cys-Gly during GSH degradation. Ueda, et al. (1997) reported that the thermal degradation of GSH in buffer solutions at low pH mainly gave rise to cyclic (Cys-Gly) (3-mercaptomethyl-2, 5-diketopiperazine) and 5-oxoproline. Ho et al. (2008) observed a more complex profile from GSH decomposition, which included residue GSH, GSH with a thiazoline ring, GSH disulfide, GSH-Cys-Gyl, and pyroglutamic acid. In Maillard reactions, mode of GSH fragmentation has been explored by Wang et al. (2012) using model systems containing xylose, thiamin, and a mixture of different combinations of GSH, GSH constituted amino acids, or dipeptide Cys-Gly (with/without glutamic acid). In their study, 3-methyl-2, 5-piperazinedione was exclusively detected in the model system containing GSH though cyclic (Cys-Gly) was produced in all systems studied. It was suggested that the dipeptide Cys-Gly could undergo intracellular cyclization to form cyclic (Cys-Gly) which after the removal of the thiol group can give rise to 3-methyl-2, 5-dipiperazinedione (Wang et al., 2012).

2-acetylpyrido [3, 4-d] imidazole (compound **65**). Among a range of volatile compounds identified in the current study, 2-acetylpyrido [3, 4-*d*] imidazole was only isolated in the RC and RCH systems. Its absence in the RCA model reaction could be possibly caused by the different extraction method. The formation of 2-acetylpyrido [3, 4-*d*] imidazole in the Maillard reaction was firstly recognized by Gi & Baltes (1993) in their model systems containing a mixture of glucose and histidine under both aqueous and dry roasted conditions. The gas fragmentation pathways of this compound were investigated in their later study (Gi & Baltes, 1995). The researchers depicted that starting from the molecular ion *m/z* 161, the separation of CH_2CO from the structure gave a base peak of *m/z* 119 which after deprotonation or protonation formed fragmentation ions *m/z* 118 or *m/z* 120. Alternatively, the removal of CO and rearrangement of the terminal methyl group could lead to the ion mass *m/z* 133 (**Appendix II, Figure 12**).

As proposed by Yaylayan & Haffenden (2003), the formation mechanism of imidazoles in the Maillard reaction can be generalized by the incorporation of nitrogen atoms to sugar-derived intermediates (α-hydroxycarbonyls & α-dicarbonyls) via the generation of α-aminocarbonyl or Amadori intermediates. The production of α-aminocarbonyls can be achieved either from the Strecker degradation of amino acids with α-dicarbonyls, or from the Amadori rearrangement of α-hydroxycarbonyls with NH_3. At this point, there are two things deserved to be highlighted here. According to Novotny et al., (2007), α-dicarbonyl and α-hydroxycarbonyl compounds formed from sugar degradations in the aqueous solution are interchangeable. In addition, α-aminocarbonyl precursors required for the generation of imidazoles are shared by the other heterocyclic volatile compounds, such as pyrazines, pyrroles, and oxazoles, in their respective Maillard formation pathways (Yaylayan & Haffenden, 2003). Under such circumstances, competition between the formations of corresponding intermediate precursors

could determine the final distributions of these aroma compounds. Based on the scheme proposed by Gi & Baltes (1993), the formation of 2-acetylpyrido [3, 4-*d*] imidazole from reaction of histidine and glucose was achieved through the Strecker degradation of histidine with pyruvaldehyde. Pyruvaldehyde is a well-known sugar degradation product. By means of retro-aldo reactions, the formation of this dicarbonyl compound was considered via 1-deoxyglucosone from glucose (Gi & Baltes, 1995), and 3-deoxyosone from ribose (Rizzi, 2004).

Taking into account the amino acid composition of carnosine, the formation of 2-acetylpyrido [3, 4-*d*] imidazole in our carnosine model systems could be reasonable. Given the fact that the α-amino group of histidine is blocked in the case of carnosine, hydrolysis of this peptide might occur upon reactions to allow the interaction between histidine and dicarbonyl compounds. However, owing to the limited information of peptides in the Maillard reaction, other reaction mechanisms related to the formation of this imidazole compound should not be excluded. It is notable that 2-acetylpyrido [3, 4-*d*] imidazole was also isolated from the volatile fraction of heated tuna meat in the presence of glucose (Gi & Baltes, 1993). It may again indicate the potential importance of this compound in foods that are rich in histidine or histidine-related constituents.

Chapter 6. Conclusions

In this study, key volatile compounds associated with cooked meat flavors were analyzed from the Maillard model systems containing ribose and low MW peptides, in this case, carnosine, GSH, and dipeptide Cys-Gly, at expected concentrations and a physiological pH condition related to fresh meat. Nitrogen-containing heterocyclic volatile compounds dominated in the reaction systems of carnosine, whilst sulfur-containing heterocyclic volatile compounds were generated in the model systems containing GSH or Cys-Gly. Several cyclic polysulfides, which have not been reported from the Maillard reaction of peptides, were found in our ribose/GSH system. The formations of 2-acetylpyrido [3, 4-*d*] imidazole, as a histidine-specific Maillard reaction product, and 3-methyl-2, 5-piperazinedione, as a DKP product of GSH degradation, were also suggested in this study. Taking into consideration the release of a great number of low MW peptides from the muscular proteins during meat storage, the impact of peptides on the final sensory quality of meat and related products by acting as α-amino group donors in the Maillard reaction could have been overlooked.

Chapter 7. Further Works

Due to the inherent limitations of our current model systems, further improvement of the study was to be mentioned in this section. In addition, potential extension of the work to other facets related to food chemistry and biological significance of low MW peptides was to be briefly suggested.

7.1. Study Improvement

7.1.1. Buffer. The use of buffer and its amount were choices of less certainty. It is established that meat itself has high buffering capacity, and pH variations are usually minor after cooking (0.2-0.5 pH units) (Meynier & Mottram, 1995). In addition, pH changes in unbuffered model systems tend to be relatively large after the reactions, and it could have a significant impact on the formation of VOCs. Therefore, an addition of buffer before reactions is generally considered in model systems. Based on the previous studies, in order to achieve an established pH value, either the reactant mixture was directly dissolved into the buffer solution, or they were firstly mixed with water to a designed volume and adjusted by the buffer afterwards. In our model systems of GSH and Cys-Gly, sodium phosphate was applied in the reactant mixtures after they were dissolved in 110 or 150 mL water. Results of pH changes (**Table 3**) showed that the buffering capacity introduced in this way was not sufficient to manipulate pH variations within the natural range of meat after cooking, therefore, the amount of buffer use should be reconsidered in the future study. However, taking into account the catalytic effect of buffers on the Maillard reaction (de Kok & Rosing, 1994), the decision should be made in caution in pursuit of neutralizing pH effects on the model systems on one hand, and retaining the natural manner of peptides in the Maillard reaction on the other hand.

7.1.2. VOC identification. Identities of certain volatile compounds cannot be finalized owing to the lack of LRI reference and/or limitation of the current alkane standard (C_8-C_{20}). Therefore, the use of an external alkane reference with a higher measuring tolerance could facilitate the detection of volatile compounds eluted at the early/late phase during GC separation. In addition, using authentic pure compounds, GC-MS/MS, and/or NMR are of value to afford definite identification of the tentative compounds.

7.2. Study Extension

7.2.1. Comparison of VOC formation from model systems between sugar/peptide and sugar/free amino acid mixture. To date, an agreement on the reactivity of peptides in the Maillard reaction has not yet achieved. Despite of the external factors built in a model system, the nature of peptides seems to be particularly important for their manners in the Maillard VOC formation. It is apparent that the degree of peptide reactivity varied depending on the chosen parameters. Based on our work, future endeavors can take place to examine the efficacy of low MW peptides in comparison to their corresponding free amino acids in the formation of pyrazines or other essential aroma compounds. Model systems containing ribose and carnosine or a mixture of β-alanine and histidine can be established in this case.

7.2.2. Use of labeled isotopic reactants in Model Maillard reaction systems.

Sugar/peptide reactions. The use of labeled isotopic reactants in model systems has several implications, such as tracing the origins of volatile compounds, facilitating the establishment of their formation pathways, as well as elucidating the behaviors of peptides in the Maillard reaction. In the case carnosine, the question of whether the generation of pyrazines was through the sugar-peptide intermediates (α-aminoketone

formation) or the Strecker degradation of peptide-derived amino acids (peptide hydrolysis), or both can be possibly clarified with the respective degree of contribution being evaluated.

Model Maillard reaction systems containing reducing sugars, peptides of question, and their corresponding free amino acid mixtures. To examine the competition between small peptides and free amino acids towards the formation of key volatile compounds, model systems containing a mixture of peptides and their constituted amino acids (with either peptides or amino acids labeled) in reactions with reducing sugars can provide a visualized comparison of the kind and abundance of volatile constituents (labeled/unlabeled) generated from the corresponding amino compounds.

Addition of peptides in a thermal-treated meat system. Using model systems is far too simple to imitate what would happen in natural meat systems upon cooking. In order to study the actual degree of flavor contribution of low MW peptides to meat flavor development, known amounts of peptides (labeled) can be added into the meat samples prior to thermal treatments. Comparison of the quantities of volatile compounds identified from the control and the system with peptide added affords a better understanding of flavor potentials of the peptides.

7.2.3. Analysis of non-volatile compounds from Maillard reaction systems containing low MW peptides using LC/GC-MS and related techniques.

Under cooking conditions. Diketopiperazines (DKPs) are cyclic dipeptide compounds generated either from the cyclodehydration of two amino acids, or from the intramolecular cyclization of dipeptides which can be hydrolyzed products of linear poly-peptides or proteins (Ginz & Engelhardt, 2000). The formation of DKPs in several thermal-treated foods and in the

model Maillard reaction systems of peptides have been reported (Ginz & Engelhardt, 2000; Rizzi, 1989; Xu et al., 2013; Yang et al., 2012). Analysis of the possible formation pathways of DKPs can provide mechanistic evidence for the cleavage of peptide bonds (oligopeptides) upon reactions. From a food point of view, DKPs are novel taste constituents due to their bitterness. However, the biological activities of some of these compounds (such as cyclic His-Pro) may render them a potential health risk, which is still a field of little exploration. Therefore, the qualitative and quantitative analysis of DKPs from Maillard reactions of peptides could be a source of reference to food manufacturers in pursuit of products with high sensory quality and safety assurance.

Under physiological conditions (at pH 7.4, 37*°C*). Further analysis of non-volatile intermediaries from Maillard reactions of low MW peptides and glucose under the physiological condition may facilitate the identification of stable biomarkers where functional peptides may be lost as amino donors.

The non-enzymatic glycation of reducing sugars to the N-terminus and amino acid side chains is one of the most common post-translation modifications of amino acids, peptides, and proteins in *vivo* (Muscat, Pischetsrieder, Maczurek, Rothemund, & Münch, 2009). It starts with the formation of a Schiff base followed by the rearrangement into Amadori or Heyns' products. Subsequent oxidative or non-oxidative reactions give rise to a great number of reactive carbonyl intermediates which can further react with peptides or proteins to form irreversible adducts usually knonw as advanced glycation end-products (AGEs) (Zhang, Ames, Smith, Baynes, & Metz, 2009). The AGE accumulation on long-lived proteins involves in the progression of age-related disorders and complications of some of the chronic diseases. The detrimental effects induced by

AGEs are not only through the alternation of protein integrity, but also through the involvement in neurological and signaling pathways (Münch, Thome, Foley, Schinzel, & Riederer, 1997). Depending on the nature of the amino compounds as well as the stability of AGE products, indications of Maillard reactions in the physiological level have several facets. Some researchers considered that AGE formation could lead to modifications in cellular metabolism when the peptides or proteins involved were biologically significant (Zeng & Davies, 2005) (as in the case of GSH and possibly Cys-Gly). Whereas some suggested that the formation of relatively inert AGEs from certain peptides could limit the potential damages caused by the browning reactions of glycated proteins in *vivo* (Ahmed, Thorpe, & Baynes, 1986) (as in the case of carnosine).

AGEs formed from the glycation of reactive carbonyls to the side chains of lysine, arginine, and cysteine residues of peptides and proteins, or from the oxidative degradation of related Amadori adducts have been characterized in both *vitro* and *vivo* (Ahmed et al., 1986; Iijima, Murata, Takahara, Shinkichi, & Fujimoto, 2000; Zeng & Davies, 2005). Specifically, Zeng & Davies (2005) identified a stable adduct, *S*-(carboxymethyl)cysteine (CMC), from the incubation of glyoxal with thiol-containing amino acids (cysteine), peptides (GSH), and proteins (creatine kinase and human serum albumin) respectively under the physiological condition. The authors pointed out that CMC could be a sensitive and unique biomarker for the glycation reactions on proteins in biological systems. In addition, the cysteine residue was a favorable glycation site for the carbonyl materials due to its stronger nucleophilic capacity compared to lysine or arginine (Zeng & Davies, 2005). Moreover, the formation of CMC is at the expense of

GSH deposit and the integrity of thiol-dependent enzymes, which in turn leads to a range of negative cellular responses at the physiological level (Zeng & Davies, 2005).

For carnosine (β-alanyl-L-histidine), besides its strong buffering and antioxidant capacities, it is also considered as an effective anti-glycation agent since it can be readily glycated in the presence of biological sugars (Hipkiss, Michaelis, & Syrris, 1995). Hipkiss et al. (1995) proposed that the protective effect of carnosine against sugar-mediated crosslinking on proteins could be due to its comparable amino acid sequence to Lys-His which was believed to be a preferable glycating-site. Hobart, Seibel, Yeargans, & Seidler (2004) verified that the imidazolium group of histidine in caronsine was the prime chemical constituent responsible for the anti-crosslinking performance of this dipeptide. Other results that were worth mentioning from their study include *1).* β-alanine itself was an effective anti-glycator at higher concentrations, indicating the importance of the primary amine at the β-position for the protective effect of this amino acid; *2).* the anti-crosslinking activity exerted by histidine was greater than that of carnosine. The reason for β-alanylation of histidine could be presumably a selection by evolution which could protect histidine from protein synthesis.

Much evidence has suggested that there could be more than one biochemical mechanism accounting for the anti-glycation property of carnosine. Besides acting as a competitive glycation site for sugars to form non-mutagenic AGE products (Hipkiss et al., 1995), carnosine may also serve as a tranglycation agent by reversing the glycated proteins at the early stage (Szwergold, 2005). To date, attention on the other histidine-containing dipeptides, such as anserine, homocarnosine, and homoanserine, has arisen dramatically due to their closely related structures and similar biological functions (Boldyrev & Severin, 1990). More recently, the identification of carnosine, anserine, and homoanserine in the post-slaughter chicken samples over storage was

achieved in our laboratory using LC-QTOF-MS (Aliani, unpublished data). Neither the glycated forms of carnosine or its analogue dipeptides of biological origin have been identified, nor have their biological significance and therapeutic indication been fully elucidated. Future investigation in this field is of great promise to develop strategies for the prevention and treatment of AGE-related complications and diseases.

7.3. Other applications

7.3.1. Gas Chromatography-Olfactometry (GC-O). It can be used as a complementary technique in combination with GC-MS to screen out the potent odorants from a model system. GC-MS is a concentration-sensitive tool to identify the volatile compounds separated in a gas chromatogram, however, it cannot differentiate the aroma compounds from a pool of volatiles. Hence, compounds that are high in aroma quality but low in concentrations may not be effectively picked up by this method. The use of GC-O coupled with AEDA method can facilitate the identification of aroma active compounds with their flavor potentials evaluated by FD factors. In this study, GC-O can be used to evaluate the aroma characteristics of the detected VOCs with unknown flavor properties.

7.3.2. Pyrazinone formation from Maillard reactions of peptides. Pyrazinones are peptide-specific volatile products whose formations from the reactions of peptides with reducing sugars or dicarbonly compounds have been reported (Van Chuyen et al., 1973; Oh, Shu, & Ho, 1992). However, they were absent under the studied condition. At this point, information of pyrazinones regarding their natural presence in foods, as well as the potential flavor and health implications is scant. Further study is of great need to expand the knowledge in this field.

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APPENDICES

APPENDIX I.

Summary of VOCs Reported in Cooked meat & Meat Products and Also Found in the Current Model Systems.

Table 1. Hydrocarbons.

Hydrocarbons	Meat systems	References
3-Methylhexane (**1**)	*sous-vide* cooked lamb	Roldán et al. (2015)
Methylcyclohexane (**2**)	*sous-vide* cooked lamb	Roldán et al. (2015)
	Fried chicken	Tang et al. (1983)
	Canned beef stew[5]	Peterson et al. (1975)
Toluene (**3**) (Methylbenzene)	Boiled chicken	Nonaka et al. (1967)
	High-pressure cooked beef	Rivas-Cañedo et al. (2011)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Roasted pork	Xie et al. (2008)
	Water boiled & roasted duck	Wu & Liou (1992)
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked salmon[3]	Methven et al. (2007)
	Cooked turkey[4]	Brunton et al. (2002)
	Canned beef stew[5]	Peterson et al. (1975)
Ethylbeneze (**4**)	*sous-vide* cooked lamb	Roldán et al. (2015)
	Roasted chicken	Noleau & Toulemonde (1986)
	Water boiled & roasted duck	Wu & Liou (1992)
	Smoked fish	Guillén & Errecalde (2002)
	Cooked beef[1]	Leo & Ames (1986)
	Canned beef stew[5]	Peterson et al. (1975)
o/m/p-Xylene (**5**) (1, 2/3/4-Dimethylbenzene)	Boiled chicken	Nonaka et al. (1967)
	sous-vide cooked lamb	Roldán et al. (2015)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Water boiled & roasted duck	Wu & Liou (1992)
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
Styrene (**6**) (Ethenylbenzene)	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Roasted pork	Xie et al. (2008)
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)

Hydrocarbons	**Meat systems**	**References**
Limonene (**21**)	Boiled chicken	Nonaka et al. (1967)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Roasted pork	Xie et al. 2008) *Flora, green, sweet*
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked turkey[4]	Brunton et al. (2002)
1,2,3/1,2,4/1,3,5-Trimethylbenzene (**8**, tentative)	Boiled chicken	Nonaka et al. (1967)
	Roasted chicken	Noleau & Toulemonde (1986)
	Boiled & roasted duck	Wu & Liou (1992)
	Roasted beef	Min et al. (1979)
1-Ethyl-2/3-methylbenzene (**8**, tentative)	Roasted beef	Min et al. (1979)
	Cooked salmon[3]	Methven et al. (2007)

Table 2. Aldehydes & Ketones.

Aldehydes & Ketones	Meat systems	References
Heptanal (**20**)	*sous-vide* cooked lamb	Roldán et al. (2015)
	Steamed fish	Tao et al (2014) *nutty, burnt*
	Boiled chicken	Nonaka et al. (1967)
	Boiled beef	Gasser & Grosch (1988) *green, fatty, oily*
	Chicken broth	
	High-pressure cooked beef	Horvat (1976)
	Roasted chicken	Rivas-Cañedo et al. (2011)
	Roasted beef	Noleau & Toulemonde (1986)
	Water boiled & roasted duck	Min et al. (1979)
	Roasted duck	Wu & Liou (1992)
	Roasted pork	Chen et al. (2009) *cheesy, fatty*
	Grilled goat meat	Xie et al. (2008) *Green, roasted, sweet*
	Boiled, roasted, & grilled pork	Madruga et al. (2009)
		Mottram (1985)
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
	Cooked beef[7]	Machiels et al. (2003) *fruity, nutty*
2, 4-Pentanedione (**13**, tentative)	Roasted chicken	Noleau & Toulemonde (1986)
	Cooked beef[1]	Leo & Ames (1986)
3-Hydroxy-2-butanone (**11**) (Acetion)	*sous-vide* cooked lamb	Roldán et al. (2015)
	Steamed fish	Tao et al. (2014)
	High-pressure cooked beef	Rivas-Cañedo et al. (2011)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Roasted pork	Xie et al. (2008) *buttery, sour*
	Roasted duck	Chen et al. (2009) *buttery, sour*
	Smoked fish	Guillén & Errecalde (2002)
	Grilled goat meat	Madruga et al. (2009)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
Benzaldehyde **(21**, tentative**)**	Boiled mutton	Nixon et al. (1979)
	Chicken broth	Horvat (1976)
	Steamed fish	Tao et al. (2014) *bitter, almond, woody, burnt*
	High-pressure cooked beef	
	Roasted chicken	Rivas-Cañedo et al. (2011)
	Roasted beef	Noleau & Toulemonde (1986)
	Roasted pork	Min et al. (1979)
	Water boiled & roasted duck	Xie et al. (2008) *roasted, almond*
	Roasted duck	Wu & Liou (1992)
	Smoked fish	Chen et al. (2009) *almond*
	Boiled, roasted, and grilled pork	Guillén & Errecalde (2002)
		Mottram (1985)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked turkey[4]	Brunton et al. (2002)

Aldehydes & Ketones	Meat systems	References
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)

Table 3. Phenols.

Phenols	Meat systems	References
Phenol (**24**)	Steamed fish	Tao et al. (2014)
	High-pressure cooked beef	Rivas-Cañedo et al. (2011)
	Roasted chicken	Noleau & Toulemonde 1986)
	Roasted beef	Min et al. (1979)
	Smoked fish	Guillén & Errecalde (2002)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Canned beef stew[5]	Peterson et al. (1975)
2/3/4-Methylphenol (**25**) (o/m/p-Cresol)	Chicken broth	Gasser & Grosch (1990) *phenolic*
	Boiled beef	Kerscher & Grosch (1997)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Smoked fish	Guillén & Errecalde (2002)
	Grilled goat meat	Madruga et al. (2009)
2,4-Bis(1,1-dimethylethyl)-phenol (**27**)	Steamed fish	Tao et al. (2014) *paint*
	Smoked fish	Guillén & Errecalde (2002)

Table 4. Furans.

Furans	Meat systems	References
Furfural (**28**) (2-Furaldehyde)	Boiled mutton	Nixon et al. (1979)
	Roasted beef	Min et al. (1979)
	Roasted pork	Xie et al. (2008) *sweet, caramel-like*
	Roasted duck	Chen et al. (2009) *almond, pungent*
	Grilled goat meat	Madruga et al. (2009)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
2-Acetylfuran (**30**) (2-Furyl methyl ketone)	Boiled mutton	Nixon et al. (1979)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Grilled pork	Mottram (1985)
	Smoked fish	Guillén & Errecalde (2002)
	Shallow fried beef	Watanabe & Sato (1972)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
2-Furanmethanol (**29**) (Furfuryl alcohol)	Roasted chicken	Noleau & Toulemonde (1986)
	Water boiled & roasted duck	Wu & Liou (1992)
	Roasted duck	Chen et al. (2009) *burnt, mild*
	Roasted pork	Xie et al. (2008)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Canned beef stew[5]	Peterson et al. (1975)

Table 5. Sulfur-containing VOCs.

Furanthiols & Thiophenes	Meat systems	References
Tetrahydro-2/3-methylthiophene **(32)**	Boiled beef	Garbusoy et al. (1976)[a]
2/3-Methylthiophene (**33**)	Chicken broth	Gasser & Grosch (1990) *sulfurous*
	Chicken broth	Horvat (1976)
	Boiled chicken	Nonaka et al. (1967)
	Boiled mutton	Nixon et al. (1979)
	Pressure-cooked beef	Wilson et al. (1973)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Grilled goat meat	Madruga et al. (2009)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
Mercaptoacetone (**34**) (Mercaptopropanone)	Canned pork[6]	Uchman & Jennings (1977)
2-Methyl-3-furanthiol (**35**)	Boiled beef	Gasser & Grosch (1988) *meat-like, sweet, sulfurous*
	Chicken broth	Gasser & Grosch (1990) *meat-like, sweet*
	Boiled beef	Kerscher & Grosch (1997)
	Boiled beef, pork, lamb, & chicken	Kerscher & Grosch (1998)
	Steam distillate of canned tuna	Withycombe & Mussinan (1988) *intense, pleasant, "beef extract" aroma*
	Pressure-cooked chicken	Farkaš et al. (1997) *meaty, sweet*
	Roasted beef	Min et al. (1979)
	Roasted duck	Chen et al. (2009) *meaty, vitamin*
2-Furfurylthiol (**36**)	Chicken broth	Gasser & Grosch (1990) *roasted*
	Boiled beef	Kerscher & Grosch (1997)
	Boiled beef, pork, lamb, & chicken	Kerscher & Grosch (1998)
	Pressure-cooked chicken	Farkaš et al. (1997) *roasted, coffee*
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted beef	Min et al. (1979)
	Roasted duck	Chen et al. (2009) *roasted, coffee-like*
	Grilled goat meat	Madruga et al. (2009)
	Grilled pork	Mottram (1985)
	Cooked salmon[3]	Methven et al. (2007)
Dihydro-3(2H)-thiophenone (**37**)	Pressure-cooked beef	Wilson et al. (1973)
Dihydro-2-methyl-3(2H)-thiophenone (**39**)	Pressure-cooked beef	Wilson et al. (1973)
	Grilled pork	Mottram (1985)
	Cooked salmon[3]	Methven et al. (2007)
2/3-Thiophenecarboxaldehyde (**40**) (2/3-Formylthiophene)	Chicken broth	Gasser & Grosch (1990) *sulfurous*
	Boiled mutton	Nixon et al. (1979)
	Pressure-cooked beef	Wilson et al. (1973)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
2/3-Thiophenemethanol (**44**)	Pressure-cooked beef	Wilson et al. (1973)

Cyclic polysulfides	**Meat systems**	**References**
Lenthionine (**50**)	Cooked ham	Thomas et al. (2014)
	Boiled mutton	Nixon et al. (1979)
Hexathiepane (**51**)	Boiled mutton	Nixon et al. (1979)
Thiazoles	**Meat systems**	**References**
Thiazole (**31**)	Pressure-cooked beef	Wilson et al. (1973)
	Roasted chicken	Noleau & Toulemonde (1986)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
2-Acetylthiazole (**41**)	Steamed fish	Tao et al. (2014) *meaty, roasted, nutty, sulfurous*
	Boiled beef	Gasser & Grosch (1988)
	Chicken broth	Gasser & Grosch (1990) *roasted*
	Pressure-cooked beef	Wilson et al. (1973)
	Water boiled & roasted duck	Wu & Liou (1992)
	Roasted duck	Chen et al. (2009) *popcorn*
	Roasted pork	Xie et al. (2008)
	Grilled goat meat	Madruga et al. (2009)
	Boiled, roasted, & grilled pork	Mottram (1985)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
	Cooked beef[7]	Machiels et al. (2003) *burnt, onion*
Benzothiazole **(47**)	Boiled beef	Gasser & Grosch (1988) *pyridine-like, metallic*
	Boiled, roasted, & grilled pork	Mottram (1985)
	Pressure-cooked beef	Wilson et al. (1973)
	Roasted pork	Xie et al. (2008)
	Roasted duck	Chen et al. (2009) *rubbery, musty*
	Grilled goat meat	Madruga et al. (2009)
	Shallow-fried beef	Watanabe & Sato (1972)
	Canned beef stew[5]	Peterson et al. (1975)
	Cooked beef[7]	Machiels et al. (2003) *burnt, meaty*

Table 6. Pyrazines & Pyridines.

Pyrazines & Pyridines	Meat systems	References
Pyrazine (**53**)	Steamed fish	Tao et al. (2014)
	Pressure-cooked beef	Mussinan et al. (1973)
	Grilled goat meat	Madruga et al. (2009)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
2-Methylpyrazine (**56**)	Chicken broth	Horvat (1976)
	Steamed fish	Tao et al., (2014)
	Pressure-cooked beef	Mussinan et al. (1973)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted pork	Xie et al. (2008) *popcorn*
	Roasted duck	Chen et al. (2009) *popcorn, roasted*
	Grilled goat meat	Madruga et al. (2009)
	Boiled, roasted, and grilled pork	Mottram (1995)
	Shallow-fried beef	Watanabe & Sato (1971)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
2, 5/6-Dimethylpyrazine (**58**)	Pressure-cooked beef	Mussinan et al. (1973)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted duck	Wu & Liou (1992)
	Roasted pork	Xie et al. (2008) *popcorn, roasted*
	Roasted duck	Chen et al. (2009) *roasted*
	Grilled goat meat	Mottram (1995)
	Boiled, roasted, and grilled pork	Madruga et al. (2009)
	Shallow-fried beef	Watanabe & Sato (1971)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)
2-Ethyl-3/5/6-methylpyrazine (**61**)	Pressure-cooked beef	Mussinan et al. (1973)
	Roasted chicken	Noleau & Toulemonde (1986)
	Roasted duck	Wu & Liou (1992)
	Grilled pork	Mottram (1995)
	Grilled goat meat	Madruga et al. (2009)
	Shallow-fried beef	Watanabe & Sato (1971)
Pyridine (**54**)	Roasted chicken	Noleau & Toulemonde (1986)
	Grilled goat meat	Madruga et al. (2009)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked pork[2]	Estevez et al. (2003)
	Cooked salmon[3]	Methven et al. (2007)
	Canned beef stew[5]	Peterson et al. (1975)

Pyrazines & Pyridines	Meat systems	References
2/3/4-Methylpyridine (**55**)	Roasted chicken	Noleau & Toulemonde (1986)
	Grilled goat meat	Madruga et al. (2009)
	Grilled pork	Mottram (1995)
	Fried chicken	Tang et al. (1983)
	Cooked beef[1]	Leo & Ames (1986)
2/3/4-Ethylpyridine (**57**)	Roasted chicken	Noleau & Toulemonde (1986)
	Grilled pork	Mottram (1985)
	Shallow-fried beef	Watanabe & Sato (1971)
	Fried chicken	Tang et al., (1983)
	Cooked beef[1]	Leo & Ames (1986)
	Cooked salmon[3]	Methven et al. (2007)
2, 3/2, 4/2, 5/2, 6/3, 4/3, 5-Dimethylpyridine (**59**)	Roasted chicken	Noleau & Toulemonde (1986)
	Grilled pork	Mottram (1995)

Note: References were arranged in order based on the severity of thermal treatments from low heat to high heat; references belong to the same type of treatments were not in order; references with superscripts were placed at the end.

[a] **Garbusoy et al. (1976):** based on the review article of Shibamoto (1980).

Cooking methods:

[1] Cooked at a surface temperature of 104℃ for 2 min followed by 171℃ for 6 min.

[2] Cooked to an internal temperature of 80℃ for 10 min.

[3] Cooked at 125℃ for 40 min.

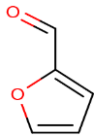
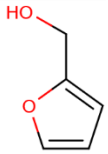
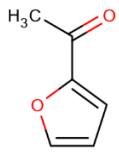
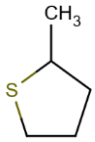
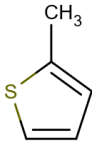
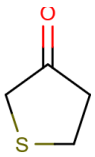
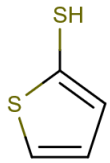
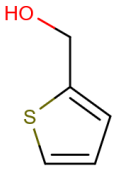
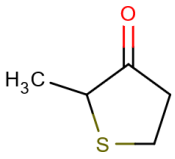
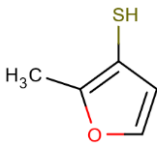
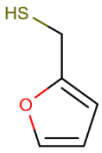
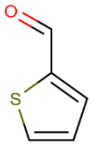
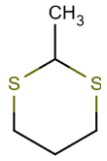
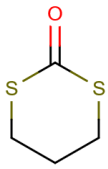
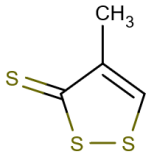
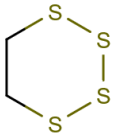
[4] Cooked at 190℃ for 20 min to an internal temperature of 85℃.

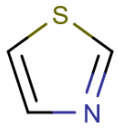
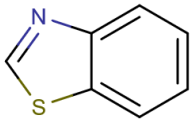
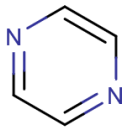
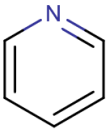
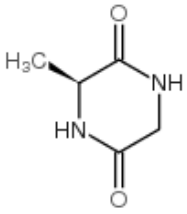
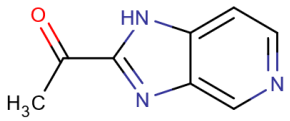
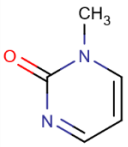
[5] Cooked at 121℃ for 75 min.

[6] Steamed-pressure heating at 121℃ for 20 min.

[7] Cooked at 150℃ in an oil bath for 25 min

Table 7. Chemical structures of some of the heterocyclic compounds (tentatively) identified in the peptide model systems in this Thesis.

Furfural (28)	2-Furanmethanol (29)	2-Acetylfuran (30)
		
tetrahydro-2-methylthiophene (32)	**2-methylthiophene (33)**	**Dihydro-3(2H)-thiophenone (37, tentative)**
		
2-thiophenethiol (38)	**2-thiophenemethanol (44, tentative)**	**Dihydro-2-methyl-3(2H)-thiophenone (39, tentative)**
		
2-Methyl-3-furanthiol (35)	**2-Furfurylthiol (36)**	**2-thiophenecarboxaldehyde (40)**
		
2-methyl-1,3-dithiane (43)	**1, 3-Dithian-2-one (45)**	**4-methyl-3H-1,2-Dithiole-3-thione (46)**
		
[1,2,3,4]Tetrathiane (48)	**Lenthionine (50)**	**Hexathiepane (51)**
		

Thiazole (31)	2-Acetylthiazole (41)	Benzothiazole (47)
		
Pyrazine (53)	**Pyridine (54)**	**3-methylpiperazine-2,5-dione (63, tentative)**
		
2-acetylpyrido[3,4-d]imidazole (65)	**1-methyl-2(1H)-pyrimidinone (60)**	
		

APPENDIX II.

Mass Spectra of Selected VOCs Identified in the Peptide Model Systems and the Related References in GC-MS Analysis.

Figure 1a. Compound 21 and Phenylglyoxal reference.

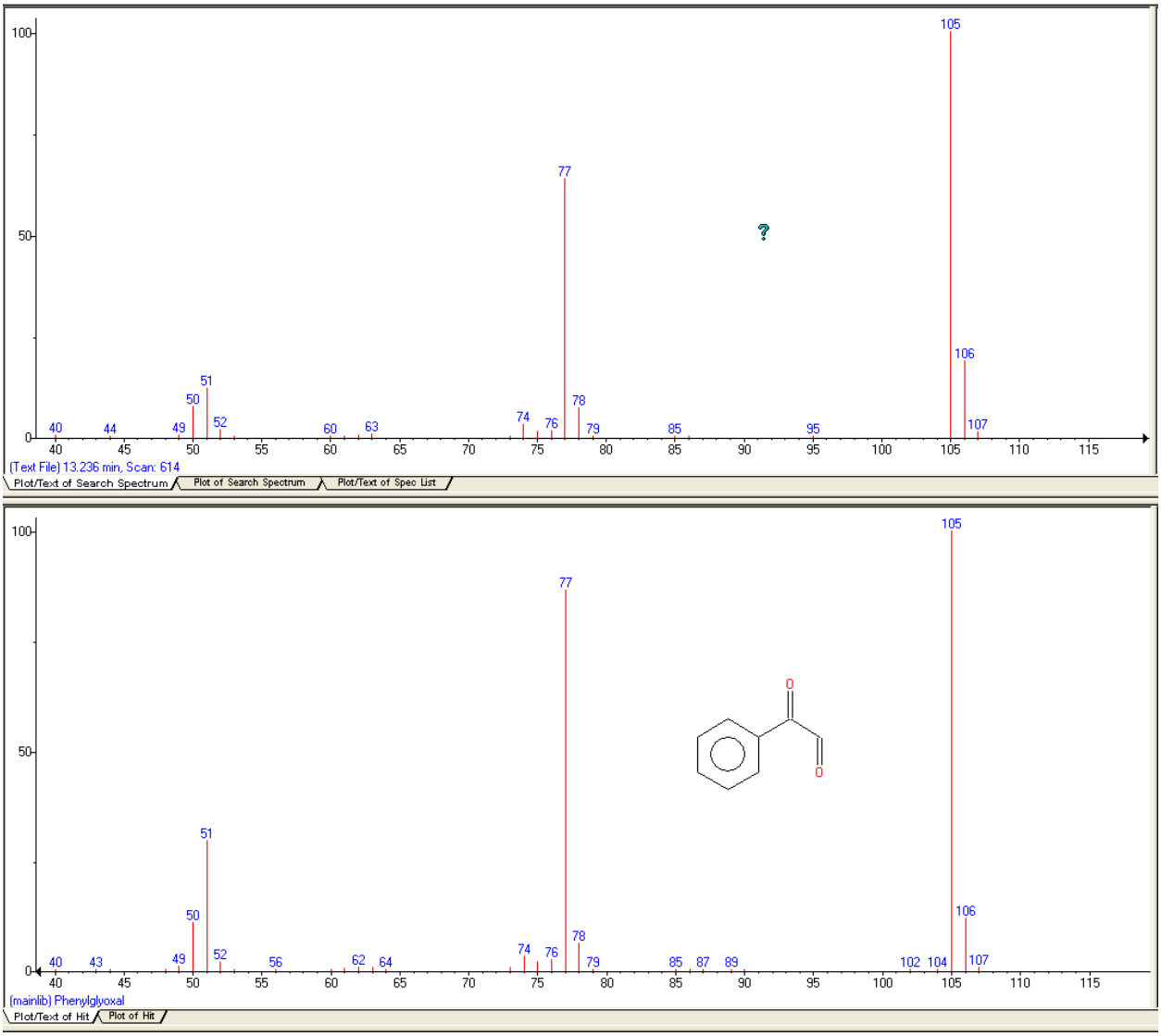


Figure 1b. Compound 21 and Benzaldehyde reference.

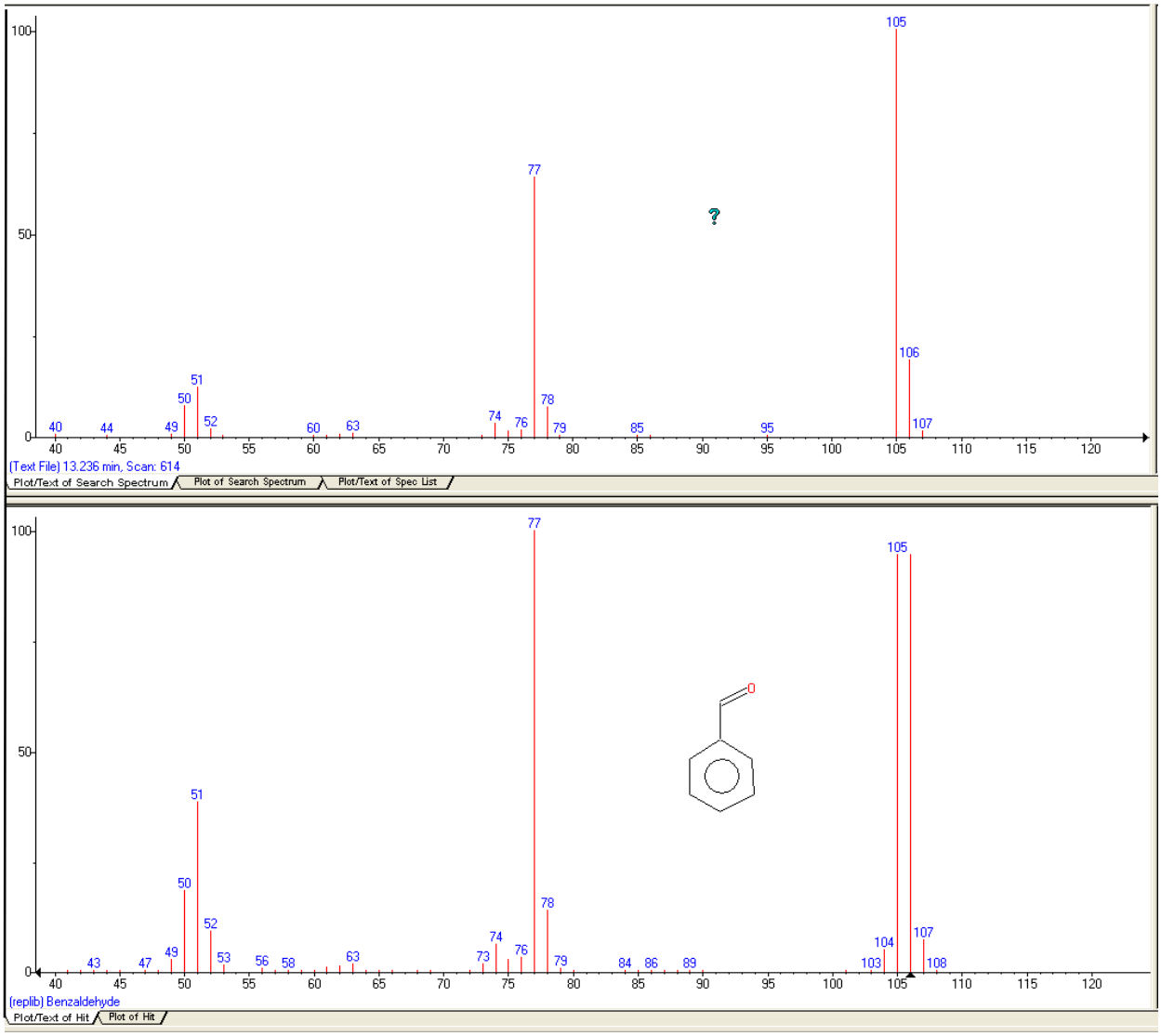


Figure 2. Compound 32 and Tetrahydro-2-methylthiophene.

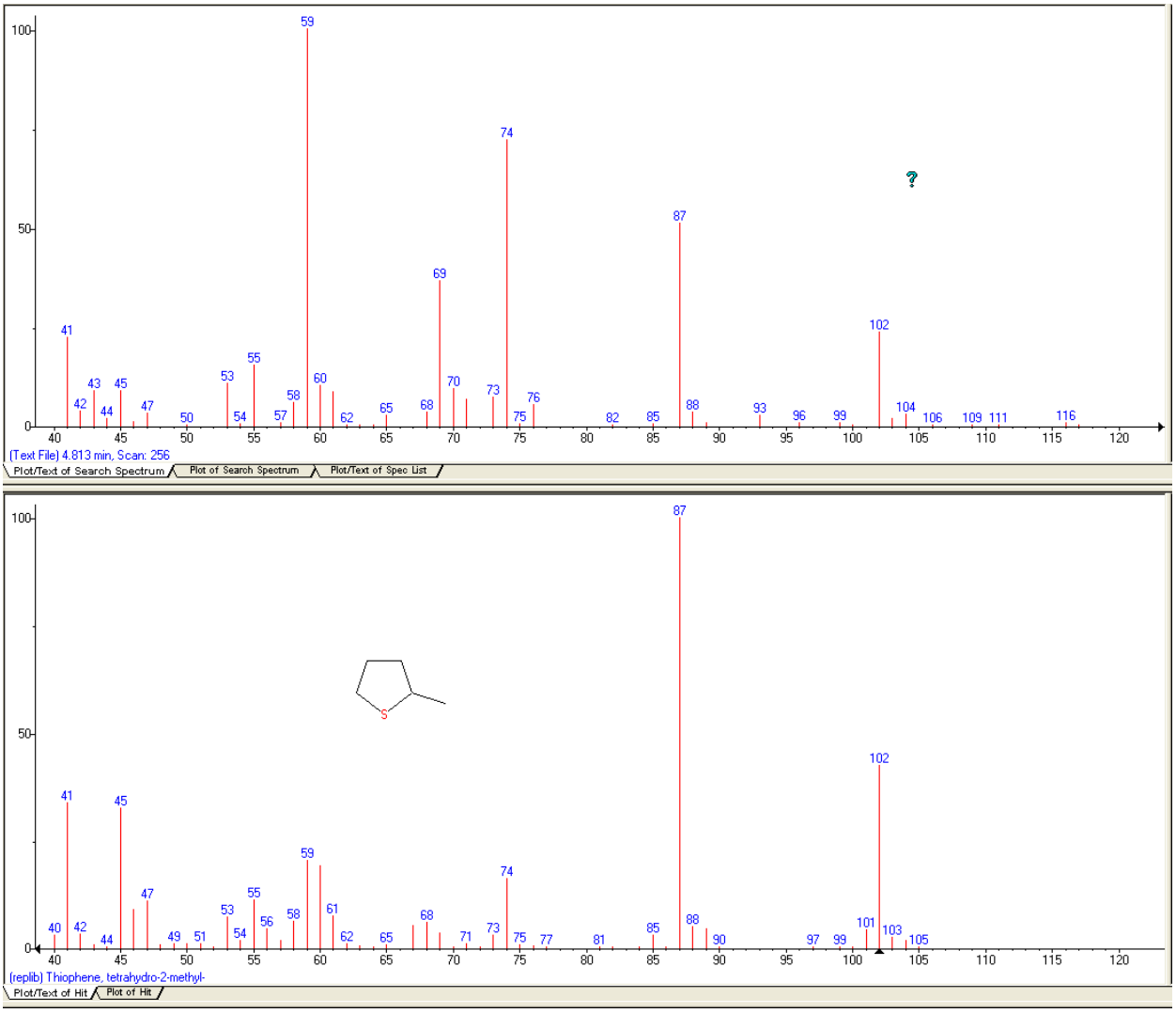


Figure 3. Compound 34 and Mercaptoacetone.

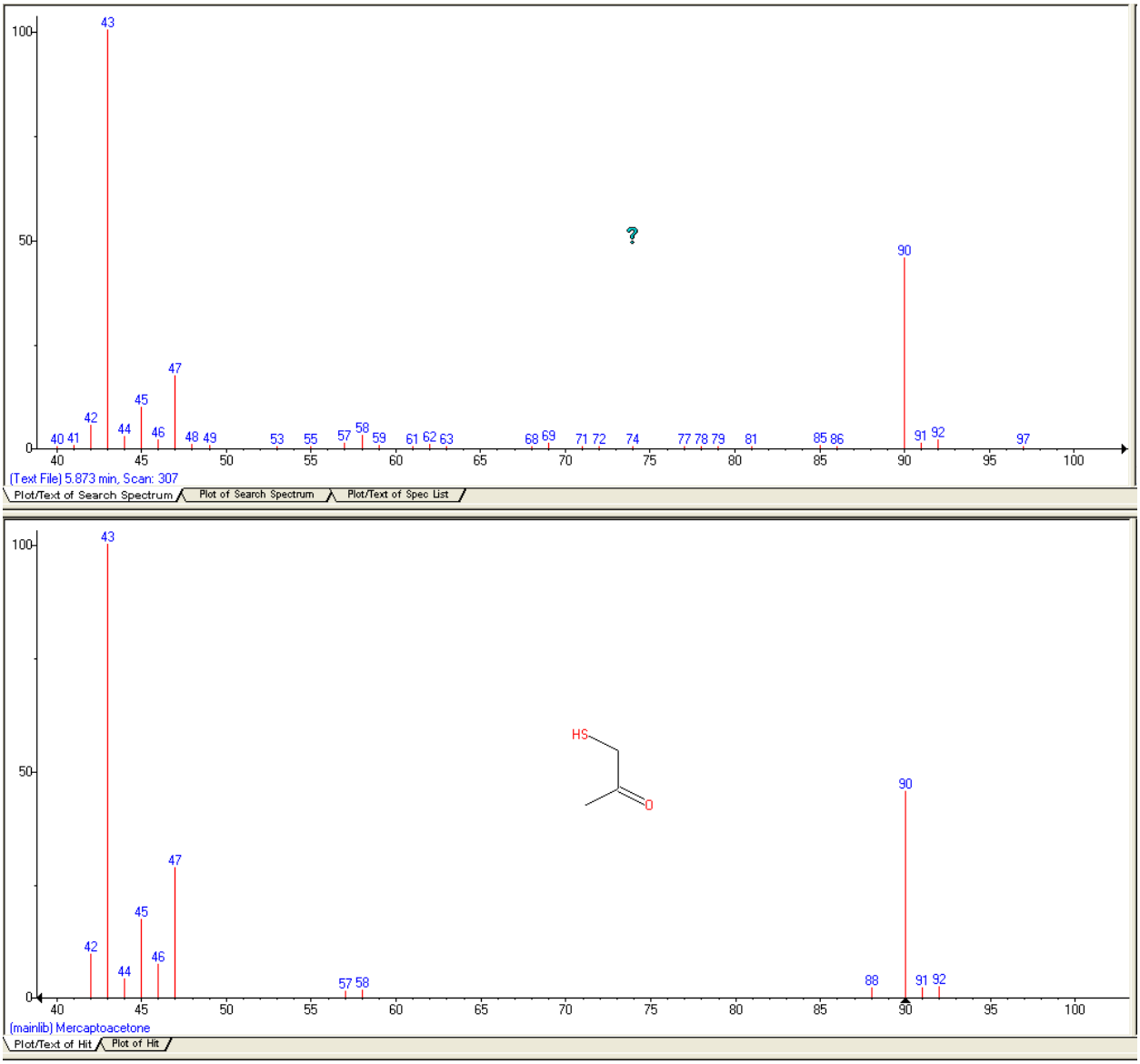


Figure 4. Compound 43 and 2-Methyl-1, 3-dithiane.

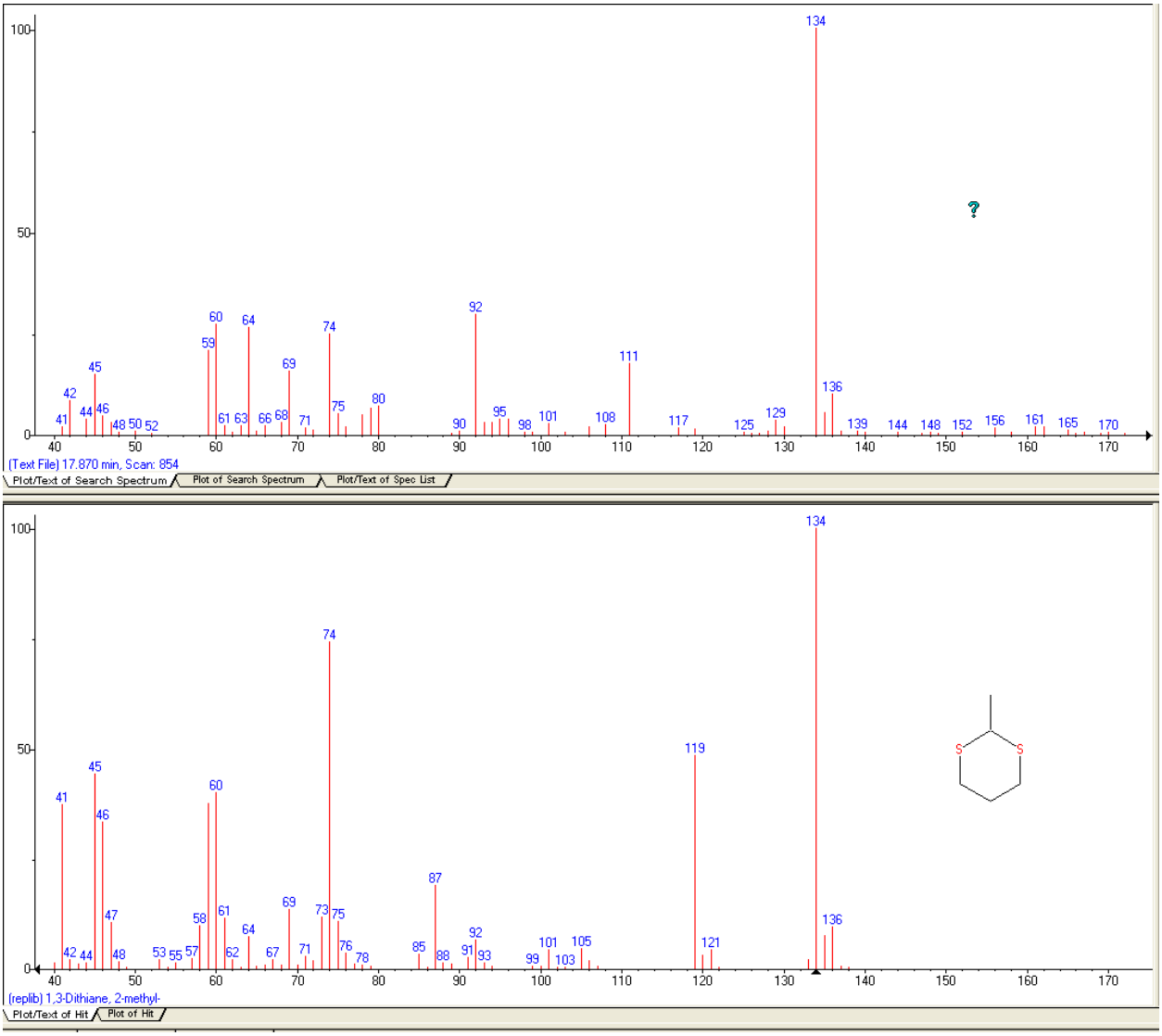


Figure 5. Compound 45 and 1, 3-Dithian-2-one.

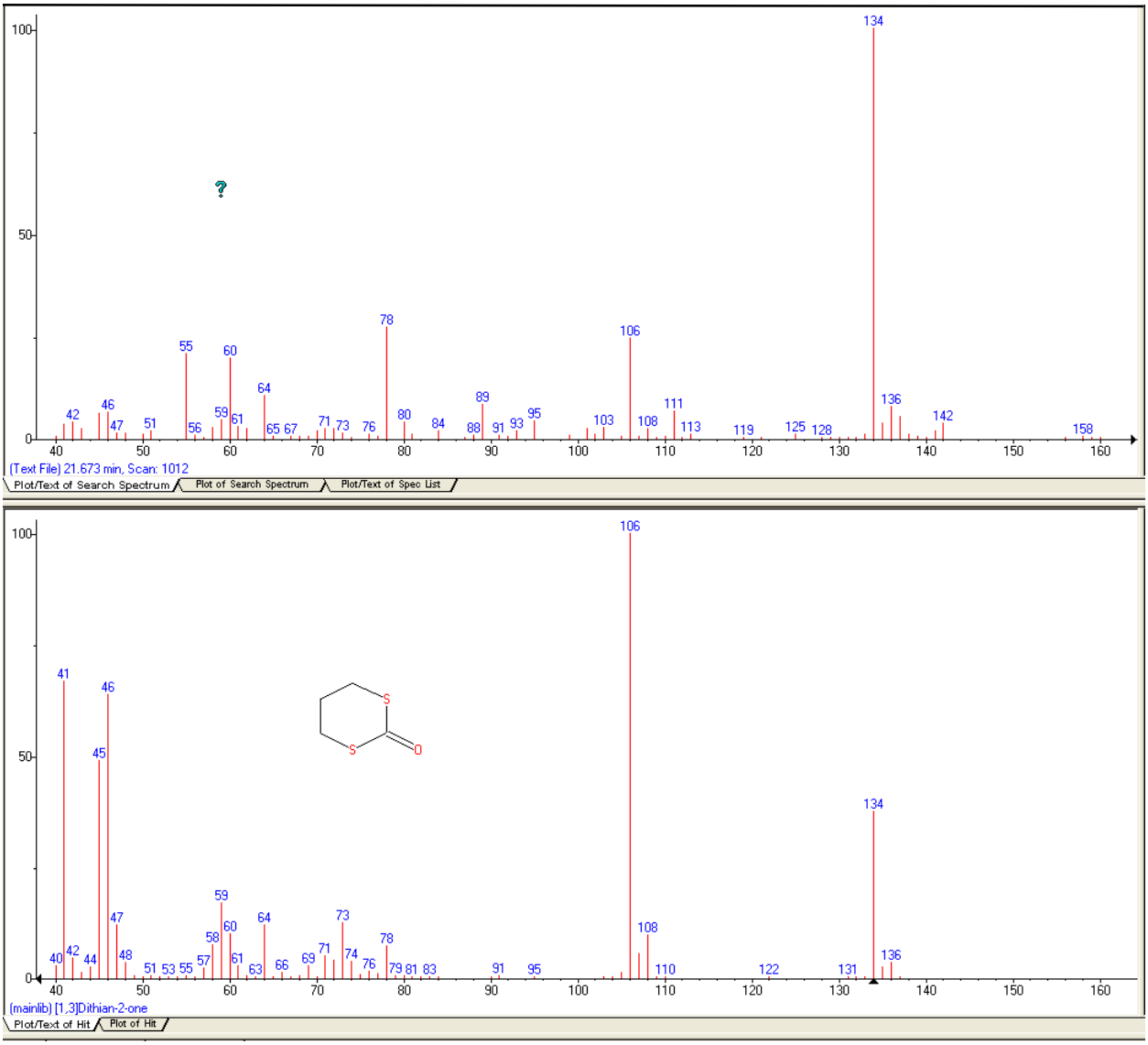


Figure 6. Compound 46 and 4-Methyl-3H-1, 2-dithiole-3-thione.

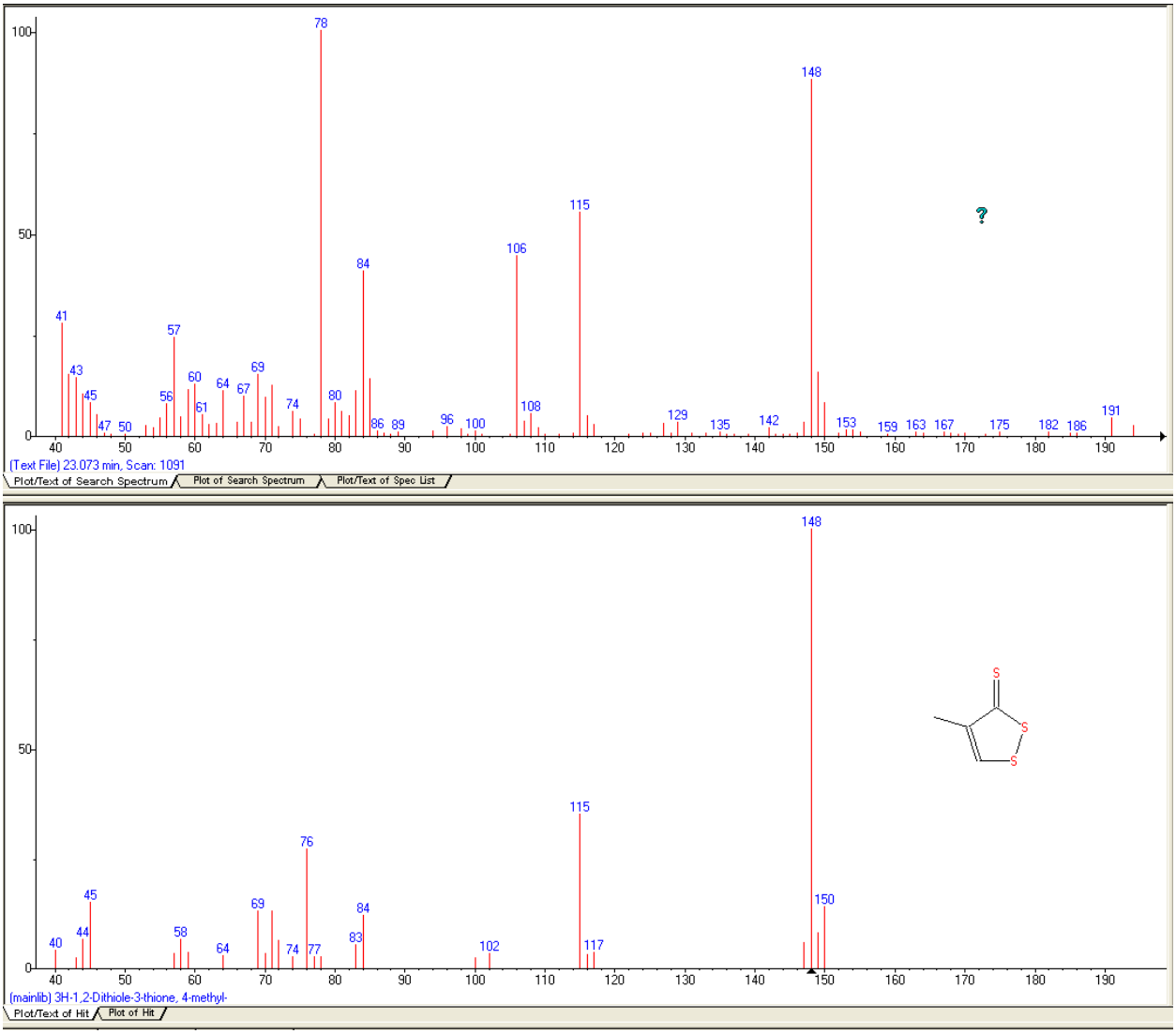


Figure 7. Compound 48 and [1, 2, 3, 4] Tetrathiane.

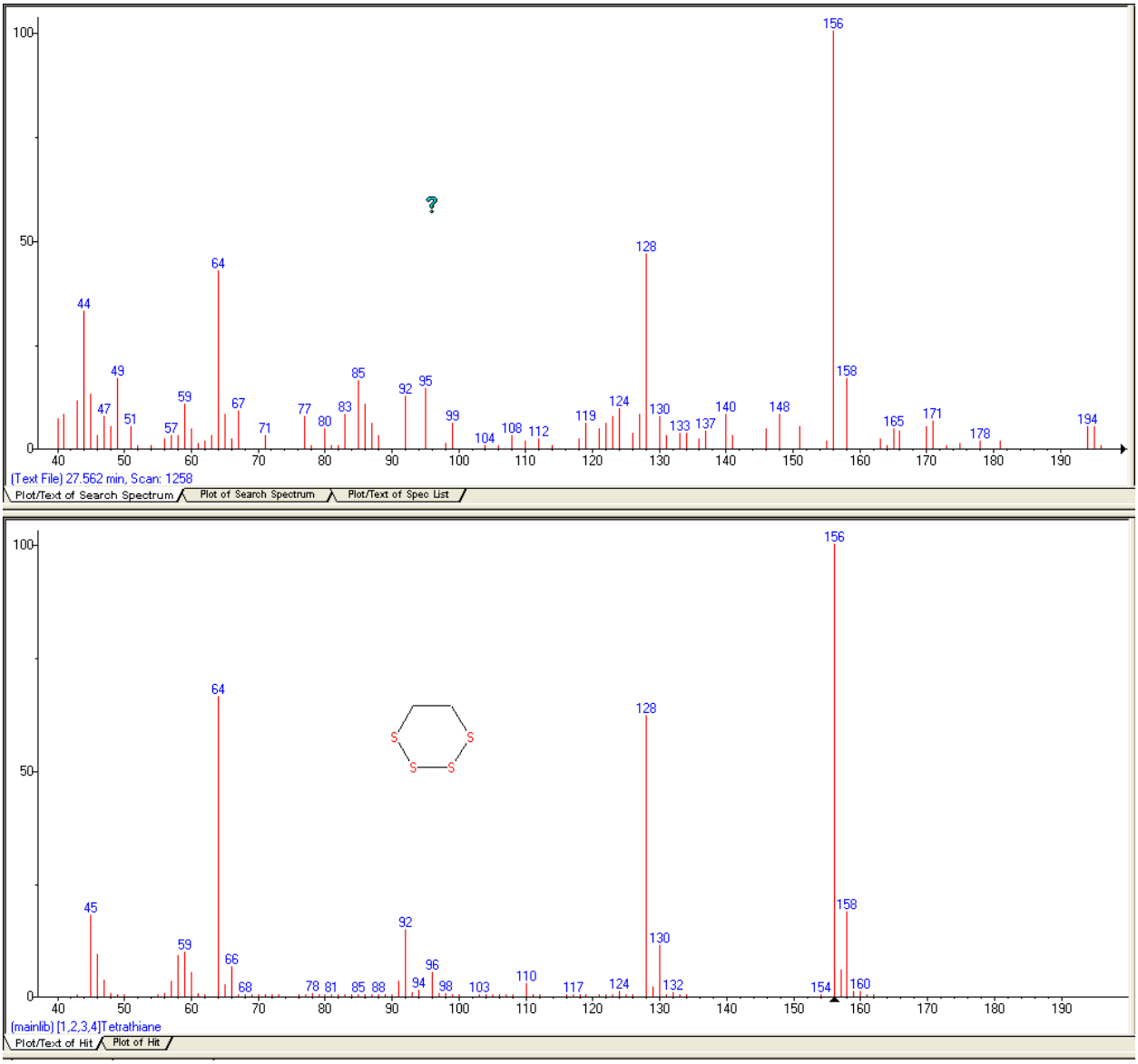


Figure 8. Compound 50 and Lenthionine.

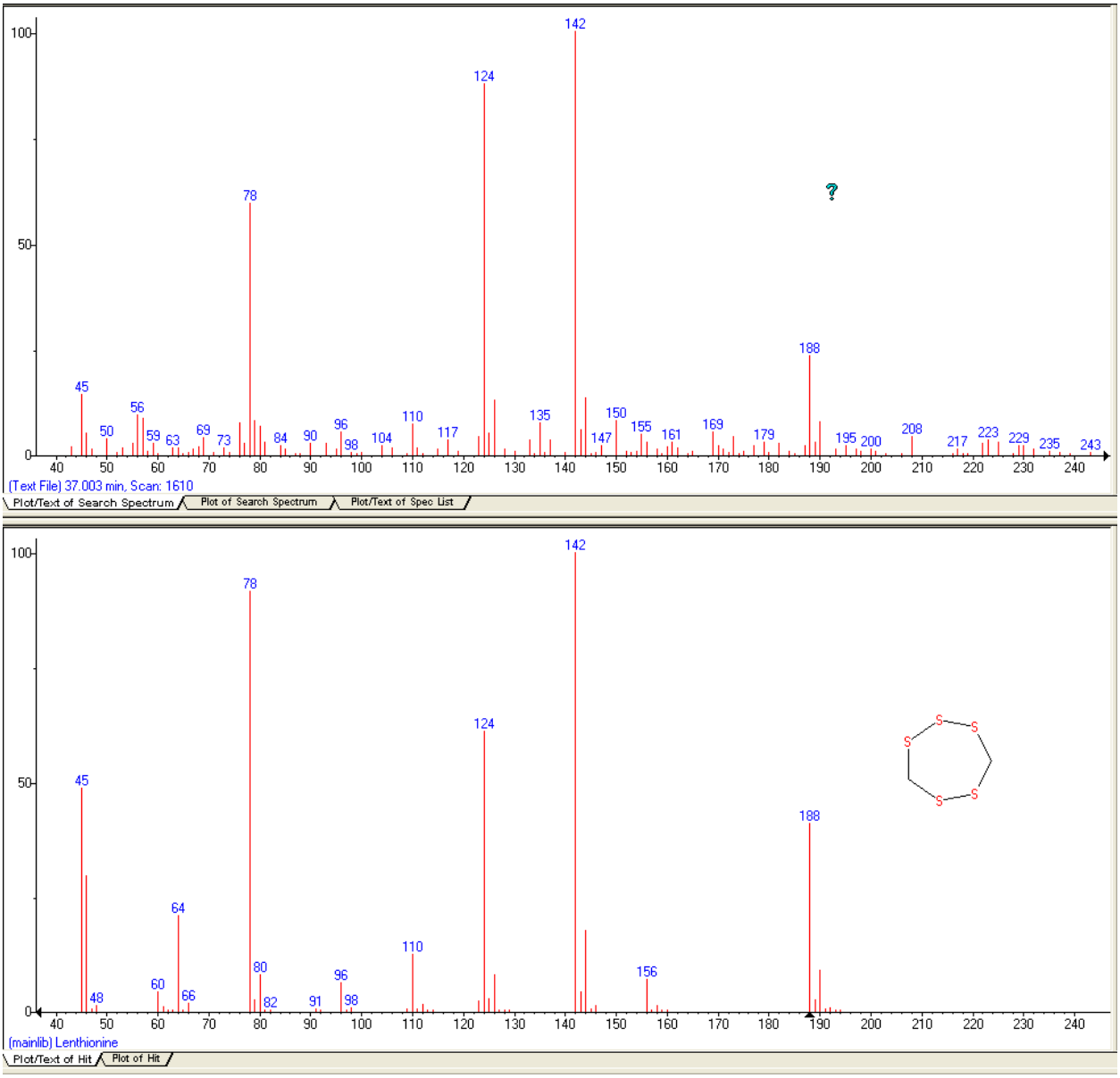


Figure 9. Compound 51 and Hexathiepane.

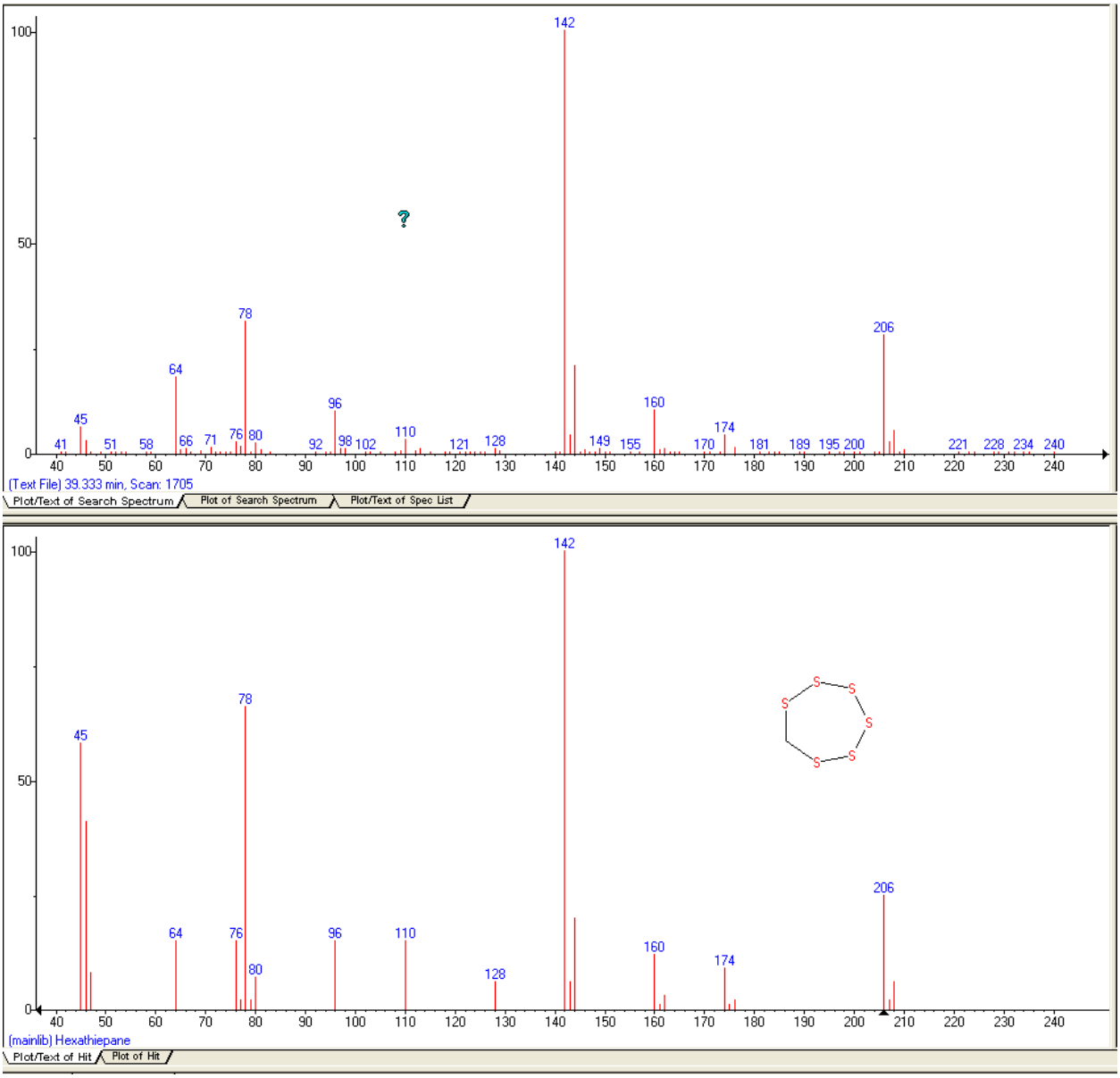


Figure 10. Compound 60 and 6-Methyl-4(1H)-pyrimidinone.

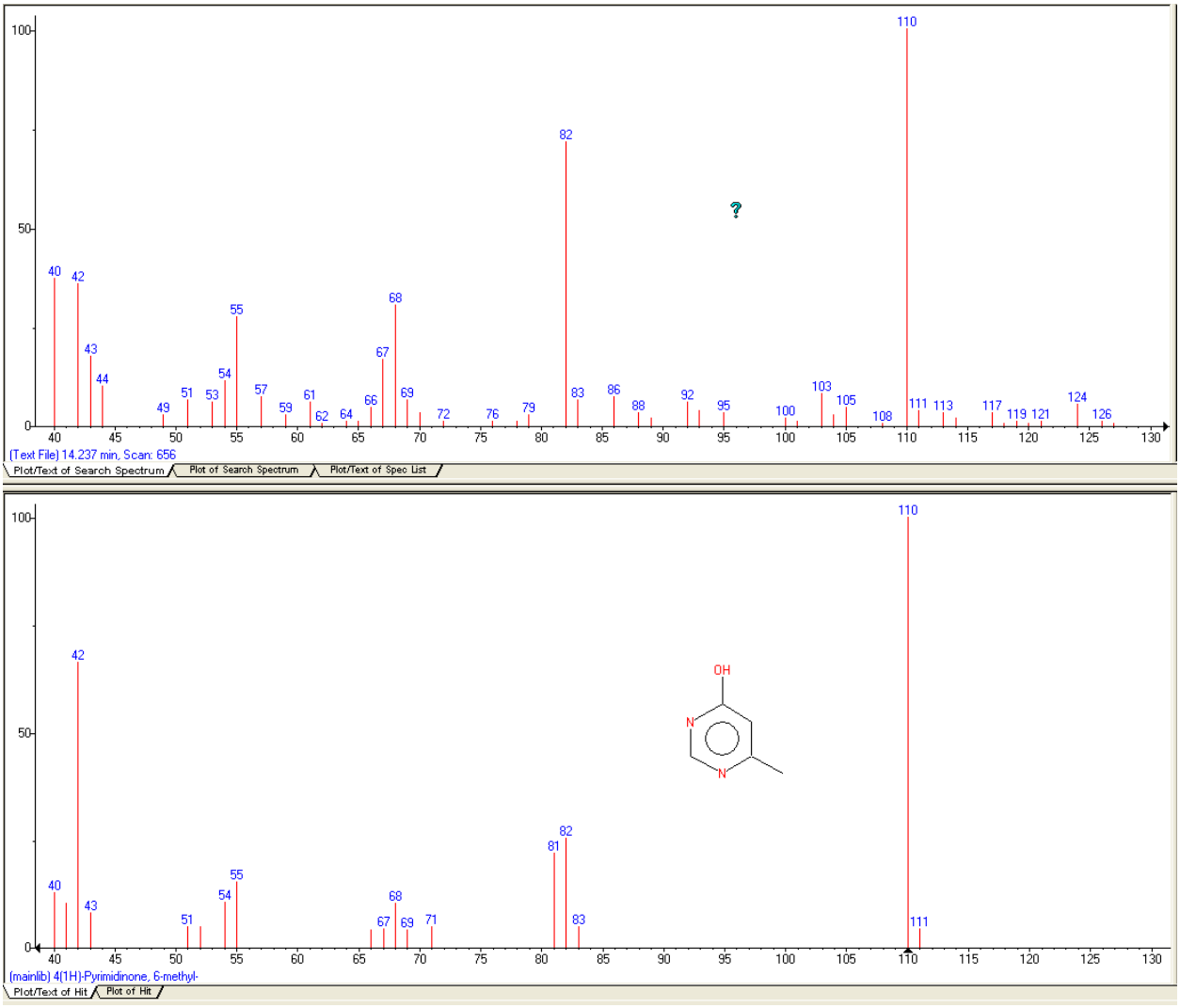


Figure 11. Compound 63 and 3-Methyl-2, 5-piperazinedione.

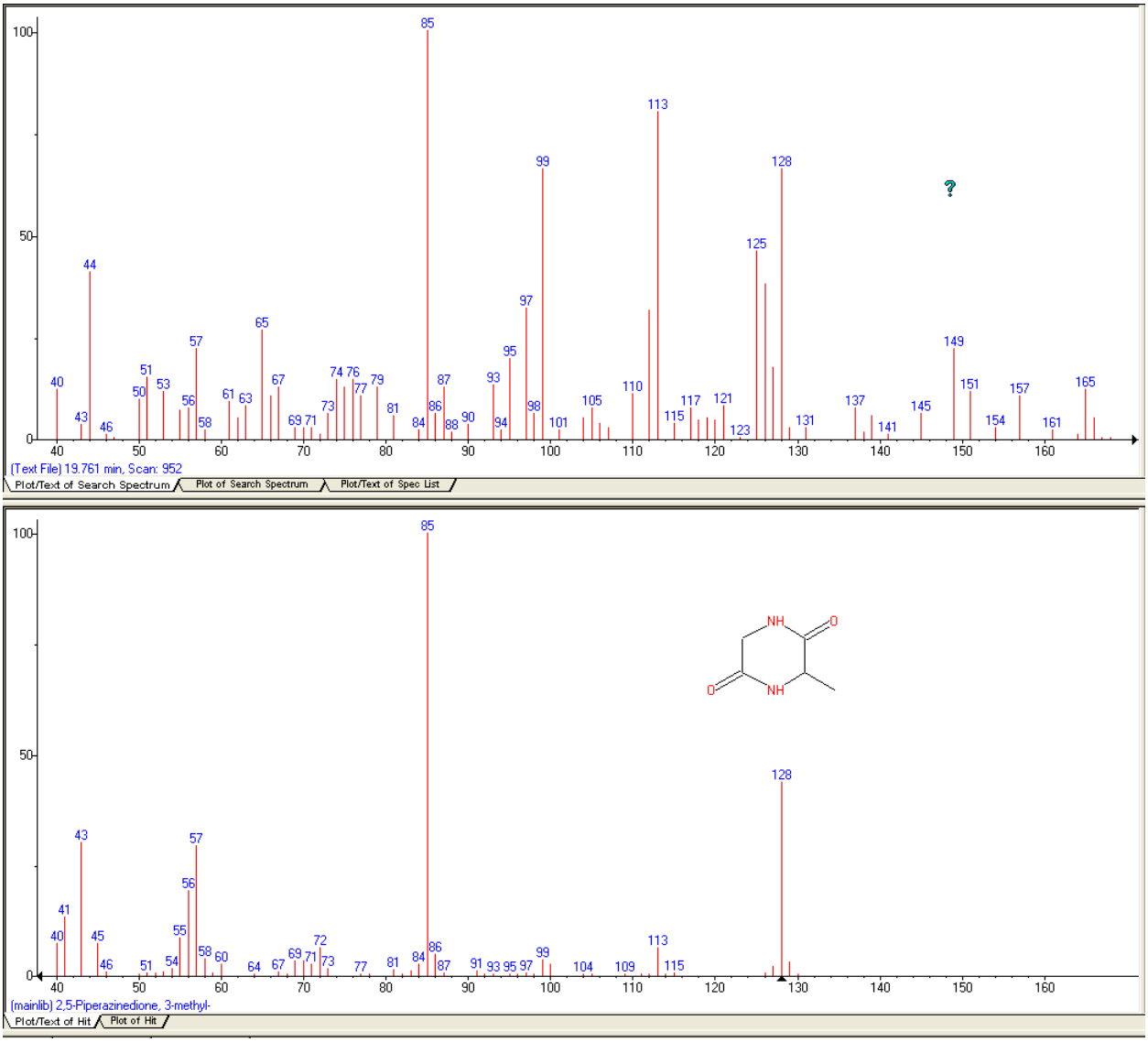
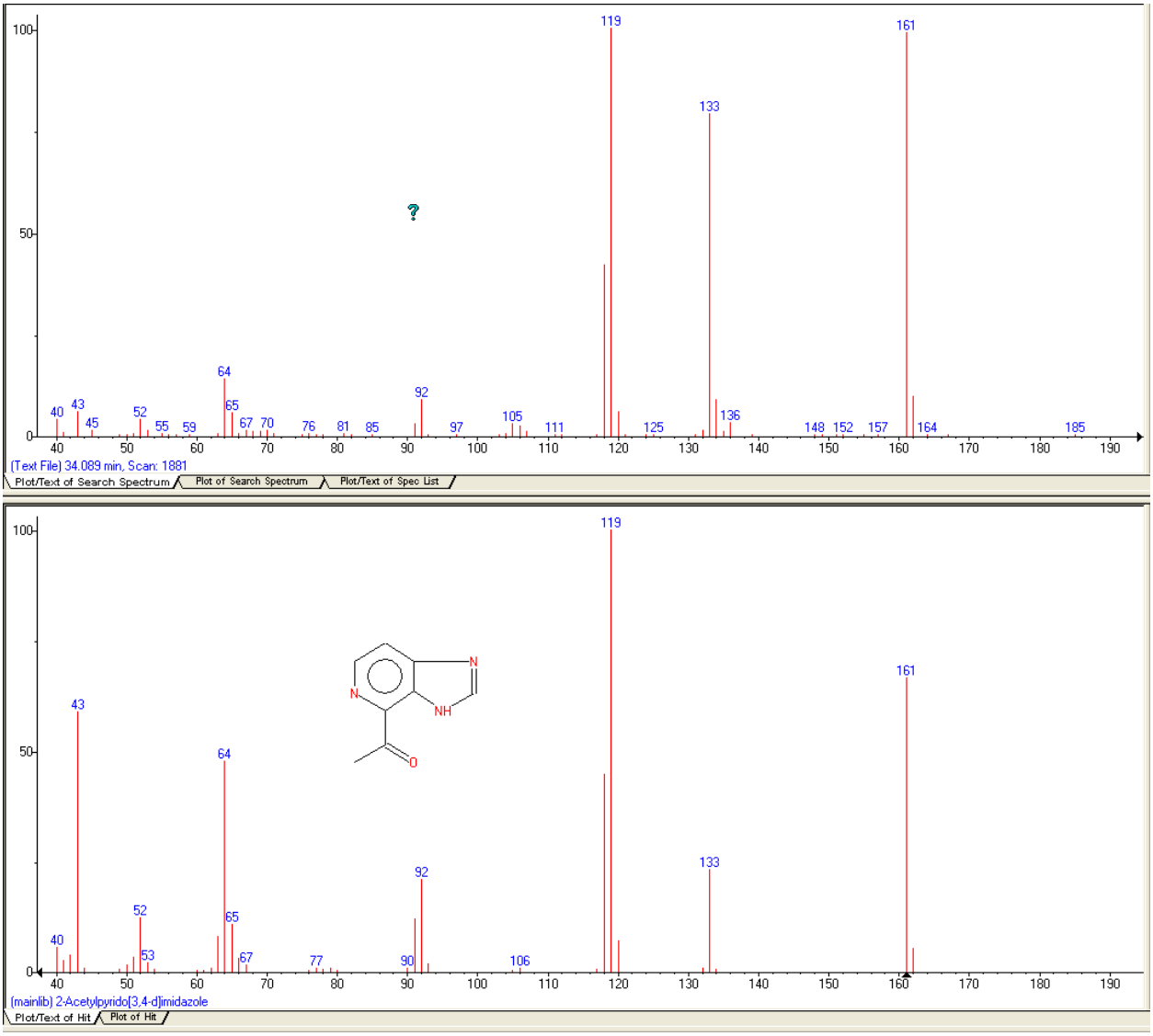


Figure 12. Compound 65 and 2-Acetylpyrido [3, 4-*d*] imidazole.



APPENDIX III.

The Average Concentrations of Selected VOCs Identified in the Peptide Model Systems.

Chart 1. Compound 11.

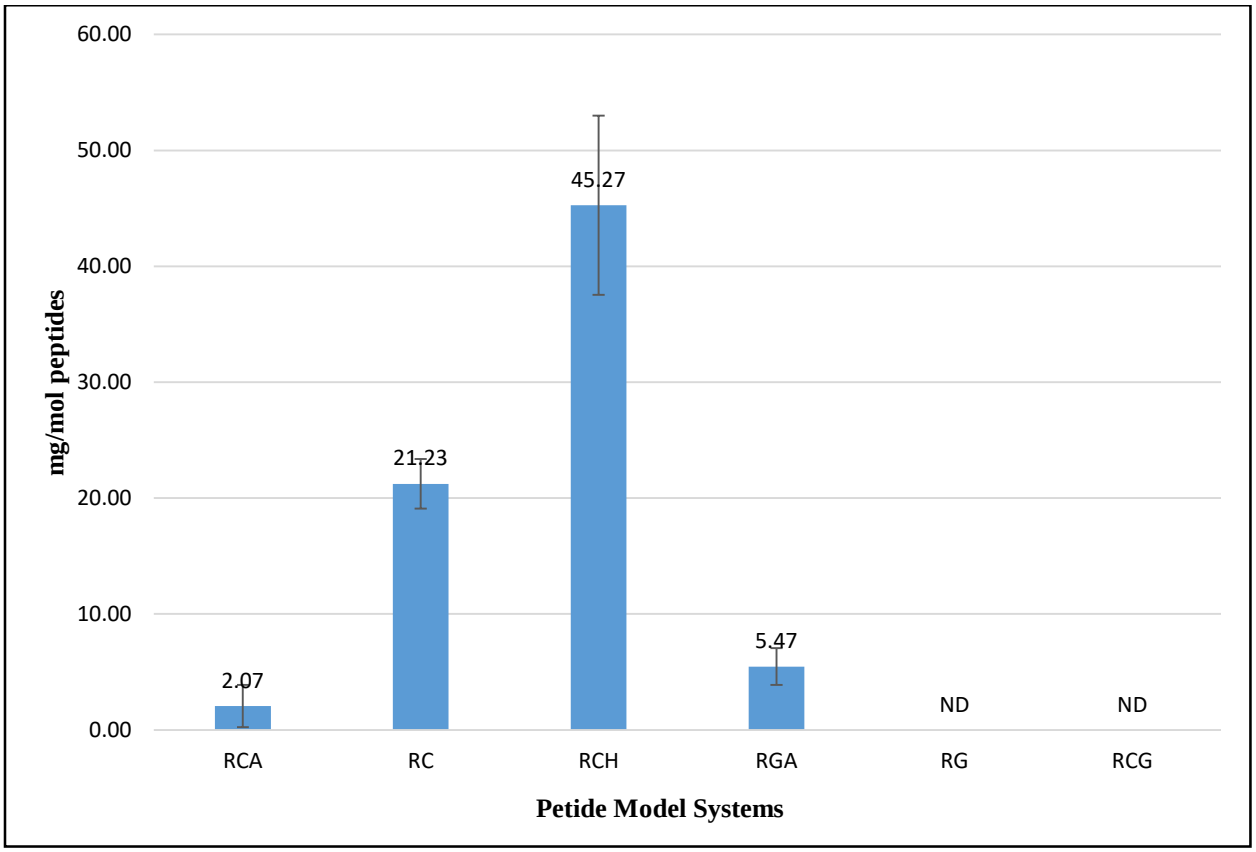


Chart 2. Compound 20.

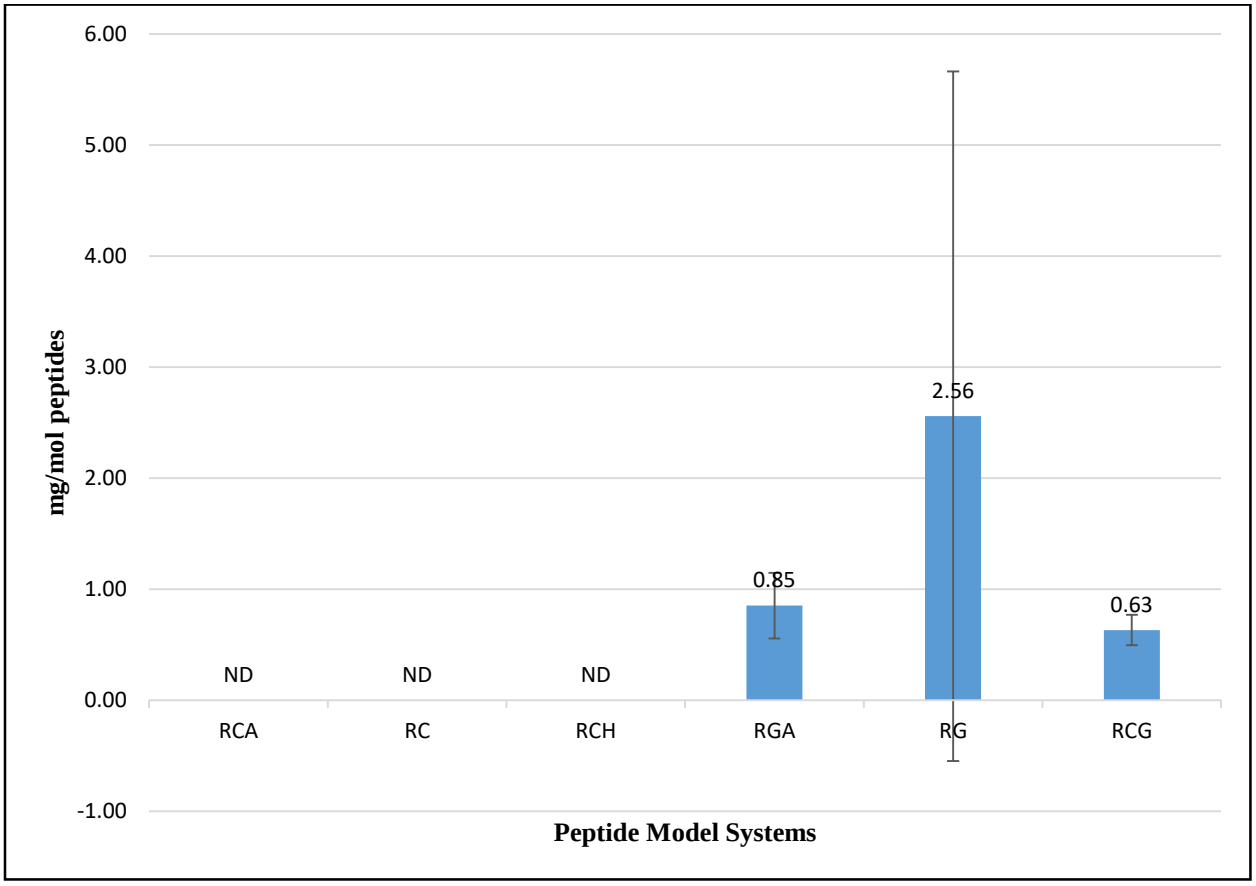


Chart 3. Compound 21.

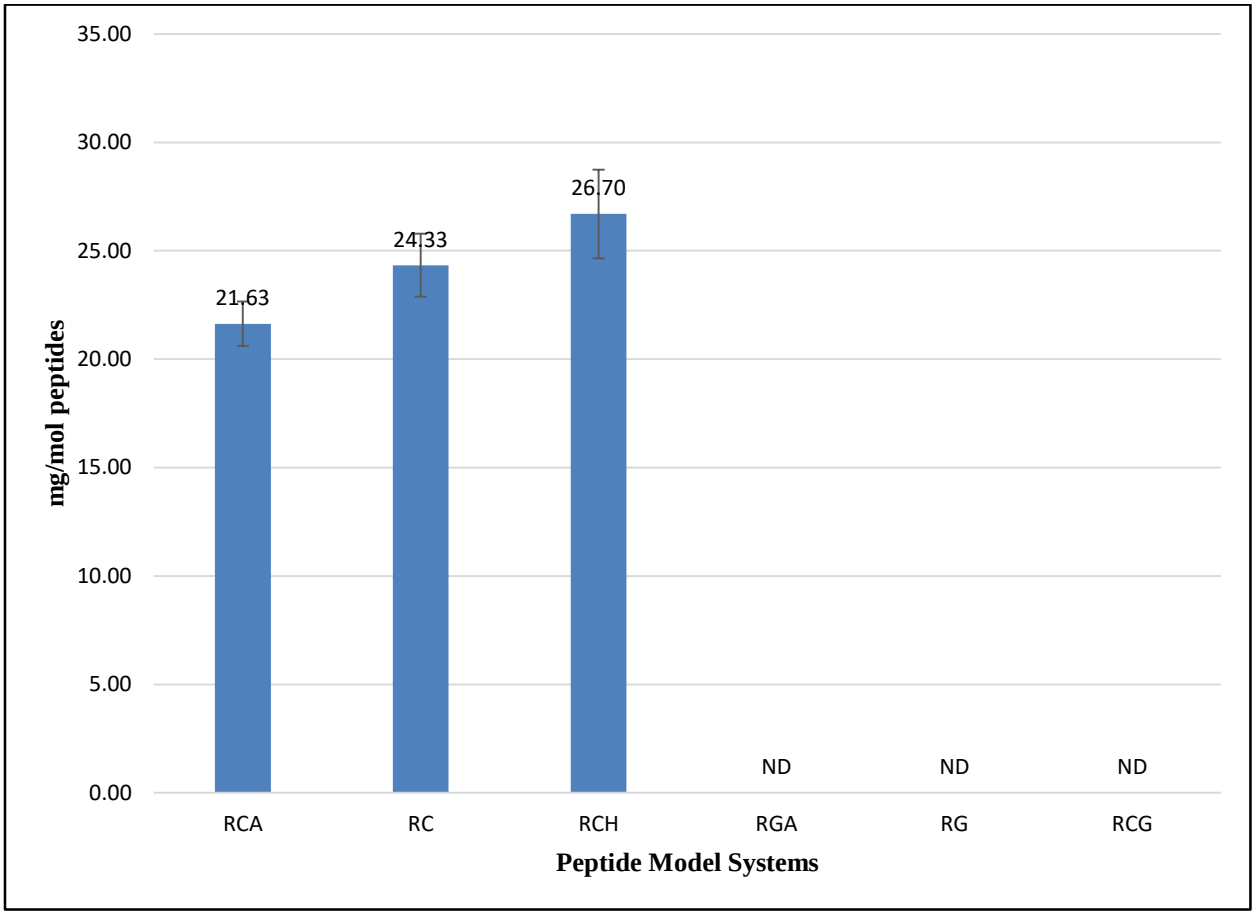


Chart 4. Compound 28.

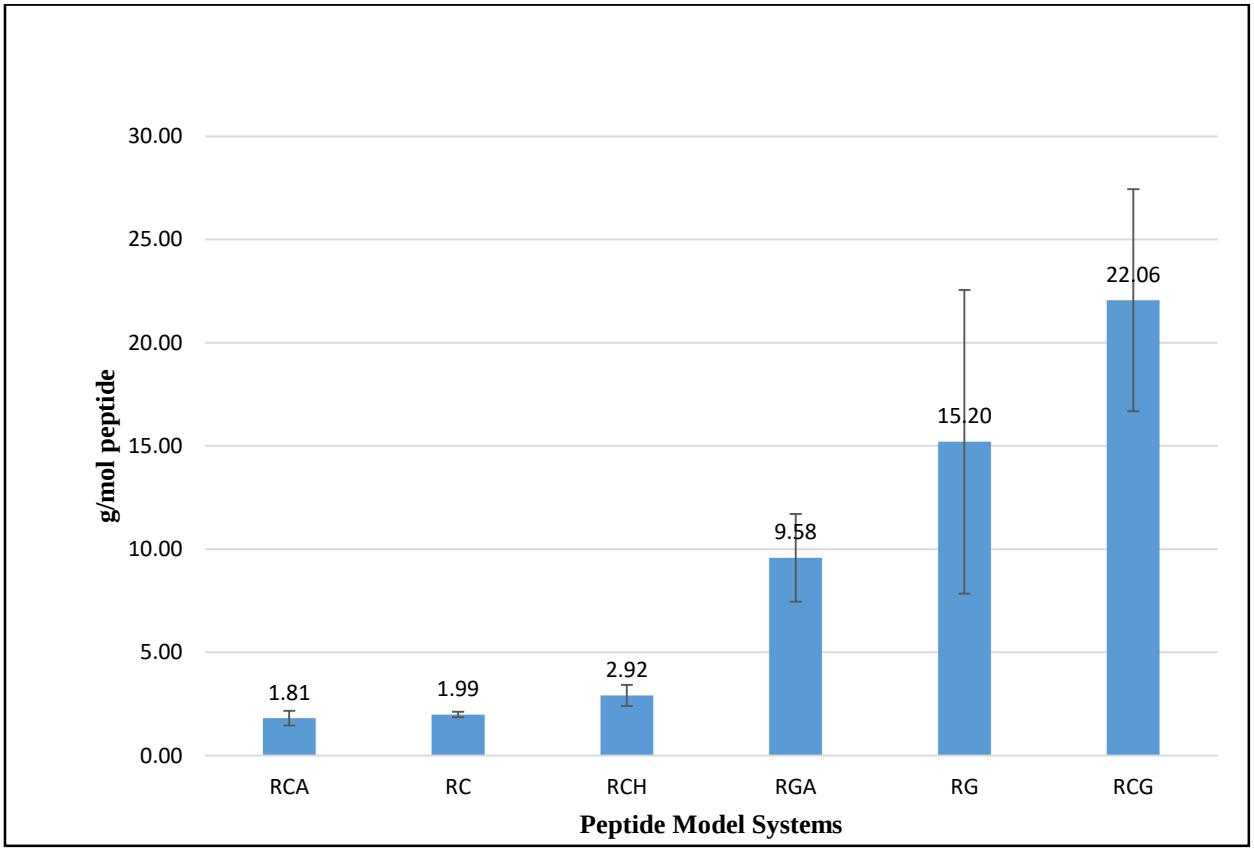


Chart 5. Compound 30.

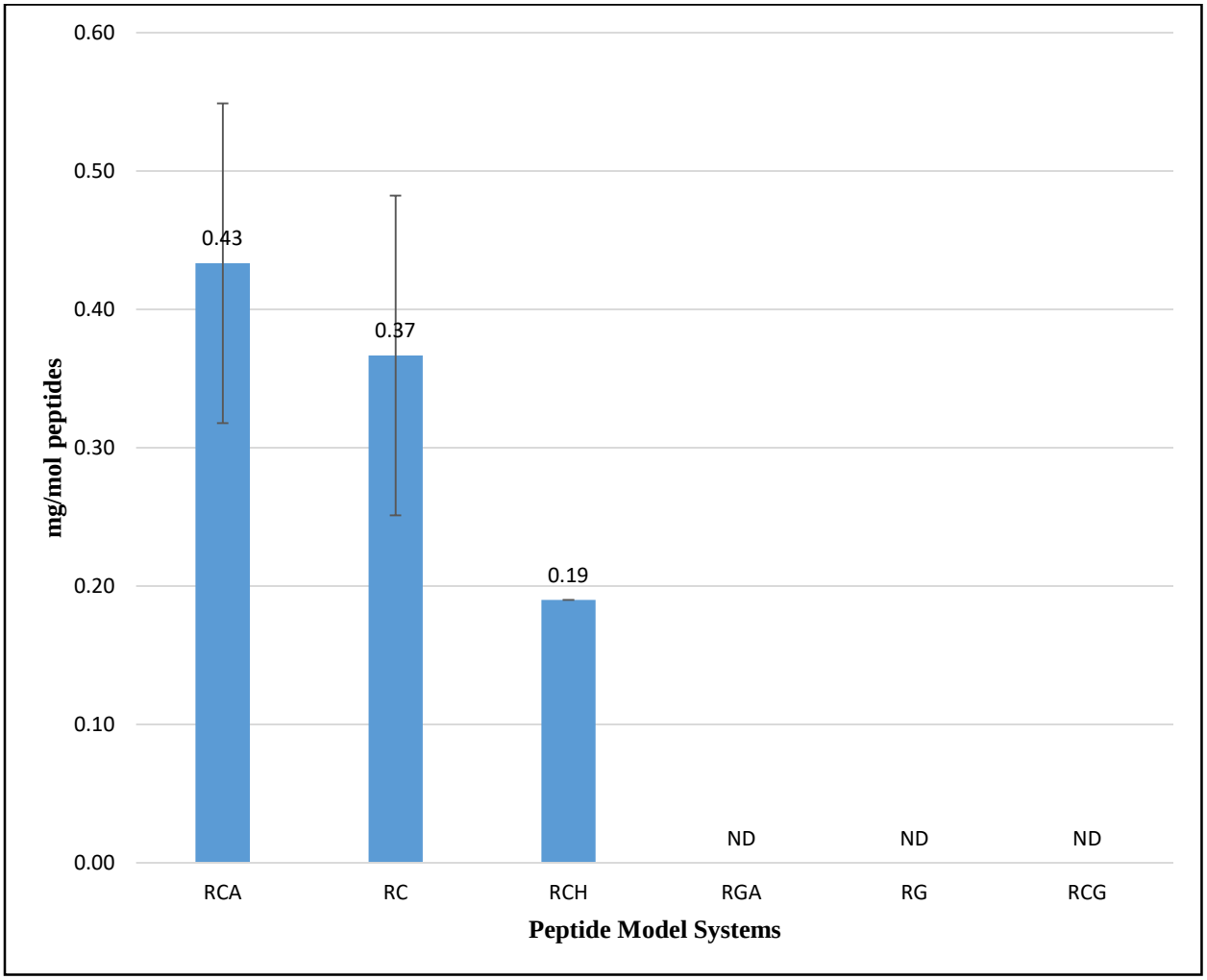


Chart 6. Compound 31.

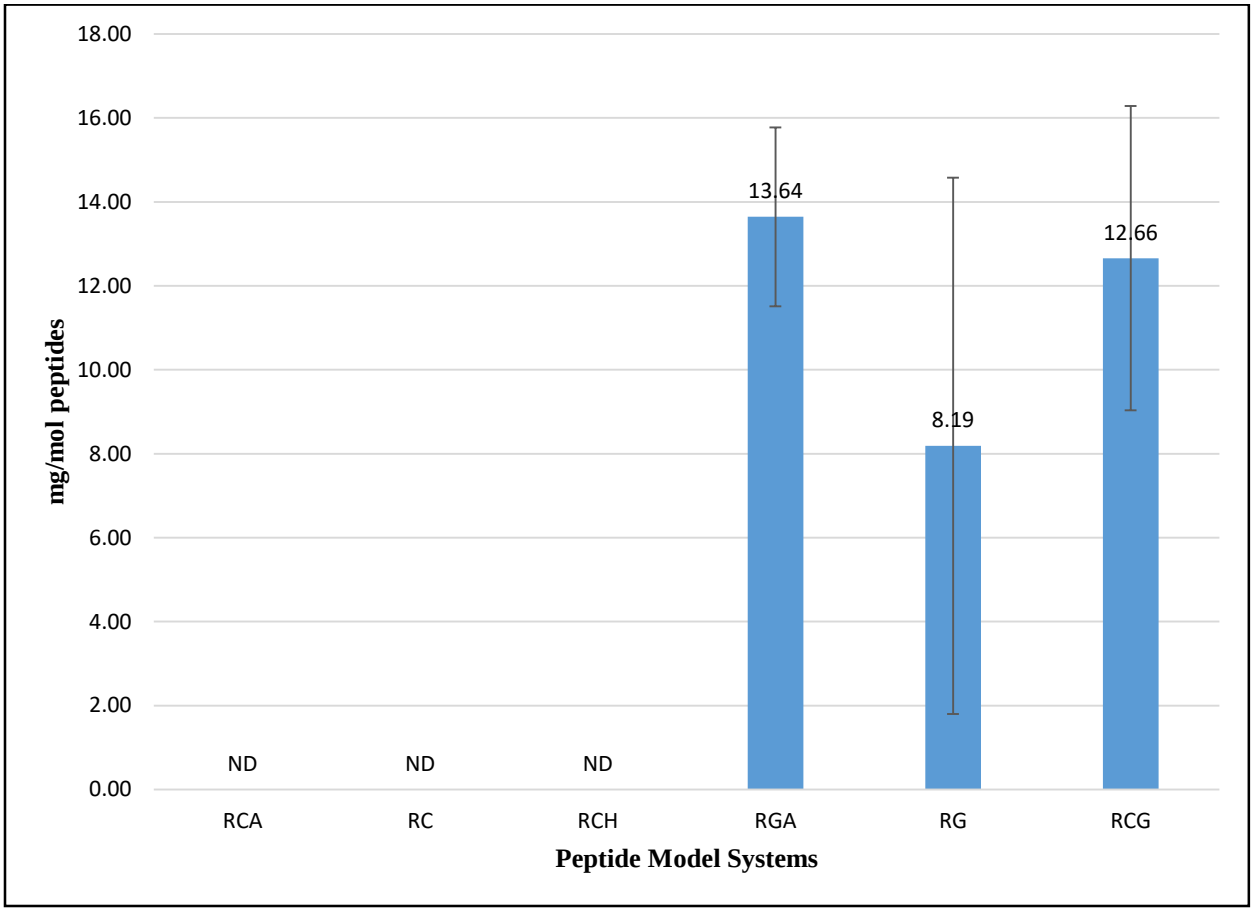


Chart 7. Compound 40.

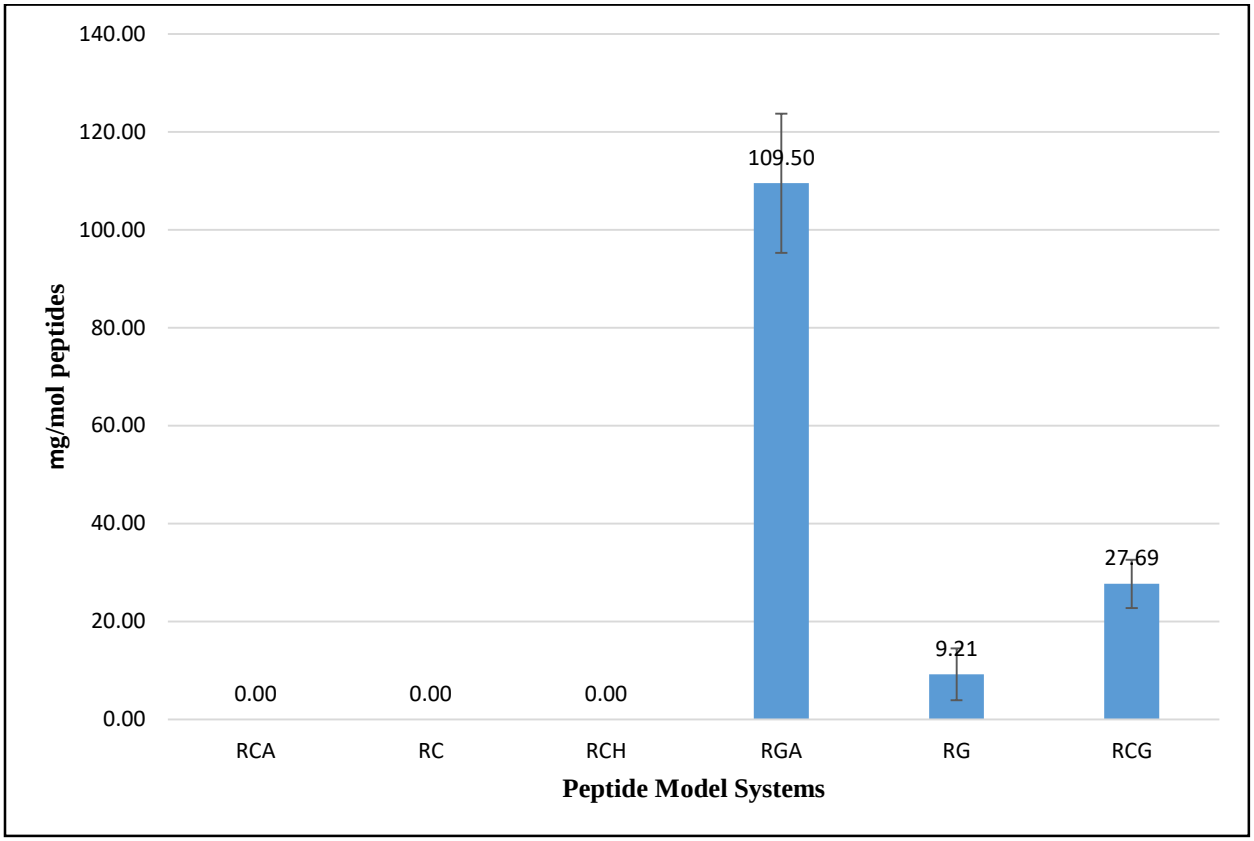


Chart 8. Compound 41.

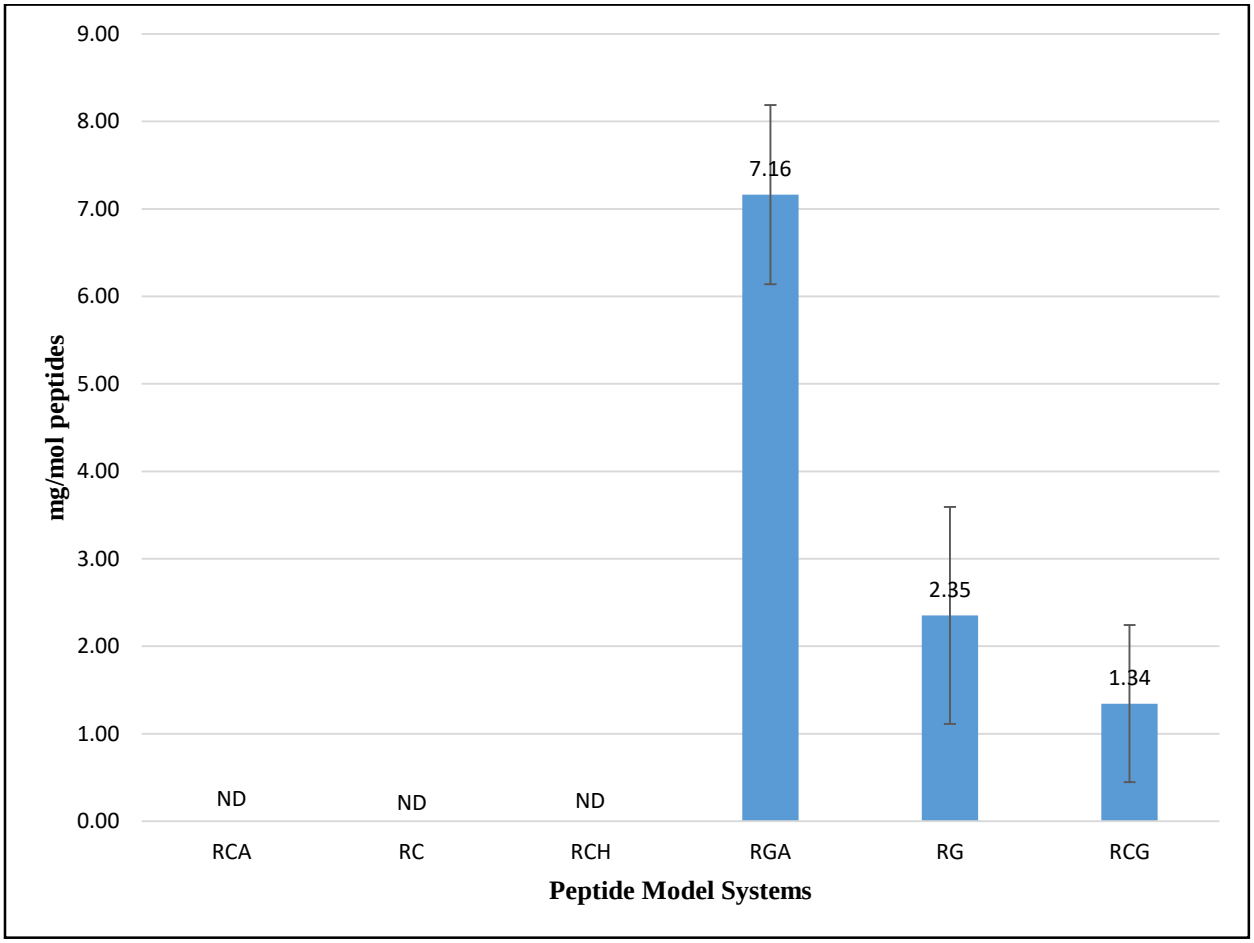
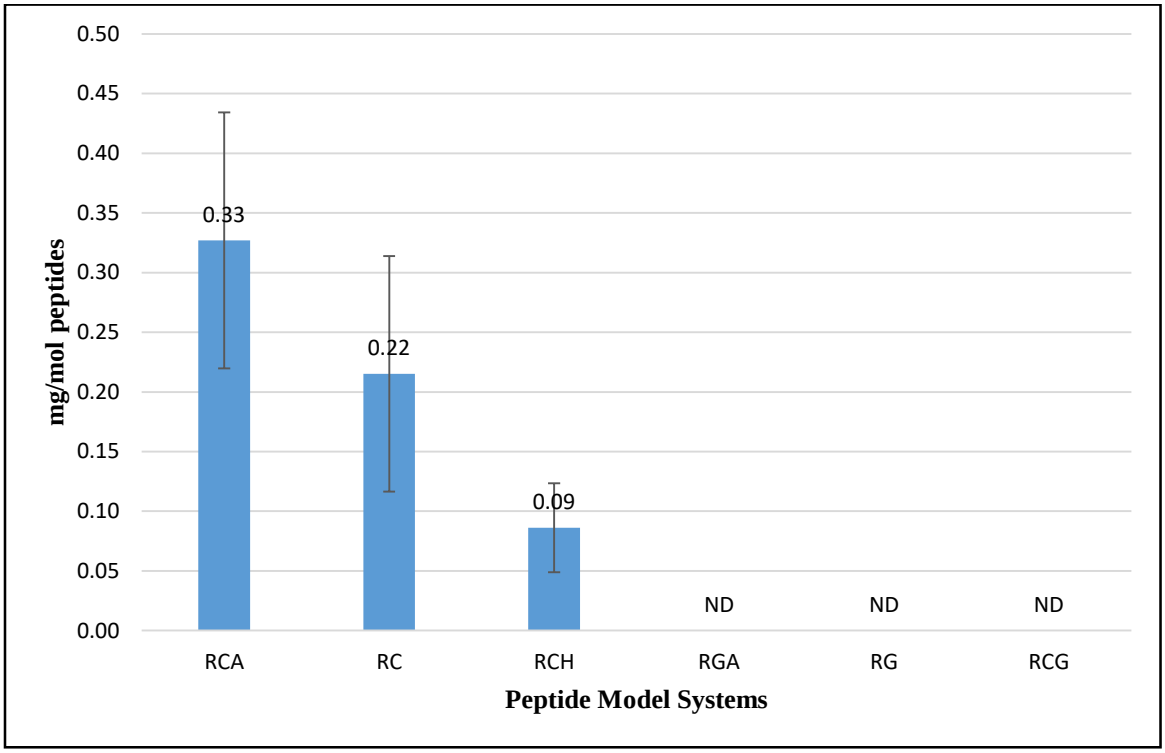


Chart 9. Compound 56.



Note:
Standard deviations are represented in the figures by the deviation bars attached to each colume.
ND: not detected in the corresponding model systems.