Chapter 41

Flavor Formation in Meat-Related Maillard Systems Containing Phospholipids

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In heat foods the main reactions by which flavors are formed are the Maillard reaction and the thermal degradation of lipids. These reactions follow complex pathways and produce reactive intermediates, both volatile and non-volatile. It has been demonstrated that lipids, in particular structural phospholipids, are essential for the characteristic flavor development in cooked meat and that the interaction of lipids with products of the Maillard reaction is an important route to flavor. When model systems containing amino acids and ribose were heated in aqueous buffer, the addition of phospholipids had a significant effect on the aroma and on the volatile products. In addition a number of heterocyclic compounds derived from lipid - Maillard interactions were found. The extent of the interaction depends on the lipid structure, with phospholipids reacting much more readily than triglycerides.

The characteristic flavors associated with cooked foods such as meat have proved particularly difficult to characterize, both for the sensory analyst and the flavor chemist. Large numbers of volatiles comprising aliphatic, aromatic and heterocyclic compounds have been identified in cooked foods, and meat, with almost 1000 volatiles, contains the largest number of compounds identified in any food or beverage. Despite this considerable research interest, the key aroma component(s) of meat remain elusive and it is probable that no simple combination of compounds is responsible for meat aroma (1,2). Meat flavor is thermally derived, since uncooked meat has little or no aroma and only a blood-like taste. The major precursors of meat flavor can be divided into two categories: water soluble components (amino acids, peptides, sugars, nucleotides, thiamine, etc) and components of lipids (principally the fatty acids). The two main types of flavor-forming reactions which occur during the cooking of meat are the Maillard reaction between amino compounds and reducing sugars, and the thermal degradation of lipids.
The Maillard Reaction in Meat Flavor

The importance of the Maillard reaction in meat flavor has long been recognized and it has formed the basis of the large number of patents for "reaction product" simulated meat flavorings. Research on cooked meat in the 1950s had shown that during cooking there were significant depletions in the levels of reducing sugars and amino acids, in particular cysteine (3,4). This led to the classic patent of Morton et al in 1960 which involved the formation of a meat-like flavor by heating a mixture of cysteine and ribose (5). Practically all the subsequent proposals for meat-like flavorings from Maillard systems involved sulfur, usually as cysteine or other sulfur-containing amino acids.

Heterocyclic compounds are dominant among the aroma compounds produced in the Maillard reaction, and sulfur-containing heterocyclics have been shown to be particularly important in meat-like flavors. In a recent review, Macleod (6) listed 78 compounds which have been reported in the literature as possessing meaty aromas; seven are aliphatic sulfur compounds, the other 71 are heterocyclic of which 65 contain sulfur. The Strecker degradation of cysteine by dicarbonyls is an extremely important route for the formation of many heterocyclic sulfur compounds; hydrogen sulfide and mercaptocetaldehyde are formed by the decarboxylation and deamination of cysteine and provide reactive intermediates for interaction with other Maillard products.

Lipid-derived Volatiles in Meat Flavor

The volatiles from cooked meat contain large numbers of aliphatic compounds including aldehydes, alcohols, ketones, hydrocarbons and acids. These are derived from lipids by thermal degradation and oxidation (7) and many may contribute to desirable flavor. In addition, the aldehydes, unsaturated alcohols and ketones produced in these reactions, as well as the parent unsaturated fatty acids, are reactive species and under cooking conditions could be expected to interact with intermediates of the Maillard reaction to produce other flavor compounds.

Meat contains neutral triglyceride lipids in the fat depots and in the intra-muscular fat cells. In addition, the polar phospholipids are important structural components of all tissues and, although they comprise less than 1% of muscle, the phospholipids contain a much higher proportion of polyunsaturated fatty acids than triglyceride lipids and therefore provide a significant source of autoxidation products. The role of phospholipids in the development of off-flavors in reheated meats (warmed-over flavor) has long been recognized (8), but until recently their possible role in the development of desirable flavors has been largely unexplored.

Lipids and the Maillard Reaction

In an examination of the contribution which lipids make to the development of aroma during the heating of meat, the phospholipids were shown to be particularly important. Consumer and taste panel studies had failed to show any relationship between the meaty flavor of lean meat and the level of fat on the carcass, apparently...
confirming early studies on meat flavor which associated meatiness with water soluble flavor precursors and species characteristics with the fat (9,10). However, the volatiles of cooked meat are dominated by lipid-derived compounds and it is difficult to believe that such compounds play no role in meat flavor. The contribution these volatiles of lipid origin make to meat aroma was investigated by observing the changes in aroma which occurred when lipids were extracted from the meat prior to cooking (11).

The inter- and intra-muscular triglyceride fats were removed from lean muscle by solvent extraction with hexane, and the material was subsequently cooked. The resulting aroma was very similar to that from the untreated material; both preparations were judged to be meaty and could not be differentiated by aroma in sensory triangle tests. However, when a more polar solvent (chloroform - methanol) was used to extract all the lipids - phospholipids and triglycerides - a highly significant difference in aroma resulted; the meaty aroma was replaced by a roasted, biscuit-like aroma. Examination of the aroma volatiles showed that both the control and the material without triglycerides had similar profiles dominated by aliphatic aldehydes and alcohols, while removal of phospholipids as well as triglycerides gave a markedly different profile; the lipid oxidation products were lost, but there was a large increase in the amounts of alkylpyrazines. This increase in alkylpyrazines, volatile products of the Maillard reaction, implied that in normal meat the lipids or their degradation products inhibit the formation of certain heterocyclic compounds by participating in the Maillard reaction.

There is increasing evidence that the interaction of lipids with the Maillard reaction is relevant to the generation of flavor in many cooked foods. For instance, the removal of lipids from coconut has been shown to cause flavor changes in the roast material (12). Uncooked coconut contained significant amounts of lactones as the main aroma components; on roasting pyrazines, pyroles and furans were also found in the aroma volatiles which added a strong nut-like aroma to the sweet aroma of the unroasted coconut. When ground coconut was defatted and then roasted, the sweet aroma due to lactones disappeared and the product possessed a burnt, nut-like aroma. A marked increase in the number and amount of Maillard reaction products, in particular pyrazines, was found.

It has been known for many years that Maillard Reaction products can behave as antioxidants in food systems (13,14), and they have been shown to inhibit warmed-over flavor development in cooked meat which is caused by the autoxidation of lipids, especially phospholipids. There has been a significant amount of research examining the Maillard reaction products and intermediates from model systems which may have antioxidative properties. However, the formation of volatile aroma compounds from the interaction of Maillard intermediates with lipid-derived compounds has received little attention.

In some recent research on flavor formation during deep-fat frying at Rutgers University, a number of heterocyclic compounds with long-chain alkyl substituents were found the volatiles of fried chicken (15) and fried potato (16). These included pyridines, thiazoles, oxazoles, trithiolanes and a pyrazine. Only the involvement of lipids or lipid degradation products in the formation of
these compounds could account for the long-chain alkyl substitution. In a study of volatiles from a heated mixture of corn components (zein, corn starch and corn oil) two alkyl substituted pyrazines (2,5-dimethyl-3-pentylpyrazine and 2-methyl-3(or 6)-pentylpyrazine) were identified (17); again these compounds must arise from a lipid-protein-carbohydrate interaction. The interaction of simple triglycerides with amino acids at high temperatures (270°C) produced fatty acid amides (18), and when beef fat was heated to 200°C with glycine, butyl- and pentylpyridines were found among the lipid degradation products (19). The conditions prevailing in all these systems were essentially low moisture and high lipid, while in meat much higher water (ca. 75%) and lower lipid (2-3%) levels exist, and this could result in different reaction pathways to volatile compounds.

Reaction of Amino Acids, Ribose and Phospholipids.

The reduction in levels of volatile Maillard reaction products observed in defatted cooked meat led to an investigation of the effect of phospholipids on the volatile products from aqueous model systems containing amino acids and sugars (20-22). Several amino acids were used including glycine, the simplest amino acid, and cysteine, a sulfur containing amino acid. Ribose was employed as the reducing sugar because of its recognized role in the production of cooked meat flavor and its high reactivity in Maillard systems. In initial work, L-α-phosphatidylcholine (lecithin) from egg yolk was selected as the phospholipid, and later studies compared other phospholipids and lipid extracts from meat. As the study originated from investigations of cooked meat flavor, the model system reactions were carried out in aqueous solution buffered with phosphate at an initial pH of 5.7 and concentrations of the reactants were selected to approximate their relative compositions in mammalian muscle. The reactions were carried out under pressure at 140°C for 1 hour in sealed glass ampoules. At the end of the reactions volatiles were collected by headspace entrainment on Tenax GC and analysed by capillary gas chromatography - mass spectrometry.

Volatiles from Amino Acid + Ribose alone. All the reaction systems produced complex mixtures of volatiles, containing large numbers of heterocyclic compounds, many of which have not been reported previously in Maillard systems. In the absence of phospholipid 74 compounds were separated and identified from the glycine system (21); furfurals and furanylketones dominated and the three largest peaks in the chromatogram were 2-furfural [1], dimethylfurfural [2] and ethylmethylfurfural [3].
Other furans included methyl-2-furfural [4], 2-acetylfuran [5], 2-propionylfuran [6], 1-(2-furanyl)-2-propanone [7] and 1-(-2-furanyl)-3-butanone [8]. Several alkylpyrazines were also found, with the 2,5-dimethyl [9] and 2-methyl-5-ethyl [10] the most abundant derivatives, and some 2-acyl-1-methylpyrroles of which only the formyl [11] and acetyl [12] derivatives were conclusively identified.

![Chemical Structures](image)

When cysteine was used instead of glycine the reaction volatiles became dominated by sulfur containing heterocyclics [22]; the alkylfurfurals and furanylketones were reduced to trace amounts and, although a series of alkylpyrazines were found, the levels were much lower than in the glycine system. A total of 118 volatile compounds were isolated from the cysteine + ribose reaction; the major components were 2-methyl-3-furanthiol [13], furanmethanethiol [14], 2-thiophenethiol [15], 2-methyl-3-thiophenethiol [16], dihydro-2-methyl-3(2H)-thiophenone [17] and 3-mercapto-2-pentanone [18]. A number of other furanethiol, thiophenethiols and thiophenones were also found including a series of acyl thiophenes analogous to the furfurals and furanylketones found in the glycine reaction. Other heterocyclic compounds identified included several alkylthiazoles, three isomeric thienothiophenes [19-21] and their methyl- and dihydro-derivatives, as well as alkyl-substituted 1,2-dithiolan-4-ones [22-24], 1,2-dithian-4-ones [25-27], 1,2,4-trithiolanes [28] and 1,2,4-trithianes [29].
Effect of Phospholipids on Reaction Volatiles. As would be expected, the inclusion of phospholipids in the reaction mixtures produced many volatiles derived from lipid degradation; these included hydrocarbons, alkylfurans, saturated and unsaturated alcohols, aldehydes and ketones. However, two other important observations were made. First, the concentrations of most of the heterocyclics, formed by the amino acid + ribose Maillard reaction, were reduced. For most of the major volatiles this reduction was of the order of 40 - 50%, but in the case of thiophenethiol and methyl-furanthiol the reduction was over 65%. This appears to support the findings that in meat and coconut, lipids exert a quenching effect on the amount of heterocyclic compounds formed in Maillard reactions during heat treatment (11,12). Second, and perhaps more important, the addition of phospholipid to the reaction mixtures resulted in the production of large amounts of compounds derived from the interaction of the lipid or its degradation products with Maillard reaction intermediates.

In the glycine reaction, 2-pentylpyridine [30] was formed. This compound has been reported in the volatiles from heated lamb fat (23), fried chicken (15) and french fried potato (16), and was a major product in the thermal interaction of valine and linoleic acid (24). It is postulated to be formed by the reaction of decadienal and ammonia, and large concentrations of 2,4-decadienal were found in the volatiles of lecithin when heated alone or in the Maillard mixtures. The addition of phospholipid to the cysteine - ribose reaction system gave over 10 times more 2-pentylpyridine than the corresponding glycine reaction. In the Strecker degradation of cysteine by dicarbonyl compounds, ammonia is known to accompany the formation of hydrogen sulfide (25,26) and this will provide a ready source of ammonia for reaction with dienals from lipid breakdown. With glycine, ammonia is not formed so readily at the slightly acidic pH employed in the reaction mixture and hence the lower concentrations of 2-pentylpyridine.

Among the most abundant components of the cysteine + ribose + lecithin reaction mixture were the 2-pentylpyridine and two long-chain alkyl substituted thiophenes: 2-pentyl- and 2-hexylthiophenes [31,32], together with a related compound with molecular formula and
mass spectrum very similar to 2-hexylthiophene but with a later retention time. This hexylthiophene isomer was tentatively identified as 2-pentyl-2H-thiapyran [33]. Closer examination of the GC-MS analysis revealed a series of 2-alkylthiophenes with n-alkyl substituents between C3 and C8 inclusive. Each had an isomeric compound with very similar mass spectrum eluting close to the next higher 2-alkylthiophene. Other lipid derived compounds included 2-hexenylthiophene [34], 1-heptanethiol and 1-octanethiol. All these compounds are probably formed by the reaction of lipid breakdown products, such as dienals, with hydrogen sulfide or mercaptoacetaldehyde obtained in the Strecker degradation of cysteine.

\[
\begin{align*}
30 & \quad \text{R} = \text{C}_5\text{H}_{11} \\
31 & \quad \text{R} = \text{C}_6\text{H}_{13} \\
32 & \quad \text{R} = \text{CH} = \text{CH} - \text{C}_4\text{H}_9 \\
33 & \quad \text{S} \quad \text{C}_5\text{H}_{11} \\
\end{align*}
\]

Hydrogen sulfide is also a key component in the formation of sulfur-containing compounds in the Maillard reaction (27). Thiophenones may be formed by the action of hydrogen sulfide on sugar degradation products such as dehydroreductones, furfural (28) or furanones (29), while thiazoles can be produced by the reaction of hydrogen sulfide with dicarbonyls and ammonia or an amino acid (30). The small amount of fufural in the cysteine mixture compared with that containing glycine may be due to its reaction with hydrogen sulfide to give furanmethanethiol. In the reaction mixtures containing lecithin, the decrease in concentrations of many of the sulfur-containing heterocyclic compounds derived from the basic cysteine + ribose reaction can be explained in terms of competition for hydrogen sulfide by lipid degradation compounds resulting in other sulfur compounds such as the alkylthiophenes and alkanethiols.

**Aroma of Reaction Mixtures** The aroma of the glycine + ribose reaction mixture after heating was described as "caramel", "sweet", "burnt sugar" while the addition of lecithin added "oily" and "chicken-like" notes. The overall odor of the cysteine containing reaction mixtures was described as "sulfurous, rubbery" but there was a distinct underlying meaty aroma. This "cooked meat", "beef" aroma was much more pronounced in the mixture containing the phospholipid. When the aromas eluting from the GC column were evaluated, the cysteine + ribose + lecithin system gave at least six areas where meaty aromas could be detected. Only one of these was detected in the absence of the phospholipid and this was attributed to 2-methyl-3-thiophenethiol; none of the other meaty odors could be assigned to particular compound identifications. The alkylthiophenes and the other identified products of the lecithin-Maillard interaction did not have meat-like aromas.
Comparison of Different Lipids

The original work on defatted meat suggested that phospholipids rather than triglycerides were important in determining meat flavor and the participation of phospholipids in the Maillard reaction between amino acids and ribose has been clearly demonstrated in the model systems. In mammalian tissue the structural phospholipids contain significant amounts of polyunsaturated fatty acids, in particular arachidonic (20:4), docosapentaenoic (22:5) and docosahexaenoic (22:6). Beef triglyceride fat contains less than 2% polyunsaturated fatty acids, mainly linoleic (18:2) and, although pork contains higher levels of linoleic acid, the triglyceride lipids of the different meat species do not contain significant amounts of C20 or C22 polyunsaturated fatty acids. Differences in fatty acid composition may be one feature of phospholipids which will result in a different behaviour from triglycerides in Maillard systems, while the other feature is the polar nature of the phosphatidyl group.

The observations on the aromas from cysteine + ribose reaction mixtures have been extended to compare the effect of different lipids: triglycerides and phospholipids extracted from beef, and commercial egg lecithin (phosphatidylcholine) and egg cephalin (phosphatidylethanolamine) (L.J. Salter; D.S Mottram, unpublished data). The inclusion of the beef triglycerides (TG) did not appear to have any effect on the aroma of the cysteine + ribose reaction mixture, which was sulfurous with an underlying meatiness. However, when beef phospholipids (PL) were used the meaty aroma increased markedly. Similarly, addition of egg lecithin (LEC) or egg cephalin (CEPH) to the cysteine + ribose reaction mixture gave increased meatiness, with the cephalin-containing mixture being judged to have the most meaty character.

The effect of these different lipids on the formation of selected volatiles has also been evaluated (Table I). Many compounds

<table>
<thead>
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<th>Compound</th>
<th>No Lipid</th>
<th>Beef TG</th>
<th>Beef PL</th>
<th>Egg LEC</th>
<th>Egg CEPH</th>
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<td>2-Furanmethanethiol</td>
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<td>1</td>
<td>11.02</td>
<td>3.97</td>
</tr>
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</table>

*a tentative identification
derived from the basic cysteine + ribose system were reduced in the presence of lipids, with the phospholipids in general having more effect than the triglycerides. The alkylthiophenes and other compounds resulting from the interaction of lipids with Maillard intermediates were only found in trace amounts in the presence of triglycerides, while considerably larger amounts were found in all the phospholipid-containing mixtures.

Conclusion

Lipids play an important part in the development of aroma in cooked foods, such as meat, by providing a source of reactive intermediates which participate in the Maillard reaction. Phospholipids appear to be more important than triglycerides. The addition of phospholipid to aqueous amino acid + ribose mixtures leads to reductions in the concentrations of heterocyclic compounds formed in the Maillard reaction. This effect could be due to lipid oxidation products reacting with simple Maillard intermediates, such as hydrogen sulfide and ammonia, to give compounds not normally found in the Maillard reaction. The precise nature of the odoriferous products obtained from lipid - Maillard interactions is dictated by the lipid structure and may depend on the fatty acid composition and the nature of any polar group attached to the lipid.

Literature Cited


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