The Maillard Reaction in Foods and Nutrition

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The Maillard Reaction and Meat Flavor

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Many desirable meat flavor volatiles are synthesized by heating water-soluble precursors such as amino acids and carbohydrates. These latter constituents interact to form intermediates which are converted to meat flavor compounds by oxidation, decarboxylation, condensation and cyclization. O-, N-, and S-heterocyclics including furans, furanones, pyrazines, thiophenes, thiazoles, thiazolines and cyclic polysulfides contribute significantly to the overall desirable aroma impression of meat. The Maillard reaction, including formation of Strecker aldehydes, hydrogen sulfide and ammonia, is important in the mechanism of formation of these compounds.

Raw meat has little desirable flavor, but each type of meat has a characteristic flavor due to the animal species and the temperature and type of cooking. Both water-soluble and lipid-soluble fractions contribute to meat flavor and the water-soluble components include precursors which upon heating are converted to volatile compounds described as "meaty."

There are two approaches to studying meat flavor: one is concerned with identification of nonvolatile precursors of flavor components and the other involves isolation and identification of volatile flavor molecules. Water-soluble meat flavor precursors encompass a number of different organic classes of compounds, including: nucleic acids, nucleotides, nucleosides, peptides, amino acids, free sugars, sugar amines, glycogen, and amines. Bender and co-workers (1,2) were perhaps the first to thoroughly examine contents of water-soluble extracts of boiled beef, which they believed contained precursors of meaty flavor. These investigators reported the presence of many low-molecular-weight nitrogen compounds. Kramlich and Pearson (3) reported that pressed fluid from raw beef produced meat flavor upon heating.

Hornstein and Crowe (4) found that flavor precursors of meat were extractable with cold water and demonstrated that lyophilized

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diffusate from cold water extracts of raw beef and pork produced meaty odors upon heating. Wood (5) demonstrated that heating at 100°C resulted in increase in inorganic phosphate and a decrease in organic phosphate and reducing substances. This was accompanied by pronounced meat odor. Heating mixtures of amino acids in the absence of reducing sugars produced neither browning nor meaty flavor. To obtain meaty aromas synthetically, a mixture of all compounds identified in beef extract was required. Wood concluded, "it is shown beyond reasonable doubt that development of the brown color and meaty flavor characteristics of these extracts is a result of the Maillard reaction."

Macy, et al. (6-10) studied the water-soluble precursors of beef, pork and lamb including the dialyzable low-molecular-weight constituents, amino acids, carbohydrates, nucleotides, and nucleosides. They studied the influence of heating on these constituents. The predominant amines in the dialyzable diffusate were alanine, anserine-carnosine and taurine, and these decreased considerably during heating. Other amino acids decreasing during heating included methylhistidine, isoleucine, leucine, methionine, cystine, serine, lysine, glycine, and glutamic acid.

Ribose-5-phosphate, ribose, glucose and fructose decreased extensively in concentration during heating at 100°C for one h. Ribose and ribose-5-phosphate decreased most readily under these conditions. Similar results were published by Wasserman and Spinelli (11), who examined the amino acids and sugars in beef diffusate after heating for one h at 125°C. They also studied changes in amino acids and sugars during heating in model systems. Wasserman (12) also concluded that Maillard browning was important for the formation of desirable flavor compounds during heating of meat, but that the relationship between compounds formed by this reaction and meat flavor was unknown.

These statements concerning the Maillard reaction are associated with the formation of 1-amino-1-deoxy-2-ketose addition products during heating of reducing sugars and amines. These addition products can then degrade by several pathways to form flavor compounds. The addition product for one pathway enolizes at C-2 - C-3 and eliminates the amine from C-1 to form methyl dicarbonyl intermediates (13,14), which further react to give fission products such as C-methyl reductones, keto aldehydes, dicarbonyl compounds and reductones (15). Important volatile flavor producing compounds include pyruvaldehyde, diacetyl and hydroxyacetone. Another pathway begins with 1,2-enediol form of the addition product with subsequent deamination at C-1 yielding 3-deoxyhexosone (16). Dehydration results in the formation of important flavor compounds such as 2-furaldehydes (furfural), a formylpyrrole, and similar compounds. Another pathway possibly beginning with the 2,3 enediol involves the enolic form of a 1-deoxy-2,3-dicarbonyl intermediate or an equilibrium enolic form which leads to formation of flavor compounds such as methylfuranones, isomaltol, and maltol (17). The acidity of the reaction medium and the basicity of the

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amine constituting the addition product (Amadori compound) are important variables for the formation of these various flavor volatiles.

Two branches of the above reaction pathways provide active reagents for the degradation of α-amino acids to aldehydes and ketones of one less carbon atom (Strecker degradation), which is another arm of the Maillard reaction. Strecker aldehydes from these reactions are important flavor compounds (18).

Further evidence of the importance of the Maillard reaction in the formation of volatile flavorants from meat precursors is gleaned by examining ingredients in reaction mixtures patented as synthetic meat constituents. Ching (19) examined 128 patents of meat flavor and found that 55 specified use of both amino acids and sugars. Amino acids were the predominant components of these mixtures, and among the amino acids, sulfur-containing cysteine and/or cystine were used in 39 mixtures. Glutamic acid was also used in 39 such mixtures.

In their recent comprehensive review of natural and synthetic meat flavors, MacLeod and Seyyedain-Ardebili (20) listed 80 patents describing "reaction products" procedures that produced meat-like flavors upon heating. Approximately one-half of these precursor mixtures included amino acids and reducing sugars. Most of the mixtures described in patented procedures for synthetic meat flavor are modeled after ingredients found in the water-soluble dialyzable fraction of fresh meat. These constituents serve as reagents for Maillard reactions.

Many investigations have been made of the volatile flavor compounds resulting from meat cookery or heating of mixtures simulating meat flavor precursors in model systems. These have been reviewed by Herz and Chang (21), Dwivedi (22), Ching (19) and MacLeod and Seyyedain-Ardebili (20). It has been estimated that approximately 600 volatiles have been identified from meat or simulated meat precursors (23). These volatiles are not only from pre-formed volatiles in meat but largely from changes of precursors by oxidation, decarboxylation, fragmentation, recombination, rearrangement, condensation, and cyclization.

Meat aroma is not the result of one chemical constituent but the sum of the sensory effects of many of these volatiles. Over 90% of the volume of volatile constituents from freshly roasted beef is from lipid, but approximately 40 percent of the volatiles from the aqueous fraction are thought to be heterocyclic compounds, many resulting from Maillard reaction products or their interactions with other ingredients.

Heterocyclic compounds as meat volatiles have been reviewed recently by Ohloff and Flament (23) and by Shibamoto (24). More comprehensive coverage of these constituents as food flavorants is currently being published (Vernin, 25).

Heterocyclic compounds contribute significantly to the overall aroma impression of meat. They include O-, N-, and S-heterocyclic structures. Meat flavor heterocyclics include furans,

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furanones, pyrazines, thiophenes, thiazoles, thiazolines, oxazolines and cyclic polysulfides. These compounds can all be formed by Maillard type interactions.

Furans and Furanones

Heterocyclics, particularly the S-containing ones, are extremely important contributors to "meaty" flavors. Ching (19) identified 11 furans and 7 furanones from reaction mixtures containing precursors responsible for beef flavor. Furfural derivatives were obtained by heating several reaction mixtures including low-molecular-weight water-extractable components from beef and simple amino acid-sugar mixtures. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone, 4-hydroxy-5-methyl-3-(2H)-furanone, and four similar compounds were identified by Ching (19) from beef by dialyzing water-soluble materials and concentrating the diffusible solutes. The diffusates were then heated with or without sugars at 130°C for 2 h. Furanones can be formed by Amadori compound pathways (17, 18). Some of these compounds were originally isolated and identified from beef by Tonsbeek, et al. (26), who stated they might be formed through interaction of ribose-5-phosphate and taurine during heating. Hexoses form 5-methylfufurals and 4-hydroxy-2,5-dimethyl-3(2H) furanone, while pentoses produce furfural and 4-hydroxy-5-methyl-3(2H) furanone. Hicks and Feather (27) and Hicks, et al. (28) synthesized the latter compound by heating amines with xylose, ribose, ribose-5-phosphate, or gluconic acid.

Although exact mechanisms have not been described for the formation of other furanoid compounds through amine-carbohydrate interactions, probably many of the 32 furans described by Ohloff and Flament (23) from meat aroma mixtures are from this source. Shibamoto (24) described many of the same components from mixtures producing meat odors.

Herz and Chang (21) examined several furan compounds which had a wide variety of aromas, but none of them were meaty. Furans that do not contain sulfur are usually fruity, nutty, and caramel-like in odor. The furanones described above have burnt pineapple and roasted chicory odors, but these contribute to overall flavor impression of meat and important N and S meat flavor compounds might be formed from them during cooking.

Thiophenes

MacLeod and Seyyedain-Ardebili (20) listed 36 thiophene derivatives as having been found during various investigations of meat or meat constituents. Ching (19) found 18 and Shibamoto (24) listed 29.

Thiophenes are extremely important in flavor and are responsible for the mild sulfurous odor of cooked meat. Numerous other thiophenes have been identified during heating of meat or meat constituents. The sulfur in thiophene may be derived from amino acids (cysteine, cystine, methionine) or from vitamin B_1.

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Probably the most important reactant in the formation of volatile meat flavor compounds is hydrogen sulfide. It can be formed by several pathways during meat cookery, but one mechanism is Strecker degradation of cysteine in the presence of a diketone as established by Kobayashi and Fujimaki (29). The cysteine condenses with the diketone and the product in turn decarboxylates to amino carbonyl compounds that can be degraded to hydrogen sulfide, ammonia and acetaldehyde. These become very reactive volatiles for the formation of many flavor compounds in meat and other foods.

Shibamoto (30) formed α-thiophenecarboxaldehyde by reacting furfural and hydrogen disulfide. This means that there probably was exchange between the S and O in the furan ring during heating.

van den Ouweland and Peer (31) made a significant contribution to the elucidation of meat flavor when they demonstrated that 4-hydroxy-5-methyl-3(2H)-furanone formed a number of "meat-like" mercapto-substituted furan and thiophene derivatives when heated in the presence of hydrogen sulfide. These authors postulated that the initial stage in forming the thiophene derivatives involves a partial substitution of the ring oxygen by sulfur to give the thio analog. Compounds having a meaty odor included 3-mercapto-2-methyl-2,3-dihydrothiophene, 3-mercapto-4-hydroxy-2-methyl-2,3-dihydrothiophene, 4-mercapto-2-methyltetrahydrothiophene, and 3-mercapto-2-methyltetrahydrothiophene. The proposed (31,32) reactions for the formation of some of these "meaty" compounds is summarized in Figure 1. Although these authors assumed that the dihydrofuranone was derived from ribose-5-phosphate via a dephosphorylation-dehydration reaction (31), there is evidence which indicates that the furanone can be formed by a reaction of aldose sugars with amines to produce Amadori products which subsequently dehydrate with amine elimination (17,28,33). The amine could be taurine, which is in ample supply in muscle (7) and was mentioned by Tonsbeek, et al. (26) in their original discovery of these compounds. None of these compounds have been identified from meat extracts, but Ching (19) identified 4-mercapto-2-methyl-4,5-dihydrothiophene from synthetic beef diffusate. The mercaptothiophenes are undoubtedly formed at low concentrations in meat.

Evers, et al. (34) identified several S-substituted furans having meaty aroma including 3-mercapto-2-methylfuran and 3-mercapto-2,5-dimethylfuran from Maillard reaction mixtures. These compounds were readily oxidized to sulfides, some of which retained meaty odors. All furans having the sulfur atom bound to the β-carbon had meaty aromas, whereas those with sulfur bound to the α-carbon had hydrogen sulfide-like odors.

Maillard reaction products formed by interaction of reducing sugar and amino acids such as α-dicarbonyl compounds, aldehydes, hydrogen sulfide, and ammonia can react further to form derivatives that have been identified from meat or its components during heating. Important reviews of sulfur compounds that might be produced by these reactions have been published by Schutte (35) and

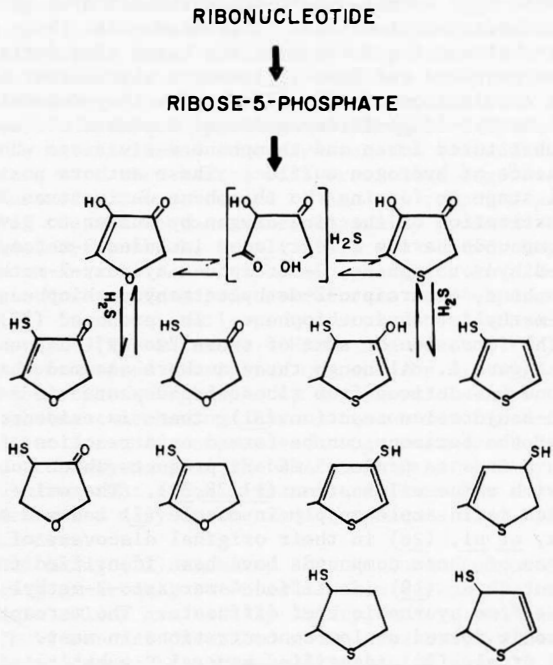

Figure 1. Reaction of 4-hydroxy-5-methyl-3(2H)-furanone with hydrogen sulfide to form mercaptofurans and mercaptothiophenes having beef-like odors (31).

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by Takken, et al. (36). Some of the important sulfur-containing compounds include thiazoles, thiazolines, and polysulfide heterocyclics.

Thiazoles and Thiazolines

Takken, et al. (36) found that these compounds could be formed by combining butane-2,3-dione (diacetyl), pentane-2,3-dione, or pyruvaldehyde with acetaldehyde, hydrogen sulfide, and ammonia. Some of the reaction pathways suggested for thiazole and thiazoline formation for these reaction ingredients are outlined in Figure 2. Thiazoles have also been identified in volatiles resulting from heating cysteine and cystine with glucose and pyruvaldehyde at 160°C (37) and by Mulders (38) who heated a similar system at 125°C and pH 5.6 for 24 h. Possible pathways for these reactions were discussed by Schutte (35). Corresponding thiazolines and thiazoles frequently coexist due to the dehydration of thiazolines during heating (39).

MacLeod and Seyyedain-Ardebili (20) listed 12 thiazoles and thiazolines that had been identified in beef samples by different investigators. Some patented compounds listed as having meaty flavor are 4-methyl-5-(2-hydroxyethyl)thiazole, 4-ethyl-5-(3-acetoxypropyl)thiazole, 2-acetyl-2-thiazoline and 2-acetyl-5-propyl-2-thiazoline. Ching (19) identified 12 thiazole derivatives in her studies of the volatile produced by heating components of meat. Important thiazoles identified in these studies included 2 and 4-acetyl derivatives. It was postulated by Tonsbeek, et al. (40) that 2-acetyl-2-thiazoline is formed by Strecker degradation of cysteine with methylglyoxal or similar compounds followed by oxidative cyclization as shown in Figure 3. This compound was isolated from 225 kg of cooked beef and had a strong odor of freshly baked bread. Other thiazoles have been isolated from meat or meat constituents which have undergone Maillard-type reactions (Wilson, et al., 41).

Polysulfide Heterocyclics

Wilson, et al. (41) also confirmed the presence of polysulfur heterocyclics in meat including thialdine (5,6-dihydro-2,4,6-trimethyl-1,3,5-dithiazine) and trithioacetone (2,2,4,4,6,6-hexamethyl-1,3,5-trithiane). Wilson (42) later discussed the possible routes of formation of some of these compounds from cysteine. Thialdine was found by Brinkman, et al. (43) in the headspace volatiles of beef broth. These workers also identified 3,5-dimethyl-1,2,4 - trithiolane from the same source. Both cis and trans isomers of this compound had previously been identified as flavor components of boiled beef by Chang, et al. (44) and Herz (45).

Chicken has also been found to be a source of polysulfide heterocyclics. Pippen and Mecchi (46) theorized that 2,4,6-tri-

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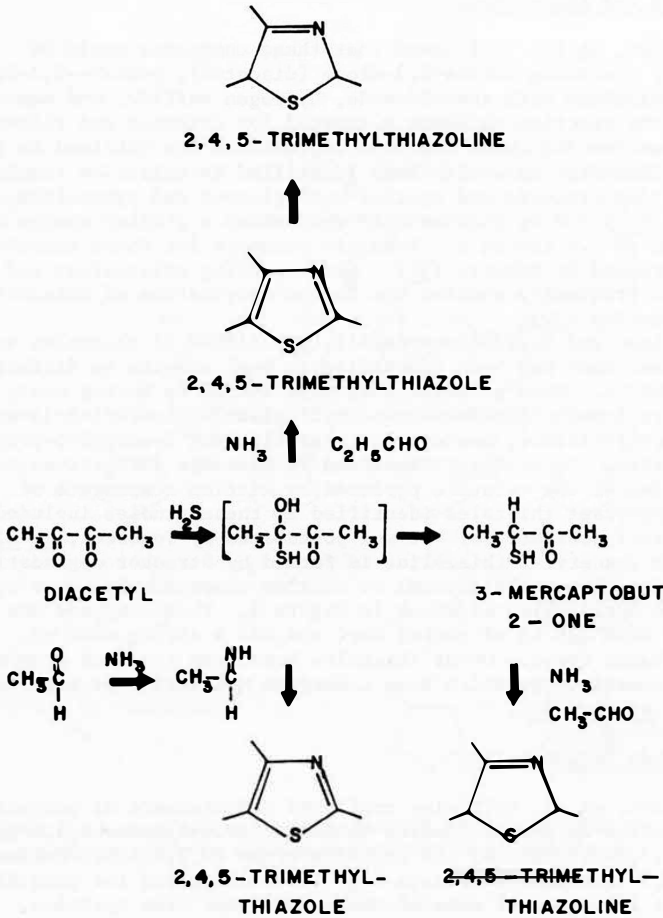

Figure 2. Reaction pathways for formation of thiazoles and thiazolines by heating Maillard degradation products of meat precursors (36).

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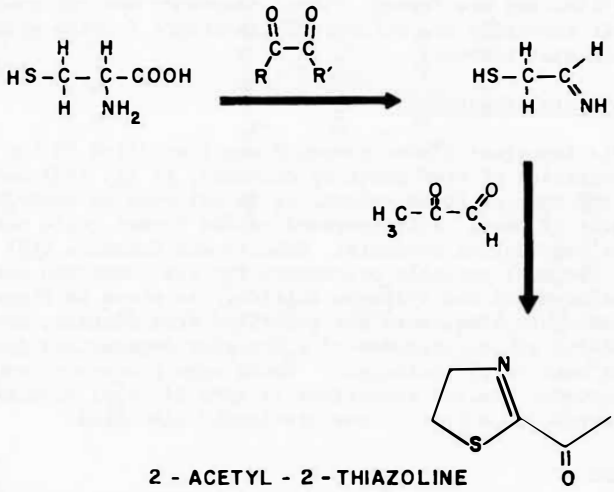

Figure 3. Formation of 2-acetyl-2-thiazoline by Strecker degradation of cysteine. (Reproduced from Ref. 40. Copyright 1971, American Chemical Society.)

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methyl-1,3,5-trithiane might be formed in chicken by indirect combinations of hydrogen sulfide and acetaldehyde in fat. The same heterocyclic was listed as a flavor volatile in chicken by Wilson and Katz (47) in their review of chicken flavor. These same workers (48) also identified this compound in lean ground beef.

The formation of 5,6-dihydro-2,4,6-trimethyl-1,3,5-dithiazine, 2,4,6-trimethyl-1,3,5-trithiane, and 3,5-dimethyl-1,2,4-trithiolane by heating of acetaldehyde, hydrogen sulfide, and ammonia was outlined by Takken and coworkers (36) and is summarized in Figure 4. Under oxidative conditions, dialkyltrithiolanes are formed; at low pH there is conversion to trialkyltrithianes; at elevated temperature isomerization into trisulfides occurs, which compounds disproportionate into di and tetrasulfides; and in the presence of ammonia, dithiazines are formed. These compounds and the conditions for their formation are of extreme importance for the production of desirable meat flavors.

1-(Methylthio)ethanethiol

This important flavor compound was identified in the headspace volatiles of beef broth by Brinkman, et al. (43) and although it has the odor of fresh onions, it is believed to contribute to the flavor of meat. This compound can be formed quite easily from Strecker degradation products. Schutte and Koenders (49) concluded that the most probable precursors for its formation were ethanal, methanethiol and hydrogen sulfide. As shown in Figure 5, these immediate precursors are generated from alanine, methionine and cysteine in the presence of a Strecker degradation dicarbonyl compound such as pyruvaldehyde. These same precursors could also interact under similar conditions to give dimethyl disulfide and 3,5-dimethyl-1,2,4-trithiolane previously discussed.

Pyrazines

The formation of pyrazines in foods has been reviewed extensively by Mega and Sizer (50). Temperature and pH are very important factors in the formation of specific pyrazines. Forty-two pyrazines have been identified in meat from various sources by these authors. MacLeod and Seyyedain-Ardebili (20) listed 49 pyrazines found in beef by various investigators. Ching (19) identified 28 pyrazines in her studies of sugar-amine reactions simulating beef flavor.

Several mechanisms have been reported for pyrazine formation by Maillard reactions (21,52,53). The carbon skeletons of pyrazines come from α-dicarbonyl (Strecker) compounds which can react with ammonia to produce α-amino ketones as described by Flament, et al. (54) which condense by dehydration and oxidize to pyrazines (Figure 6), or the dicarbonyl compounds can initiate Strecker degradation of amino acids to form α-amino ketones which are hydrolyzed to carbonyl amines, condensed and are oxidized to substituted

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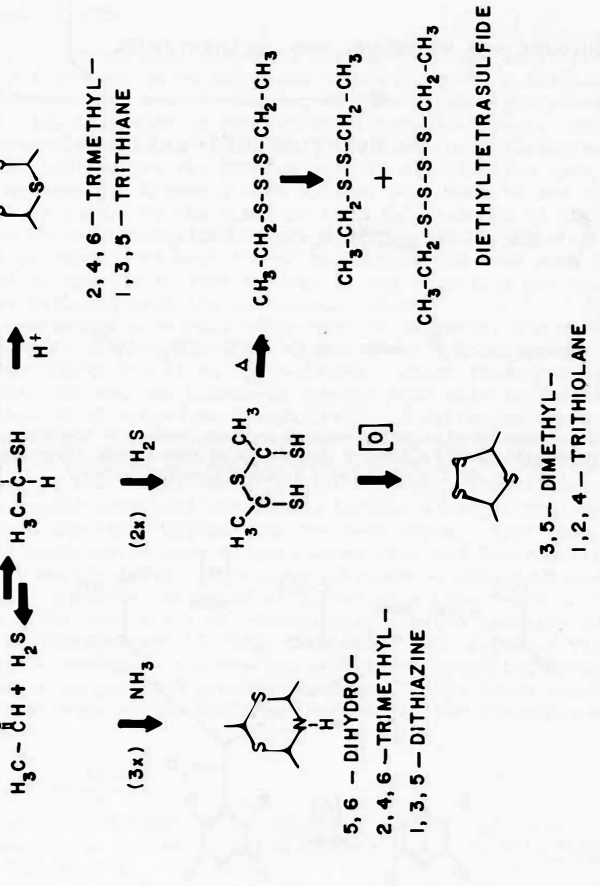

Figure 4. Formation of important meat flavor polysulfide heterocyclics by heating acetaldehyde and hydrogen sulfide (36).

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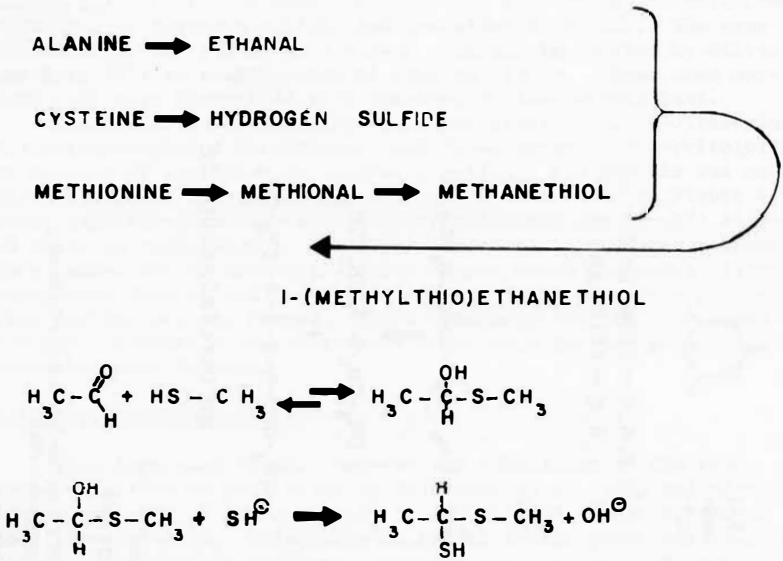

Figure 5. Formation of secondary reaction products involved in production of 1-(methylthio)ethanethiol by Strecker degradation of amino acids. (Reproduced from Ref. 49. Copyright 1972, American Chemical Society.)

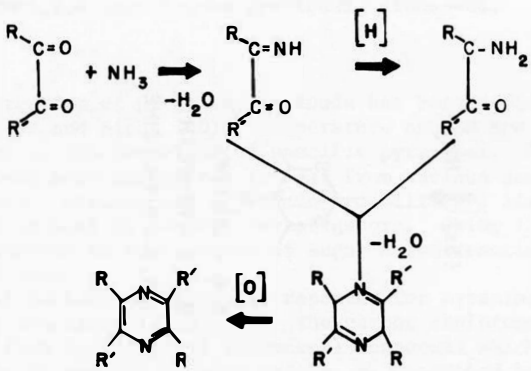

Figure 6. Interaction of Maillard reaction products to form pyrazines (54).

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pyrazines. The latter mechanism was proposed by Hodge and Osman (55).

Some meat flavor-contributing pyrazines patented for use for synthetic purposes are methylpyrazine, 5,7-dihydro-5,7-dimethyl-furo-(3,4-b)pyrazine, and 5-methyl-(7H)-cyclopenta[b]pyrazine (20).

Present Outlook

Even though many compounds discussed in the above presentation are thought to be important in meat flavor, a delicate blend of these compounds and other ingredients at the appropriate concentration is needed to synthesize acceptable flavor. In view of the possible instability of the flavor compounds themselves, precursors that supply the precise mixture of volatiles upon heating will be needed. Attempts have already been made to use this approach as judged by the numerous patented mixtures of precursors listed in the literature. More effort should be given to the quantitative aspects of meat flavor production and work must be continued on the qualitative aspects of the volatiles and the appropriate Maillard reaction precursors chosen.

One method presently being used to determine the contribution of volatile compounds to food flavor is to combine sensory analyses with GC/MS fractionation technics. Using these combined procedures, Persson and coworkers (56-59) were able to correlate acceptability of canned beef with levels of different compounds in the product. Pearson and coworkers (60,61,62) have used a similar method to optimize the ingredients responsible for desirable beef aroma. In their most recent publication (62) they report that heterocyclic compounds containing oxygen, nitrogen and sulfur were the most important ingredients for beef aroma. Hydrophilic compounds contributed more to meat aroma than did hydrophobic compounds, verifying the importance of water-soluble compounds in meat flavor. Important mixtures of ingredients were found to be hydrogen sulfide with 2-acetyl-3-methylpyrazine and hydrogen sulfide with 4-hydroxy-5-methyl-3(2H)-furanone. This approach will ultimately be useful in solving the problem of producing desirable meat flavors from purified precursors and certainly lends support for the importance of the Maillard reaction in the formation of these flavors.

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