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EFFECT OF LIPIDS ON FLAVOR DEVELOPMENT IN A MAILLARD REACTION MODEL SYSTEM

by

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ABSTRACT

EFFECT OF LIPIDS ON FLAVOR DEVELOPMENT IN A MAILLARD REACTION MODEL SYSTEM

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Maillard reactions between amino acids and reducing sugars occur in foods during cooking, and volatile compounds developed in these reactions contribute to the flavor. Sulfur-containing compounds generated via the Maillard reaction are important to meat flavor. However, the formation of these compounds is influenced by the presence of lipids during heating. This study investigated the effect of phospholipids of different degrees of unsaturation on flavor development in a Maillard model system containing cysteine and ribose. The influence of antioxidants on this system was also studied.

A headspace analysis method was developed using Tenax traps to collect volatiles which were subsequently desorbed into a solvent by a combination of heat and solvent extraction. More than 90 compounds were isolated from a Maillard model system containing cysteine and ribose heated at 140°C for 1 h. Thirty-one compounds were identified by their Kovats indices and mass spectrometry. Compounds identified included thiophenes, thiophenone, thiopyran, bicyclic thiophenes, thiazole, disulfides, heterocyclic thiols, thiolanone, mercapto carbonyls, methylthio acetates, and non-sulfur-containing compounds such as furan derivatives and sugar-derived carbonyls. Fifteen of them were selected as markers to evaluate the effect of lipids on the Maillard reaction.

Cysteine, ribose and phospholipids were heated at 140°C for 1 h to produce Maillard reaction volatiles. Sensory evaluation of the headspace of the heated reaction mixtures revealed that the aromas developed in the systems containing only cysteine and

ribose were more meaty and sulfury than those detected in systems containing phospholipids. More meaty and sulfury aromas were generated in the reaction mixtures containing phosphatidylcholine-distearoyl than in the systems containing phosphatidylcholine-dioleoyl. However, no discernible differences in aroma were observed between systems containing phosphatidylethanolamine and phosphatidylcholine.

Phospholipids suppressed the formation of selected Maillard reaction products. In addition, the degree of unsaturation of the phospholipids influenced the extent to which Maillard reaction volatiles were generated. However, the effects of phospholipids on the formation of volatile compounds was not related to the amino moieties of the phospholipids. Suppression of the formation of Maillard volatiles was also observed in model systems containing aldehydes.

Phospholipids isolated from nitrite-cured and uncured hams were heated in a model system with cysteine and ribose. The formation of Maillard reaction volatiles was affected less in the presence of phospholipids from cured hams. When antioxidants were added to the model systems of cysteine, ribose and phosphatidylcholine, the impact of the phospholipids on the Maillard reaction was reduced. A sensory evaluation of volatiles produced from the cysteine-ribose-phosphatidylcholine system indicated flavor differences as a result of adding antioxidants to the systems. These observations were confirmed by gas chromatographic - mass spectrometric analyses.

Carbonyl compounds were predominant in the flavor profiles of both cured and uncured hams. Of the carbonyl compounds identified, pentanal and hexanal were more abundant in the uncured hams. The addition of nitrite to hams reduced the formation of these carbonyl compounds. Two nitriles and two thiazoles were also detected in the hams cured with nitrite.

Dedicated to My Parents, My Sister and My Lovely Wife.

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TABLE OF CONTENT

CHAPTER 1

ANALYSIS OF VOLATILE COMPOUNDS PRODUCED FROM THE MAILLARD REACTION BETWEEN CYSTEINE AND RIBOSE

CHAPTER 2

EFFECTS OF PHOSPHOLIPIDS AND ALDEHYDES ON THE FORMATION OF MAILLARD REACTION VOLATILES IN MODEL SYSTEMS CONTAINING CYSTEINE AND RIBOSE

CHAPTER 3

THE EFFECTS OF STABILIZED LIPIDS ON THE FORMATION OF FLAVOR VOLATILES IN A HEATED MODEL SYSTEM CONTAINING CYSTEINE AND RIBOSE

CHAPTER 4

FLAVOR PROFILES OF CURED AND UNCURED HAMS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

FIGURES Page

REVIEW OF LITERATURE

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

INTRODUCTION

Flavor is one of the important sensory attributes that contribute to the overall acceptability of meat products. Raw meat itself has only a blood-like odor. However, it is a rich reservoir of non-volatile compounds with taste tactile properties, flavor enhancers, and aroma precursors. Flavor is perceived as the simultaneous stimulation of taste and odor senses caused by the volatile chemicals present in cooked meats (Shahidi, 1989).

Cooked meat flavor is principally derived from non-volatile water-soluble precursors (Hornstein and Crowe, 1960; Macey et al., 1964a, b; Hornstein and Wasserman, 1987; Shahidi, 1989, Mottram, 1994c), and fat-soluble constituents (Sanders et al., 1966; Sink, 1973; Shahidi, 1989). The heating process develops complex components which evoke characteristic sensory responses (Wasserman, 1979). On cooking, free amino acids and reducing sugars (e.g., glucose or fructose produced by glycogen breakdown) together with other low molecular weight water-soluble materials, react with one another to produce flavor compounds. The reaction between amino acids and reducing sugars is defined as the Maillard reaction. Products from one reaction may often become the precursors for others. Thus, the Maillard reaction and its subsequent reactions lead to the formation of a large number of volatile components, which are contributory to cooked meat flavor development (Shahidi et al., 1986). The oxygen-, nitrogen- and sulfur- containing heterocyclic volatiles contribute significantly to the overall desirable flavor of meat (Bailey, 1983; Shahidi, 1989; Werkhoff et al., 1993). In particular, sulfur compounds play a major role in meat flavor due to their distinctive olfactory

properties and generally low taste thresholds (Güntert et al., 1990). Although many volatiles are derived via lipid oxidation, it is believed that compounds produced from the water-soluble precursors are the major contributors to meat flavor (Nursten, 1986; Shahidi, 1989; Mottram, 1991).

Lipid is a major component in muscle foods and provides characteristic flavors after cooking (Hornstein and Crowe, 1960). Lipids are important in flavor development because of the oxidation products generated during cooking (Mottram and Edwards, 1983; Shahidi, 1989). Lipids also serve as a depot for fat-soluble compounds which are volatilized during heating and strongly affect flavor (Shahidi, 1989). Additionally, lipid oxidation products, such as carbonyl compounds, contribute to species-specific meat flavors (Mottram et al., 1982; Mottram and Edwards, 1983; Shahidi, 1989). For example, the "goaty" or "chickeny" flavors are strongly related to carbonyls, both qualitatively and quantitatively (Shahidi, 1989).

Lipids also are involved in the Maillard reaction. Suppression of Maillard reaction products was observed when polyunsaturated lipids, e.g., phospholipids were heated in a model system containing an amino acid and a reducing sugar. These results indicated that lipid oxidation derivatives may participate in the Maillard reaction (Mottram and Whitfield, 1987b; Whitfield et al., 1988; Farmer et al., 1989; Farmer and Mottram, 1990a, 1994). It was concluded that Maillard reaction intermediates (e.g., hydrogen sulfide or ammonia) react with carbonyl compounds derived from lipid oxidation, thus reducing the amounts of Maillard reaction products formed (Salter et al., 1988; Farmer and Mottram, 1990a).

It is generally believed that the curing process alters the flavor of fresh muscle by changing either the nature of the flavor volatiles, or the quantities of the individual volatiles formed. This modification is achieved through either the

addition of extraneous compounds such as salt or sugar, or by the reduction of flavor-producing components (Swain, 1972). The so-called cured meat flavor has actually been defined as the basic meat flavor which describes the flavor derived from precursors other than triacylglycerols (Cross and Ziegler, 1965). The differences in the volatile compounds contributing to cured and uncured meat flavor are quantitative rather than qualitative. For example, the concentrations of aldehydes are smaller in cured meats (Cross and Ziegler, 1965; Ramarathnam et al., 1991a, b), an observation which has been ascribed to lipid-nitrite interactions (Swain, 1972; Mottram, 1985a; Freybler, 1989). Oxidation of the unsaturated lipids results in the formation of carbonyl compounds which have been implicated as significant contributors to the flavor of uncured meat products, but not to cured meat flavor (Ramarathnam et al., 1991a).

The role of nitrite in cured meat flavor development has not been clearly defined. Nitrite functions as an antioxidant, thus preventing the formation of carbonyl compounds through lipid oxidation. Based on the studies of Farmer and Mottram (1990a), it is possible that nitrite stabilization of lipids in cured meats (Freybler et al., 1993) may play a significant role in modifying the flavor compounds generated by the Maillard reaction in heat-processed meat products.

This study is based on the hypothesis that the difference in the flavors of cured and uncured meat is due, in part, to the differences in the concentrations of carbonyl compounds and Maillard reaction heterocyclic compounds. Carbonyl compounds, generated through thermal processing (cooking), may modify the Maillard reaction and subsequently suppress the formation of heterocyclic compounds. Stabilization of meat lipids through the addition of nitrite should theoretically produce fewer carbonyl compounds during the cooking of meat. Therefore, the Maillard reaction between amino acids and reducing sugars should

proceed without competition from the carbonyl compounds and generate the optimum quantities of Maillard reaction products.

Specific objectives of this study are:

1. To develop a dynamic headspace analytical technique for the collection and identification of Maillard reaction products in a model system containing ribose and cysteine.
2. To identify some Maillard reaction volatile compounds as marker compounds in the studies of the impact of lipids on the formation of Maillard reaction products.
3. To determine the effect of the presence of lipids of varying degrees of unsaturation on Maillard reaction volatiles produced from heating a mixture of cysteine and ribose in a model system.
4. To ascertain the role of nitrite in cured meat flavor by characterizing the flavor profiles obtained on heating cysteine and ribose with phospholipids isolated from cured and uncured hams.
5. To investigate the effects of antioxidants on the formation of Maillard reaction products in a model system containing cysteine, ribose and phospholipids.
6. To compare the total flavor profiles of cured and uncured meat products.

LITERATURE REVIEW

Chemistry of Meat Flavor

General aspects of meat flavor

Flavor is defined as the sensation caused by those properties of any substance taken into the mouth which stimulate the senses of taste and smell, and also the general pain, tactile and temperature receptors in the mouth (Farmer, 1992). Odor and taste are the main contributors to flavor (Shahidi et al., 1986). Flavor is an essential sensory aspect of the overall acceptability of meat products. It is perceived as the simultaneous stimulation of taste and odor senses by the high molecular weight components and volatile compounds present in cooked meats. The flavor volatiles have a significant effect on the sensory acceptability of food even before it is consumed (Shahidi, 1989).

Meat has always constituted an important part of our diet. It is an excellent source of protein and provides certain essential vitamins and minerals. Its unique sensory properties make it the focus of culinary art in many cultures. Meat may account for nearly one-third of the total food expenditure in western societies, and therefore flavor becomes a very important component of its eating quality. Many studies have focused on understanding the chemistry of meat flavor. The very desirable characteristics of meat flavor have also been sought in the production of simulated meat flavorings, and these are of significant commercial importance in convenience and processed foods as well as in meat substitutes (Mottram, 1991).

Although a number of factors are known to influence the flavor of meat, no single group has been assigned a major role. Genetic as well as environmental factors are probably the most important (Shahidi et al., 1986). Of the former, the "species flavor" has the most pronounced effect. Diet is the most important

environmental factor, the type of diet and nutrient source exhibiting significant influence on the flavor of muscle foods (Shahidi et al., 1986; Monahan, 1992; Monahan et al., 1993). The amount and type of protein in the diet have little effect, but lipids do make a significant contribution (Sink, 1979). The method of cooking also affects the formation of meat flavor. Boiling, roasting, frying, and pressure-cooking, contribute significantly to the volatile compounds that are formed in the heating process and hence relate to differences in overall meat flavor (Shahidi et al., 1986).

Precursors of meat flavor

Meat flavor is principally derived from non-volatile precursors which are both water-soluble (Hornstein and Crowe, 1960; Macey et al., 1964a, b; Hornstein and Wasserman, 1987) and fat-soluble (Sanders et al., 1966; Sink, 1973; Lawrie, 1974; Shahidi, 1989). The development of meat flavor begins with raw meat which exhibits a simple blood-like odor (Shahidi et al., 1986). During cooking, a series of reactions occur between non-volatile components of the lean and adipose tissues that evoke characteristic sensory responses (Wasserman, 1979; Mottram, 1991).

The flavor of cooked meats is due to a combination of thermal degradation products of sugars, amino acids and nucleotides, and Maillard reaction and lipid oxidation products (Shahidi et al., 1986; Shahidi, 1989; Mottram, 1991). Meat tissue consists primarily of water, proteins, fats and carbohydrates, plus lesser quantities of non-protein nitrogen-containing compounds, minerals, trace amounts of vitamins and organic substances. On heating, these compounds react with one another to produce complex volatile mixtures that contribute to meat flavor (Hornstein and Wasserman, 1987). Though free amino acids constitute only about 0.1% of the fresh weight of beef muscle, these components appear to be

responsible for the flavor substances formed by the Maillard reaction on heating (Shahidi et al., 1986). Therefore, meat flavor precursors are low molecular weight water-soluble compounds. Higher molecular weight muscle fibrils and sarcoplasmic proteins are thought to be unimportant (Mottram, 1991). The amino acid and carbohydrate compositions of lean meat from different animals (beef, pork or lamb) are similar; and thus, similar aromas are produced when lean tissue extracts of the various species are heated (Hornstein and Crowe, 1960, 1963; Hornstein and Wasserman 1987; Shahidi, 1989; Mottram, 1991). Hornstein and Wasserman (1987) reported that no meaty aroma was produced on heating individually amino acid and reducing sugar fractions obtained from ion-exchange chromatographic separation of meat dialysis diffusates. When the two fractions were heated together, a meaty aroma was generated, thus demonstrating the importance of the Maillard reaction. The main flavor precursors are regarded as free sugars, nucleotide-bound sugars, free amino acids, peptides, nucleotides, glycopeptides, creatine and creatinine (Mottram, 1991).

The role of lipids in meat, both adipose tissue and fat contained within the lean portion, is to provide the species-characteristic aroma. Hornstein and Crowe (1963) and Hornstein and Wasserman (1987) found that, while aqueous extracts of beef, pork and lamb had similar aromas after heating; however, heating the fats yielded species-specific aromas. These investigators concluded that lipids provide volatile compounds with the characteristic flavors of different species, and that the lean gave a basic meaty flavor common to all species.

The interaction between compounds derived from both the adipose and lean tissues of meat may also contribute to flavor. The direct reaction of free amino groups of proteins and carbonyl compounds derived from lipid oxidation could produce odorous components (Wasserman and Spinelli, 1972). Pippen and

Mecchi (1969) suggested that hydrogen sulfide derived from sulfur amino acids reacts with carbonyls produced from the thermal degradation of lipids to form volatile compounds which contribute to chicken aroma. The involvement of phospholipids, the essential structural components of all cells, has been well documented in the development of warmed-over-flavor (Pearson et al., 1977). However, phospholipids also play an important role in the formation of desirable flavor. Mottram and Edwards (1983) demonstrated that lipids were essential for the development of cooked meat aroma, and that the phospholipids alone could provide sufficient lipid for meat aroma production. Removal of triacylglycerols from meat had only a minor effect on the formation of oxidative volatile compounds during cooking. However, the additional removal of phospholipids changed the aroma from a meaty to a roast or biscuit-like note. This change caused a significant increase in certain heterocyclic volatile compounds such as alkylpyrazines, alkylthiazoles and thiophenes (Whitfield et al., 1988). As the primary source of the heterocyclic compounds in food is the Maillard reaction, it appears that the involvement of phospholipids or their decomposition products in the Maillard reaction may play an important role in the development of the characteristic aroma of cooked meat (Mottram, 1991).

Reactions leading to meat flavor production

Many components in meat undergo a series of physical and chemical changes when heated, that are governed by temperature, duration of heating, and water content (Wasserman, 1979). Heat serves many functions including releasing flavor precursors from fat, allowing intimate mixing of fat and water-soluble components, and accelerating non-enzymatic browning reactions (Herz and Chang, 1970). The primary reactions during cooking that lead to the generation of cooked

meat flavor include pyrolysis of amino acids and peptides, carbohydrate degradation, interaction of reducing sugars with amino acids and peptides, degradation of thiamine, and thermal degradation of lipids (MacLeod and Seyyedian-Ardebili, 1981). More complicated reactions arise by secondary reactions which occur between the products of the initial reactions, and which give rise to a vast number of volatile compounds contributing to meat flavor (Mottram, 1991). Some of the reactions that produce flavor compounds are discussed briefly below:

A. Sugar degradation.

When sugars are heated, caramelized flavors are formed; but, in general, relatively high temperatures are required. At temperature above 150°C, a sugar molecule may lose a unit of water and form an anhydride. The anhydride from pentose can be further dehydrated to a furfural (Feather and Harris, 1973). Subsequent heating results in the formation of many odorous compounds, including furan derivatives, carbonyl compounds, and both aliphatic and aromatic hydrocarbons (Feather and Harris, 1973). Though the concentrations of sugars in meat are low, caramelization reactions can possibly occur on the surface of roasted and grilled meat when dehydration is accomplished (Mottram, 1991).

A major group of sugar degradation products, the furanones, are produced by aqueous degradation. For example, 2,5-dimethyl-4-hydroxyl-3(2H)-furanone results from the base-catalyzed degradation of fructose in a boiling aqueous solution (van den Ouweland et al., 1975). Hydroxymethylfuranones further react with hydrogen sulfide to produce volatiles with meaty aromas (Figure 1).

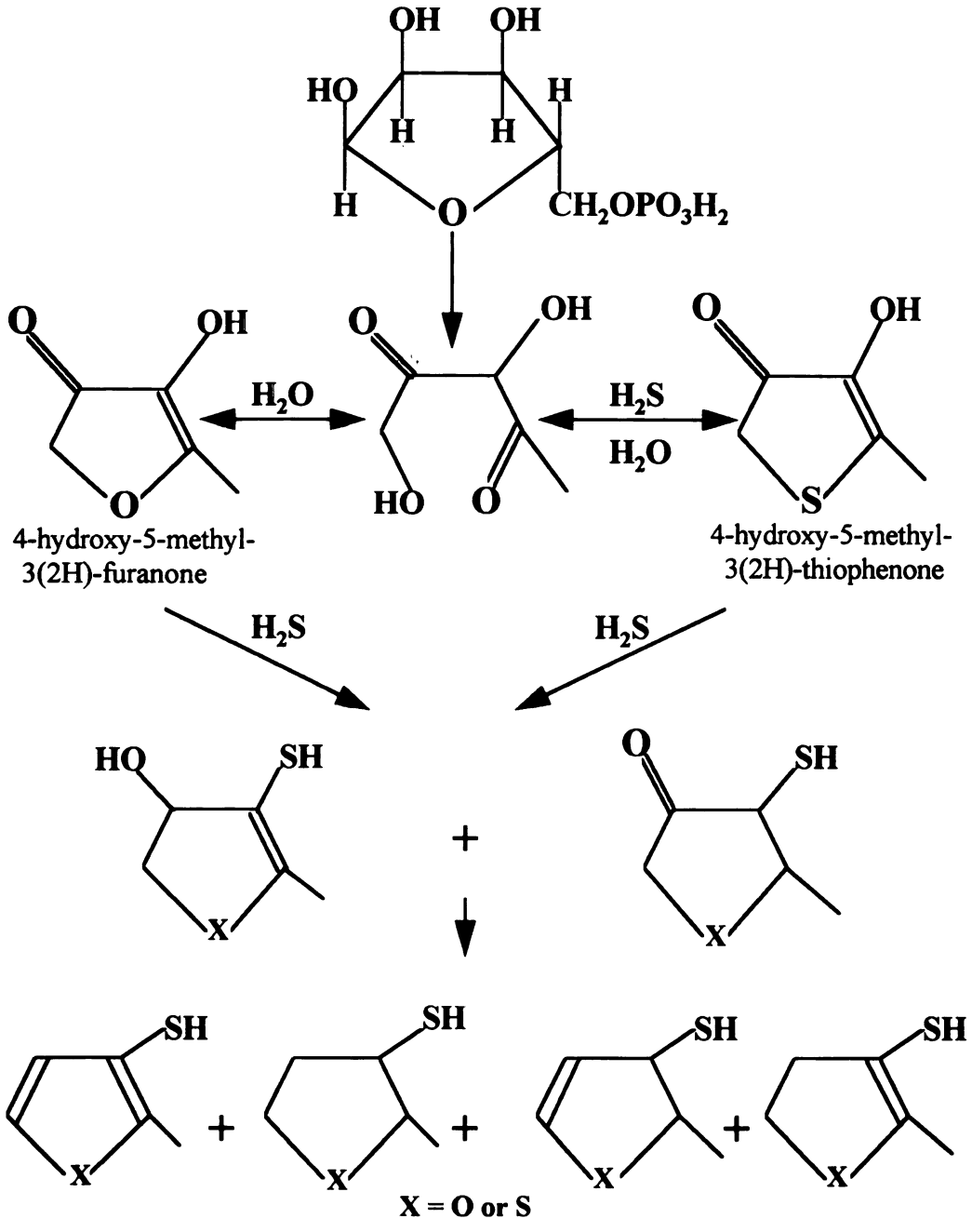


Figure 1 Formation of furan and thiophene derivatives from the reaction of 5-methyl-4-hydroxy-3(2H)-furanone with hydrogen sulfide (van den Ouweland et al., 1975).

B. Maillard reaction

The Maillard reaction between reducing sugars and amino acids is one of the most important routes to flavor compounds in cooked foods. The Maillard reaction does not require the high temperatures normally associated with sugar caramelization and protein pyrolysis (Hurrel, 1982). However, the reaction rate increases markedly with temperature, and the formation of flavor compounds generally occurs at elevated temperatures. The Maillard reaction occurs in aqueous systems, but it proceeds more readily in dried and concentrated foods with lower moisture contents (Hurrel, 1982). Therefore, flavor volatiles produced in meat by the Maillard reaction tend to be associated with those parts of the meat that have been dehydrated by the heat source (van den Ouweland et al., 1978). The Maillard reaction and its involvement in meat flavor formation will be discussed in more detail in a later section.

C. Strecker degradation.

The Strecker degradation is one of the most important elements associated with the Maillard reaction. It involves the oxidative deamination and decarboxylation of an α-amino acid in the presence of a dicarbonyl compound. The degradation results in the formation of an aldehyde containing one carbon atom less than the original amino acid and an aminoketone. Aminoketones can rearrange to produce several heterocyclic compounds such as pyrazines, oxazoles and thiazoles (MacLeod and Seyyedian-Ardebili, 1981; Vernin and Párkányi, 1982; Baines and Mlotkiewicz, 1984). Further details will be provided in a later section.

D. Lipid oxidation.

An important route of meat flavor generation during cooking is the thermally-induced oxidation/degradation of the unsaturated acyl chains in lipids. However, oxidation of these unsaturated fatty acids is also responsible for the development of some undesirable off flavors usually associated with rancidity (Mottram, 1991). Lipid oxidation during cooking plays an important role in the development of cooked meat aroma, and its oxidative products have been found predominantly in lightly grilled or boiled meat (Mottram et al., 1982; Mottram and Edwards, 1983). The role of lipid oxidation in meat flavor formation will be discussed in more detail later.

Flavor compounds in meats

In general, the flavor of cooked meat is due to a mixture of compounds including non-volatile or water-soluble compounds with taste-tactile characteristics, potentiators or synergists that enhance the flavor contributions of other agents, and volatiles which give rise to the odor properties (Shahidi et al., 1986). Nitrogen, sulfur and oxygen heterocyclic compounds, along with noncyclic sulfur compounds and hydrocarbons, are the predominant "meaty" flavor volatiles (Table 1). Heterocyclic aroma compounds in meat primarily arise from interactions between mono- and dicarbonyl compounds with hydrogen sulfide and ammonia. These carbonyl compounds are derived from the Maillard reaction, lipid oxidation, and aldolization reactions. The low-molecular weight intermediates, such as hydrogen sulfide and ammonia, are produced by thermal degradation of sulfur-containing amino acids, and pyrolysis of amino acids, respectively (Bailey and Einig, 1989).

Table 1. Volatile compounds with "meaty" aroma[a]

Compound	Number	Example	Flavor note
Sulfides Thiols (acyclic)	7	Mercaptan Methylthioethane	meaty (1-5 ppb) onion
(hydro) Furan with sulfur containing side chain	17	Furfuryl thiol; 5-methyl	meaty (0.5 - 1 ppb) sulfurous (> 1 ppb)
Thiophenes	11	Thiophene-2-methyl -3-thiol	roast meat
di-, tri-Thiolanes	5	1,2,4-Trithiolane	roast meat
Trithianes	3	1,3,5-Trithiane 2,4,6-Trimethyl trithiane	meaty
Thiazol(in)es	14	Thiazole	meaty, nutty, pyridine-like
Thialdines	4	Thialdines	meaty, roast beef
Pyrazine-furan sulfide	3	Furfurythio-2-(3-methyl) pyrazine	cooked meat (< 1 ppb)
Furans	2	Furan 2-Methylfuran	meaty, pleasant, slightly sulfurous sickly
Oxazol(in)es	2	Oxazole 2,4,5-Trimethyl oxazole	boiled beef, nutty, sweet, green
Ketones	4	Cyclopentanone 3-Methyl	roast beef
Hydrocarbons	1	n-Octane	meaty
Miscellaneous	5	2-Ethyl thiophenol	

[a] Shahidi, 1989.

Qualitative information on the presence of volatile compounds in different species is available in the literature (Table 2). For example, the proportion of sulfur compounds in beef (20% of the total number of compounds reported) is much higher than that in other meats. Few furans have been found in lamb, yet lamb contains more carboxylic acids than other species. The volatiles in chicken flavor contain a higher proportion of lipid oxidation derivatives (hydrocarbons, alcohols, carbonyl compounds), with aldehydes and ketones particularly abundant. Additionally, a large numbers of alcohols and phenols in cured pork are mainly due to phenols derived from the smoking of the meat (Shahidi et al., 1986; Mottram, 1991).

Over 100 hydrocarbons, mainly branched alkanes, alkenes and alicyclics have been reported in beef volatiles (MacLeod and Ames, 1986, 1987; Umano and Shibamoto, 1987; Vercellotti et al., 1987). Two classes of compounds that are believed to make important contributions to characteristic beef aroma have been recently reported by MacLeod and Ames (1987). One of these is a series of methyl-substituted cyclopentanones, cyclopentenones and cyclohexenones. The other group is the sulfur-substituted furans such as 2-methyl-3-(methylthio)furan, 2-methyl-3-furanthiol and the corresponding disulfides.

Volatiles from pork, including fried bacon and other cured and uncured pork products, are mainly aliphatic compounds, with aldehydes, acids and esters being the most abundant (Mottram et al., 1982, 1984; Mottram, 1984, 1985b; Ho et al., 1983). Some heterocyclic compounds have been reported in grilled pork and fried bacon, including pyrazines, pyridines, thiophenes, thiazoles, and oxazoles. Many of them have also been reported in beef flavor (Mottram, 1991). Phenols

Table 2. Volatile compounds of different chemical classes reported in cooked meats[a]

Compounds	beef	pork		lamb	chicken
		cured	uncured	mutton	
Hydrocarbons	193	39	37	43	84
Alcohols and phenols	82	64	25	20	53
Aldehydes	65	38	41	39	83
Ketones	76	32	31	20	53
Carboxylic acids	24	29	30	51	22
Esters	59	21	33	11	16
Lactones	38	8	12	14	24
Furans and pyrans	47	16	28	5	16
Pyrroles and pyridines	39	12	16	19	24
Pyrazines	51	22	44	16	22
Other nitrogen compounds	28	22	9	2	7
Oxazoles and oxazolines	13	3	1	4	5
Non-heterocyclic sulfur compounds	72	20	17	7	17
Thiophenes	35	4	15	2	7
Thiazoles and thiazolines	29	6	17	13	18
Other heterocyclic sulfur compounds	13	4	1	4	6
Miscellaneous compounds	16	7	4	1	11
Total	880	347	361	271	468

[a] Mottram, 1991.

and guaiacols have been found in bacon, and are believed to originate from the smoke treatment. A series of organic nitrates and nitriles have been reported in boiled bacon, and arosed from the interaction of sodium nitrite with lipids (Mottram et al., 1984; Mottram, 1984). Many of the compounds reported in chicken flavor have also been found in other meats, including hydrocarbons, acids, lactones, saturated aliphatic alcohols and ketones, pyrazines and oxazoles (Tang et al., 1983; Hartmen et al., 1984). The most noticeable feature of chicken flavor is the large number of unsaturated aliphatic aldehydes, alcohols and ketones found in both roast chicken meat and fat (Mottram, 1991). These meats contain significant numbers of thiazoles. Thiazoles have low odor threshold values, green and nutty aromas. These compounds may be important in both meaty aroma and the differences in aroma between species (Mottram, 1991).

The Importance of the Maillard Reaction to Meat Flavor

The Maillard reaction is one of the primary routes for the development of flavor associated with cooked meat and other heat-processed foods such as bread, cereal products, and roasted coffee (Apriyantono and Ames, 1990; Knoch and Baltes, 1992; Tressl et al., 1993; Mottram and Whitfield, 1994). The reaction is named after the French chemist Louis Maillard, who first described the formation of browning pigments or melanoidins upon heating a solution of glucose and glycine (Maillard, 1912). The Maillard reaction in food products is important for a number of reasons: it is involved in the production of color and flavor compounds, it reduces the nutritive value of foods by the involvement of essential amino acids such as lysine, it is also involved in the formation of heterocyclic aromatic amines, some of which are mutagenic and carcinogenic, and it may impart antioxidant

properties to heat processed foods (Nursten, 1986; O'Brien and Morrissey, 1989; Skog, 1993, Vernin et al., 1993). The carbonyl-amino reaction occurs widely during the heating or prolonged storage of foodstuffs, and is a major source of browning and flavor production (Hurrell, 1982). In food systems, the reactions normally occur between reducing sugars and amino acids or proteins, although aldehydes formed from lipid oxidation may react in a similar way with amino acids or proteins (Kwon et al., 1965; Montgomery and Day, 1965).

Chemistry of the Maillard reaction

The Maillard reaction can be divided into three stages. The initial stage involves a sugar-amine condensation reaction to form a glycosylamine which then undergoes rearrangement. The intermediate stage involves the dehydration of sugar, either by loss of three molecules of water to form furfural or by loss of two water to produce reductones, fission, mainly by dealdolization, and the Strecker degradation. The final stage consists of the conversion of carbonyl compounds into high molecular weight products such as melanoidins (Hodge, 1953). A simplified scheme of the Maillard reaction is shown in Figure 2 (Hodge, 1967).

I. Initial stage of the Maillard reaction

The first step involves the addition of the amine to the carbonyl group of a reducing sugar, followed by elimination of water to form a Schiff base. The Schiff base cyclizes to an N-substituted glycosylamine which undergoes an acid-catalyzed Amadori rearrangement to form the N-substituted-1-amino-1-deoxy-2-ketose (Figure 3). Amadori products are predominantly degraded to deoxyosones which are reactive α-dicarbonyl compounds and are involved in the formation of

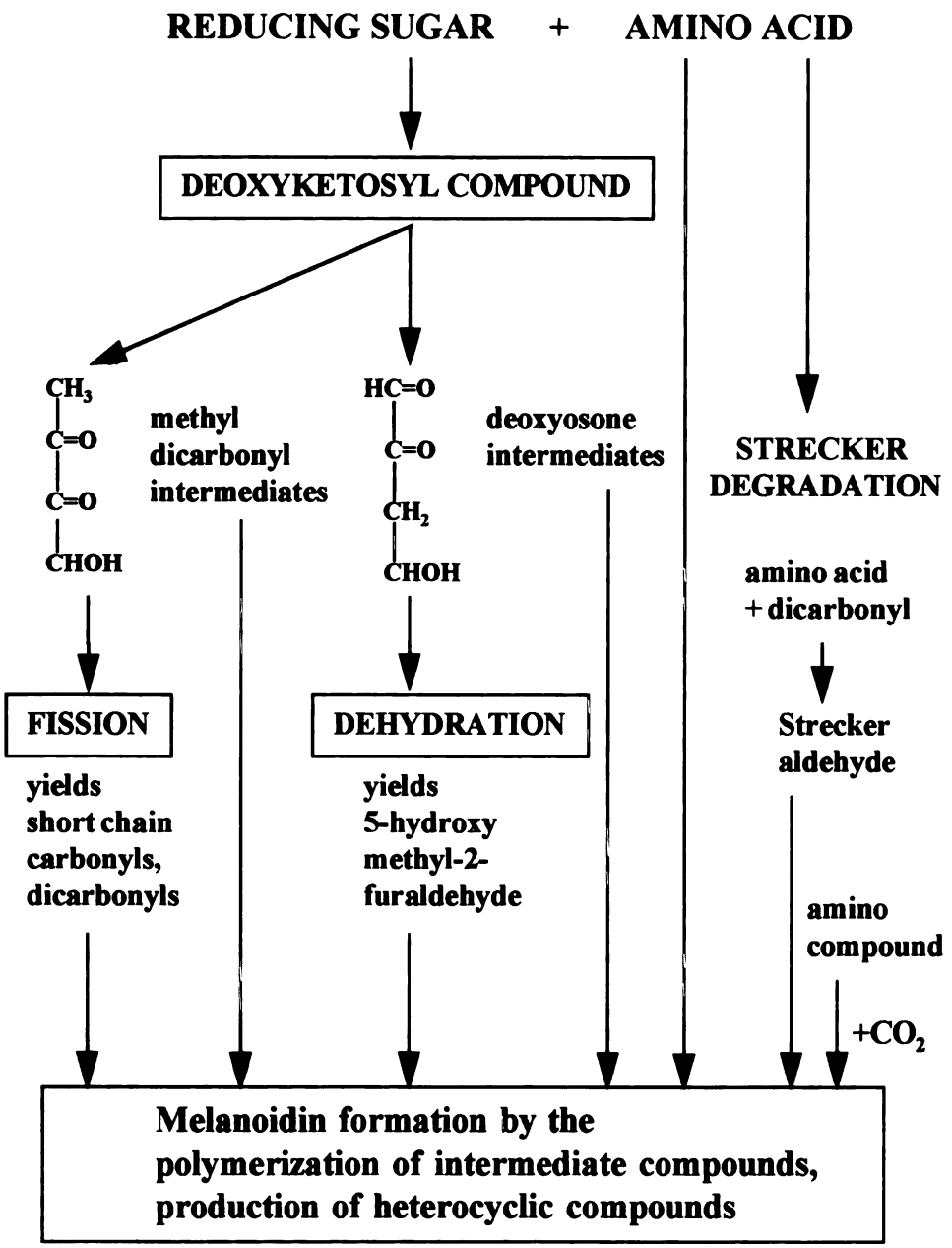


Figure 2 Simplified scheme of the Maillard reaction (Hodge, 1967).

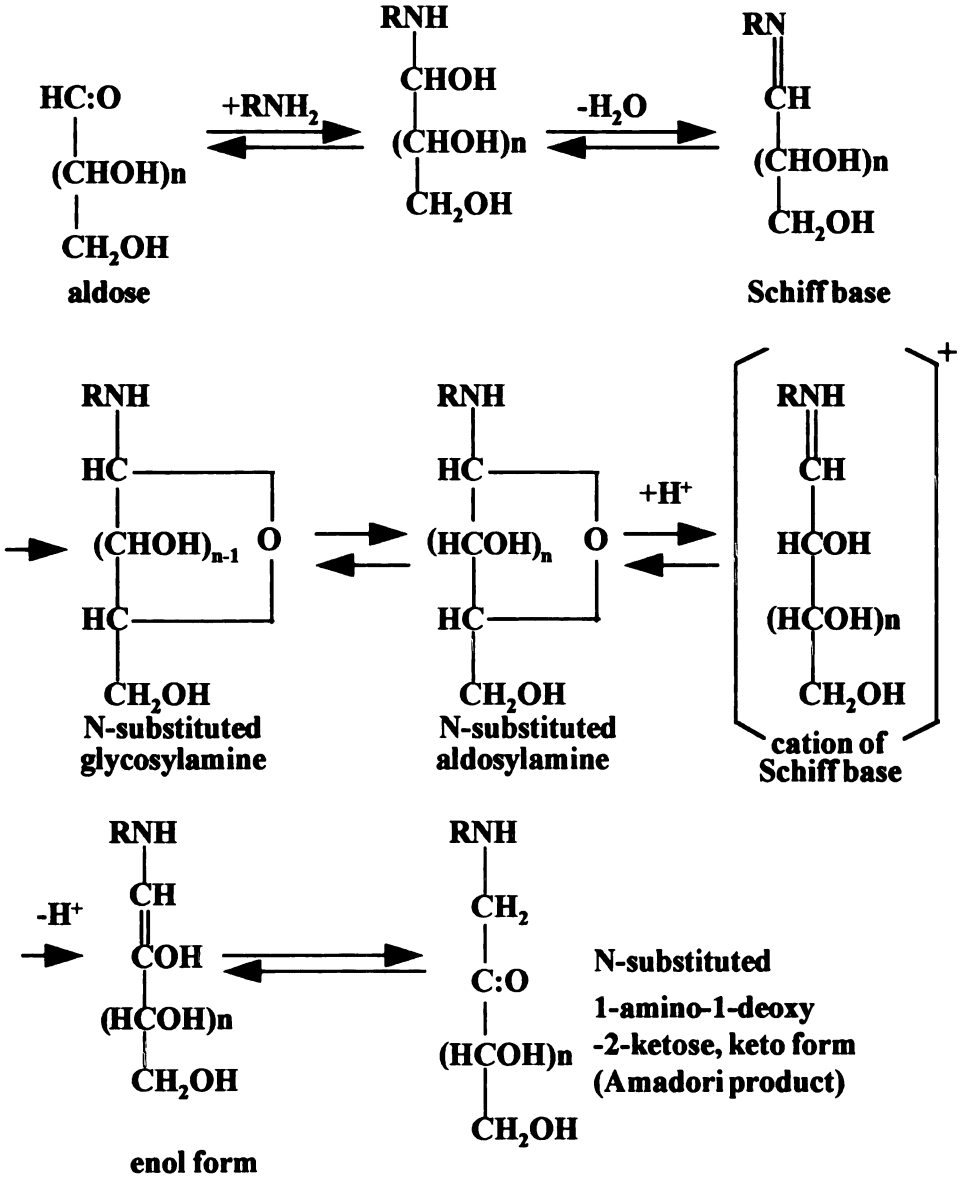


Figure 3. Initial steps of the Maillard reaction (Hodge, 1953).

many heterocyclic volatile compounds (Ledl, 1990).

The sugar-amine condensation is the first step in the so-called catalysis of amino compounds. After condensation and rearrangement, the sugar moiety is dehydrated and easily polymerizable, and unsaturated compounds are formed in which the amine moiety is labile (Hodge, 1953). In the condensation step, the amines act as a nucleophiles, their activities depending on the pH of the system (Ledl, 1990). Amines catalyze the enolization of sugar at pH values greater than 8, with the resultant production of low molecular weight fragments (Ledl, 1990). These early Maillard reactions do not cause browning or produce flavor compounds in food systems, although they may severely reduce the nutritive value (Hurrell and Carpenter, 1974).

H. Intermediate stage of the Maillard reaction

There are three main pathways in the advanced Maillard reaction, and all lead to the production of brown pigments or melanoidins. Two of these pathways begin with the Amadori compounds, while the third pathway is the Strecker degradation (Figure 2).

In the first pathway, the Amadori product undergoes 2,3-enolization which eliminates the amine from the C-1 position to form a methyl dicarbonyl intermediate (Hodge, 1953). This compound undergoes fission to produce C-methyl aldehydes, keto aldehydes, dicarbonyls and reductones (Hodge, 1967). The reaction products also include some flavor compounds such as acetaldehyde, pyruvaldehyde, diacetyl and acetic acid (Hurrel, 1982). In the second pathway, 3-deoxyosone, a compound formed from the enol form of the Amadori product by the elimination of the hydroxyl group at C-3, may lose three molecules of water to

yield furfural (Hodge, 1953). The reactions which follow the formation of these intermediates may result in the production of dark brown nitrogen-containing pigments. The reactions involved are the aldol condensation and aldehyde-amino polymerization, and result in the formation of nitrogen-containing heterocyclic compounds such as pyrazines, pyrroles and pyridines (Hodge, 1953). These compounds are largely responsible for the roasted, bready and nutty flavor of heated foods.

Strecker degradation

The Strecker degradation is a reaction where the amino acids undergo oxidative decarboxylation and deamination to produce carbon dioxide and Strecker aldehydes (Baltes et al., 1989). The reaction involves the oxidative degradation of free amino acids by the α-dicarbonyls and other conjugated dicarbonyl compounds produced by the breakdown of the Amadori product (Hurrel, 1982). Generally, the Strecker degradation takes place at temperature above 100^{o}C, due to the requirement of a relative high activation energy associated with the amino acid decarboxylation step (Rizzi, 1987).

The aldehydes formed from the Strecker degradation are a source of browning precursors. They can condense with themselves, with sugar fragments such as furfural and other dehydration products, and with aldimines and ketimines to form brown pigments (Hodge, 1953). With the liberation of carbon dioxide, aldehydes produced via the Strecker degradation contain one carbon less than the amino acid (Hodge, 1953). Maillard (1912) concluded that the carbon dioxide liberated in the Strecker degradation was from the carboxyl group of the α-amino acid, but not from the sugar radical.

With cysteine, the Strecker degradation yields hydrogen sulfide (Figure 4). Other than elemental sulfur, hydrogen sulfide is the simplest sulfur-containing chemical which can be obtained. In general, this chemical plays an important role in the formation of sulfur-containing heterocyclic compounds such as mercaptoketones, thiazoles and thiazolines, all of which contribute to meat and poultry flavor. It can also react with ammonia, aldehydes, dicarbonyl compounds, or carbonyls derived from lipid oxidation (Katz, 1981). Though the quantities of these heterocyclic sulfur compounds are not significant, they play an important role in the aroma of cooked meats due to their low threshold values (Guntert et al., 1990).

III. Final stage of the Maillard reaction

The major reactions involved in the final stage of the Maillard reaction are aldol condensation, aldehyde-amine polymerization, and the formation of heterocyclic compounds such as pyrroles, imidazoles, pyridines and pyrazines (Hodge, 1953).

With amine catalysts present, aldehdyes undergo an aldol condensation to produce melanoidins (Hodge, 1953). The condensation proceeds initially through the formation of the ordinary aldol between two molecules, then intramolecular condensation takes place to yield 2,5-dimethyl-*p*-quinone. The intermediate compound then decomposes to form color pigments at room temperature (Hodge, 1953). In the subsequent steps, reactions involve the interaction of furfural, furfuranones, and dicarbonyl compounds with other reactive products such as ammonia, hydrogen sulfide, and thiols. These reactions lead to the formation of

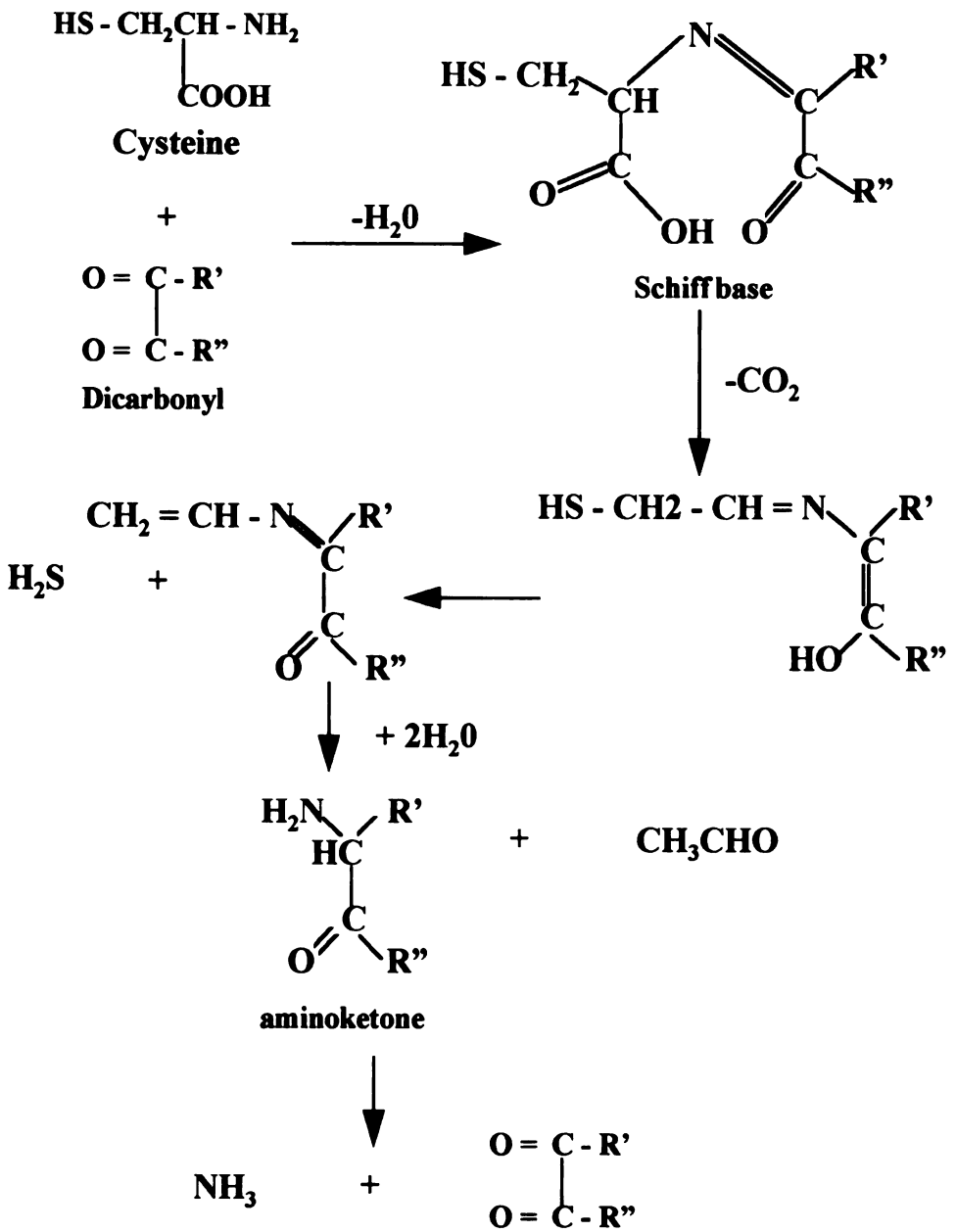


Figure 4. Strecker degradation of cysteine (Kobayashi and Fujimaki, 1965).

many important classes of heterocyclic flavor compounds such as pyrazines, thiazoles, thiazolines, oxazoles, thiophenes, and other sulfur-containing compounds (Mottram, 1991).

Factors influencing the Maillard reaction in foods

A. Duration and temperature of heating

Temperature has the greatest influence on the Maillard reaction, an increase in temperature resulting in an increased reaction rate (Leahy and Reineccius, 1989; Shu and Ho, 1989; Shaw and Ho, 1989; Ames and Apriyantono, 1994a; Spanier and Drum-Boylston, 1994). However, the Maillard reaction can also occur in foods stored at refrigerated temperatures (Whitfield, 1992). The Maillard reaction is also affected by the duration of heating. For example, Apriyantono and Ames. (1990) found that the amounts of furans, pyrroles, and furfural from the Lickens and Nickerson extraction increased in a model system containing lysine and xylose, and then decreased after 2 h. It is suggested that the time and temperature of heating influence the advanced stage of the Maillard reaction (e.g., the Strecker degradation), because high energy is required in such reactions (Hurrell, 1982).

B. Water content

The Maillard reaction and the Strecker degradation have maximum reaction rates at the intermediate moisture level in most food products (Eichner and Karel, 1972). The rate of the Maillard reaction increases from the dry state, starting at critical water activities of 0.2 - 0.3 for most foods to a maximum at water activities of 0.5 - 0.8, then decreases at higher water activities (Baisier and Labuza, 1992; Labuza and Baisier, 1992). The reaction rate follows zero-order kinetics, with the

rate constants being drastically reduced with the addition of small amounts of water (Peterson et al., 1994). The decreased reaction rates at higher water activities have generally been attributed to the dilution of the reactants. The decreased reaction rate at low water activities (< 0.1) has been ascribed to an increasing diffusion resistance which lowers the mobility of the reactants (Labuza et al., 1970). Therefore, the optimum Maillard reaction conditions are determined by the amount of water and state of water binding in a distinct system, and by the mobility of reactants (Mottram and Whitfield, 1995).

Since moisture loss is a typical feature of thermally processed foods, the water content strongly affects volatile production through the thermal reaction. Zhang et al. (1994) determined that the greatest concentrations of volatiles were produced in model food systems with water contents ranging from 20% to 50%. When the water content was greater than 50%, smaller quantities of volatile compounds were produced, which might be due to the substrate dilution effect or the inhibition of condensation steps (Zhang et al., 1994).

C. pH

Model systems studies have established that the Maillard reaction is affected by pH as both carbonyl and amino groups have the potential to be charged or uncharged depending on the hydrogen concentration of the system. For example, greatest quantities of pyrazine were observed at pH 9 - 10 (Bemis-Young et al., 1993). The pH of the system influences the extent to which the Amadori rearrangement products degrade by 1,2-enolization (Baltes et al., 1989; Ames and Apriyantono, 1994a). As the pH increases, the quantities of colored and polymeric compounds increase (Mauron, 1981; Ames and Apriyantono, 1994b). Alkaline conditions favor the formation of pyrazines, pyridines and some aliphatic sulfur

compounds including 2-acetylthiazole and dimethyl disulfide (Leahy and Reineccius, 1989; Mottram, 1990; Ames and Apriyantono, 1994a). However, the production of 2-furfural, furanthiols, methional and thermal degradation products of cysteine such as 2-thiophenethiol and trithiacycloheptene compounds, is reduced with increasing pH (Shu et al., 1985; Mottram, 1990). Some heterocyclic sulfur compounds (e. g., 1,2-dithia-4-one and furanmethanol) are largely unaffected by variation in pH (Mottram, 1990). In meat systems, meat is characterized by a pH value in the range of 5.5 - 6.0 and a high buffering capacity, therefore, very little change in pH occurs during cooking (Mottram, 1990). Thus, sulfur-containing heterocyclic volatile compounds may be predominant in meat products in which pH values are low (Ames and Apriyantono, 1994a).

D. Sugars

Only reducing sugars can take part in the Maillard reaction as they provide the necessary carbonyl groups (Hurrell, 1982). At 37°C and 15 % moisture, the order of reactivity of reducing sugars is ribose > xylose > glucose > lactose. Moreover, aldopentoses are more reactive than aldohexoses (Lewis and Lea, 1950).

The Maillard reaction is also influenced by the relative concentrations of sugars and amino acids (Warmbier et al., 1976; Shaw and Ho, 1989). The reaction rate increases linearly as the ratio of reducing sugar to free amino acid increased from 0.5 to 3.0. The rate does not change above this range. As the ratio increases, more sugar molecules may be in close proximity to the amino groups and overcome the diffusion barrier caused by the viscosity, thus increasing the reaction rate (Warmbier et al., 1976).

Maillard reaction products and meat aroma

The main types of heat-induced reactions leading to the formation of meat flavor volatiles from non-volatile precursors have been summarized as follows (van den Ouwland et al., 1978; Baltes et al., 1989):

1. Pyrolysis of amino acids and peptides.
2. Sugar fragmentation.
3. Interactions involving sugars, peptides, amino acids, or their degradation products (Maillard reaction).
4. Degradation and reaction of ribonucleotides.
5. Reaction of hydrogen sulfide, ammonia, and thiols with non-volatile and volatile components.
6. Oxidation, hydrolysis, dehydration, and decarboxylation of lipids.
7. Thiamin degradation.

Although many volatiles in meat flavor are derived by lipid oxidation, many researchers believe that the volatiles produced from water-soluble precursors may be the major contributors to meat flavor, especially heterocyclic compounds arising from the Maillard reaction (Bailey, 1983; Fors, 1983). Many researchers have concluded that oxygen-, nitrogen- and sulfur- containing heterocyclic compounds contribute significantly to the overall desirable flavor of meat (Bailey, 1983; Whitfield et al., 1988; Salter et al., 1988; Farmer et al., 1989). Lactones, acyclic sulfur-containing compounds (e.g. mercaptans and sulfides), non-aromatic heterocyclic compounds containing sulfur, nitrogen and oxygen (e.g. hydrofuranoids) and aromatic heterocyclic compounds containing sulfur, nitrogen and oxygen (e.g. pyrazines, thiazoles and furans) are probably the dominant contributors to meat flavor. In particular, sulfur compounds play a major role in

meat flavor due to their distinctive olfactory properties and generally low flavor thresholds (Guntert et al., 1990).

Classification of flavor compounds produced from the Maillard reaction

The aroma compounds produced in the Maillard reaction can be classified into three groups as follows (Nursten , 1986):

A. Simple sugar dehydration or fragmentation products:

furans

pyrones

cyclopentenes

carbonyl compounds

acids

B. Simple amino acid degradation products:

aldehydes

sulfur-containing compounds (including hydrogen sulfide, methanethiol)

nitrogen-containing compounds (including ammonia, amies)

C. Volatiles produced by further interaction:

pyrroles, pyridines, pyrazines

imidazoles, oxazloles

thiazole, thiophene

di-, trithiolanes

di-, trithianes

furanthiols

Oxygen-containing flavor compound from the Maillard reaction.

Furans with oxygenated substituents such as furfural and furanones, contribute to flavor of all heated foods and are among the most abundant products of the Maillard reaction. These oxygenated furans generally impart caramel-like, sweet or fruity characteristics to foods (Mottram, 1994a). However, they are important intermediates in the formation of other flavor compounds including thiophenes, furanthiols and other sulfur containing compounds (Ruther and Baltes, 1994).

Aliphatic carbonyl compounds, such as diacetyl which has a butter-like aroma, may contribute to the aromas derived from Maillard reaction. Many of the Strecker aldehydes also have characteristic sensory notes. For example, 3-methylbutanal which is derived from leucine, has a malt flavor (Mottram, 1994a).

Nitrogen-containing compounds.

Pyrazines are important aroma compounds and are believed to contribute to the pleasant and desirable flavor of many foods (Leahy and Reineccius, 1989; Maga, 1992; Weenen et al., 1994). Several mechanisms have been proposed for the formation of pyrazines in cooked foods including their genesis from α-aminoketones. Aminoketones undergo self-condensation or condensation with other aminoketones to form a dihydropyrazine which is further oxidized to the pyrazine (Figure 5). The alkylpyrazines generally have nutty, roast aromas with some eliciting earthy or potato-like notes. However, the odor threshold values of the mono-, di-, tri- and tetramethylpyrazines are all relative high (> 1 ppm), and these pyrazines probably only play minor roles in food aromas (Fors, 1983).

The other major nitrogen-containing compounds in food aromas are the pyrroles. Pyrroles have been reported to contribute to the caramel-like, sweet and

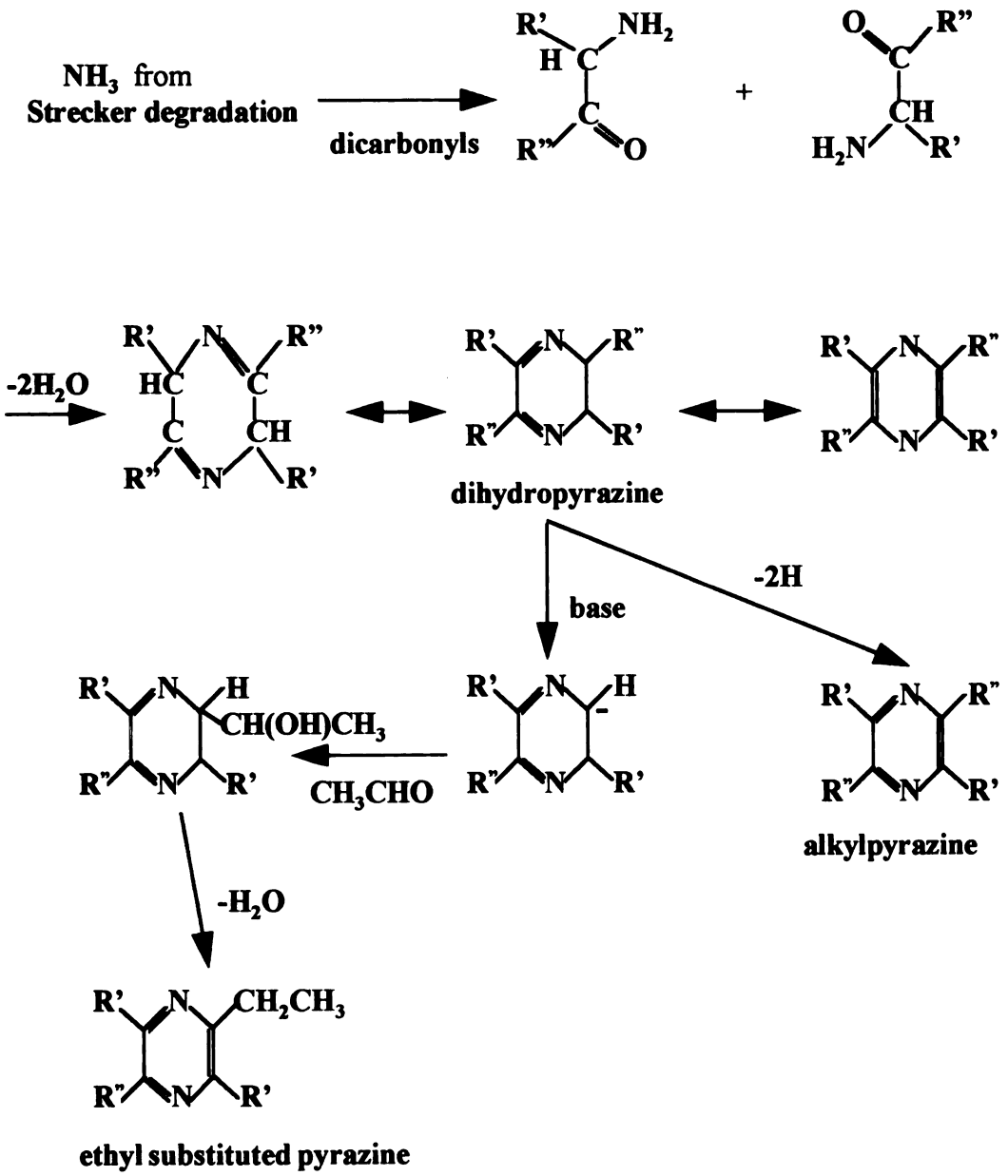


Figure 5. Pathway for the formation of pyrazine (Maga, 1992).

corn-like flavor (Fors, 1983), and may be important in defining roast beef aroma (MacLeod and Coppock, 1977). They may be formed from the reaction of 3-deoxyketose with ammonia or an amino compound followed by dehydration and ring closure (Mottram, 1991).

Sulfur-containing compounds.

Sulfur-containing compounds contribute both pleasant and unpleasant aroma to many foods. In meat, both aliphatic and heterocyclic sulfur compounds impart the characteristic flavor. MacLeod (1986) reported that 78 sulfur-containing compounds possess meat-like aroma, including 7 aliphatic and 65 heterocyclic sulfur compounds .

Thiazoles and thiazolines are more profilic in food flavors than their oxygenated analogues such as oxazoles and oxazolines, and have lower threshold values (Mottram, 1994a, b). Both groups of compounds are important constituents of food aroma, especially in roast, grilled or fried meat products (Maga, 1975; Mottram 1991). Some dialkylthiazoles have been reported in roast pork that are not present in other meats, while beef and chicken contain more trialkylthiazoles (Tang et al., 1983). The thermal degradation of thiamine is one of the sources of thiazole in heated foods. The formation of thiazole involves the reaction of hydrogen sulfide and ammonia with aliphatic aldehydes and dicarbonyl compounds (Figure 6), which is closely related to the pathway of oxazole (Mottram, 1994a).

A number of polysulfur heterocyclic compounds containing two or three sulfur atoms in five or six membered ring have been identified as meat flavor volatiles (Mottram, 1994a; Mottram and Madruga, 1994; Mottram et al., 1995).

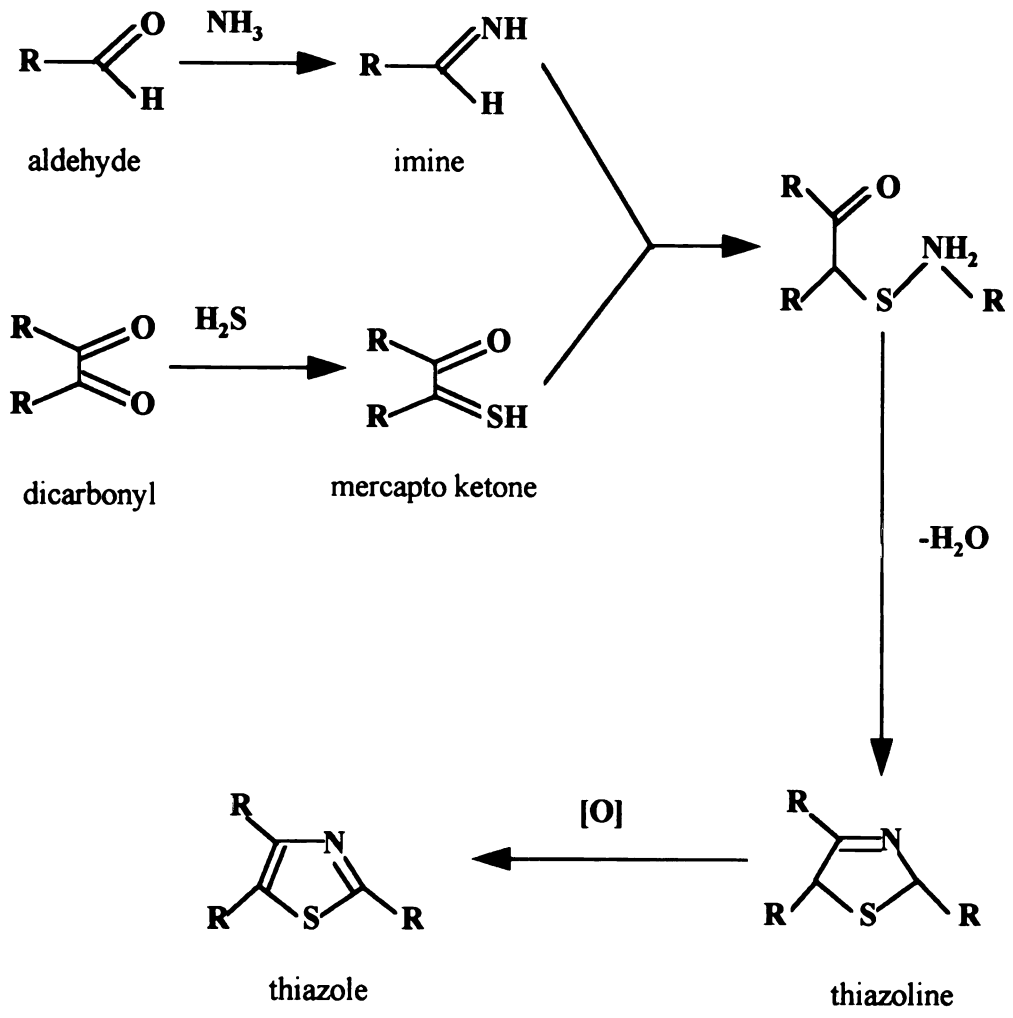


Figure 6. Formation of thiazole and thiazoline form the intermediates of the Maillard reaction (Vernin and Párkányi, 1982).

The most frequently found is 3, 5-dimethyl-1, 2, 4-trithiolane which was first isolated from boiled beef. Other sulfur-heterocyclics found in meat include trithioacetaldehyde, trithioacetone and thialdine (Mottram, 1991).

Thiophenes have odor threshold values in the low part per billion (ng/g) ranges. Thus, they are considered to be major contributors to the aroma of foods (Mottram, 1994a). Thiophenes with a thiol group in the 3-position possess meaty aroma characteristics. Several acylthiophenes have been reported in pork liver, but 2-acetylthiophene has been found only in chicken (Mottram, 1991). The thiophenes with long alkyl chains may arise from lipid sources, probably through the interaction of hydrogen sulfide with unsaturated fatty acids or their oxidation products. These compounds have been formed upon heating a model system containing cysteine, ribose and phospholipid (Whitfield et al., 1988; Farmer et al., 1989; Farmer and Mottram, 1990a). A pathway has been proposed that involves the addition of hydrogen sulfur across the 4-ene group in 2,4-dienal, followed by ring closure and dehydration (Mottram and Salter, 1989).

Role of Lipids in Meat Flavor

Significance of lipid oxidation in flavor formation

Lipids, proteins and carbohydrates are the major structural components of living cells. Lipids not only play a vital role in the metabolism of cells by providing a source of energy via oxidation, but also are an important source of food flavors (Ho and Chen, 1994). Lipids serve as a depot for fat-soluble compounds which are volatilized when foods are heated. This particular function of lipids strongly affects the flavor of cooked meats (Shahidi, 1989). In addition,

lipids may act as a solvent for the accumulation of flavor compounds during meat processing and cooking (Mottram and Edwards, 1983). Lipid oxidation products can also contribute to species-specific meat flavors, in that a "chickeny" or a "beefy" flavor is strongly associated with special carbonyl compounds (Shahidi, 1989).

There are many catalytic systems in foods that oxidize lipids such as light, temperature, enzymes, metal and metalproteins, and microorganisms (Vercellotti et al., 1992). As lipids oxidize, they form hydroperoxides which are susceptible to further oxidation or decomposition to secondary reaction products (Figure 7). Lipid oxidation affects the flavor, aroma, taste, nutritional value and overall quality of foods (Vercellotti et al., 1992; Ho and Chen, 1994); however, only the volatile lipid oxidation compounds impart the desirable meat aroma. Mottram et al. (1982) reported that alcohols and aldehydes were the predominant components in the volatile isolated from cooked beef and pork. These investigators further (1983) investigated the roles of triacylglycerols and phospholipids in the formation of meat aroma, and observed that the removal of the triacylglycerols had little effect on the aroma of the cooked meat or on the pattern of volatile compounds observed using gas chromatography - mass spectrometry. The additional extraction of phospholipids, however, eliminated the "meaty" character of the odor and resulted in a marked alteration of the volatile components. It was concluded that the presence of lipids is necessary for the development of the full meaty aroma. Phospholipids, being more susceptible to oxidation, appear to provide sufficient flavor formation, while triacylglycerols apparently are not essential. This is due to the unsaturated carbon chains of the lipids reacting with oxygen to form a number

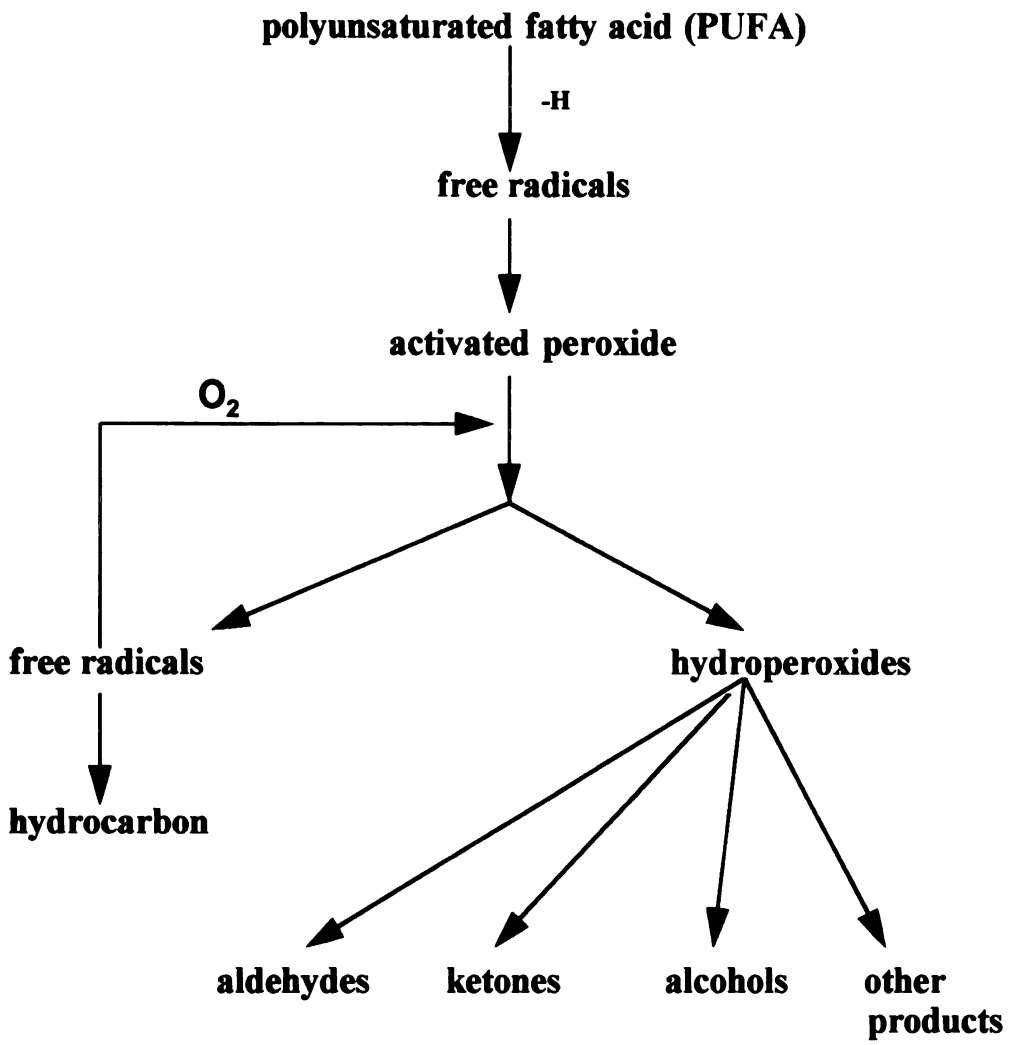


Figure 7. Mechanism of lipid oxidation and formation of carbonyl compounds (Shahidi, 1989).

of volatile compounds which can contribute either a desirable or an undesirable flavor (Mottram, 1987).

Mechanism of lipid oxidation

Lipid oxidation is generally considered to be a free radical process. The reaction of unsaturated lipids with oxygen to form hydroperoxides involves three basic steps, initiation, propagation and termination, as shown below (Frankel, 1980):

Initiation:

$$RH \xrightarrow{\text{initiator (heat, light)}} R\bullet$$

Propagation:

$$R\bullet + O_2 \longrightarrow ROO\bullet \quad \text{(fast)}$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \quad \text{(slow)}$$

$$ROOH \longrightarrow RO\bullet + OH\bullet$$

Termination:

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$$

$$ROO\bullet + R\bullet \longrightarrow ROOR$$

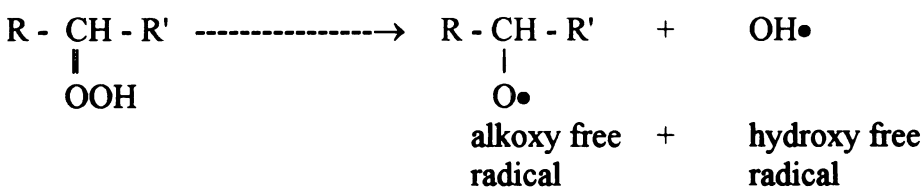
$$R\bullet + R\bullet \longrightarrow R\text{-}R$$

$$R\bullet\ (ROO\bullet + RO\bullet + OH\bullet) + AH \longrightarrow RH\ (ROOH + ROH + H_2O) + A\bullet$$

Initiation occurs when a labile hydrogen atom is abstracted from a fatty acid molecule (RH) in the presence of an initiator (such as heat or light) to form a carbon-centered alkyl radical (R•). This alkyl radical reacts rapidly with oxygen to form a peroxy radical (ROO•). The peroxy radical in turn is capable of abstracting

a hydrogen atom from another unsaturated fatty acid, and hence propagates the chain reaction. The propagation results in the formation of hydroperoxides (ROOH) which then decompose to a variety of secondary products. The termination step involves the interaction of free radicals, e.g. R• and ROO•, to form non-initiating and non-propagating products (Gray and Crackel, 1992; Ho and Chen, 1994). In the presence of antioxidants (AH), the chain-breaking antioxidants may donate hydrogen atoms to free radical species (R•, ROO•, RO•), and form less reactive products (e.g. RH, ROH, ROOH, A•), thus, terminate the propagating reaction (Monahan, 1992).

The primary site for oxidative attack is the methylene group (CH_2) adjacent to a double bond, with the initial step involving abstraction of a labile hydrogen from the allyl methylene group (Mottram, 1991). The resulting radical undergoes rearrangement prior to reaction with oxygen, giving rise to a number of different hydroperoxides (Figure 8). Hydroperoxides are the primary initial products of lipid oxidation, and are essentially odorless (Paquette et al., 1985). Degradation of hydroperoxides involves homolytic cleavage of the -OOH group, giving rise to an alkoxy radical and a hydroxyl radical (shown as below).



The alkoxy radical then undergoes β-scission on the C-C bond, with the formation of an aldehyde and an alkyl or vinyl radical (Mottram, 1991; Ho and Chen, 1994). A general reaction scheme for the formation of volatile aldehydes,

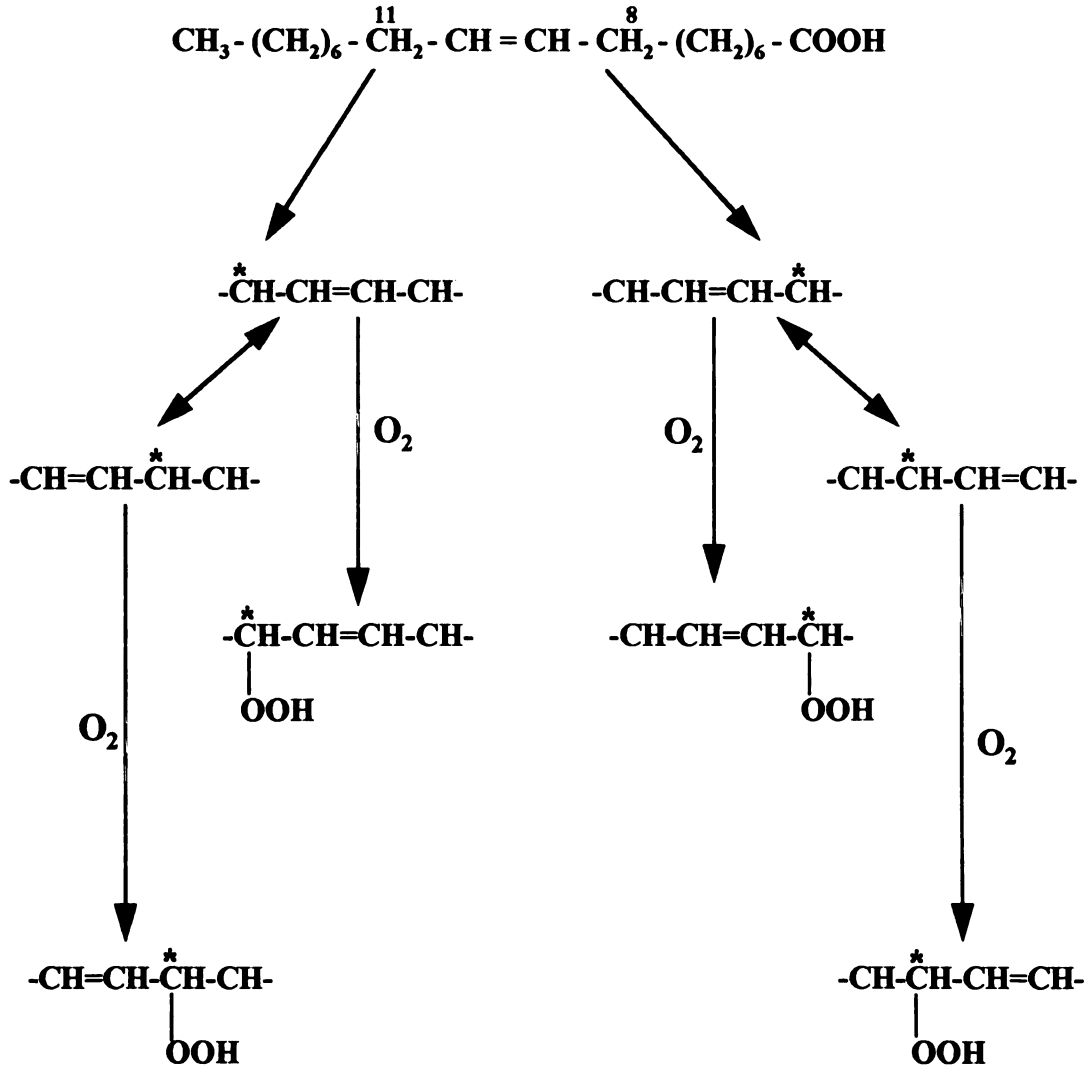


Figure 8. Hydroperoxides formed by the oxidation of oleic acid (Mottram, 1987).

alkenes, alkanes and alcohols is illustrated in Figure 9. These secondary lipid oxidation products are the main contributors to off-flavor in meats (Gray and Crackel, 1992).

Lipid oxidation during cooking

Lipids in meat undergo oxidation during cooking to produce a range of volatile compounds. Compounds found from the reaction are dominated by aldehydes, alcohols, furans and hydrocarbons, and are similar to those arising from the autoxidation of stored fats (Mottram, 1987).

In general, volatiles produced from the thermal oxidation of lipids in cooked meats contribute to the desirable flavor and do not impart off-flavors. However, if the off-flavors have already developed before cooking, they can't be removed by the cooking process (Mottram, 1987). Thus, subtle differences exist in the mechanisms of oxidation occurring during thermal processing and storage. These may be explained by the formation of hydroperoxides. The amounts of hydroperoxides in heated fat are always small because they are extremely heat labile. At low temperatures, hydroperoxides are more stable and may be found in significant high concentrations before degrading to other volatile compounds. This will lead to different proportions of the various radical intermediates, and will result in differences in the relative amounts of volatiles and varieties of compounds formed. A breakdown mechanism unique to the heated system is the oxidation of saturated lipids which does not occur at storage temperatures and will give compounds not produced by low temperature oxidation. These two systems will lead to the generation of a number of volatiles; however, both qualitative and

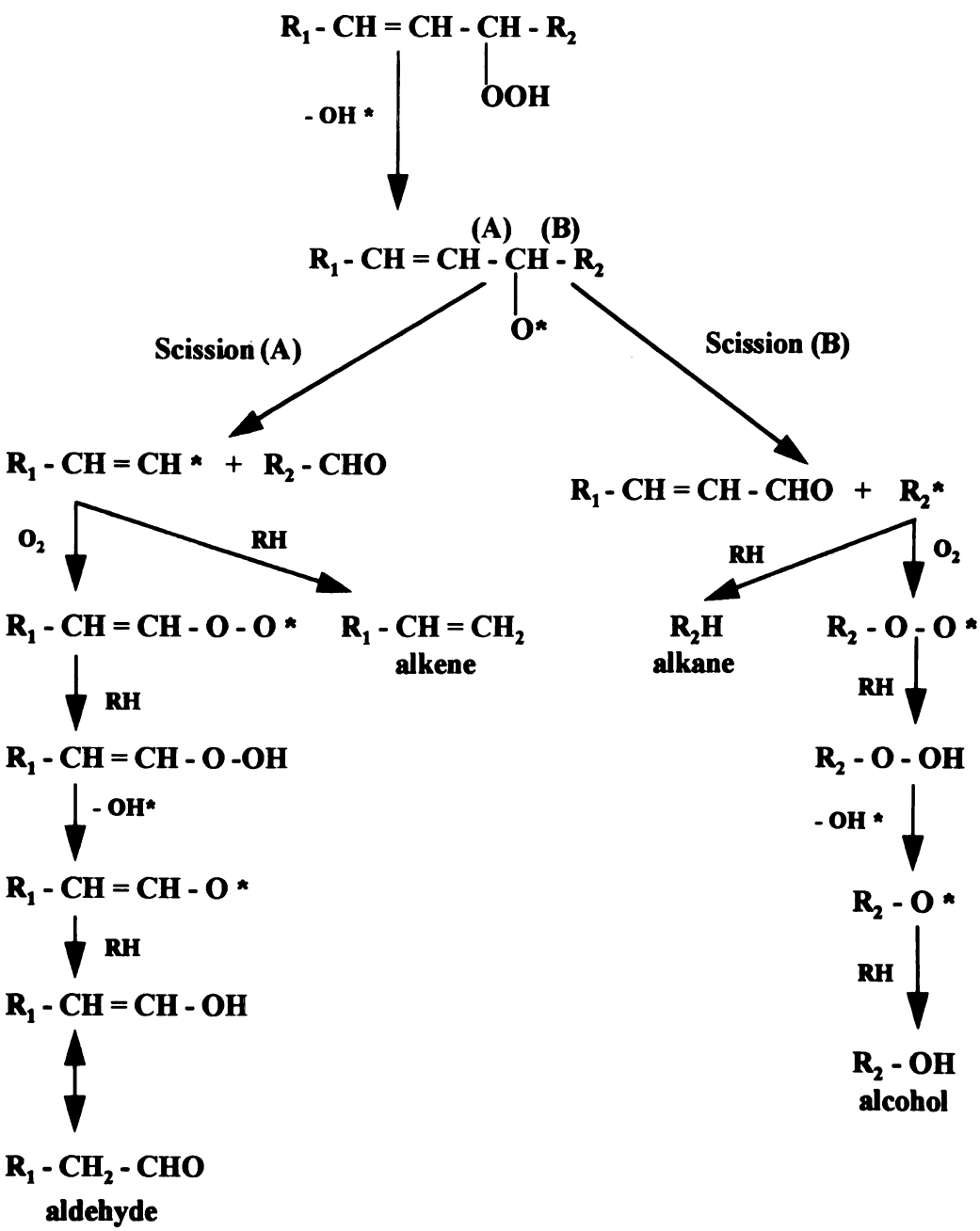


Figure 9. General reaction pathway for the hemolytic cleavage of hydroperoxides of unsaturated lipids (Ho and Chen, 1994).

quantitative are different (Mottram, 1987).

Interaction of Lipids in the Maillard Reaction

The main sources of flavor formation in heated foods are the Maillard reaction and the thermal degradation of lipids. Lipids, particularly the structural phospholipids, are essential for the development of the characteristic flavor in cooked meat, while the interaction of the products of lipid oxidation with Maillard reaction products is also an important route to flavor development (Mottram and Salter, 1989). The precise lipid composition of a food will affect the balance of the many flavor-forming reactions occurring during cooking and hence will influence the overall flavor and aroma of the food (Farmer and Mottram, 1994).

Though the interaction of lipid oxidation and Maillard reaction products plays an important role in meat flavor, lipids are capable of suppressing the formation of certain heterocyclic compounds by competing with sugar-derived dicarbonyl for the Maillard reaction intermediates (Farmer and Mottram, 1990a, 1994). Mottram and Whitfield (1987b) found that the removal of phospholipids, as well as triacylglycerols from meat products before cooking, resulted in marked differences in aroma and an increase in the concentrations of volatile heterocyclic compounds, particularly alkylpyrazines. The enhanced formation of these compounds implied that lipids or their degradation products may suppress their formation by participating in the Maillard reaction. This effect could be due to lipid oxidation products reacting with Strecker degradation products such as ammonia and hydrogen sulfide, thus reducing the extent of the Maillard reaction (Mottram and Whitfield, 1987b). There is also evidence of a distinct flavor change

in cooked food caused by the interaction of lipids with Maillard reaction products. For instance, Saittagaroon et al. (1984) found that defatted ground coconut lost its characteristic sweet aroma after roasting, and developed a burnt, nut-like aroma. The uncooked coconut contained significant amounts of lactones which provided the sweet aroma note. On roasting, pyrazines, pyrroles and furans were formed and added a strong nut-like characteristic to the sweet aroma of the unroasted coconut.

Studies of the involvement of lipid oxidation in the Maillard reaction

The formation of volatiles from the interaction between lipid and its oxidation derivatives with Maillard reaction intermediates have been studied in detail in many model systems. Examples are as follows:

A. Interaction of amino acid degradation products with carbonyl compounds.

The involvement of lipid oxidation carbonyls in the Maillard reaction was first reported by Barch (1952), who claimed that the reaction of gaseous hydrogen sulfide (a specific Strecker degradation product from cysteine) with an aliphatic aldehyde developed from lipid oxidation led to the formation of flavoring compounds. Though compounds responsible for flavor were not identified, onion- and bacon-like flavors were produced by this reaction. Other researchers have investigated the reactions of acetaldehyde with hydrogen sulfide and ammonia; acetaldehyde or propanal with hydrogen sulfide, methanethiol, ethanethiol or propanethiol; and 2-hexenal or 2,4-decadienal with hydrogen sulfide and methanethiol (Boelens et al., 1974). Volatile compounds generated from the reaction of saturated aldehydes with hydrogen sulfide at atmospheric pressure and temperature included dioxathianes and trithianes. Many of the compounds

synthesized in these studies have been found in flavor extracts, particularly those from alliums and boiled meat and beef broth (Boelens et al., 1974).

An extension of this study was the reaction of pentanal or hexanal, common lipid oxidation products, with ammonium sulfide (Hwang et al., 1986). Forty-seven compounds, including forty-two containing at least one hetero atom, were identified, and the most abundant compounds were 2,3,5-trialkylpyridines. Many of the compounds formed are present in the volatile extracts of fried chicken (Hwang et al., 1986).

The identification of heterocyclic compounds from reactions between fatty aldehydes and Maillard reaction products indicates that they could be formed in foods during cooking. Studies have shown that aldehydes arising via lipid oxidation, viz. pentanal, hexanal, 2-hexenal, 2-decenal and 2,4-decadienal, can readily enter into these reactions (Whitfield, 1992).

B. Interaction between amino acids and carbonyl compounds.

When heptanal was used to react with glycine at temperatures between 10 and 55°C, 2-alkenals were the only carbonyl compounds found (Montgomery and Day, 1965). It was suggested that the amino group of glycine acted as a basic catalyst in an aldol condensation reaction. These investigators concluded that the reaction could lead to the polymerization of carbonyl compounds generated during lipid oxidation, which in turn would lead to the depletion of carbonyl flavor components in some foods.

Zhang and Ho (1989) studied the volatiles produced from deep-fat frying foods by heating a mixture of 2,4-decadienal with cysteine in an aqueous system. A total of 45 compounds was obtained, including a large number of long-chain alkyl-substituted heterocyclic compounds such as thiophenes, pyridines, thiazoles,

and other sulfur-containing compounds. It was concluded that short-chain aldehydes such as acetaldehyde, butanal and hexanal, were probably derived from the decomposition of 2,4-decadienal. These aldehydes then participated in the formation of the heterocyclic compounds, although the mechanism is unclear.

The variety of volatile compounds formed during the reaction of aliphatic aldehydes with amino acids suggests that these reactions could be an important part of the interactions of Maillard reaction products and lipids. Many studies have shown the ease with which carbonyl compounds react with amino acids to form heterocyclic compounds (Ho et al., 1989; Zhang and Ho, 1989). In addition, those reactions involving 2,4-decadienal showed that the carbonyl compounds can be converted into a number of volatile compounds through degradation and condensation reactions (Whitfield, 1992). Future studies in this field should study the reaction of other major lipid oxidation products such as 2-alkenals and 1-alken-3-ones with amino acids. Such reactions could lead to the identification of many new potent flavor compounds, as yet not detected in cooked or processed foods (Whitfield, 1992).

C. Interaction of Maillard reaction products and triacylglycerols or phospholipids in model systems.

Ledl and Severin (1973) heated cysteine, xylose and tributyrin at 200°C to study the volatiles formed from the interaction of Maillard reaction products with lipids. Sixty-nine compounds were identified including 19 with sulfur, 13 with nitrogen, and 22 with both sulfur and nitrogen. Compounds identified included furans or furanones, pyrroles, pyridines, thiophenes, thiazoles, and a number of other heterocyclic compounds. The detection of five propylthiazoles and one propenylthiazole also indicated that the triacylglycerol components of the reaction

mixture could be involved in the formation of these compounds. Model system studies of the effect of lipids on the formation of flavor of roasted cocoa were investigated using different amino acids, fructose in water and deodorized cocoa-butter heated at 120°C for 3 h (Arnolidi et al., 1987, 1990). A total of 78 compounds including furans, pyrroles and pyrazines were identified; however, they did not report any information on the possible interaction between Maillard reaction products and lipids.

The effect of phospholipids on the formation of volatile heterocyclic compounds produced during the cooking of meat was investigated using model systems containing glycine, lysine or cysteine, and ribose that were heated in the presence and absence of egg yolk lecithin (Mottram and Whitfield, 1987, Whitfield et al., 1988). In the simple Maillard reaction mixtures (i.e., those without lecithin), alkylpyrazines were the predominant volatile products when either glycine or lysine was used as the nitrogen source. In the system with cysteine, sulfur-containing heterocyclic compounds such as alkylthiophenes and alkylthiazoles, were predominant. When lecithin was added to these Maillard reaction mixtures, the quantity of the heterocyclic compounds formed was decreased (Whitfield et al., 1988).

In general, the addition of lecithin to simple Maillard reaction mixtures exerted some quenching effect on the production of heterocyclic volatiles. For example, smaller quantities of the alkylpyrazines were produced in the presence of lipids; the trend was more obvious with the alkylthiophenes and alkylthiazoles (Whitfield et al., 1988). The decrease was due to preferential reaction of free ammonia or the amino acid nitrogen with compounds derived from lipid oxidation rather than with sugar-derived carbonyls. The aliphatic aldehydes were considered to be the most likely compounds to react with ammonia and amino acids to form

polymeric materials. In a continuation of these studies, efforts were made to investigate the effect of lipid composition on the production of specific compounds from the Maillard / lipid interaction (Mottram and Salter, 1989). It was found that the fatty acid composition of phospholipids, as well as the phosphatidyl group, would produce different outcome from that of triacylglycerols in defatted meat.

Farmer and Mottram (1990a) heated cysteine and ribose in the presence of beef triacylglycerols and three phospholipids (phosphatidylcholine (PC), phosphatidylethanolamine (PE) and beef phospholipid) to study the interaction between lipids and Maillard reaction products. They classified four groups of compounds which were produced as follows:

[A] *Compounds formed only in the presence of lipid*:

The greatest differences between the effects of the four lipids were observed for those compounds which required lipid precursors for their formation. Such compounds, e.g., 2-pentylpyridine, 2-alkylthiophenes, 2-akenylthiophens, were present in greater quantities in the systems containing the phospholipids. Of the three phospholipids investigated, systems containing PC produced the greatest amounts of the compounds.

[B] *Compounds produced from the reaction of cysteine and ribose that were suppressed by the presence of lipids*:

(a). Phospholipids showed greater suppression than beef triacylglycerols.

Heterocyclic compounds, e.g. mercaptocarbonyls, tended to be reduced considerably by phospholipids. These compounds were probably formed by the reaction of hydrogen sulfide with dicarbonyl or α,ß-unsaturated

carbonyl compounds (Takken et al., 1976). Reduction in their formation may be due to the removal of hydrogen sulfide from solution by its reaction with the polyunsaturated fatty acids of the phospholipids.

(b). PE and beef phospholipids showed greater suppression than beef triacylglycerols.

The quantities of some thiol-substituted compounds were markedly reduced in the presence of PC and beef triacylglycerols, and suppressed even more in the presence of PE and beef phospholipids. Compounds classified in this group include 3-furanthiols and 2-methyl-3-furanthiols.

(c). Beef triacylglycerols showed greater suppression than phospholipids.

Compounds suppressed include 2,3-dihydro-6-methylthieno[2,3]furan and isomers of thienothiophenes, as well as a series of methyl-substituted thienothiophenes and dihydrothienothiophenes. The amounts of some of these substances were reduced by phospholipids, but they were all strongly suppressed by the presence of triacylglycerols.

(d). Triacylglycerols caused similar reductions to phospholipids.

In contrast to the above compounds, only small variations between the different lipids were observed for other compounds. For example, with 3(2H)-thiophenones the effects of lipid were relative small, although there was a consistent trend towards reduced levels in the presence of all the lipids.

[C] *Compounds largely unaffected by lipid*:

Three dithianones were included in this group and their mechanism of formation has been proposed in relation to the thiophenones (Hartman et al., 1984). Other compounds such as certain thiazoles were reduced slightly by the addition of lipid, especially PC (Whitfield et al., 1988); however, the differences were small and within the range of standard deviation. Thus, no clear tendencies can be inferred.

[D] *Compounds not included in previous categories*:

2-furfural was markedly influenced by the presence of the various lipids. Concentrations were approximately halved by the addition of PC or PE, while beef triacylglycerols and beef phospholipids gave lesser extents of suppression (Whitfield et al., 1988). The addition of lipids would not be expected to affect the formation of furfural from ribose; however, in the presence of lipid degradation products further reactions may occur to form polymeric materials or volatile fragmentation products (Shibamoto and Bernhard, 1977). Two ethylmethyloxazoles also displayed patterns similar to that of 2-furfural.

Possible mechanisms for the interaction of lipid oxidation products with Maillard reaction products

The most likely mechanism by which lipids can interact in the Maillard reaction may be summarized as follows:

1. The reaction of lipid-derived carbonyl products with the amino group(s) of an amino acid and the ammonia produced by its Strecker degradation.

2. The reaction of the amino group of ethanolamine (in phosphatidylethanolamine) with lipid-derived carbonyl compounds.
3. The interaction of free radicals from peroxidized lipids in the Maillard reaction.
4. The reaction of hydroxyl and carbonyl lipid oxidation products with free hydrogen sulfide which is derived from the Strecker degradation of cysteine (Mottram and Salter, 1989; Farmer and Mottram, 1990a; Whitfield, 1992).

The different effects of the triacylglycerols and phospholipids may be due to the dissimilarities in fatty acid composition and the polar moieties of the phospholipids (Farmer and Mottram, 1990a). Less than 2% of the fatty acids in beef triacylglycerols contain two or more double bonds, while in beef phospholipids, at least 20% of the fatty acids are polyunsaturated (Farmer and Mottram, 1990a). Thus, triacylglycerols lack the most reactive precursors for both the formation of long chain heterocyclic compounds and for the reaction with Maillard reaction intermediates such as free hydrogen sulfide and ammonia. The participation of triacylglycerols in the Maillard reaction may also differ from that of phospholipids due to the fact that triacylglycerols are less miscible with the aqueous Maillard reactants than the phospholipids. It is possible that these physical effects limit the participation of triacylglycerols in the Maillard reaction in both model systems and meat (Farmer and Mottram, 1990a).

Cured Meat Flavor

Cured meats are distinctively attractive to consumers because of their pink color, characteristic texture and unique flavor. The curing process is achieved by adding a number of curing agents to the meat, each ingredient imparting its own unique characteristics (Gray and Pearson, 1984). The origin of the use of nitrite in the curing process is lost in history, but it is believed that the preservation of meat with salt has been used for many centuries. Kramlich et al. (1973) stated that salting of fish as a means of preservation can be traced back to before 3500 BC, and the use of nitrite was discovered probably through the presence of impurities in the salt. Currently, meat curing involves the addition of sodium nitrite and salt along with sugar, some reducing agents such as sodium erythorbate, phosphates, and seasonings to impart the characteristic properties to cured meat products.

Role of nitrite in cured meats

Nitrite is a unique curing ingredient that imparts many characteristics to cured meats, such as color, texture, and flavor. In addition, it has a potent antibacterial effect.

Nitrite produces nitric oxide in the absence of light and air, which then combines with myoglobin to form nitric oxide metmyoglobin. This compound is then reduced to nitric oxide myoglobin and is connected to nitrosohemochromogen under the influence of smoke or heat. Nitrosohemochromogen is the typical cured meat pigment which imparts the desirable pink color to cured meat (Kramlich et al., 1973; MacDougall, et al., 1975).

Nitrite also improves the texture of cured meat (Eakes et al., 1975) and has been reported to increase the firmness of country-style hams. Nitrite serves as an antimicrobial agent and prevents the outgrowth of *Clostridium botulinum* spores and the subsequent formation of a lethal toxin, particularly under conditions of mishandling (Hotchkiss and Gassens, 1987). In the aspect of cured flavor, Cho and Bratzler (1970) and MacDougall et al. (1975) suggested that nitrite reacts with tissue components such as thiol- and amino-containing compounds, to produce hitherto unidentified products which might contribute to the unique flavor. Moreover, nitrite provides oxidative stability to meat by preventing lipid oxidation.

Antioxidant role of nitrite in cured meats

A number of studies have shown that nitrite retards lipid oxidation or the development of warmed-over flavor (WOF) in cooked meat and meat products (Sato and Hegarty, 1971; Swain, 1972; Fooladi et al., 1979; Gray and Pearson, 1984; Ramarathnam et al., 1991a, b). Sato and Hegarty (1971) found that WOF was completely eliminated by adding nitrite at a level of 2000 mg/kg meat. Fooladi et al. (1979) confirmed the effect by demonstrating that nitrite (156 mg/kg) inhibited WOF in cooked meat with a 2-fold reduction in TBA (2-thiobarbituric acid) values for beef and chicken, and a 5-fold reduction in pork. Recently, Ramarathnam et al. (1991a) reported that the concentration of hexanal which is produced from lipid oxidation was approximately 400 times greater in uncured pork compared to cured pork. These results confirm the data of many previous studies that formation of hexanal was suppressed in the presence of nitrite (Cross and Ziegler, 1965; Swain, 1972).

The mechanism by which nitrite prevents or retards lipid oxidation in cured meat is not fully understood. Results suggest that more one mechanism is involved

(Gray and Pearson, 1994). Four possible mechanisms have been proposed as follows:

1. Stabilization of the unsaturated fatty acids by forming nitrite-derived antioxidants such as S-nitrosocysteine or nitrogen oxide myoglobin (Kanner et al., 1980).
2. Formation of a strong complex with the heme pigments, thereby, preventing the release of ferrous ion during processing / cooking with its attendant catalysis of the propagation stage of lipid oxidation (Igene et al., 1985).
3. "Chelation" of metal ions, e.g., ferrous ions, thus rendering them unavailable for catalysis of oxidation reaction (Morrissey and Tichivangana, 1985).
4. Stabilization of polyunsaturated lipids within the membranes (Freybler et al., 1993).

Formation of nitrite-derived antioxidants

The proposed mechanism involves the formation of certain nitrosyl compounds. For example, nitrogen oxide myoglobin can quench free radicals, thus lowering the amounts of prooxidants in the meat (Kanner et al., 1980). Other nitrite reaction products such as S-nitrosocysteine also possess antioxidant properties. Kanner (1979) demonstrated the antioxidant potential of this compound in an aqueous linoleate model system. Kanner et al. (1984) found that both hemin nitroxide and cysteine-iron-nitroxide have antioxidant properties. These compounds are believed to act in a similar manner as nitric oxide myoglobin by quenching free radicals.

Stabilization of the heme pigments

Zipser et al. (1964) first proposed that nitrite can form a stable complex with the iron porphyrins in heated meat systems, thus, preventing heme-catalyzed lipid oxidation. A study of the effect of nitrite on the stability of heme pigments in beef extract was acomplished by Morrissey and Tichivagana (1985). They reported that extracts without nitrite contained greater amounts of free irons after heating; however, beef extracts with nitrite were not found any changes in the amounts of free or heme irons after heating. They also suggested that nitrite reacted with myoglobin to form nitric oxide myoglobin, and a corresponding stable complex of nitric oxide hemochromogen was formed after heating, thus preventing the release of free iron.

Chelation of metal ions

It has also been proposed that nitrite can inhibit the lipid oxidation by chelating trace metals (MacDonald et al., 1980). When a small amount of nitrite (< 25 mg/kg) was added to a system containing linoleic acid, phosphate buffer, Tween 20 and ferrous iron, the amount of lipid oxidation was greatly reduced. Morrissey and Tichivangana (1985) also postulated that nitrite probably formed inactive 'chelates' or complexes with nonhaem iron, copper and cobalt. These studies used water-extracted muscle fibers to which was then added the various prooxidants (ferrous, copper and cobalt irons) and nitrite. The results indicated that nitrite at concentrations as low as 20 mg/kg, caused a significant reduction in TBA values. Maximum inhibition of lipid oxidation was observed when 50 mg/kg was used. Thus, the investigators concluded that nitrite must react with these ions, thus preventing lipid oxidation.

Stabilization of lipids

Pearson et al. (1977) suggested that nitrite stabilizes the membrane lipids in cured meat, thereby inhibiting lipid oxidation. Goutefongea et al. (1977) demonstrated that nitrite reacted with unsaturated lipids, and reported the amount of binding of nitrite was related to the degree of unsaturation of the lipids. They hypothesized that nitrite or a derivative reacted with one or more of the carbon-carbon double bonds in the adipose tissues. Liu et al. (1988) further investigated the stabilization of lipids in cured meats and concluded that nitrite reacted with unsaturated fatty acids to form nitro-nitrosite derivatives (Figure 10). Freybler et al. (1993) reacted unsaturated lipids with dinitrogen trioxide and found an increase of stability of these lipids to peroxidative changes. They also reported that phospholipids, and microsomes and mitochondria from cured pork were less susceptible to metmyoglobin / hydrogen peroxide-catalyzed peroxidation than their counterparts from nitrite-free pork samples. These results indicate that nitrite reacts with unsaturated lipids to form nitro-nitroso derivatives, thus stabilizing the lipids toward peroxidation changes (Freybler et al., 1993).

Chemistry of cured meat flavor

Although nitrite is clearly associated with cured meat flavor, the chemical changes for the unique flavor are not entirely understood (Gray and Pearson, 1984). Consequently, many researchers have attempted to identify the volatile compounds isolated from cured and uncured meat (Swain, 1972; Bailey and Swain, 1973; Mottram and Rhodes, 1973; MacDougall et al., 1975; Gray et al., 1981; Shahidi, 1989; Neol et al., 1990; Ramarathnam et al., 1991a, b, 1993a, b, 1994).

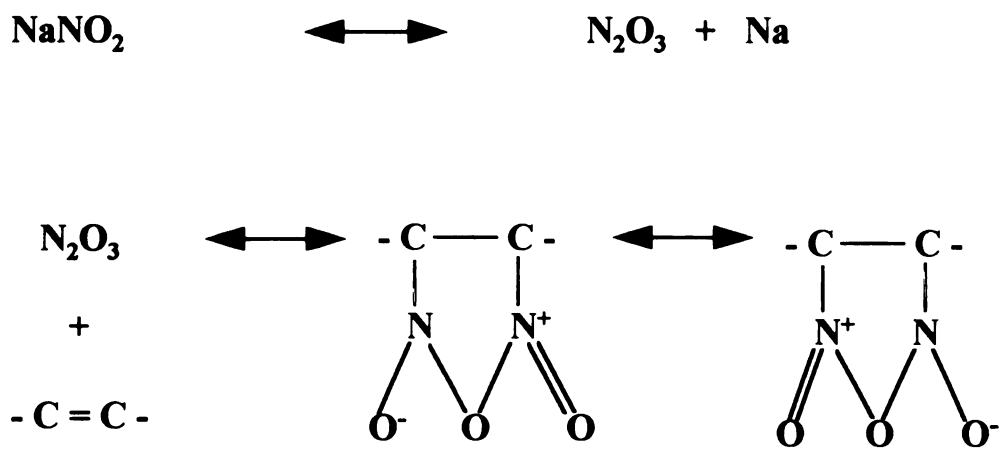
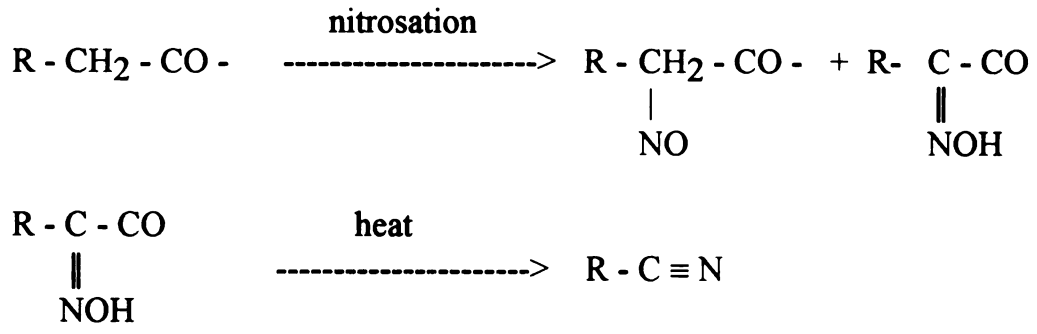


Figure 10. Mechanism for the formation of nitrite with polyunsaturated lipids (Liu et al., 1988)

Cross and Ziegler (1965) analyzed the volatile compounds from both uncured and cured ham and found quantitative differences. Pentanal and hexanal were present in appreciable quantities in the uncured product, whereas the cured hams contained smaller amounts. It was suggested that the smaller concentrations of these aldehydes in cured meats were responsible, in part, for the flavor differences between uncured and cured products. The investigators also found that the volatiles, after passing through a solution of 2,4-dinitrophenylhydrazine, still had a characteristic cured ham aroma regardless of whether they originated from cured or uncured ham. They concluded that "cured-meat" flavor was composed of essentially the same constituents that were generated by a combination of several reactions, in addition to suppression of lipid oxidation. Similar results were observed by Swain (1972), who also found that volatiles from hams treated with and without nitrite were qualitatively similar but quantitatively different.

Several alkyl nitrates, and aryl- and alkane-nitriles have been found in pork cured with nitrite (Mottram et al., 1973, 1978, 1984a, b, 1985a). The most likely origin of these compounds is from the reaction between fatty acids and sodium nitrite. The thermal oxidation of unsaturated fatty acids generally proceeds via a free radical mechanisms, thus alkyl nitrates could result from the participation of nitrous acid, or a free radical derived from nitrite, in these decomposition reactions (Mottram, 1984b). A mechanism for the formation of nitriles from the interaction between nitrite and lipids involves the C-nitrosation of a methylene group in the aliphatic chain of a fatty acid. The reaction is activated by an adjacent carbonyl, carboxyl or similar group. Thermal degradation of the resulting oxime could give the CN group as shown bellows.



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CHAPTER 1

ANALYSIS OF VOLATILES PRODUCED FROM THE MAILLARD REACTION BETWEEN CYSTEINE AND RIBOSE

ABSTRACT

A headspace collection technique was developed in this study. Volatile compounds produced in a Maillard model system containing cysteine and ribose were collected in a Tenax trap and then desorbed into ethyl acetate by a combination of heat and solvent elution. Percent recoveries of the internal standards, butyl acetate, methyl docanoate, thiazole, furfuryl mercaptan and 1-(2-thienyl)-propanone were 90%, 82%, 89%, 85% and 72%, respectively. More than 90 compounds were isolated, thirty-one of which were identified via Kovats indices and mass spectrometry. The compounds identified included thiophenes (8), thiophenone (1), thiopyran (bi-heterocyclic compound) (1), bicyclic thiophenes (3), thiazole (1), disulfides (4), heterocyclic thiols (3), thiolanone (1), mercapto carbonyls (2), methylthio acetates (2), and non-sulfur-containing compounds such as furan derivatives (3) and sugar-derived carbonyls (2). Fifteen of these compounds were selected as markers in subsequent studies to evaluate the effect of lipids on the Maillard reaction. This selection was based on previous literature findings from model systems involving lipids and their contribution to meat flavor.

INTRODUCTION

The Maillard reaction is one of the most important routes for the generation of flavor compounds in cooked foods. It is responsible for the production of many heterocyclic compounds with distinctive aromas and low thresholds (Mottram, 1994a). The Maillard reaction can be conveniently divided into three stages. The initial reaction is the condensation of an amino acid and a reducing sugar to form a glycosylamine which then undergoes the Amadori rearrangement. This is followed by the dehydration of intermediate endiol structures to form furan derivatives and dicarbonyl compounds. The final stage involves the conversion of the furan and dicarbonyl intermediates into aroma compounds by reaction with other intermediates such as amino compounds or Strecker degradation products (Baltes et al., 1989; Mottram 1994a).

More than 1000 volatile compounds developed in the Maillard reaction have been isolated. These aroma compounds have been classified into three groups based on the origin of the complex mixture of volatiles (Nursten 1981; 1986).

I. *Compounds derived from "simple" sugar dehydration / fragmentation.*

The predominant products consist of furans and furanones, pyranones, cyclopentenones and cyclopentanones with or without hydroxyl groups, and some aliphatic carbonyl compounds. Many of these compounds possess aroma and may contribute to food flavor. However, their roles are more important in serving as precursors for other flavor compounds.

II. *Compounds produced from "simple" amino acid degradation.*

This group comprises simple aldehydes, hydrogen sulfide or amino compounds which are formed via the Strecker degradation.

III. *Volatiles compounds generated by further interactions.*

Pyrroles, pyridines, pyrazines, imidazoles, oxazoles, thiazoles, thiophenes, di- and trithiolanes, di- and trithianes, furanthiols and compounds from aldol condensations are primary products in this group. These compounds are important to the flavor of meat products, and are particular, the sulfur-containing heterocyclic compounds (Mottram 1991; 1994a, b).

Model systems containing amino aicds and reducing sugars have been investigated extensively to determine the major compounds contributing to meat flavor (Whitfield, 1992). In an early attempt to synthesize meat flavor, cysteine, and amino acids or hydrolyzed proteins were heated with a pentose or hexose (Morton et al., 1960). Farmer and Mottram (1990a) established a model system containing cysteine and ribose to study the effect of three phospholipids and a triacylglycerol on the formation of Maillard reaction volatiles. The Maillard reaction products were collected by a purge-and-trap method, and the trapped compounds were then thermally desorbed into a gas chromatograph / mass spectrometer (GC / MS) system via a "Unijector" inject port. Solid carbon dioxide was also used to condense the volatile compounds in the front end of the GC column. Sixty volatile compounds were identified and used to demonstrate the effect of lipids on aroma production.

The purpose of this study was to develop a dynamic headspace technique to collect the Maillard reaction volatiles, and to isolate and identify these compounds using gas chromatography / mass spectrometry. Another objective was to identify

some Maillard reaction products to be used as "marker" compounds for the subsequent investigation of the role of lipids in modifing Maillard reaction flavors.

EXPERIMENTAL

Materials

Cysteine and ribose, straight chain alkanes (C_8 - C_{24}), butyl acetate and methyl decanoate were purchased from Sigma Chemical Co. (St. Louis, MO). Ethyl acetate, a solvent for the extraction of volatile compounds, and disodium hydrogen phosphate and sodium dihydrogen phosphate for phosphate buffer preparation, were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Tenax GC (60/80 mesh) was obtained from Alltech Associates Inc. (Deerfield, IL). A "flavors and fragrances kit" containing 24 heterocyclic compounds (Appendix A) and three sulfur-compounds (1-(2-thienyl)-propanone, 2-methyl thiophene, and furfuryl mercaptan) were used as standard compounds, and purchased from Aldrich Chemical Co. (Milwaukee, WI). The reagents and solvents used in this study were analytical and/or HPLC grade.

Preparation of Maillard reaction mixtures and collection of volatile compounds

Cysteine (0.61g) and ribose (0.75 g) were dissolved in 2 ml phosphate buffer (0.5M, pH 5.7), and placed in 10 ml clear glass vials (25 mm dia x 54 mm length, mouth 20 mm o.d. x 13 mm i.d.) (Pierce Chemical Co., Rockford, IL). The vials were flushed with nitrogen for 2 min before sealing with Tuf-bond Teflon-silicon discs and aluminum caps (Pierce Chemical Co., Rockford, IL). The reaction mixtures were heated at 140^{o}C in an autoclave under a pressure of 0.28

MPa for 1 h. After heating, the vials containing the Maillard reaction products were stored in a freezer (-10°C) prior to volatile collection.

Tenax traps were prepared by packing 200 mg of the Tenax GC porous polymer into a glass tube (5 mm i.d. x 70 mm length x 1 mm thick, custom made in the Glass Shop, Department of Chemistry, Michigan State University) and plugging both ends with glass wool. The Tenax traps were conditioned overnight in an oven at 180°C, with a nitrogen flow (30 ml / min) prior to volatile collection.

For the collection of the volatile compounds, the contents of the reaction vials (ca. 2 ml) were transferred to a 500-ml two-neck flask (Kontes, Vineland, NJ) containing 18 ml phosphate buffer (pH 5.7) (Figure 1). The flask was fitted with a L-shape glass joint (Kontes, Vineland, NJ), and the Tenax trap was attached by a stainless steel Swagelok reducing union (Cajon Ultra-Torr® fitting, Cajon, Macedonia, OH) to the outlet of the joint. During collection, the flask was heated at 60°C using a heating mantle, and the Tenax trap was maintained at room temperature (approximately, 20°C ± 2°C). The volatile compounds were swept into the Tenax trap using a stream of nitrogen (30 ml /min), and the collection was continued for 1 h. The flask was then removed and the Tenax trap directly connected to the nitrogen supply for 5 min to remove water vapor (Whitfield et al., 1988; Farmer et al., 1990a).

Desorption of trapped volatile compounds from the Tenax trap

A new desorption technique was developed in this study which involved the simultaneous use of heat and solvent extraction. This method utilized the properties of the Tenax polymer whose affinity for volatile compounds decreases at high temperatures. Solvent extraction was also used to elute the trapped compounds as it has been reported that there is no decomposition of Tenax in the

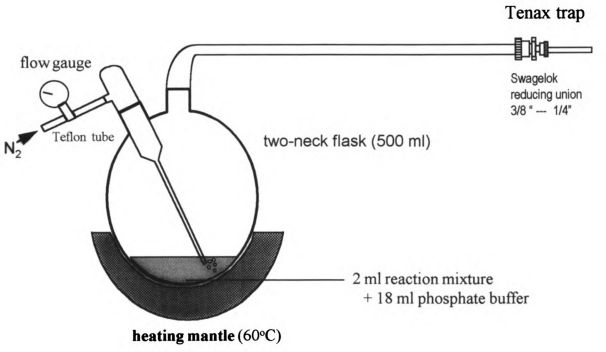

Figure 1. Apparatus used for the collection of the volatile compounds generated by heating cysteine and ribose at 140°C for 1 h.

absence of heat (Toyoda et al., 1993). In this study, the desorption process involved heating at 150°C (which is approximately 100°C lower than the temperature usually employed in commercial thermal desorption equipment) with simultaneous flushing of the Tenax trap with a stream of nitrogen saturated with solvent (ethyl acetate). The released compounds were then collected in a cold trap.

During desorption, the Tenax trap was connected between a solvent (ethyl acetate) source and a cold trap (Figure 2). The Tenax trap was wrapped with a heating strip and heated at 150°C. The nitrogen was passed through the ethyl acetate reservoir (30 - 40 ml/min), and then the ethyl acetate - nitrogen gas was introduced into the heated Tenax trap. The nitrogen stream was dried by passing through a desiccant (anhydrous calcium sulfate) (W. A. Hammond Drierite Co., Xenia, OH) before entering the ethyl acetate reservoir. The desorbed flavor compounds were then condensed in a cold trap using dry ice - acetone. The desorption process was continued for 30 min, after which the ethyl acetate extract was concentrated to 0.1 ml under nitrogen. The concentrate was transferred to a screw-cap vial and stored in a refrigerator prior to gas chromatographic (GC) / mass spectrometric (MS) analysis.

To measure the recovery of the trapped compounds, solution of ester compounds (butyl acetate and methyl decanoate, 1 μg each in 1 ml ethanol) and sulfur-containing heterocyclics (thiazole, furfuryl mercaptan and 1-(2-thienyl)-propanone, 5 μg each in 1 ml ethanol) were prepared. An aliquot (1 μl) of the internal standard solution was deposited into the trap using a 10 μl syringe (Hamilton, Reno, NV), and the solvent was removed by purging with nitrogen (30 ml / min) for 5 min. The recovery of each internal standard was calculated by comparing the relative GC peak areas of the compounds in the original standard

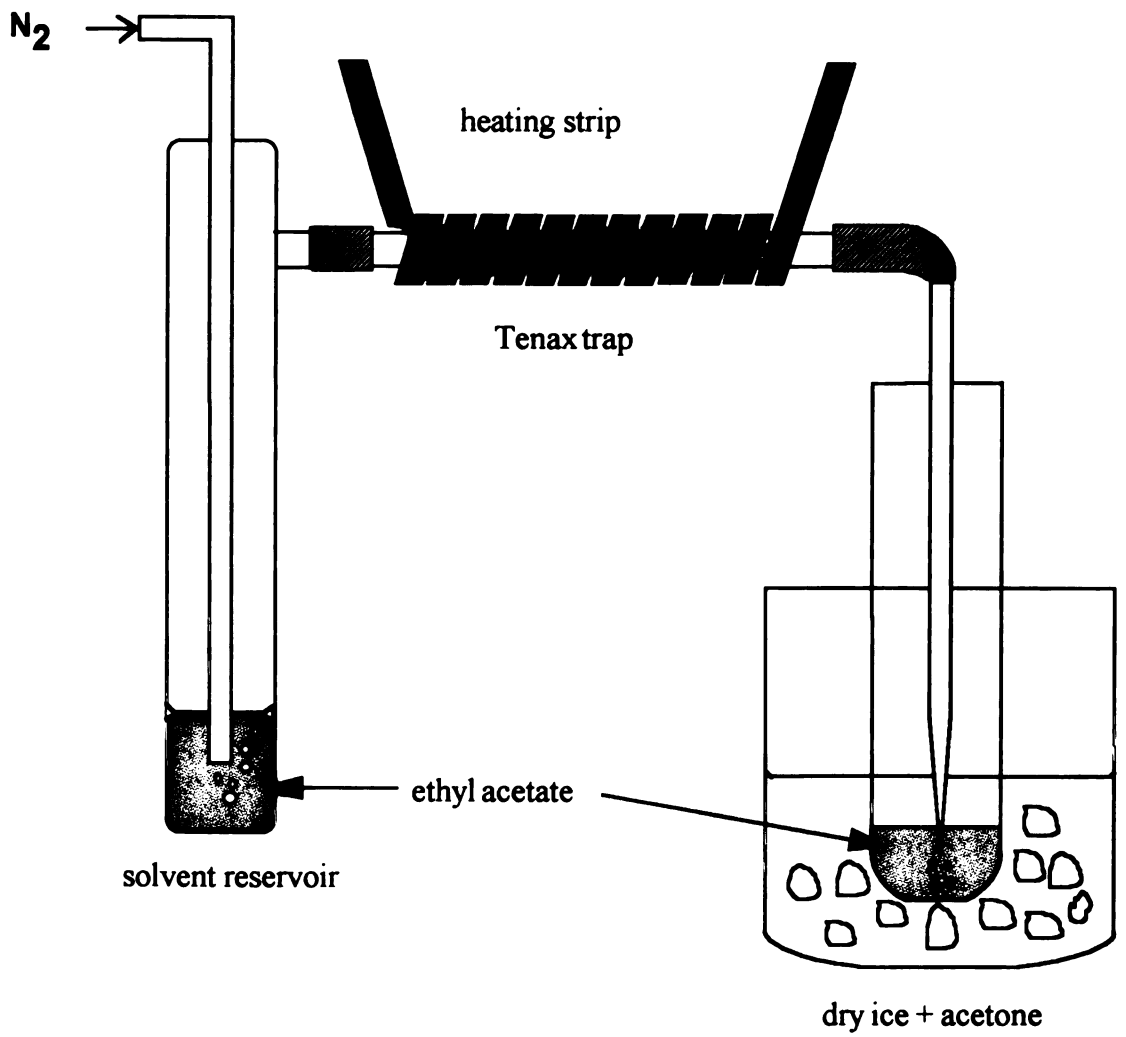

Figure 2. Desorption of the volatile Maillard reaction compounds from the Tenax trap. The Tenax trap was simultaneously heated at 150°C and extracted by an ethyl acetate-saturated nitrogen flow (30 ml/min) for 30 min. The solvent and desorbed volatile compounds were collected in dry ice - acetone cold trap.

solution to those of the compounds recovered from the Tenax traps. To measure the Kovats index (KI) of a compound, straight-chain alkanes (C_8 - C_{24}, 15 ng each) were added to the internal standard solution before applying to the Tenax traps. The KI of each compound was calculated using the retention times of the compound and the two adjacent n-alkanes.

Gas chromatographic - mass spectrometric analysis (GC / MS)

The volatile compounds were separated and identified using a HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) interfaced with a HP 5970 MSD (mass selective detector) mass spectrometer. The GC / MS system was equipped with a HP 59970 Chemstation Data System. A Carbowax 20M fused silica capillary column (60m x 0.32 mm i.d., Supelco Inc., Bellefonte, PA) was used for the separation of the volatile compounds. The GC oven temperature was programmed as follows: initial temperature, 40°C; initial time, 5 min; rate 2°C / min; final temperature, 200°C; final time, 10 min. Helium was used as a carrier gas with a flow rate of 10 ml/min. The temperatures of the injection port and transfer lines were both set at 220°C.

The MS was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250°C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 sec over a mass range of 40-300 was employed. The GC / MS data were monitored, stored and analyzed using a HP Chemstation data system.

The Maillard reaction products were identified by comparing their mass spectra and KI's to those of authentic standard compounds. Two mass spectral libraries were used in the study. One was the INRA Mass Spectra Library -

"INRAMASS", - a computer program created by R. Almanza (Laboratoire de Recherche sue les Aromes, Dijon, France) and donated by Professor D. S. Mottram (University of Reading, Reading , UK). The other was the NIST / EPA / MSDC Mass Spectral Database purchased from the ACS Publication Co., (Washington, DC). Where reference compounds were not available, identifications were made on the basis of mass spectral data.

RESULTS AND DISCUSSION

Development of analytical procedures for the identification of volatile compounds in a heated Maillard reaction system

A new desorption technique was developed by combining thermal desorption and solvent extraction. The desorption efficiency of the method was determined by measuring the recovery of internal standards. For ester compounds, the recoveries were 90 ± 8% and 82 ± 6% for butyl acetate and methyl decanoate, respectively. Recoveries of sulfur-containing compounds were 89 ± 5% , 85 ± 4% and 72 ± 7% for thaizole, furfuryl mercaptan and 1-(2-thienyl)-propanone, respectively. The difference in the recoveries obtained may be due to the relative affinities of the compounds for the Tenax absorbent.

The volatile Maillard reaction compounds were collected by a purge-and-trap method using Tenax trap as the absorbent (Whitfield et al., 1988). As the flavor/aroma compounds in food products are often present at concentrations too small to permit direct analysis by GC, a preconcentration step is normally required. Tenax (p-2,6-diphenyl-p-phenylene oxide), a porous polymer absorbent, has been widely used to concentrate headspace volatiles because of its sensitivity

and mobility (Toyoda et al., 1993; MacCaffrey et al., 1994). This absorbent has been reported to have low affinities for organic compounds at high temperatures (Buchholz et al., 1980; Barnes et al., 1981). Therefore, thermal desorption is routinely used to release the absorbed volatile compounds. A direct-inject type of thermal desorption system requires substantial heating (at temperatures in excess of 250°C) to completely desorb the trapped volatile compounds. This process frequently causes isomerization and decomposition of heat labile compounds (Toyoda et al., 1993). The solvent desorption method described here utilizes a lower temperature in order to minimize such changes. Solvent extraction, however, produces a dilution effect and results in the non-detection of compounds present in trace amounts. The desorption technique developed in this study combined thermal desorption and solvent extraction, and provided excellent recoveries of the internal standards. Additionally, no Tenax breakdown products (such as phenyl compounds) were observed using the method. Therefore, this new desorption procedure was applied in all subsequent studies.

Isolation and identification of Maillard reaction products

Cysteine and ribose were used as reactants to produce Maillard reaction products. The choice of cysteine as the Maillard reactant was based on the observation that sulfur-containing compounds are dominant in meat flavor, and that hydrogen sulfide, formed from the Strecker degradation of this amino acid, is essential for the formation of these compounds (Mottram, 1991).

More than 90 compounds were isolated from the reaction mixture of cysteine and ribose heated at 140°C for 1h. Thirty-one compounds were identified (Table 1), confirmation of their identities being achieved by Kovats indices (KI)

Table 1. Maillard reaction products isolated from a model system of cysteine and ribose heated at 140°C for 1 h.

Compound	MW	KI	KI (ref)	Relative amount (%)[a]	Confirmation[b]
cyclopentanone	84	1237		2.69	MS
thiazole	85	1247	1248	0.78	KI, MS[c]
methylthiol acetate	90	1332		1.67	MS
furfural	96	1453	1455	9.68	KI, MS
2,5-furandione	98	1371		0.55	MS
4,5-dihydro-2-methylthiophene	100	1145	1142	0.82	KI, MS
2-ethyl butanal	100	1286		4.73	MS
dihydro-3(2H)-thiophenone	102	1558	1556	0.19	KI, MS
3-thiolanone	102	1592		1.32	MS
2-formyl thiophene	112	1700	1688	0.23	KI, MS
furfuryl mercaptan	114	1423	1426	18.82	KI, MS[c]
2-methyl-3-furanthiol	114	1296	1295	13.82	KI, MS
tetrahydro-4H-thiopyran-4-one	116	1705		0.82	MS
3-thiophenethiol	116	1576	1581	10.32	KI, MS
2-mercapto-3-pentanone	118	1481		1.20	MS
3-mercapto-2-pentanone	118	1353	1350	16.47	KI, MS
2-acetyl thiophene	126	1775	1772	0.06	KI, MS[c]
3-methyl-2-formylthiophene	126	1813	1813	0.16	KI, MS
2-ethyl tetrahydrothiophene	130	1654		0.07	MS

Table 1. continued.

ethyl 2-(methylthio)-acetate	134	1328		3.54	MS
1-(2-furyl)-3-butanone	138	1653	1657	0.29	KI, MS
1-(2-thineyl)-2-propanone	140	1846	1842	0.58	KI, MS[c]
dimethyl formylthiophene	140	1924		0.06	MS
3-ethyl-2-formylthiophene	140	1869	1871	0.20	KI, MS
dihydrothienothiophene	142	2029	2022	0.03	KI, MS
methyl dihydrothienothiophene	156	2109	2103	0.22	KI, MS
dimethyl dihydrothienothiophene	170	2082	2077	0.01	KI, MS
Disulfide compounds					
3-[(2-methyl-3-furyl)dithio]-2-butanone	216	2147		0.15	MS
bis-(2-methyl-3-furyl) disulfide	226	2120	2115	0.53	KI, MS
2-methyl-3-[(2-furylmethyl) dithio] furan	226	2233		0.05	MS
3-[(2-methyl-3-furyl) dithio]-2-pentanone	230	2174		0.08	MS

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[a] The relative amount was calculated by expressing the peak area of each compound as a percent of the total peak area. Numbers represent the average of 3 replications.

[b] KI : Kovats index.
MS : confirmed by MS using the standard mass spectrum library.

[c] Confirmation was made by comparing the reference compounds.

and mass spectral data. The relative amount of each compound was calculated by expressing its peak area as a percentage of the total peak area of the isolated compounds. An identical model system was used by Farmer and Mottram (1990a) to study the volatile compounds in meat flavor. Fewer compounds were identified in this study than what was reported by Farmer and Mottram (1990a). These investigators isolated 60 compounds and subsequently used them to compare the effects of beef triacylglycerols, beef phospholipids, egg phosphatidylethanolamine and egg phsphatidylcholine on the production of Maillard reaction volatiles from heated cysteine and ribose. The qualitative differences in the compounds isolated in the two studies is due to the different experimental procedures involved. Farmer and Mottram (1990a) used an "Unijector" injector port linked with a cryogenic trap in the front end of the GC column to concentrate the volatile compounds. When solvent is used in the final extraction (concentration phase), many flavor compounds present in very low concentrations will be diluted. The present study, however, was not intended to repeat the definitive investigations of Farmer and Mottram (1990a), but rather to select "marker" compounds so that the effect on stabilized lipids in the Maillard reaction could be determined.

Compounds identified included thiazole, furan derivatives, thiophenes and thiophenones, heterocyclic thiols, mercapto carbonyls, disulfides and nonsulfur-containing compounds (Table 1). Sulfur-containing compounds (26) were predominant in the isolate. Twelve thiophenes or their derivatives were confirmed. These compounds are widely present in beef products and many have been reported to have odor threshold values in the μg / kg range (Mottram, 1991). Of the thiophene compounds isolated, an alkylthiophene (4,5-dihydro-2-methyl thiophene), six acylthiophenes (2-formyl thiophene, 2-acetyl thiophene, 3-methyl-2-formyl thiophene, 1-(2-thienyl)-2-propanone, a dimethyl formyl thiophene and

3-ethyl-2-formyl thiophene), one thiophenone (dihydro-3(2H)-thiophenone) and three bicyclic compounds (dihydrothienothiophene, methyl dihydrothienothiophene and dimethyl dihydrothienothiophene) were reported by Farmer and Mottram (1990a). These compounds were produced in a heated (140°C, 1 h) model system containing cysteine and ribose; however, their formation were noticeably suppressed in the presence of lipids. These investigators assumed that carbonyl compounds produced by the thermal oxidation of lipids may compete with sugar-derived dicarbonyls for free hydrogen sulfide (a Maillard reaction intermediate), thus reducing the production of Maillard reaction volatile compounds. One thiophene compound, 2-ethyl tetradihydrothiophene, was not reported by Farmer and Mottram (1990a). However, this compound is unlikely to play an important role in subsequent studies because it was only present in a relatively small amount (0.07%).

Other sulfur-containing compounds identified in this study were one thiazole, one thiolanone, three heterocyclic thiols (furfuryl mercaptan, 2-methyl-3-furanthiol, 3-thiophenethiol), and four disulfide compounds (Table 1). Though thiazole was present in only very small concentrations (0.78%), it is important to meat flavor and has been reported in many meat products (Mottram, 1991). Several mechanisms have been proposed for the formation of thiazoles. A major route involves the reaction of hydrogen sulfide and ammonia with aliphatic aldehydes and 1,2-dicarbonyl compounds (Figure 3) by a mechanism similar to that for oxazole formation (Mottram, 1991). Smaller amounts of thiazole were produced in a Maillard reaction mixture containing lipids; however, the difference in quantities formed in the presence and absence of lipids was small and within the

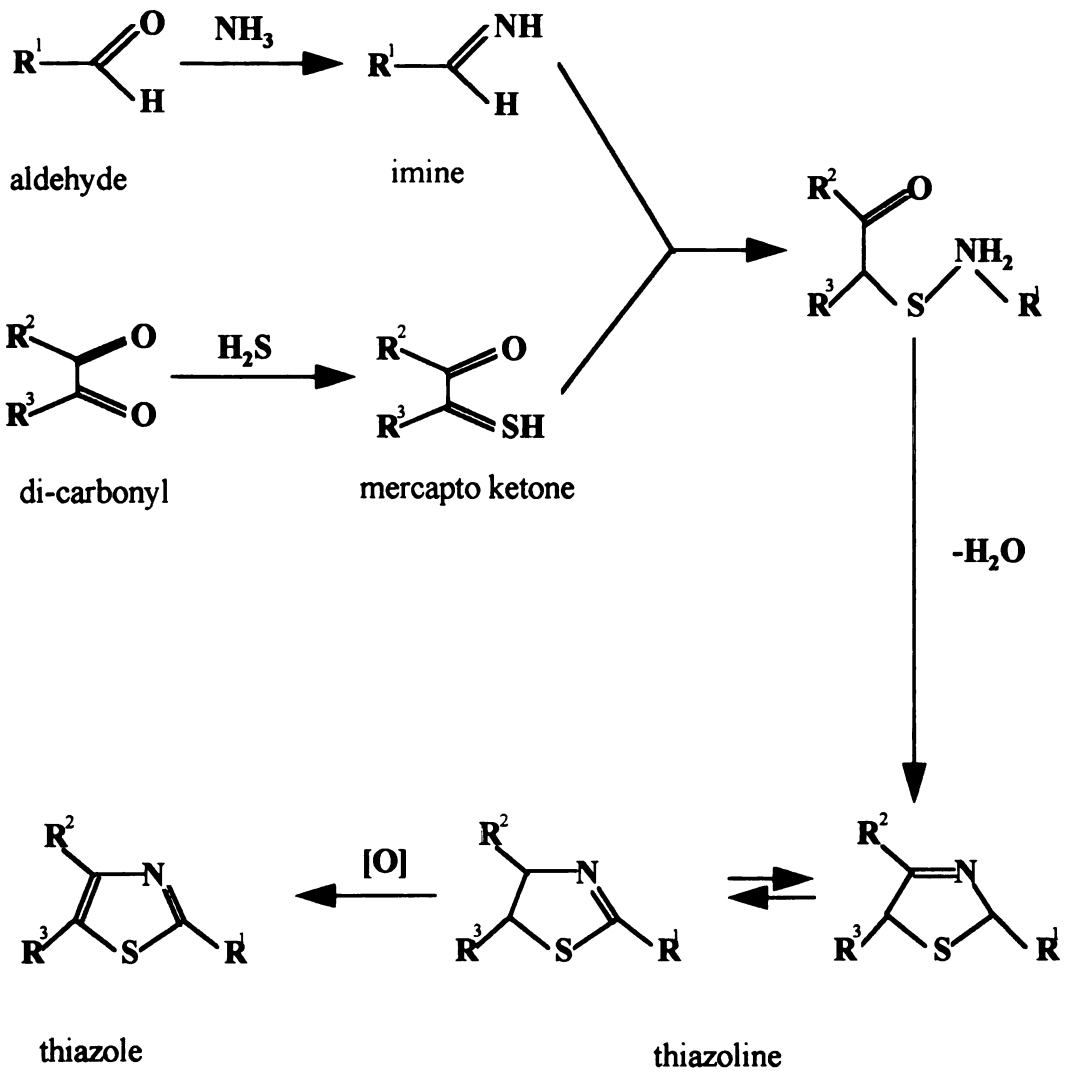


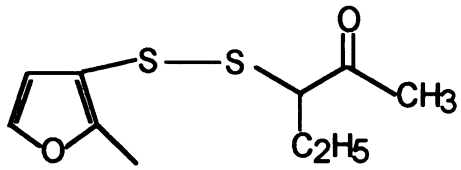
Figure 3. Formation of thiazoles and thiazolines from intermediates of the Maillard reaction.

range of standard deviation (Farmer and Mottram, 1990a).

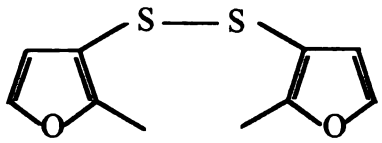
Heterocyclic thiol compounds were the most abundant compounds formed in the system containing cysteine and ribose (Table 1). Furfuryl mercaptan was the most dominant (18.82 %) compound in the model system and is believed to be a product of the reaction between furfural and hydrogen sulfide (Farmer and Mottram, 1990a). Two other heterocyclic thiols, 2-methyl-3-furanthiol and 3-thiophenethiol, are also very important because of the quantities produced (13.82 % and 10.32 %, respectively). These compounds are reported to be important contributors to meat aroma. Furans and thiophenes with a thiol substitution in the 3-position possess meat-like aromas (Mottram and Madruga, 1994). However, the quantities of these heterocyclic thiol compounds are also reduced when lipids are introduced into the model system of cysteine and ribose (Farmer and Mottram, 1990a).

Four disulfide compounds were identified in the present study (Figure 4), albeit in low concentrations (total relative amounts less than 1%, Table 1). However, they are very important contributors to meaty aroma (Farmer and Mottram, 1990b; Farmer and Patterson, 1991; Mottram and Whitfield, 1994; Mottram et al., 1995). The disulfide compounds are reportedly derived from 2-methyl-3-thiophenethiol, 2-methyl-3-furanthiol and 2-furylmethanethiol (Werkhoff et al., 1990; Farmer and Mottram, 1990b; Mottram et al., 1995). Farmer et al. (1990b, 1991) reported that phospholipids were involved in the formation of these disulfide compounds, and reduced the quantities formed in the model system.

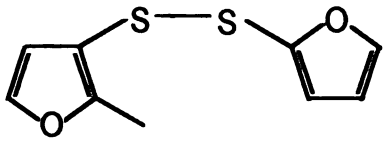
Several non-heterocyclic sulfur-containing compounds were also identified. Among these were mercapto carbonyls (2-mercapo-3-pentanone and 3-mercapto-



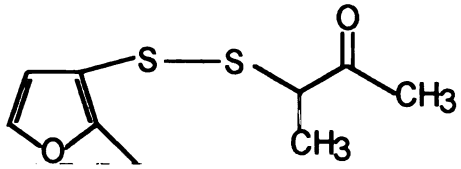
3-[(2methyl-3-furyl) dithio]-2-pentanone (meaty, boiled meat, roast beef)[a]



bis (2-methyl-3-furyl) disulfide (beefy, meaty, meat soup)[a]



2-methyl-3-[(2-furylmethyl) dithio] furan (meat, burnt meat, roast meat)[a]



3-[(2methyl-3-furyl) dithio]-2-butanone (meaty, burnt meat)[a]

Figure 4. Structures and sensorial properties of disulfide compounds isolated from a model system containing cysteine and ribose.
[a] (Mottram et al., 1995).

2-pentanone) and methylthiol acetates (methylthiol acetate and ethyl 2-(methylthiol) acetate). The second most abundant compound produced in the model system was 3-mercapto-2-pentanone (16.47%) and possessed a sulfury note (Mottram et al., 1995). Farmer and Mottram (1990a) concluded that mercapto carbonyls were derived from the reaction of hydrogen sulfide and dicarbonyls, and that their formation tended to be reduced considerably by the presence of phospholipids in a model system. Two methylthio acetates were also identified, and were present at approximately 5.0 % of the total amount. The majority of methyl sulfides are generated from the reaction of hydrogen sulfide or methanethiol with aliphatic aldehydes or alcohols, and their further reactions give rise to a range of acyclic and heterocyclic compounds (MacLeod, 1986). However, these methylthio acetates are believed to contribute to the aroma of meat (Mottram, 1991).

Three furan-related compounds were identified: furfural, 2,5-furandione and 1-(2-furyl)-3-butanone. These compounds represented more than 10.5% of the total amount of compounds isolated in the model system (Table 1). Furfural is a degradation product of ribose and its formation is also affected negatively by the presence of phospholipids (Farmer and Mottram, 1990a). The other two compounds, 2,5-furandione and 1-(2-furyl)-3-butanone, were not reported by Farmer and Mottram (1990a); however, they could be derived from furfural. Two other non-heterocyclic compounds, cyclopentanone and 2-ethyl butanal, were identified and could be derived from sugar degradation (Nursten, 1986).

No pyrazines were isolated in this study. This could be explained by the fact that the formation of pyrazine is favored by alkaline conditions (Maga, 1992). The model system study was performed in an aqueous phase with a pH value of

5.6 immediately after heating. The slightly acidic reaction medium could lead to minimal formation of pyrazines.

Selection of marker compounds for use in subsequent studies

In subsequent studies, the effect of phospholipids on the generation of Maillard reaction volatiles in a model system containing cysteine and ribose will be investigated. Therefore, some compounds isolated in this model system and shown to be influenced by the presence of lipids by Farmer and Mottram (1990a) were selected as "marker" compounds. Fifteen compounds were selected, 14 of which contain sulfur (Table 2). The role of sulfur-containing heterocyclic compounds in meat flavor is well documented (Mottram, 1991). Their contribution is due to their meaty notes and low threshold levels. The formation of the majority of these compounds is affected by the presence of lipids. Lipid-derived carbonyls compete for free hydrogen sulfide with sugar-derived dicarbonyls and reduces / suppresses the formation of Maillard reaction volatiles (Farmer and Mottram, 1990a; Whitfield, 1992). Therefore, these compounds will be used to assess the effect of unsaturated fatty acids on the suppression of Maillard reaction products in model systems containing phospholipids.

CONCLUSION

A new technique for the analysis of volatiles was developed using a combination of heating and solvent extraction to desorb volatiles from Tenax

Table 2. Sensorial properties of Maillard reaction volatiles selected as marker compounds to study the effect of phospholipids on volatile production.

Compound	Odor description	Source
Thiophenes		
2-acetyl thiophene	roasted[1,2]	chicken, beef, pork[2,3]
1-(2-thienyl)-2-propanone	creamy, caramel-like[1]	beef[2]
dimethyl formylthiophene	nutty, meaty[1]	
3-ethyl-2-formylthiophene	nutty, meaty[1]	
Bicyclic compounds		
dihydrothienothiophene	meaty, sulfury[1,3]	
Thiazoles		
thiazole	meaty, nutty[1,2]	chicken, beef, pork[2,3]
Disulfides		
3-[(2-methyl-3-furyl) dithio]-2-butanone	meaty, burnt meat[4]	
bis-(2-methyl-3-furyl) disulfide	beefy, meaty, broth[4]	
2-methyl-3-[(2-furylmethyl) dithio] furan	meat, burnt meat, roast beef[4]	
3-[(2-methyl-3-furyl) dithio]-2-pentanone	meaty, broiled meat, roast beef[4]	
Heterocyclic thiols		
furfuryl mercaptan	sweet, caramel-like[1]	beef[3]
2-methyl-3-furanthiol	beef broth, roast meat[1]	
3-thiophenethiol	meaty[1]	
Mercapto carbonyls		
3-mercapto-2-pentanone	sulfurous, rotten meat[3]	
Non-sulfur compounds		
furfural	pungent but sweet,[3]	mutton, beef, pork[3]

[1] Fors, 1983. [2] Shahidi et al., 1986. [3] Mottram, 1991. [4] Mottram et al., 1995.

traps. Thirty-one compounds were identified in a mdoel system containing cysteine and ribose. Of these, fifteen compounds were selected as "marker" compounds. The selection was based on literature reports of compounds whose formation in similar model systems was suppressed by the presence of lipids.

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CHAPTER 2

EFFECTS OF PHOSPHOLIPIDS AND ALDEHYDES ON THE FORMATION OF MAILLARD REACTION VOLATILES IN MODEL SYSTEMS CONTAINING CYSTEINE AND RIBOSE

ABSTRACT

Cysteine and ribose were heated to produce Maillard reaction volatiles, and fifteen compounds were selected as "markers" to study the influence of lipids on their formation. Sensory assessment revealed that the main difference between the aromas developed in model systems containing cysteine and ribose was that more meaty and sulfury notes were detected in those systems which did not contain phospholipids. No discernible differences in aroma were observed between samples containing phosphatidylethanolamine (PE) and phosphatidylcholine (PC). More meaty and sulfury aromas were generated in the reaction mixture containing PC-distearoyl than in the system containing PC-dioleoyl.

Phospholipids suppressed the formation of selected Maillard reaction products. In addition, the degree of unsaturation of the phospholipids influenced the extent to which Maillard reaction volatiles were generated. However, the effects of phospholipids on the formation of volatile compounds was not related to the amino moieties of the phospholipids. Suppression of the formation of Maillard volatiles was also observed in model systems containing aldehydes.

INTRODUCTION

Maillard-derived heterocyclic compounds are key components in the desirable flavors of processed and cooked foods, particularly meat products. The primary source of these compounds is the Maillard reaction between amino acids and reducing sugars (Whitfield et al., 1988). The Maillard reaction does not require high temperatures and readily produces aroma compounds at temperatures associated with the cooking of food. It also occurs much more readily at low moisture levels; thus, flavor compounds generated in meat by the Maillard reaction tend to be associated with the dehydrated areas during cooking (Mottram, 1994c).

Recently, model system studies have implicated lipids in the Maillard reaction, thus affecting the production of certain heterocyclic compounds (Salter et al., 1988; Whitfield et al., 1988; Farmer et al., 1989; Farmer and Mottram, 1990a, b). When lipids were added to model systems containing cysteine and ribose, a noticeable quenching effect on the formation of Maillard reaction products was observed. Many of those compounds whose mechanism of formation was susceptible to lipid intervention, were affected more by the presence of a phospholipid than by a triacylglycerol (Farmer and Mottram, 1990a). A mechanism by which phospholipids suppress the formation of Maillard reaction products in model system was proposed by Farmer and Mottram (1990a). It was hypothesized that carbonyl compounds formed by the thermal oxidation of lipids competed with the reducing sugars for Maillard reaction intermediates such as hydrogen sulfide and ammonia, thus reducing the formation of heterocyclic compounds. Because carbonyl compounds are derived from lipid oxidation, the

degree of unsaturation of lipids may play an important role in this lipid - Maillard interaction.

Compounds isolated from a heated Maillard model system containing cysteine and ribose in the presence of beef triacylglycerols (BTG), beef phospholipids (BPL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were classified into four categories by Farmer and Mottram (1990a). These categories are:

[A] ***Compounds formed only in the presence of lipids.***

The greatest differences between the effects of lipids were observed among those compounds which required lipid precursors for their formation.

[B] ***Compounds suppressed by the presence of lipids.***

These compounds were further subdivided based on the effects of different lipids on their formation.

(a) Phospholipids showed greater suppression than triacylglycerols.

(b) PE and BPL showed greater suppression than BTG and PC.

(c) Triacylglycerols showed greater suppression than phospholipids.

(d) Triacylglycerols caused similar reductions to phospholipids.

[C] ***Compounds largely unaffected by the nature of lipids.***

Compounds such as certain thiazoles showed some reduction on the addition of lipid, especially PC (Whitfield et al., 1988); however, the differences were small. Thus, no clear trends were inferred.

[D] ***Compounds not included in the previous categories.***

The four lipids influenced the formation of 2-furfural by different extents. The final concentration was approximately halved by the addition of PC or PE to the model systems, while BPL and BTG suppresse to a lesser extent (Whitfield et al., 1988).

As previous results have shown, the formation of Maillard reaction products is influenced by the presence of lipids in a Maillard model system. The degree of lipid unsaturation may play an important role in this interaction. Thus, this study was designed to address the effects of adding lipids of different degrees of unsaturation to the model system on the production of Maillard reaction volatiles. The investigation involved heating phospholipids with different fatty acids with cysteine and ribose. The influence of lipid derived carbonyls on the Maillard reaction was also determined by including selected aldehydes in the model systems.

EXPERIMENTAL

Materials

Cysteine and ribose, two phosphatidylethanolamines (PE) (L-α-phosphatidylethanolamine: dioleoyl and L-α-phosphatidylethanolamine, distearoyl) and three phosphatidylcholines (PC) (L-α-phosphatidylcholine, dilinoleoyl, L-α-phosphatidylcholine, dioleoyl and L-α-phosphatidylcholine, distearoyl) were purchased from Sigma Chemical Co. (St. Louis, MO). Hexanal and dodecanal were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Ethyl acetate and sodium phosphates (monobasic and dibasic) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Tenax GC was obtained from Alltech Associates Inc. (Deerfield, IL). All the reagents and solvents used in this study were analytical and/or HPLC grade.

Preparation of Maillard reaction volatiles

Cysteine and ribose were heated together in the presence of phospholipids / aldehydes in a phosphate buffer. Cysteine and ribose served as the control system. Each reaction mixture was prepared in triplicate.

The reaction mixtures were prepared by dissolving cysteine (0.61g) and ribose (0.75 g) in 2 ml phosphate buffer (0.5M, pH 5.7) in a 10 ml clear glass vial (Pierce Chemical Co., Rockford, IL). For the phospholipid systems, 100 mg of each phospholipid were added to the reaction mixture. The vials were immersed in an ultrasonic bath (Branson 2000, Danbury, CT), and held at 50°C to disperse the lipids prior to heating. These reaction vials were then flushed with a stream of nitrogen (30 ml/min) for 2 min, and sealed using Tuf-bond Teflon-silicon discs and aluminum caps (Pierce Chemical Co., Rockford, IL). The reactants were heated at 140°C in an autoclave under a pressure of 0.28 MPa for 1 h. After heating, the vials containing the Maillard reaction products were stored in a freezer (-10°C) prior to the volatile collection.

For the aldehyde systems, hexanal (0.1 and 1 mmole) and dodecanal (0.1, 0.5, 1 and 2 mmole) were added to the glass vials containing cysteine and ribose. The reaction vials were sonicated for 2 min and flushed with nitrogen prior to sealing. The Maillard reaction was carried out at 140°C in an autoclave under a pressure for 1 h. The vials were then cooled and stored in a freezer prior to analysis.

Isolation and identification of Maillard reaction volatiles

Volatile compounds developed from the Maillard reaction were collected using a purge-and-trap procedure, and then desorbed into ethyl acetate using a technique combining thermal desorption-solvent extraction. Tenax traps were used

to collect the volatile compounds, and were conditioned overnight at 180°C with nitrogen flowing at 30 ml / min prior to volatile collection.

The contents of the reaction vials (ca. 2 ml) were transferred to a two-neck flask (500 ml, Kontes, Vineland, NJ) containing 18 ml phosphate buffer (0.5M, pH 5.7). The flask was fitted with a L-shape glass joint (Kontes, Vineland, NJ), and a Tenax trap was attached by a stainless steel Swagelok reducing union (Cajon Ultra-Torr® fitting, Cajon, Macedonia, OH) to the outlet of the joint. During collection, the flask containing the Maillard reaction mixture was heated at 60°C in a heating mantle, and the Tenax trap was maintained at room temperature (approximately 20°C ± 2°C). Volatile compounds were swept into the Tenax trap using a stream of nitrogen (30 ml /min), and the collection was continued for 1 h. At the end of the collection period, the Tenax trap was directly connected to the nitrogen supply for 5 min to remove water vapor. A more detailed description of this procedure is provided in Chapter 1.

Desorption of the trapped volatile compounds from the Tenax trap

During desorption, the Tenax trap was connected between a solvent (ethyl acetate) source and a cold trap. The Tenax trap was wrapped with a heating strip and heated at 150°C. The nitrogen was passed through the ethyl acetate reservoir (30 - 40 ml/min), and then the ethyl acetate - nitrogen gas was introduced into the heated Tenax trap. The nitrogen stream was maintained water-free by passing through a desiccant (anhydrous calcium sulfate) (W. A. Hammond Drierite Co., Xenia, OH) before passing through the ethyl acetate reservoir. The desorbed volatile compounds were then condensed in a cold trap using dry ice - acetone. The desorption process was continued for 30 min, after which the ethyl acetate extract was concentrated to 0.1 ml under nitrogen. The concentrate was transferred

to a screw-cap vial and stored in a refrigerator prior to gas chromatographic / mass spectrometric analysis.

Gas chromatography - mass spectrometry (GC / MS)

The volatile compounds were identified using a HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) interfaced with a HP 5970 MSD (Mass Selective Detector) mass spectrometer. The GC / MS system was equipped with a HP 59970 Chemstation Data System. A Carbowax 20M fused silica capillary column (60m x 0.32 mm id, Supelco Inc., Bellefonte, PA) was used to separate the compounds. The GC oven temperature was programmed as follows: the initial temperature was set at 40^{o}C for 5 min, the temperature was increased at a rate 2^{o}C / min to a final temperature of 200^{o}C, and maintained for 10 min. Helium was the carrier gas with a flow rate of 10 ml/min. The temperatures of the injection port and transfer lines were both set at 220^{o}C.

The mass spectrometer was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250^{o}C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 sec over a mass range of 40-300 was employed. The GC / MS data were monitored, stored and analyzed using an HP Chemstation data system. Two mass spectral libraries, the INRA Mass Spectra Library - "INRAMASS" (Laboratoire de Recherche sue les Aromes, Dijon, France) and the NIST / EPA / MSDC Mass Spectral Database (ACS Publication Co., Washington, DC), were used to identify the compounds isolated.

Evaluation of odor characteristics

The odor characteristics of each sample were determined by three flavor chemists in the Department of Food Science and Human Nutrition, Michigan State University. When each sample was opened for volatile collection, the headspace in the reaction vials was sniffed by the panelists to assess the odor. They were asked to describe freely the odor characteristics and their comments were noted (Farmer and Mottram, 1990a).

Determination of the effects of phospholipids and aldehydes on the Maillard reaction

The effects of phospholipids and aldehydes on the formation of Maillard reaction volatiles were determined by comparing the relative amounts of each compound produced in these systems to those in the controls. The calculation of the relative amounts was accomplished by using the peak areas on the gas chromatograms. The peak area of each compound from the control system (cysteine and ribose heated alone) was standardized as 100%, and the relative amounts of the same compound generated from the reaction mixture containing either phospholipid or aldehyde was determined as:

$$\text{relative amount (\%)} = \frac{\text{peak area (with phospholipids or aldehydes)}}{\text{peak area (control)}} \text{ X } 100\ \%$$

RESULTS AND DISCUSSION

Sensory evaluation of compounds developed from cysteine and ribose in the presence of phospholipids

A summary of the odor descriptions attributed to the reaction mixtures is presented in Table 1. The control reaction mixtures, i.e., cysteine and ribose, had pleasant meaty aromas; however, sulfury notes were also detected. Upon adding phospholipids to the reaction mixtures, the intensities of the meaty and sulfury notes were decreased. No discernible differences between samples containing phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were noticed. More meaty and sulfury aromas were generated from the reaction mixtures containing PC-distearoyl than the systems containing PC-dioleoyl. These results imply that the susceptibility of the fatty acid to oxidation may play a major role in determining the nature of the aroma compounds developed in the Maillard reaction.

These observations were similar to those previously reported by Farmer and Mottram (1990a), who concluded that the major difference between the odors of reaction mixtures with and without lipids was in the relative intensities of the various meaty and sulfury notes. Thus, lipids, in particular phospholipids, may influence the Maillard reaction and the subsequent development of key flavor compounds, thus affecting the intensity of the flavor generated.

Table 1. Summary of odor descriptions attributed to the headspace of cysteine and ribose heated in the presence of phospholipids.

REACTION MIXTURE	DESCRIPTORS
cysteine + ribose	pleasant, brothy-beefy, slightly sulfury
cysteine + ribose + phosphatidylethanolamine - dioleoyl	pleasant, slightly meaty, slightly sweet, slightly "cooked green pepper"
cysteine + ribose + phosphatidylcholine - dioleoyl	pleasant, slightly meaty, slightly sweet, slightly caramelization
cysteine + ribose + phosphatidylcholine - distearoyl	pleasant, slightly meaty, sweet, slightly sulfury

Effect of phospholipids on the formation of Maillard reaction volatiles from heated cysteine and ribose

More than 30 compounds developed from the reaction of cysteine and ribose were identified. This number is smaller than those identified by Farmer and Mottram (1990a) using a similar model system. This may be due to the different equipment employed in these studies. Farmer and Mottram (1990a) used an "Unijector" (Scientific Glass Engineering Ltd, Milton Keynes) in place of a conventional GC injector, and a cold trap (solid carbon dioxide) to increase the efficiency of desorption. However, the present study was not intended to repeat the definitive studies of Farmer and Mottram (1990a), but rather to determine the effects of phospholipids on the formation of selected Maillard reaction products. To do this, fifteen compounds whose formation was modified by the presence of lipids were selected as "marker" compounds (Whitfield et al., 1988; Farmer et al., 1989; Farmer and Mottram, 1990a, b; Mottram et al., 1995). These marker compounds were considered adequate to study the effects of lipids and their oxidation products on the Maillard reaction.

The formation of the selected compounds was influenced by the presence of PE (Table 2). In general, their formation of the selected compounds was reduced when PE was present in the reaction mixtures. The relative amounts of the majority of the marker comopunds were suppressed by up to 25 % in the presence of PE-distearoyl (Table 2). Heterocyclic thiols, 3-mercapto-2-pentanone, and furfural were noticeably more reduced than were thiazole, dihydrothienothiophene, 1-(2-thienyl)-2-propanone and three disulfides. The quantities of the latter compounds were reduced by less than 10 %.

Table 2. Relative amounts of selected Maillard reaction volatiles from the reaction of cysteine and ribose in the presence of phosphatidylethanolamines.

Compound	PE-distearoyl	PE-dioleoyl
Thiophenes		
2-acetyl thiophene	----[a]	----
1-(2-thienyl)-2-propanone	90[b]	60
dimethyl formylthiophene	----	----
3-ethyl-2-formylthiophene	----	----
Bicyclic compounds		
dihydrothienothiophene	95	98
Thiazoles		
thiazole	87	79
Disulfides		
3-[(2-methyl-3-furyl) dithio]-2-butanone	99	90
bis-(2-methyl-3-furyl) disulfide	74	82
2-methyl-3-[(2-furylmethyl) dithio] furan	96	88
3-[(2-methyl-3-furyl) dithio]-2-pentanone	99	79
Heterocyclic thiols		
furfuryl mercaptan	76	77
2-methyl-3-furanthiol	77	50
3-thiophenethiol	81	55
Mercapto carbonyls		
3-mercapto-2-pentanone	75	33
Non-sulfur compounds		
furfural	75	75

Table 2. (continued)

[a] "----" indicates not detected.

[b] The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction mixture without phospholipid (i.e., control). The calculation was accomplished by standardizing the peak area of the selected compound in the control system as 100%. The peak area of the Maillard reaction product was then divided by the peak area of the same compound in the control.

Similar suppressive effects were found in the Maillard reaction system containing PE-dioleoyl (Table 3). However, more discernible reductions in the formation of some compounds were observed with the more unsaturated phospholipid. Only dihydrothienothiophene and 3-[(2-methyl-3-furyl) dithio]-2-butanone were reduced by less than 10 % by this phospholipid, while the amount of 3-mercapto-2-pentanone formed was approximately one third of that produced in the control model system. Two heterocyclic thiols, 1-(2-thienyl)-2-propanone and 3-[(2-methyl-3-furyl) dithio]-2-pentanone, were also considerately reduced, while the remaining compounds listed in the Table 2 suffered smaller reductions. There was no apparent difference in the formation of dihydrothienothiophene, furfuryl mercaptan and furfural between the two PEs. These results indicated that lipids, particularly those that are unsaturated, suppress the formation of selected Maillard reaction volatiles developed from the reaction of cysteine and ribose. The data generated also confirmed the observations of Farmer and Mottram (1990a) that phospholipids from egg and beef sources have a greater suppressive effect on the Maillard reaction than do triacylglycerols.

Phosphatidylcholine (PC) also suppressed the formation of selected Maillard reaction products when included in the model system containing cysteine and ribose (Table 3). Three PCs with different degrees of unsaturation were used, and each influenced the formation of Maillard reaction volatiles to a different extent.

Compounds developed in the system containing PC-distearoyl were reduced by 11 - 93% relative to the control. Several compounds such as 2-acetyl thiophene, dimethyl formylthiophene and 3-ethyl-2-formylthiophene, were not detected in

Table 3. Relative amounts of selected Maillard reaction volatiles from the reaction of cysteine and ribose in the presence of phosphatidylcholines.

Compound	PC-distearoyl	PC-dioleoyl	PC-dilinoleoyl
Thiophenes			
2-acetyl thiophene	----[a]	----	----
1-(2-thienyl)-2-propanone	80[b]	75	12
dimethyl formylthiophene	----	----	----
3-ethyl-2-formylthiophene	----	----	----
Bicyclic compounds			
dihydrothienothiophene	85	77	----
Thiazoles			
thiazole	83	89	84
Disulfides			
3-[(2-methyl-3-furyl) dithio]-2-butanone	79	59	20
bis-(2-methyl-3-furyl) disulfide	63	37	15
2-methyl-3-[(2-furylmethyl) dithio] furan	93	73	66
3-[(2-methyl-3-furyl) dithio]-2-pentanone	88	78	81
Heterocyclic thiols			
furfuryl mercaptan	76	75	76
2-methyl-3-furanthiol	11	----	----
3-thiophenethiol	76	78	78
Mercapto carbonyls			
3-mercapto-2-pentanone	76	57	----
Non-sulfur compounds			
furfural	75	82	79

Table 3. (continued)

[a] "----" indicates not detected.

[b] The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction mixture without phospholipid (i.e., control). The calculation was accomplished by standardizing the peak area of the selected compound in the control as 100%. The peak area of the Maillard reaction product was then divided by the peak area of the same compound in the control.

this system. The compound suppressed to the greatest extent was 2-methyl-3-furanthiol (11 % of control), while the smallest suppression was observed for 2-methyl-3-[(2-furylmethyl) dithio] furan (93% of control). The relative amounts of the remaining selected compounds generally ranged between 75 - 90%.

The data in Table 3 also show that the extent of suppression of the Maillard reaction volatiles in the presence of PCs increased with increasing unsaturation of the fatty acid moieties. Greater suppression was observed in the systems containing PC-dioleoyl or PC-dilinoleoyl compared to the systems with PC-distearoyl (Table 3). Three compounds, dihydrothienothiophene, 2-methyl-3-furanthiol and 3-mercapto-3-pentanone, were not detected in the presence of PC-dilinoleoyl, while 2-methyl-3-furanthiol was not detected in the reaction mixture containing PC-dioleoyl. Noticeable decreases in the formation of 1-(2-thienyl)-2-propanone and three disulfides were observed with PC-dioleoyl.

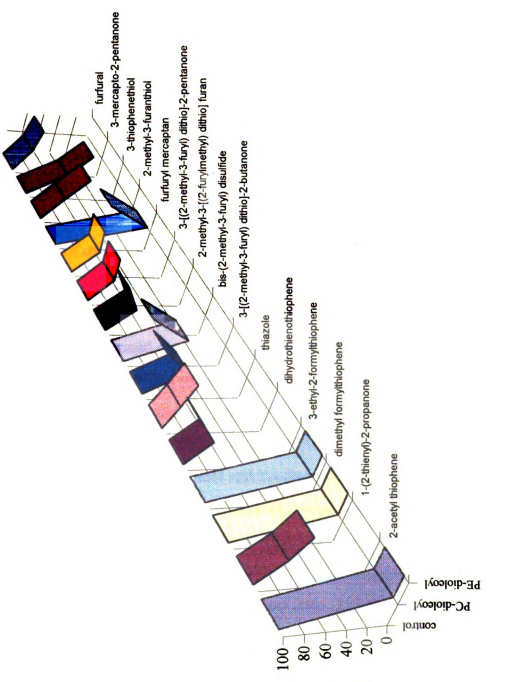
These studies with PEs and PCs indicate that the generation of Maillard reaction products from cysteine and ribose is influenced by the presence of lipids. Greater suppressive effects were observed with increasing unsaturation of the phospholipids. Similar results were reported by Farmer and Mottram (1990a) who demonstrated that lipids were capable of reducing the formation of Maillard reaction products. They also reported that beef phospholipids exhibited a greater suppressive effect than did beef triacylglycerols, and suggested that this could be due to the difference in the fatty acid composition of the lipids. These investigators also suggested the most likely pathways by which lipids could interact in the Maillard reaction. Lipid-derived carbonyls could react with the amino groups of amino acids and free ammonia produced by the Strecker degradation, while hydroxyl and carbonyl products derived via lipid oxidation could react with free hydrogen sulfide.

Results in this study indicate that the formation of Maillard reaction compounds is impacted by the presence of phospholipids. The greater influence of phospholipids with more unsaturation implies that thermal oxidation during the heating of the model systems may be the reason for this effect. To verify this hypothesis further, the effect of aldehydes on the formation of Maillard reaction products was investigated.

The effects of various phospholipids on the Maillard reaction involving cysteine and ribose

As described previously, both PE and PC exerted a quchening effect on the formation of Maillard reaction products (Tables 2 and 3). However, dissimilarities in the behavior of PE and PC with the same degree of unsaturation were observed. Greater suppression of the formation of three disulfides, 2-methyl-3-furanthiol and dihydrothienothiophene was observed in the presence of PC-dioleoyl compared to PE-dioleoyl (Figure 1). However, smaller amounts of 1-(2-thienyl)-2-propanone, thiazole, 3-thiophenethiol, 3-mercapto-2-pentanone and furfural were formed in the system containing PE-dioleoyl. One long-chain alkylthiophene, 2-hexylthiophene, was detected in the reaction systems containing PC-dioleoyl and PE-dioleoyl. The route of formation for this chemical was suggested to involve the reaction of 2,4-decadienal and hydrogen sulfide. Therefore, it was classified in the category of compounds formed only in the presence of lipids (Farmer and Mottram, 1990a). The amount of 2-hexylthiophene isolated from PE-dioleoyl was approximately 40% of that from PC-dioleoyl. Farmer and Mottram (1990a) also reported that a greater amount of this compound was formed in the Maillard

Figure 1. Relative amounts of selected Maillard reaction volatiles isolated from the reaction mixture of cysteine and ribose in the presence of phospholipids compared to cysteine and ribose alone.


Relative amount
100
80
60
40
20
0
control
PC-diolcoyl
PE-diolcoyl
2-acetyl thiophene
1-(2-thienyl)-2-propanone
dimethyl formylthiophene
3-ethyl-2-formylthiophene
dihydrothienothiophene
thiazole
3-[(2-methyl-3-furyl) dithio]-2-butanone
bis-(2-methyl-3-furyl) disulfide
2-methyl-3-[(2-furylmethyl) dithio] furan
3-[(2-methyl-3-furyl) dithio)-2-pentanone
furfuryl mercaptan
2-methyl-3-furanthiol
3-thiophenethiol
3-mercapto-2-pentanone
furfural

reaction system in the presence of egg PC compared to egg PE.

These was no apparent trend in the suppression of the formation of Maillard reaction products in the two phospholipid systems. Similar results were reported in a study of the browning reaction of various unsaturated fatty acids heated with either ethanolamine or choline (Husain et al., 1986). It was shown that the resultant color intensity increased with the degree of fatty acid unsaturation in the presence of both ethanolamine and choline. However, for the same fatty acid, choline gave greater browning intensity than ethanolamine. Ethanolamine showed much greater reactivity than choline when heated with the conjugated carbonyl derivatives of methyl linoleate. This is due to the reaction of the amino group of ethanolamine and carbonyls to yield a Schiff's base and subsequently the colored products (Husain et al., 1986). Since the amino group of ethanolamine in PE may react with sugar-derived dicarbonyls (Farmer and Mottram, 1990a), greater suppression of the Maillard reaction compounds formed in the presence of PE may be predicted. In a previous study, egg PE and egg PC were used in a Maillard system containing cysteine and ribose (Farmer and Mottram 1990a). Greater quantities of 1-heptanol, 1-octanol and 2,4-decadienal were produced from the thermal degradation of PC than PE, and thus might cause greater suppression of the Maillard reaction. Therefore, the suppressive effects of phospholipids on the formation of Maillard reaction products may be due to a number of factors, the most important being the formation of lipid-derived carbonyl compounds.

Effects of aldehydes on the production of Maillard reaction volatiles in a heated cysteine and ribose model

The results of the phospholipid study indicate that phospholipids have a quenching effect on the formation of Maillard reaction products in a heated model

system containing cysteine and ribose. It is assumed that the unsaturated lipid moieties undergo thermal oxidation during heating of the model system, and that the carbonyl compounds produced may be involved in suppressing the Maillard reaction.

Two aldehydes, hexanal and dodecanal, were used to investigate the effects of lipid oxidation products on the Maillard reaction containing cysteine and ribose. The formation of selected Maillard reaction products was influenced by the addition of hexanal in the model system (Table 4). Four disulfides, three thiophenes, dihydrothienothiophene and one heterocyclic compounds were not detected in the presence of 0.1 mmole of hexanal. One of the thiophene compounds, 1-(2-thienyl)-2-propanone, was remarkably influenced by the difference concentrations of hexanal used. However, increasing the amount of hexanal in the model system containing cysteine and ribose, resulted in a similar suppression of the formation of selected Maillard reaction volatiles (Table 4). Generally, these results confirm that lipid oxidation, or more accurately carbonyl compounds derived by thermal oxidation, influences the formation of Maillard volatiles.

In a similar investigation, a longer chain aldehyde, dodecanal, was also heated with cysteine and ribose in a model system. Thiophenes and thienothiophene was not detected in any of the systems with dodecanal (Table 5). It was also observed that the greater the concentration of dodecanal in the model system, the greater the reduction of the quantities produced. The most obvious difference was observed between the model systems containing 0.1 and 0.5 mmole of dodecanal (Table 5). Disulfide compounds were sensitive to the presence of

Table 4. Relative amounts of selected Maillard reaction volatiles from the reaction of cysteine and ribose in the presence of hexanal.

Compound	0.1 (mmole)	1 (mmole)
Thiophenes		
2-acetyl thiophene	----[a]	----
1-(2-thienyl)-2-propanone	105[b]	29
dimethyl formylthiophene	----	----
3-ethyl-2-formylthiophene	----	----
Bicyclic compounds		
dihydrothienothiophene	----	----
Thiazoles		
thiazole	84	92
Disulfides		
3-[(2-methyl-3-furyl) dithio]-2-butanone	----	----
bis-(2-methyl-3-furyl) disulfide	----	----
2-methyl-3-[(2-furylmethyl) dithio] furan	----	----
3-[(2-methyl-3-furyl) dithio]-2-pentanone	----	----
Heterocyclic thiols		
furfuryl mercaptan	57	36
2-methyl-3-furanthiol	71	----
3-thiophenethiol	----	45
Mercapto carbonyls		
3-mercapto-2-pentanone	38	58
Non-sulfur compounds		
furfural	61	63

Table 4. (continued)

[a] "----" indicates not detected.

[b] The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction mixture without hexanal (i.e., control). The calculation was accomplished by standardizing the peak area of the selected compound in the control as 100%. The peak area of the Maillard reaction product was then divided by the peak area of the same compound in the control.

Table 5. Relative amounts of Maillard reaction volatiles from the reaction of cysteine and ribose in the presence of dodecanal.

Compound	0.1 (mmole)	0.5	1	2
Thiophenes				
2-acetyl thiophene	----[a]	----	----	----
1-(2-thienyl)-2-propanone	----	----	----	----
dimethyl formylthiophene	----	----	----	----
3-ethyl-2-formylthiophene	----	----	----	----
Bicyclic compounds				
dihydrothienothiophene	----	----	----	----
Thiazoles				
thiazole	104[b]	39	34	27
Disulfides				
3-[(2-methyl-3-furyl) dithio]-2-butanone	23	14	----	----
bis-(2-methyl-3-furyl) disulfide	25	19	----	----
2-methyl-3-[(2-furylmethyl) dithio] furan	----	----	----	----
3-[(2-methyl-3-furyl) dithio]-2-pentanone	----	----	----	----
Heterocyclic thiols				
furfuryl mercaptan	71	35	43	16
2-methyl-3-furanthiol	98	28	29	5
3-thiophenethiol	58	43	31	21
Mercapto carbonyls				
3-mercapto-2-pentanone	46	27	11	4
Non-sulfur compounds				
furfural	39	36	23	16

Table 5. (continued)

a "----" indicates not detected.

b The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction mixture without dodecanal (i.e., control). The calculation was accomplished by standardizing the peak area of the selected compound in the control as 100%. The peak area of the Maillard reaction product was then divided by the peak area of the same compound in the control.

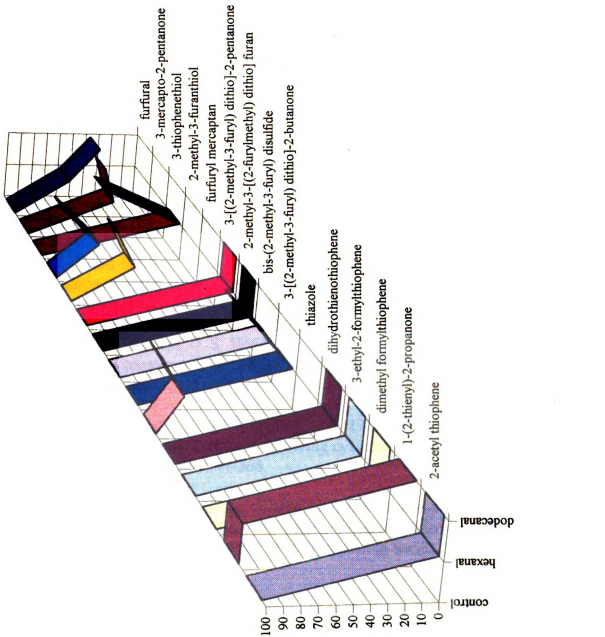
dodecanal, none being detected when 1 mmole was added to the model system. Thiazole was found in a relatively high amount in the system containing 0.1 mmole of dodecanal.

The suppressive effects of different aldehydes on the formation of selected Maillard reaction volatiles in a model system of cysteine and ribose are presented in Figure 2. Both hexanal and dodecanal suppressed of the formation of selected Maillard reaction products. Two disulfides and four thiophenes were not detected in both systems, and, to some degrees, hexanal produced more suppression of the selected Maillard reaction volatiles than dodecanal at same concentration (0.1 mmole) (Figure 2).

CONCLUSION

These studies show that the susceptibility of phospholipids to thermal oxidation (i.e., degree of unsaturation) influences the extent that lipids suppress the formation of Maillard reaction volatiles in model systems containing cysteine and ribose. The inclusion of aldehydes in the model system also resulted in suppression of the production of Maillard reaction volatiles, giving indirect evidence that thermal oxidation of lipids is involved in this suppressive effect. It is our hypothesis that the stabilization of lipids in meat products such as through the use of nitrite as a curing adjunct (Freybler et al., 1993), could reduce the effect of lipids and/or their oxidation carbonyl products on the development of flavor

Figure 2. Relative amounts of selected Maillard reaction volatiles isolated from the reaction mixture of cysteine and ribose in the presence of 0.1 mmole aldehydes compared to cyseine and ribose alone.


Relative amount
100
90
80
70
60
50
40
30
20
10
0
control
hexanal
dodecanal
2-acetyl thiophene
1-(2-thienyl)-2-propanone
dimethyl formylthiophene
3-ethyl-2-formylthiophene
dihydrothienothiophene
thiazole
3-[(2-methyl-3-furyl) dithio]-2-butanone
bis-(2-methyl-3-furyl) disulfide
2-methyl-3-[(2-furylmethyl) dithio] furan
3-[(2-methyl-3-furyl) dithio]-2-pentanone
furfuryl mercaptan
2-methyl-3-furanthiol
3-thiophenethiol
3-mercapto-2-pentanone
furfural

compounds via the Maillard reaction. The stabilization of lipids could contribute, in part, to the difference in the flavors of cured and uncured meats. A study of the influence of the nitrite-stabilized phospholipids and antioxidants on the Maillard reaction is reported in the next section (Chapter 3).

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CHAPTER 3

THE EFFECTS OF NITRITE-STABILIZED LIPIDS ON THE FORMATION OF FLAVOR VOLATILES FROM A HEATED MODEL SYSTEM INVOLVING CYSTEINE AND RIBOSE

ABSTRACT

Phospholipids (PLs) were isolated from cured and uncured hams and heated in a model system with cysteine and ribose. Nitrite stabilization of the phospholipids was confirmed by heating them with morpholine and measuring the formation of the corresponding N-nitrosamine. The concentrations of N-nitrosomorpholine produced by PLs from cured and uncured hams were significantly different ($p < 0.005$). Volatiles produced by heating cysteine and ribose were less suppressed in the presence of PLs from cured hams. When antioxidants were added to the model systems of cysteine, ribose and phosphatidylcholine before heating, less suppression of the Maillard reaction was also observed. It was concluded that nitrite not only prevents lipid oxidation, but also modifies the formation of Maillard flavor compounds. This modification of the Maillard reaction may be partially responsible for the difference in flavors of cured and uncured meats. A sensory evaluation of volatiles produced from the cysteine-ribose-phosphatidylcholine system indicated flavor differences as a result of adding antioxidants to the system. These observations were confirmed by gas chromatographic analysis

INTRODUCTION

The Maillard reaction is a complex reaction which yields both high molecular weight browning products and volatile aroma compounds (Hodge, 1953). Among the latter are heterocyclic compounds which play a major role in meat flavor (Mottram, 1994).

Recently, the interaction between lipids and Maillard reaction mixtures (cysteine and ribose) in a heated model system has been studied extensively (Whitfield et al., 1988; Farmer et al., 1989; Farmer and Mottram, 1990a). These studies established that lipids or their degradation products can alter the composition of the volatile products formed, both qualitatively and quantitatively. In general, the addition of phospholipids to the simple Maillard reaction mixtures exerted a quenching effect on the production of the heterocyclic compounds derived solely from the reaction of the amino acids with ribose (Whitfield, 1992). For example, alkylpyrazines were produced in slightly smaller concentrations in the presence of the lipids. The trend was more obvious with many of alkylthiophenes and alkylthiazoles (Whitfield et al., 1988; Farmer and Mottram, 1990a). The observed reduction in the concentrations of the alkylpyrazines, alkylthiazoles and alkylthiophenes in the reactions containing phospholipids was thought to be due to the preferential reaction of free hydrogen sulfide or ammonia with compounds derived from the lipids (Whitfield et al., 1988; Farmer and Mottram, 1990a). Phospholipids in meat systems contain unsaturated fatty acids, particularly those with three or more double bonds. The unsaturated moieties undergo oxidative breakdown during heating to give carbonyl compounds which could compete with sugar-derived carbonyls for Maillard reaction intermediates

such as free hydrogen sulfide. This will affect the relative proportions of the heterocyclic compounds produced from the Maillard reaction (Farmer and Mottram, 1990a; Mottram, 1994).

The use of antioxidants to stabilize lipids in a heated model system containing cysteine and ribose may minimize the effect of lipid oxidation on the formation of heterocyclic compounds. It is well established that nitrite functions as an antioxidant in meats (Gray and Crackel, 1992), and several mechanisms have been proposed to explain its antioxidant effects:

1. Nitrite forms stable complexes with the heme pigment in meat systems, thereby inhibiting heme-catalyzed lipid oxidation (Igene et al., 1985).
2. Nitrite chelates metal ions such as ferrous ions, thus rendering these ions unavailable for catalysis of oxidation (Morrissey and Tichivangana, 1985).
3. Nitrite forms certain compounds, such as nitrite oxide - myoglobin, which can quench free radicals (Kanner et al., 1980).
4. Nitrite stabilizes unsaturated lipids in meat systems (Freybler et al., 1993).

Stabilization of the porphyrin ring during cooking appears to be the most plausible mechanism (Gray and Pearson, 1987). The mechanism involving nitrite addition to the double bonds of unsaturated fatty acids also merits consideration (Freybler et al., 1993). The antioxidant activities of nitrite may modify the flavor compounds generated by the Maillard reaction in heat-processed meat products, and could explain, in part, the difference in the flavors of cured and uncured meats. There is considerable evidence that smaller quantities of carbonyl compounds are formed in nitrite-cured meats during cooking compared to those that are nitrite-free (Bailey and Swain, 1973; Shahidi, 1989; Gray and Pearson, 1994; Ramarathnam et al., 1991a, b; Ramarathnam and Rubin, 1994).

Consequently, less suppression or quenching of the Maillard reaction may be obtained, resulting in the generation of a more intense meaty aroma.

The purpose of this study was to determine the effect of nitrite-stabilized phospholipids on the formation of the Maillard reaction products in model systems containing cysteine and ribose. Another objective was to investigate the effect of adding antioxidants to the model system of cysteine, ribose and phospholipids on the formation of flavor compounds.

EXPERIMENTAL

Materials

Pork ham samples were obtained from a local commercial processor within 48 hr of slaughter. Chemicals used in the curing brine were salt (International Salt Co., Clark Summit, PA), sodium ascorbate (Sigma Chemical Co., St. Louis, MO), sodium nitrite (J. T. Baker Inc., Phillipsburg, NJ) and sodium tripolyphosphate (Stauffer Chemical Co., Westport, CT). Anhydrous sodium sulfate, calcium phosphate and dichloromethane were purchased from Mallinckrodt Inc. (Paris, KY), and the solvent was redistilled before using. Celite 545 and methanol were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Phosphatidylcholine (egg yolk, type III-E) and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). tert-Butylhydroquinone (Tenox® TBHQ, food-grade antioxidant) was obtained from Eastman Chemical Products Co. (Kingsport, TN). All the chemicals and solvents used were reagent grade.

Preparation of cured hams

Three pork leg-quarter samples (ca. 50 lb each) were processed in the Meat Laboratory, Michigan State University. Each sample was divided into three equal portions for the different curing processes. The composition of the curing brines is listed in the Table 1.

The pork samples were stitch pumped with the curing brine to 120% (wt/wt) of their green weights. The pumped hams were then placed in large plastic containers. The hams were transferred to a cooler set at 2-4°C and allowed to equilibrate for overnight. The cured samples were then removed from the cooler and freshened using cold running water. The hams were then placed in stockinettes and hung in the smokehouse. Samples were placed in an Elec-Trol laboratory smokehouse (Drying System Inc., Chicago, IL), and processed under the following conditions:

1. 140°F (dry bulb temperature) / 110°F (wet bulb temperature) (60°C/43°C) for 2 hr.
2. 160°F / 130°F (71°C/54°C) for 2-4 hr.
3. 175°F / 155°F (79°C/68°C) until a temperature of 152°F (67°C) was reached.

After cooking, the hams were held overnight in a cooler (approximately 4°C) prior to slicing and packing. The hams were sliced into 1 in (2.54 cm) slices, and each slice was packed in a polyethylene - laminated nylon pouch (Koch, Kansas City, MO). These pouches were 90 μm thick and had a water vapor transmission

Table 1. Composition of the curing brines used to process hams.

Ingredients	weight (g/kg)		
	brine 1	brine 2	brine 3
water	882.9	882.9	882.9
salt	100.0	100.0	100.0
sugar	34.0	34.0	34.0
sodium tripolyphosphate	15.0	15.0	15.0
sodium ascorbate	1.3	1.3	1.3
sodium nitrite (mg /kg)	0	156	1560

rate of 0.041 ml / m^2•day•mmHg and an oxygen transmission rate of 0.124 ml / m^2•day•mmHg at 22.7°C and 50% RH. The packaged ham samples were sealed under vacuum and stored in a freezer (-15°C) until required for analysis.

Quantitation of phospholipids in hams

Total phospholipids in the hams were quantitatively isolated using the dry-column procedure of Marmer et al. (1981). The hams were trimmed of excess adipose tissue prior to lipid extraction. The lean portions were ground using a food processor (Model K5-A, Kitchen Aid, Troy, Ohio). A 5g sample was ground with 20g anhydrous sodium sulfate and 15g Celite 545 in a porcelain mortar until a free-flow powder was produced. The mixture was then transferred to a glass chromatography column (16 mm i.d. x 25 cm with 8 mm i.d. x 5 cm drip tip, Kontes, Vineyard, NJ) containing 10g of a Celite / calcium phosphate mixture (9:1 w/w), and lightly tamped to obtain a uniform bed using a glass rod.

The neutral lipids were eluted first with 150 ml dichloromethane, followed by the phospholipids (polar fraction) using 150 ml of the solvent mixture of dichloromethane / methanol (9:1 v/v). The solvent extracts were concentrated to approximately 5 ml using a rotary vacuum evaporator (Büchi Rotavapor, Büchi Inc., Postfach, Switzerland). The concentrates were quantitatively transferred into 4-ml glass vials (Kimble Opticlear glass vials, 15 mm o.d. x 45 mm, Supelco Inc., Bellefonte, PA), and flushed with nitrogen to remove the solvent. After weighing, the vials were immediately sealed with screw-caps and stored in a freezer (-15°C) until required for further analyses.

Nitrosation of morpholine by phospholipids extracted from ham

To confirm that nitrite reacted with the phospholipids to form nitro-nitrosite derivatives, a secondary amine (morpholine) was heated with the extracted phospholipids to form the corresponding N-nitrosamine (Freybler et al., 1993).

Aliquots (180 μg) of the phospholipids were transferred to a glass ampule to which were added morpholine (500 μg) and 0.6 μl heptane. Six replications of each reaction mixture were prepared. The ampules were flushed with nitrogen for 5 min, flame-sealed, and heated in a Reactitherm Heating Module (Pierce Chemical Company, Rockford, IL) at 130 ^{o}C for 30 min. The reaction mixtures were then allowed to cool, and the contents in each ampule dissolved in 1 ml dichloromethane (Ross et al., 1987).

Quantitation of N-nitrosomorpholine was achieved using a GC (Varian Model 3700) / thermal energy analyzer system (TEA) (Thermal Electron Corporation Model 502, Waltham, Mass.) linked to a Hewlett Packard 3390a integrator (Freybler et al., 1993). A glass chromatographic column (2 mm i.d. x 3 m) packed with 10% Carbowax 20m TPA on 80/100 Chromosorb WHP was used for analysis. The GC operating conditions were programmed as follows: an initial temperature of 140^{o}C for 1 min, followed by temperature increase up to 180 ^{o}C at a rate of 15 ^{o}C/min, holding at this temperature for 7 min. A 5μl aliquot of the concentrate was injected. A standard solution of N-nitrosomorpholine (NMOR) (0.1 μg / ml) was used for quantitation of the N-nitrosamine produced.

Preparation and isolation of Maillard reaction volatiles

Cysteine and ribose were heated together in the presence and absence of phospholipids in a phosphate buffer. Each reaction mixture was prepared in triplicate.

The reaction mixtures were prepared by dissolving cysteine (0.61g) and ribose (0.75 g) in 2 ml phosphate buffer (0.5M, pH 5.7) in a 10 ml clear glass vial (Pierce Chemical Co., Rockford, IL). Phospholipids (100 mg) were weighed into the reaction vials which were then were immersed in an ultrasonic bath (Branson 2000, Danbury, CT). The reaction mixtures were held at 45°C until the reactants were uniformly dispersed throughout the aqueous phase. The vials were flushed with a stream of nitrogen (30 ml/min) for 2 min prior to sealing with a Tuf-bond Teflon-silicon disc and aluminum cap (Pierce Chemical Co., Rockford, IL). The reaction vials were heated at 140°C in an autoclave under a pressure of 0.28 MPa for 1 h. After heating, they were stored in a freezer (-10°C) prior to volatile collection.

To study the effect of antioxidants on flavor production, a model system containing phosphatidylcholine (PC) (100 mg), cysteine (0.61g) and ribose (0.75g) in 2 ml phosphate buffer (0.5M, pH 5.7) was heated at 140°C. Three antioxidants: α-tocopherol, TBHQ, and sodium nitrite (150 μg/g lipid) were investigated and added individually to the reaction mixture. Three replications were carried out for each antioxidant.

The aroma generated in these model systems was evaluated by three flavor chemists in the Department of Food Science and Human Nutrition, Michigan State University. Each sample was presented to the panelists in the reaction vial at room temperature (22 ± 2°C). The panelists were asked to open the vials and sniff the headspace. The panels were allowed to describe the odor characteristics freely.

Volatile compounds were collected using the purge-and-trap procedure which was described in Chapter 1. The contents of the reaction vials (ca. 2 ml) were transferred to a 500-ml two-neck flask (Kontes, Vineland, NJ) containing 18 ml phosphate buffer (0.5M, pH 5.7). The flask was fitted with a L-shape glass

joint (Kontes, Vineland, NJ), and the Tenax trap was attached by a stainless steel Swagelok reducing union (Cajon Ultra-Torr® fitting, Cajon, Macedonia, OH) to the outlet of the joint. During collection, the flask was heated at 60°C using a heating mantle, and the Tenax trap was maintained at room temperature (approximately, 20°C ± 2°C). The volatile compounds were swept into the Tenax trap using a stream of nitrogen (30 ml /min), and the collection was continued for 1 h. The flask was then removed, and the Tenax trap was connected directly to the nitrogen supply for 5 min to remove water vapor (Whitfield et al., 1988; Farmer et al., 1990a).

For the desorption of volatile compounds, the Tenax trap was connected between a solvent (ethyl acetate) source and a cold trap. The nitrogen was passed through the ethyl acetate reservoir (30 - 40 ml/min), and the ethyl acetate - nitrogen gas was introduced into the heated (150°C) Tenax trap. The desorbed volatile compounds were then condensed in a dry ice / acetone cold trap. The desorption process was continued for 30 min, after which the ethyl acetate extract was concentrated to 0.1 ml under nitrogen. The concentrate was transferred to a screw-cap vial and stored in a refrigerator prior to gas chromatographic / mass spectrometeric (GC/MS) analysis.

The volatile compounds were separated and identified using a HP 5890 GC (Hewlett Packard, Avondale, PA) interfaced with a HP 5970 MSD (Mass Select Detector) MS. The GC / MS system was equipped with a HP 59970 Chemstation Data System. A Carbowax 20M fused silica capillary column (60m x 0.32 mm id, Supelco Inc., Bellefonte, PA) was used for the separation of the volatile compounds. The GC oven temperature was programmed initially at 40°C for 5 min, and increased to 200°C at a rate of 2°C / min, then held at this temperature for 10 min. Helium was used as a carrier gas with a flow rate of 10 ml/min. The

temperatures of the injection port and transfer lines were both set at 220°C. The MS was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250°C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 sec over a mass range of 40-300 was employed. The GC / MS data were monitored and stored in the HP Chemstation data system.

The Maillard reaction products were identified by comparing their mass spectra and Kovats indices (KI) to those of authentic standard compounds. Two mass spectral libraries, the INRA Mass Spectra Library - "INRAMASS" (Laboratoire de Recherche sue les Aromes, Dijon, France) and the NIST / EPA / MSDC Mass Spectral Database (ACS Publication Co., Washington, DC), were used to confirm the identity of selected compounds.

Statistical analysis

A one way ANOVA (Analysis of Variance) was used to determine the significance of the differences of the phospholipid contents in the hams and the amounts of NMOR produced by the various phospholipids. The significance between treatment means was determined by the Bonferroni-t test for non-orthogonal comparisons (Gill, 1978). Six replications were performed in each treatment, and the data were calculated using the MSTATC microcomputer statistical program (Michigan State University, 1991).

RESULTS AND DISCUSSION

Quantitation of phospholipids in ham samples

The quantities of phospholipids extracted from cured and uncured hams are listed in Table 2. The phospholipid contents of the lean portion of the hams were in the range 0.8 - 1.2 % (w/w). The phospholipid contents in hams cured with 150 and 1500 mg/kg nitrite averaged of 1.2% ± 0.2% (w/w), while the uncured hams appeared to contain smaller quantities (0.8% ± 0.2%, w/w). The phospholipid contents expressed as a percentage of the total lipids were also determined (Table 2). An average of 10.6 % ± 3.4% was obtained for the nitrite-free hams, while the percentages of phospholipids in the nitrite-cured hams averaged 13.0 ± 3.3 and 13.2 ± 2.7% respectively for the hams cured with 150 and 1500 (mg nitrite / kg meat), respectively.

The concentrations of phospholipids in the lean tissue of meats are relatively constant (Bodwell et al., 1986). Their proportions vary as a function of the total lipids in muscle (Anderson, 1988). As the total lipid content of meat decreases from 12% to 1%, the percentage of phospholipid in the total fat increases from less than 10% to over 80% (Weihrauch et al., 1983). For example, Anderson (1988) reported that the phospholipid contents in lean tissues of beef and pork represented approximately 12% and 15% of the total lipid, respectively. These values are comparable to those reported in Table 2.

Nitrosation of morpholine

To confirm that the stabilization of the phospholipids was due to the

Table 2. Lipid contents[1] of hams cured with varying levels of nitrite.

Nitrite target level (mg/kg ham)	Phospholipids		Total lipids		phospholipid %[2]
	wt[3]	%	wt	%	
0 (control)	0.04 ± 0.01	0.8 ± 0.2	0.39 ± 0.10	7.75 ± 0.02	10.6 ± 3.4
150	0.06 ± 0.01	1.2 ± 0.2	0.46 ± 0.04	9.20 ± 0.01	13.0 ± 3.3
1500	0.06 ± 0.01	1.2 ± 0.2	0.44 ± 0.04	8.97 ± 0.01	13.2 ± 2.7

[1] Lipid contents are expressed as an average wt ± standard deviation (S.D.) (g) in a 5 g ham sample. Two slices were taken from each ham sample. There were three hams for each level of nitrite. Each determination was performed in duplicate.

[2] Phospholipid contents are expressed as a percentage of the total lipid content.

[3] No significant difference in the average phospholipid weights between hams cured with different levels of nitrite was obtained (Gill, 1978).

interaction between nitrite / oxides of nitrogen and the double bonds of the unsaturated fatty acids, phospholipids extracted from nitrite-free and nitrite-cured hams were heated with morpholine to form the corresponding N-nitroso compound. Nitrite, per se, is not a nitrosating agent. However, a number of nitrite derivatives can nitrosate amines under certain conditions. Oxides of nitrogen (NOx) are believed to be responsible for N-nitrosamine formation in cured meat products. Liu et al. (1988) proposed that dinitrogen trioxide is formed when nitrite is added to cured meat and can react with the carbon-carbon double bonds of unsaturated lipids to form nitro-nitroso derivatives. This derivative can decompose during cooking to release the NOx compounds which are capable of nitrosating secondary amines.

Significant differences ($p < 0.005$) in NMOR formation was observed using phospholipids from the various hams (Table 3). The greatest quantity of NMOR was formed (1858.60 ± 451.23 μg / g lipid) by the phospholipids extracted from the hams cured with 1500 mg / kg nitrite. Phospholipids from the hams cured with 150 mg / kg nitrite produced 695.99 ± 319.00 μg NMOR / g lipid. However, NMOR was not detected when phospholipids from the uncured hams were heated with morpholine.

Results of this study clearly indicate that phospholipids extracted from the nitrite-cured hams were capable of nitrosating morpholine, whereas those from the uncured samples could not. Similar results were obtained by Freybler et al. (1993). When phospholipids from nitrite-cured pork (156 mg / kg) were heated with morpholine, a significantly ($p < 0.05$) greater amount of NMOR (1433.5 ± 82.5 μg / g lipid) was produced compared to that formed by phospholipids from

Table 3. Formation of N-nitrosomorpholine on heating morpholine with phospholipids extracted from hams cured with different levels of nitrite[1].

Sample	μg N-nitrosamine / g lipid
phospholipids from uncured ham	not detected[a]
phospholipids from 150 mg / kg nitrite ham	695.99 ± 319.00[b] (337.96 - 950.00)
phospholipids from 1500 mg / kg nitrite ham	1858.60 ± 451.23[c] (1353.58 - 2222.22)

1 Values (i.e., the means of 6 replications) having different superscripts within column are significantly different ($p < 0.005$) in a one-way ANOVA analysis (Gill, 1978).

uncured pork (226.8 ± 79.4 μg / g lipid). These investigators concluded that nitrite stabilizes the double bonds of unsaturated fatty acids against peroxidative changes through the formation of nitro-nitroso derivatives.

Volatiles generated from the Maillard reaction in the presence of phospholipids

The formation of selected Maillard reaction volatiles was reduced when phospholipids from uncured were present in the model system containing cysteine and ribose (Table 4). Fifteen volatile compounds generated from the reaction of cysteine and ribose upon heating were previously identified in order to study the effect of ham phospholipids on the Maillard reaction (Chapter 2). Formation of these compounds in an identical model system were reported to be influenced by the presence of phospholipids (Farmer and Mottram, 1990a, b), an observation that was confirmed in Chapter 2.

Most of the selected Maillard reaction volatiles were reduced in the presence of phospholipids from uncured hams, the relative amounts being in the range of 12 - 84 % compared to those in the phospholipid-free control. Furthermore, thiazole and two thiophenes were not detected in this system (Table 4). The amounts of a bicyclic compound, heterocyclic thiols, two disulfides, 2-mercapto-3-pentanone and furfural were reduced to less than 30%, while two disulfides were reduced to approximately 55% (Table 4).

A smaller effect on the formation of Maillard reaction volatiles was observed when phospholipids from the cured hams were heated with cysteine and ribose (Table 4). An obvious difference was noticed in the formation of thiazole. Concentrations of this compound in the systems containing phospholipids from

Table 4. Relative amounts of selected Maillard reaction volatiles from the reaction of cysteine and ribose in the presence of phospholipids extracted from hams.

Compound	phospholipids		
	nitrite-free ham	nitrite-cured (150 mg/kg)	nitrite-cured (1500 mg/kg)
Thiophenes			
2-acetyl thiophene	----[a]	----	----
1-(2-thienyl)-2-propanone	80[b]	88	82
dimethyl formylthiophene	----	----	----
3-ethyl-2-formylthiophene	84	84	87
Bicyclic compounds			
dihydrothienothiophene	24	25	31
Thiazoles			
thiazole	----	39	48
Disulfides			
3-[(2-methyl-3-furyl) dithio]-2-butanone	20	27	33
bis-(2-methyl-3-furyl) disulfide	26	37	35
2-methyl-3-[(2-furylmethyl) dithio] furan	58	63	66
3-[(2-methyl-3-furyl) dithio]-2-pentanone	53	56	89
Heterocyclic thiols			
furfuryl mercaptan	25	31	36
2-methyl-3-furanthiol	12	27	28
3-thiophenethiol	18	40	38
Mercapto carbonyls			
3-mercapto-2-pentanone	25	30	33
Non-sulfur compounds			
furfural	24	24	22

Table 4. (continued)

a "----" indicates not detected.

b The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction mixture without phospholipid (i.e. control). The calculation was accomplished by standardizing the peak area of the selected compound in the control as 100%. The peak area of the Maillard reaction product was then divided by the peak area of the same compound in the control system.

hams cured with 150 and 1500 mg /kg nitrite were 61% and 52% of the concentration in the control system (i.e. cysteine and ribose). Thiazole was not detected in the system containing phospholipids from the uncured ham (Table 4).Two thiophenes (1-(2-thienyl)-2-propane and 3-ethyl-2-formyl thiophene) were not detected in both the cured ham phospholipid systems. Similar results were obtained for phospholipids from uncured hams. Greater quantities of disulfides, heterocyclic thiols and mercapto carbonyl compounds were observed in the reactions with phospholipids extracted from the nitrite-cured hams compared to those with phospholipids from nitrite-free samples (Table 4). No apparent difference in furfural formation was observed between systems.

Previous results have indicated that the formation of selected Maillard reaction volatiles were suppressed in the presence of phospholipids (Farmer and Mottram, 1990a, b). It was proposed that this quenching / suppression was due to lipid-derived carbonyls reacting with the amino group of cysteine and/or free ammonia or hydrogen sulfide from the Strecker degradation. As nitrite stabilizes the unsaturated fatty acids in phospholipids (Freybler et al., 1993), it was anticipated that the effect of phospholipids from nitrite-cured hams on the Maillard reaction would be less than phospholipids from the nitrite-free hams. This was confirmed by the results in Table 4. This study also revealed that smaller quantities of lipid oxidation products, notably hexanal (Shahidi et al., 1987), were produced in those systems containing the nitrite-stabilized phospholipids (Table 5). Hexanal and three long-chain alkyl heterocyclic compounds were isolated from the reaction between cysteine, ribose and phospholipids. Hexanal is a major lipid oxidation carbonyl product and is used routinely to monitor the extent of lipid

Table 5. Relative amounts of lipid oxidation products and heterocyclic compounds derived from the reaction of cysteine and ribose in the presence of phospholipids extracted from hams cured with different levels of nitrites.

Compound	Phospholipids		
	nitrite-free ham	nitrite-cured (150 mg/kg)	nitrite-cured (1500 mg/kg)
hexanal	100	67[a]	28
2-pentyl furan	100	40	48
2-pentyl thiophene	100	----[b]	12
2-hexyl thiophene	100	----	3

[a] The numbers represent the average percent (3 replications) of the relative amounts of the compounds formed compared to the same compound isolated from the system containing phospholipids from uncured hams. The calculation was accomplished by standardizing the peak area of selected compound from the system containing phospholipids from the uncured hams as 100%. The peak area of the volatile compound was then divided by the peak area of same compound in the uncured ham system.

[b] "----": not detected.

oxidation in foods (Frankel, 1991; Shahidi and Pegg, 1994). It has been proposed that the long-chain alkyl thiophenes are derived from the reaction between hydrogen sulfide and the corresponding furans at high temperatures (Vernin et al., 1982). A more probable route is the reaction of hydrogen sulfide with dienals (Farmer and Mottram, 1990a), and the alkyl substituent orginated from the omega end of fatty acid chain (Farmer and Whitfield, 1993). Suggested routes for the formation of long-chain alkyl heterocyclic compounds from 2,4-decadienal, a thermal oxidation product of unsaturated fatty acids, are shown in Figure 1. As 2,4-decadienal, like hexanal, is known to be produced from linoleic acid (Frankel, 1991), it is expected that smaller quantities of this aldehyde would be produced in the system containing nitrite-stabilized lipids. Thus, it is expected that smaller concentrations of 2-pentyl furan, 2-pentyl thiophene and 2-hexyl thiophene were produced in the model systems containing phospholipids from nitrite-cured hams relative to systems containing phospholipids from the uncured hams (Table 5).

The results indicated that the production of selected Maillard reaction volatiles is quenched to a lesser extent by phospholipids that have been stabilized by the addition of nitrite during curing of hams. It has also been observed that smaller quantities of carbonyl compounds, i.e., pentanal and hexanal, are produced in the model system containing the nitrite-stabilized phospholipids. Therefore, it is evident that when lipid oxidation is minimized through the stabilization of lipids by nitrite, the extents of quenching/suppression of the Maillard reaction is also minimized. Consequently, the antioxidant role of nitrite in cured meats may play an important role in the formation of cured meat flavor, i.e., by balancing the degree of interaction between lipid oxidation and products of the Maillard reaction.

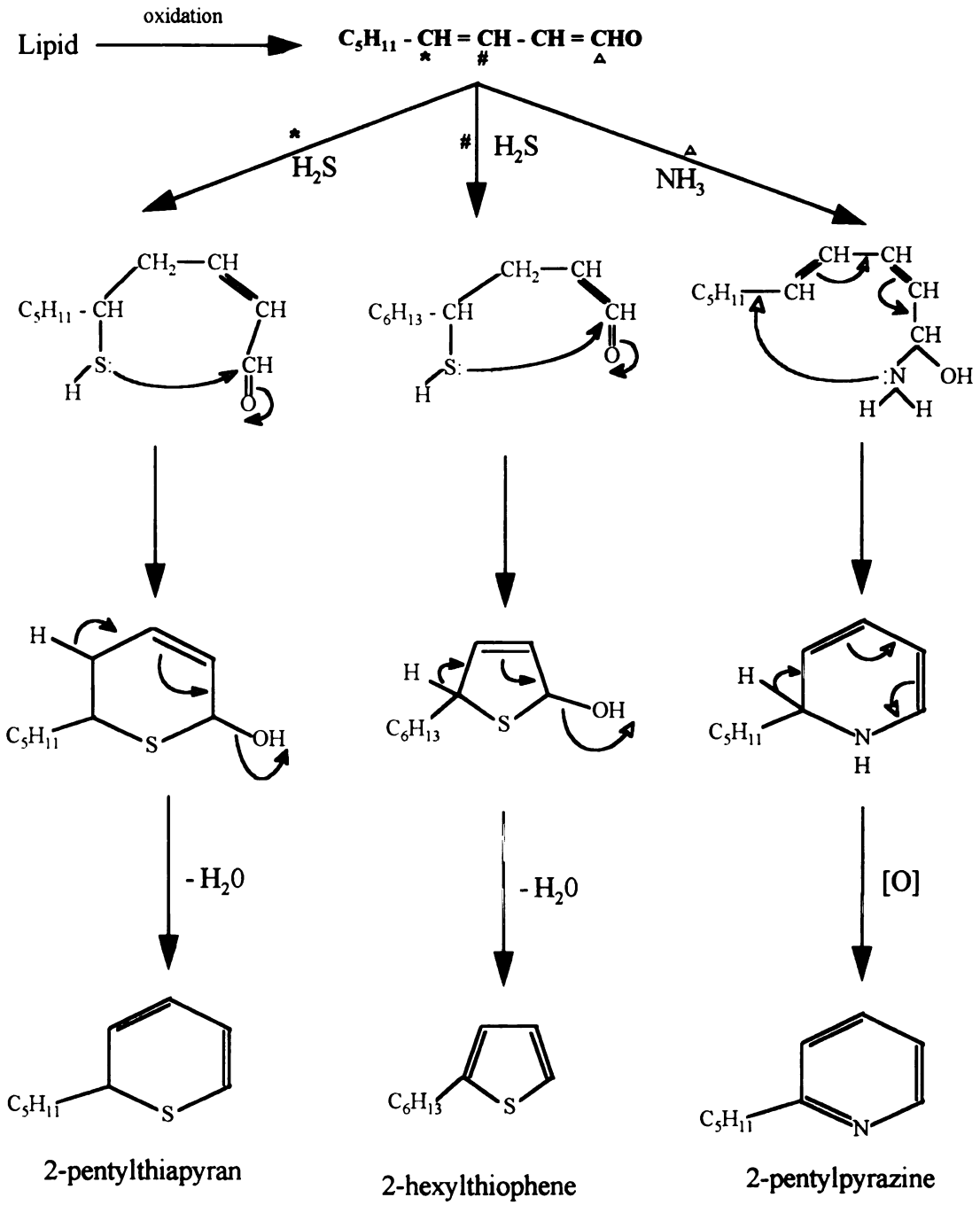


Figure 1. Suggested routes for the formation of 2-pentylthiapyran, 2-hexylthiophene and 2-pentylpyridine from 2,4-decadienal (Farmer and Mottram, 1990a).

Effect of antioxidants on the interaction between the Maillard reaction and lipids in a heated model system

The previous results indicated that nitrite stabilization of phospholipids from cured hams minimizes the suppression of selected Maillard reaction volatiles by thermal oxidation of phospholipids. Thus, antioxidants (e.g., α-tocopherol and TBHQ) when added to a model system containing cysteine, ribose and phospholipids, should reduce the extent of lipid oxidation and ultimately the effect of the carbonyls on the Maillard reaction.

The antioxidants provided different degrees of modification of the lipid-Maillard interaction (Table 6). Compounds including two disulfides, 2-methyl-3-furanthiol, mercapto carbonyl and furfural were produced in similar quantities in systems with different antioxidants. However, nitrite had a greater effect in minimizing the suppression of thiazole, two disulfides and two heterocylic thiols than did α-tocopherol and TBHQ (Table 6). Hexanal concentrations were smallest in the systems containing α-tocopherol, but the lipid-derived long-chain thiophene (2-hexylthiophene) was also present in the greatest amounts in these systems.

A sensory evaluation of the odor of systems containing cysteine, ribose and phosphatidylcholine with different antioxidants revealed no obvious difference, through the use of the antioxidants (Table 7). All samples had slightly meaty aroma; however, one panelist noted that the most meaty note was in the system containing TBHQ.

The antioxidants provided different degrees of influence on lipid oxidation and subsequently in the formation of the Maillard reaction products (Table 6). There was no obvious trend on the formation of volatile compounds from heating

Table 6. Relative amounts of selected Maillard reaction volatiles from the reaction of cysteine and ribose in the presence phosphatidylcholine and antioxidants.

Compound	antioxidants (0.015%, w/w PC)		
	nitrite	α-tocopherol	TBHQ
Thiazoles			
thiazole	42[a]	----[b]	----
Disulfides			
3-[(2-methyl-3-furyl) dithio]-2-butanone	35	7	7
bis-(2-methyl-3-furyl) disulfide	20	6	12
2-methyl-3-[(2-furylmethyl) dithio] furan	25	27	24
3-[(2-methyl-3-furyl) dithio]-2-pentanone	46	49	26
Heterocyclic thiols			
furfuryl mercaptan	33	15	20
2-methyl-3-furanthiol	38	27	40
3-thiophenethiol	50	33	37
Mercapto carbonyls			
3-mercapto-2-pentanone	41	43	44
Non-sulfur compounds			
furfural	31	34	32
Compounds related to lipid oxidation			
hexanal	100	78[c]	97
2-hexylthiophene	100	214	192

[a] The number represents the average percent (3 replications) of the compound compared to the same compound isolated from the reaction of cysteine and ribose alone (i.e. control). The calculation was accomplished by standardizing the peak area of the selected compound in the control as 100%. The peak area of Maillard reaction product was then divided by the peak area of same compound from the control.

[b] "----" means did not detected.

[c] Numbers indicated the relative amounts of compounds isolated from heated cysteine and ribose in the presence of antioxidants compared with nitrite where the peak areas were standardized as 100%.

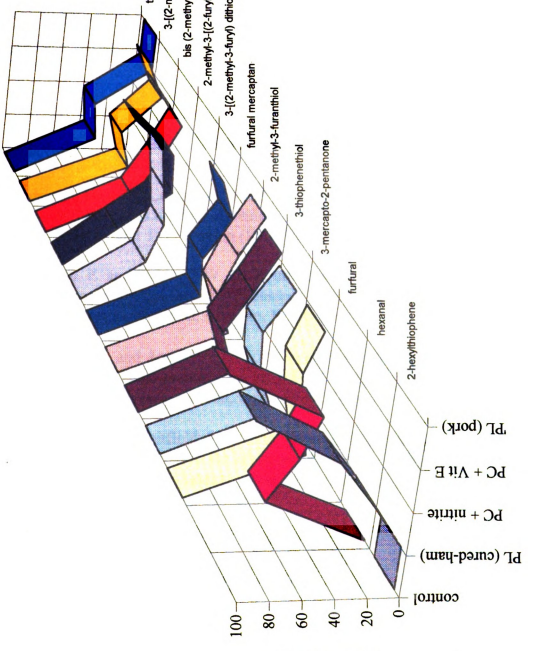
Table 7. Summary of odor descriptions attributed to the headspace of heated cysteine and ribose in the presence of phosphatidylcholine and antioxidants.

Reaction mixture	Descriptors
cysteine + ribose + 100 mg phosphatidylcholine + 0.015% (w/w PC) nitrite	slightly meaty, slightly vinegar
cysteine + ribose + 100 mg phosphatidylcholine + 0.015% (w/w PC) α-tocopherol	slightly meaty, slightly sweet
cysteine + ribose + 100 mg phosphatidylcholine + 0.015% (w/w PC) TBHQ	slightly meaty most meaty/ beefy in all

cysteine, ribose and PC with different antioxidants. However, the relative amounts of the selected Maillard reaction products in the various model systems were in the order: control (cysteine and ribose) > control + phospholipids from nitrite-cured (150 mg/kg) ham, control+ PC + 0.015 % nitrite (w/w PC), control + PC + 0.015% α-tocopherol (w/w PC) > control + phospholipids from nitrite-free hams; however, no differences were apparent in the quantities formed in the following systems: control + phospholipids from nitrite-cured (150 mg/kg) ham, control + PC + 0.015 % nitrite (w/w PC), and control + PC + 0.015% α-tocopherol (w/w PC) (Figure 2). The greatest quantities of hexanal and 2-hexylthiophene were found in the system containing phospholipids from the uncured hams. The results of this study indicate that antioxidants inhibit lipid oxidation, and, thus, minimize the quenching of the formation of Maillard reaction products.

These results indicate that phospholipids from uncured hams suppress the formation of Maillard reaction volatiles in a model system containing cysteine and ribose. This suppression is caused by oxidation of the fatty acids (Farmer and Mottram, 1990a). If the involvement of lipids is due to their thermal oxidation during the reaction process, then a "stabilized" lipid such as that shown by Freybler et al. (1993) for phospholipids in cured pork, would have a smaller impact on the production of Maillard reaction volatiles than phospholipids from uncured meats. This hypothesis was established by the results using phospholipids from hams cured with different levels of nitrite. The use of nitrite or other antioxidants (such as α-tocopherol and TBHQ) minimizes the suppression of the formation of Maillard reaction products in the model systems. The results confirm the assumption that prevention of lipid oxidation leads to less quenching of the Maillard reaction volatiles.

Figure 2. Relative amounts of selected Maillard reaction volatiles isolated from model systems containing cysteine and ribose in the presence of phospholipids with or without antioxidants.
(**Cotnrol**: cysteine and ribose heated alone; **PL (cured-ham)**: control + phospholipids from 150 (mg/kg) nitrite-cured hams; **PC + nitrite**: control + phosphatidylcholine (PC) + 0.015% nitrite (w/w PC); **PC + Vit E**: control + PC + 0.015% α-tocopherol (w/w PC); **PL (pork)**: control + phospholipids from nitrite-free hams).


Relative amount
100
80
60
40
20
0
control
PL (cured-ham)
PC + nitrite
PC + Vit E
PL (pork)
2-hexylthiophene
hexanal
furfural
3-mercapto-2-pentanone
3-thiophenethiol
2-methyl-3-furanthiol
furfural mercaptan
3-[(2-methyl-3-furyl) dithio]-2-pentanone
2-methyl-3-[(2-furylmethyl) dithio] furan
bis (2-methyl-3-furyl) disulfide
3-[(2-methyl-3-furyl) dithio]-2-butanone
thiazole

CONCLUSION

Results in this study indicate that prevention of lipid oxidation may minimize the suppression of the Maillard reaction products in the model systems. Thus, it may be concluded that the importance of nitrite in cured meat flavor is due, in part, to its modefication of the relative concentrations of Maillard reaction products and lipid carbonyl compounds.

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CHAPTER 4

FLAVOR PROFILES OF CURED AND UNCURED HAMS

ABSTRACT

Flavor compounds from uncured and cured hams were collected by Tenax traps and then directly desorbed into a gas chromatograph - mass spectrometer system via a thermal desorption system. Carbonyl compounds were predominant in the flavor profiles of both cured and uncured hams. Of the carbonyl compounds identified, pentanal and hexanal were the more abundant in the uncured hams. The addition of nitrite to hams reduced the formation of these carbonyl compounds. Two nitriles and two thiazoles were also detected in the hams cured with nitrite.

INTRODUCTION

The curing of meat is an ancient art which originated in salting, one of the first methods used for preserving meat (Gray and Pearson, 1984). Nitrite is the most important ingredient in curing brines and imparts the characteristic flavor and pink color to cured meats. It has an antimicrobial effect and prevents the outgrowth of *Clostridium botulinum* spores. It also serves as an antioxidant by minimizing lipid oxidative changes during storage of meat.

Although nitrite is closely associated with cured meat flavor, the chemical changes that are responsible for the unique aroma are not clearly understood (Gray and Pearson, 1984). Noel et al. (1990) studied the role of nitrite in flavor development in uncooked cured meat products via a sensory evaluation, and found that the flavor of the samples containing nitrite was significantly different from that of nitrite-free samples. They concluded that nitrite plays a preeminent role in the development of the unique flavor notes in cured meats.

The general chemical composition of volatile compounds in cured and uncured pork was summarized by Shahidi et al. (1986). They indicated that the total number (118) of carbonyl compounds and hydrocarbons in uncured pork was greater than that of cured pork (45). The number of sulfur-containing compounds in uncured and cured pork was essentially the same. However, the concentration of sulfur-containing compounds in cured pork was approximately two and half times greater than the concentrations of similar compounds in uncured pork. The types of sulfur-containing compounds were also different. Trithiahexanes and thiophenes were predominant in uncured pork; however, more mercaptans, alkyl-sulfides, -disulfides and -trisulfides were isolated from cured pork. Ramarathnam

and Rubin (1994) reported the presence of 30 aldehydes, 20 carboxylic acids, 9 alcohols, 9 esters, 6 furans, 4 hydrocarbons, 12 ketones, a dimethylphenol, a 2-methylpyridine, a 2-methylpyrazine, 3 nitrogen-containing compounds, 31 sulfur-containing compounds, 6 nitriles and 4 nitrates in nitrite-cured hams. However, they concluded that no single group of volatile compounds was responsible for the unique cured meat flavor.

Many studies have indicated that carbonyl compounds make a significant contribution to uncured meat flavor (Hornstein and Crowe, 1960; Cross and Ziegler, 1965; Bailey and Swain, 1973; Shahidi et al, 1986; Shahidi, 1989). However, the nature of cured meat flavor, which is made apparent by suppression of lipid oxidation by nitrite and which is the basic meat flavor of cooked meat, remains a mystery (Rubin and Shahidi, 1988).

The presence of phospholipids in model systems containing cysteine and ribose suppressed the formation of selected Maillard reaction volatiles (Chapter 2). The stabilization of the phospholipids by the addition of antioxidants such as nitrite, α-tocopherol and tert-butylhydroquinone (TBHQ) reduced the quenching effect of unsaturated lipids (Chapter 3). Nitrite prevents lipid oxidation in cured meat products and thus may minimize the negative impact of lipids on the development of the Maillard reaction products. Therefore, the uniqueness of cured meat flavor may be due, in part, to the presence of greater amounts of Maillard reaction products, along with the formation of fewer carbonyl compounds.

The objective of this study was to investigate the differences in the flavor profiles of cured and uncured hams, particularly with the respect to the relative concentrations of carbonyl compounds and Maillard reaction products. The effect of different concentrations of nitrite in the hams on the formation of selected Maillard reaction volatiles was also studied.

EXPERIMENTAL

Materials

Hams were obtained from a local processor within 48 hr of slaughter and processed in the Meat Laboratory (Michigan State University, East Lansing, MI. Detailed were provided in Chapter 3). Sodium phosphate (monobasic and dibasic) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). All the reagents and solvents used in this study were analytical and/or HPLC grade.

Preparation of cooked ham samples

Hams, processed to contain 0, 150, 1500 mg nitrite /kg meat, were sliced into a 1/4" (0.6 cm) thick slices and cooked in an electric frying pan set at 350^{o}F (177^{o}C) for 5 min on each side. The cooked slices were allowed to cool to room temperature, and homogenized in a Waring blender (New Hartford, CT). The samples were transferred to low density polyethylene pouches (8" x 10", 75 μm thickness, Whirl-Pak, Fisher Scientific Co., Fair Lawn, NJ) and stored in a freezer (- 10^{o}C) prior to use.

Collection of flavor compounds

Volatile compounds were collected using a purge-and-trap procedure. Cooked hams (50 g, homogenized) were placed in a two-neck flask (500 ml, Kontes, Vineland, NJ) containing 150 ml phosphate buffer (0.5M, pH 5.7). The flask was fitted with a L-shape glass joint (Kontes, Vineland, NJ), and a Tenax trap (glass lined stainless steel tube containing 200 mg Tenax, 4 mm i.d. x 100 mm) threaded on both ends , Scientific Instrument Services, Ringoes, NJ) was

attached by a stainless steel Swagelok reducing union (Cajun Ultra-Torr® fitting, Cajun, Macedonia, OH) to the outlet of the joint. Tenax traps were conditioned overnight at 180°C with nitrogen flowing at 30 ml / min prior to volatile collection. During collection, the flask containing the ham sample was heated at 60°C in a heating mantle, while the Tenax trap was maintained at room temperature (approximately 20°C ± 2°C). Volatile compounds were swept into the Tenax trap using a stream of nitrogen (30 ml /min), and the collection was continued for 1 h. At the end of the collection period, the Tenax trap was directly connected to the nitrogen supply for 5 min to remove water vapor. More details of this procedure were provided in Chapter 1.

Gas chromatography - mass spectrometric analysis

The trapped flavor compounds were directly desorbed into a gas chromatograph - mass spectrometer (GC / MS) system using a thermal desorption unit (Short Path Thermal Desorption, Scientific Instrument Services, Ringoes, NJ). For desorption, the Tenax trap was fitted with a syringe needle and attached to the desorption unit (Figure 1). This permitted the trapped volatile compounds to be heated by the desorption heater block and desorbed from the Tenax absorbent into the injection port of the gas chamber (GC) via the shortest path possible, i.e. direct injection into the GC via a syringe (Figure 2). The trapped flavor compounds in the Tenax trap were heated at 250°C with nitrogen flushing (10 ml/min) for 5 min.

The GC / MS analysis was simultaneously started with the desorption process. The flavor compounds were separated and identified using a HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) interfaced with a HP 5970 MSD

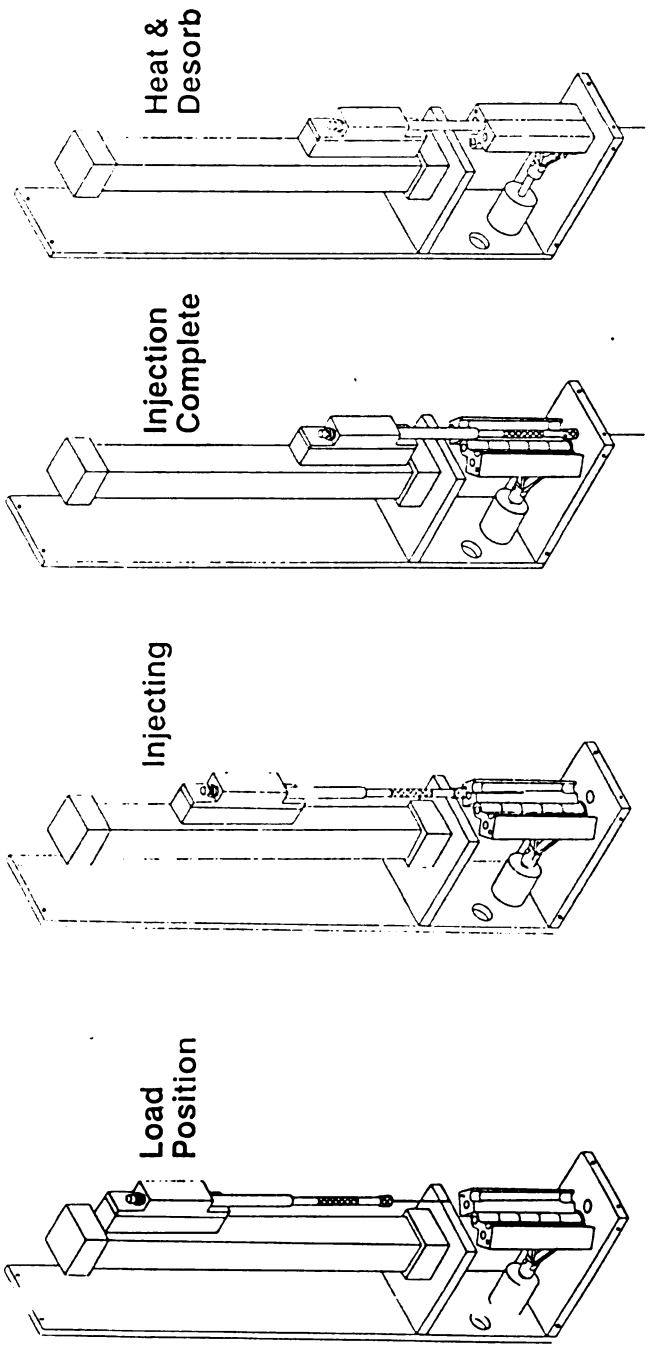

Figure 1. **The scheme of the Short Path Thermal Desorption Unit and the procedures for the desorption of trapped volatile compounds into a GC/MS**

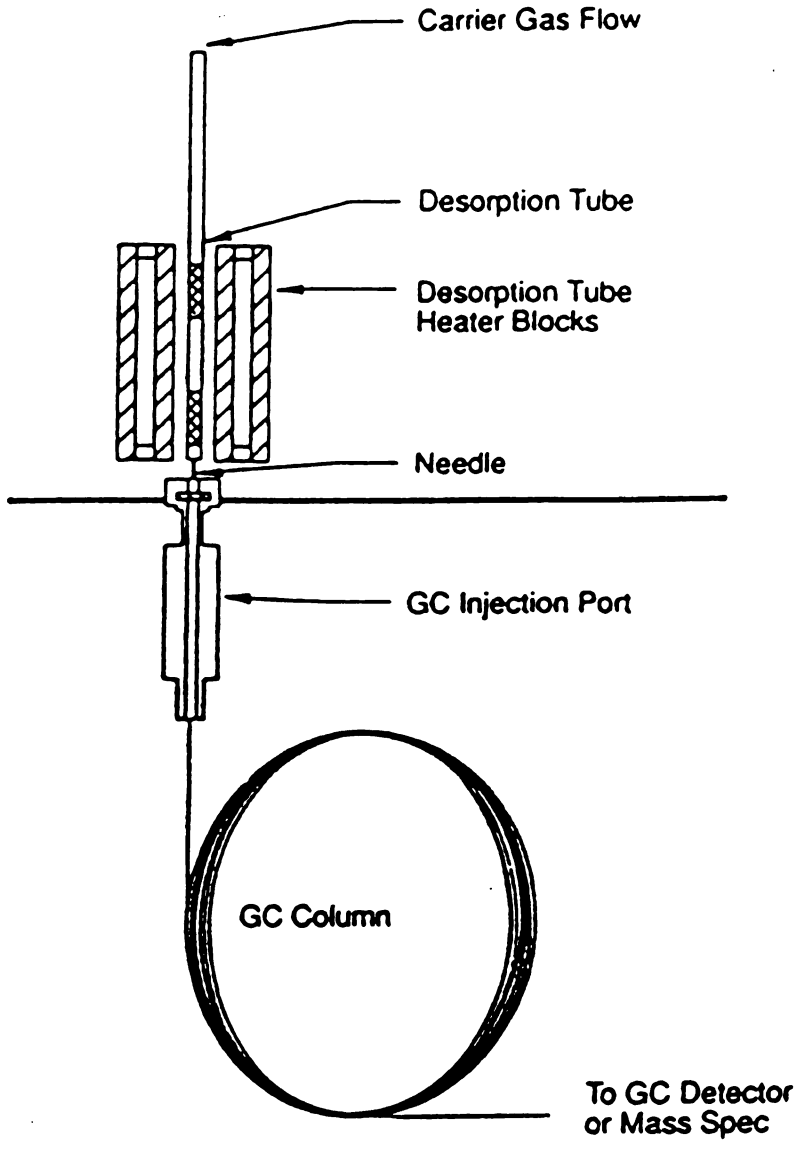

Figure 2. A schematic diagram of the procedure used to desorb the trapped volatile compounds from the Tenax trap. The trapped flavor compounds were thermally desorbed from the Tenax trap and swept into the injection port of the GC via a needle.

(Mass Selective Detector) mass spectrometer. The GC / MS system was equipped with a HP 59970 Chemstation Data System. A Carbowax 20M fused silica capillary column (60m x 0.32 mm id, Supelco Inc., Bellefonte, PA) was used to separate the compounds. The GC oven temperature was programmed as follows: the initial temperature was set at 40°C for 5 min, the temperature was increased at a rate 2°C / min to a final temperature of 200°C, and maintained for 10 min. Helium was the carrier gas with a flow rate of 10 ml/min. The temperatures of the injection port and transfer lines were both set at 220°C.

The mass spectrometer was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250°C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 sec over a mass range of 40-300 was employed. The GC / MS data were monitored, stored and analyzed using an HP Chemstation data system. Two mass spectral libraries, the INRA Mass Spectra Library - "INRAMASS" (Laboratoire de Recherche sue les Aromes, Dijon, France) and the NIST / EPA / MSDC Mass Spectral Database (ACS Publication Co., Washington, DC), were used to identify the compounds isolated.

RESULTS AND DISCUSSION

Volatile compounds isolated from nitrite-free hams

Volatile compounds were collected by the Tenax traps and directly desorbed into the GC via a thermal desorption system. The major advantage of using direct thermal desorption was the reduction of loss of trace compounds. The

concentrations of free amino acids and reducing sugars in meats are much smaller than those used in the model systems, thus much smaller concentrations of Maillard reaction volatiles were expected to be generated in the cooked meat. The use of the thermal desorption system was an attempt to provide a more efficient system for the analysis of the volatile compounds than the system used in the model system studies.

The volatiles collected from the cooked uncured ham contained more than 75 compounds, twelve of which were identified and used to study the differences in uncured and cured meat flavor profiles. The volatile compounds identified were dominated by carbonyl compounds (Table 1). Pentanal and hexanal were the primary compounds, and are derived from the oxidation of unsaturated fatty acids in meats (Cross and Ziegler, 1965). One sulfur-containing compound, ethylthioacetate, was also identified. This compound, reported in cured hams by Golovnya et al. (1982), represented less than 1% of the total volatiles. 1-Octene, the only hydrocarbon isolated from the uncured hams, was also identified in an uncured pork sample by Mottram et al. (1982).

Very few Maillard reaction products were identified in the ham samples. As discussed by Farmer and Mottram (1990a) and confirmed in Chapters 2 and 3, lipid oxidation suppresses the formation of Maillard reaction volatiles in model systems containing cysteine and ribose. However, the failure to detect the Maillard reaction products in the uncured ham samples may be due to the lack of sensitivity of the analytical procedure. In addition, the amount of sample (50 g) could also play an important role. Ramarathnam et al. (1993) used a similar system containing 250-400 g of ground meat in distilled water (meat-to-water ratio was

Table 1. Volatile compounds isolated from uncured and nitrite-cured ham by purge-and-trap method.

Compound	MW	KI	Ham (nitrite mg/kg) 0	150	1500
pentanal	86	982	100	33[a]	33
3-methyl-1-butanol	88	1120	100	60	37
5-hexen-2-one	98	1413	100	----	----
hexanal	100	1090	100	36	30
benzonitrile	103	1605	----[b]	100	130
ethylthioacetate	104	1792	100	105	131
benzaldehyde	106	1524	100	165	145
2,3-dimethyl-2-cyclopenten-1-one	110	1642	100	83	----
1-octene	112	1359	100	97	98
2-methyl-tetrahydrothiophene-1-one	116	1521	100	----	----
methyl bezonitrile	117	1917	----	100	336
acetyl thiazole	127	1714	----	100	120
benzothiazole	135	1972	----	100	180
1-nonen-3-ol	142	1510	100	44	43
2,4-decadienal	152	1816	100	38	15
decyl acetate	200	1675	100	6	5

a The numbers represent the average percent (3 replications) of the compound in the cured ham compared to the same compound isolated from the uncured ham. The calculation was accomplished by standardizing the peak area of the flavor compound from the uncured ham as 100%, and expressing the peak area of the same compound in cured hams as a percentage. When the compound was not detected in the uncured ham, the percentage was expressed by comparing to 150 (mg/kg) nitrite-cured ham (i.e. peak area standardized as 100%).

b "----" : indicates not detected.

4:1 w/w) to collect the volatile compounds from uncured and cured pork. They, too, did not isolate any sulfur-containing compounds. They reported some nitrogen-containing Maillard reaction compounds such as pyridines, pyrazines and pyrroles, but these compounds were present at concentrations less than 0.4 part per million in the cured products. Thus, the smaller sample size used in this study did not permit the detection of compounds that are formed in only small amounts.

Flavor profile of cooked cured hams

The volatile compounds isolated from the cured hams (150 and 1500 mg/kg) were also dominated by carbonyl compounds. However, their concentrations were considerably smaller than those isolated from the uncured hams (Table 1). The concentrations of pentanal and hexanal were approximately 30-36 % of those in the uncured hams. Increasing the nitrite level in cured hams reduced the formation of the carbonyls (Table 1).

Similar results were reported by Cross and Ziegler (1965) regarding the concentrations of pentanal and hexanal in cured and uncured meats. Pentanal and hexanal were present in appreciable quantities in the uncured products, but were barely detectable in the volatiles of the cured meats. Other carbonyls derived from lipid oxidation were also reduced by the presence of nitrite in cured meat (Ramarathnam et al., 1991a, b). Nitrite serves as an antioxidant and prevents lipid oxidation in meats during cooking and subsequent storage (Gray and Pearson, 1994). However, carbonyl compounds are still predominant in the cured meat flavor profiles, Bailey and Swain (1973) reported that the volatile compounds from ham treated with and without nitrite were qualitatively similar, but quantitatively different. The presence and absence of certain carbonyls, or the differences in their concentrations, can be a major contributing factor to the differences in the aromas

of cured and uncured hams. Carbonyl compounds have been implicated as significant contributors to the flavor of uncured meat, but not to cured meat flavor (Ramarathnam and Rubin, 1994).

Greater amounts of benzaldehyde were isolated from cured hams compared to the uncured samples (Table 1). The quantities were 65% and 45% greater in the 150 and 1500 (mg/kg) nitrite-cured hams relative to the uncured hams. However, Ramarathnam et al. (1993, 1994) reported smaller concentrations of benzaldehyde in cured pork than in uncured meat. The differences in the two studies may be due to the relatively small amounts present in the cured hams, thus a possible instrumental error may be involved.

Two nitriles (benzonitrile and methyl benzonitrile) and two thiazoles (2-acetyl thiazole and benzothiazole) were isolated from the cured hams (Table 1). The amounts of these compounds increased with increasing nitrite content. Nitrile compounds were also reported in the volatiles of cooked cured pork by Mottram (1984b; 1985). The origin of these nitrogen compounds is from the reaction of fatty acids and sodium nitrite (Mottram, 1985). Benzonitrile appear to have a low odor threshold with almond-like aromas similar to that of benzaldehyde. Thus, nitriles may contribute to the characteristic cured meat flavor (Mottram, 1984b). Both thiazole compounds identified in this study were previously reported in bacon aroma (Mottram, 1984a), and possess strong nutty-roasted and smoky to meaty notes (Fors, 1983). These compounds may also contribute to the unique cured meat flavor.

The curing process simply modifies the composition of the volatile constituents. Cured meat flavor is believed to be the basic meat flavor (Noel et al., 1990), and possibly is derived from Maillard reaction products. The failure to detect the selected Maillard reaction volatiles which were isolated from a model

system (Chapter 1) could be due to several factors. First, the sample size was inadequate to produce appreciable concentrations of volatile compounds for the flavor collection studies. The free amino acid and reducing sugar contents in meats are too small to provide sufficient Maillard reaction products for adequate analysis. Much greater concentrations of these reactants were used in the model system. Second, the temperatures and time of cooking also play an important role in the formation of the Maillard reaction products in meats. Though the Maillard reaction may occur at refrigerated temperatures (Hurrel, 1982), temperature has the greatest influence on the Maillard reaction (Leahy and Reineccius, 1989; Shu et al., 1989; Shaw and Ho., 1989; Ames and Apriyantono, 1994). The Maillard reaction may be initiated at temperatures greater than 90°C and the reaction rate is markedly increased as the temperature rises (Hurrel, 1982). Hams were fried at 350°F for 5 min per side; however, the internal temperature of the ham samples would be much lower than this. Thus, smaller concentrations of Maillard reaction products would be produced. Future studies of cooked meat flavor profiles should employ better and more efficient methods for collecting and identifying trace volatiles compounds in meat products. The use of the cryogenic traps to concentrate the volatile compounds is a necessity.

CONCLUSION

In our hypothesis, carbonyl compounds derived from lipid oxidation may suppress the formation of Maillard reaction products which contribute to the basic meat aroma. Nitrite functions as an antioxidant in cured meat, thus preventing lipid oxidation and benefiting the formation of Maillard reaction volatiles. Though, the

quantities of the Maillard reaction volatiles isolated from the ham samples were too small for positive identification, it is still our speculation that the inhibition of lipid oxidation will reduce quenching of the formation of Maillard reaction products. Model system studies confirmed this hypothesis. However, it remains to be proven in cured meat systems.

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SUMMARY AND CONCLUSIONS

A series of studies were conducted to investigate the effect of lipids on the formation of Maillard reaction products in a model system containing cysteine and ribose. Maillard reaction products are major contributors to many food flavors; however, the formation of these compounds in the model systems are influenced by the presence of lipids because lipid oxidation-derived carbonyls compete with sugar-derived dicarbonyl compounds for hydrogen sulfide and ammonia.

A new technique for the analysis of volatiles was developed using Tenax traps to collect volatile compounds which were subsequently desorbed into a solvent by a combination of heat and solvent extraction. Percent recoveries of five internal standards were greater than 70%. More than 90 compounds were isolated from the heated model system using this method. Thirty-one compounds were identified via the mass spectral library softwares. Of these, fifteen compounds were selected as "marker" compounds. This selection was based on literature reports of compounds whose formation in similar model systems was suppressed by the presence of lipids.

The effect of the degree of unsaturation of phospholipids on the formation of Maillard reaction volatile compounds in a model system containing cysteine and ribose were studied. The extent of suppression or quenching of the formation of Maillard reaction products was related to the increase of unsaturation in the phospholipids. However, the suppressive effect was not related to the amino moieties of the phospholipids. The inclusion of aldehydes in the model system suppressed of the production of Maillard reaction volatiles, thus providing indirect evidence that thermal oxidation of lipids is involved in this suppressive effect. It is

our hypothesis that the stabilization of lipids in meat products, such as through the use of nitrite as a curing adjunct, could reduce the effect of lipids and/or their oxidation carbonyl products on the development of flavor compounds via the Maillard reaction. The stabilization of lipids could contribute, in part, to the difference in the flavors of cured and uncured meats.

The influence of nitrite-stabilized lipids on the formation of Maillard reaction volatiles in a model system containing cysteine and ribose was investigated. Nitrite stabilization of the phospholipids was confirmed by the formation of N-nitrosomorpholine on heating the phospholipids isolated from hams with morpholine. A significant difference ($p < 0.005$) in the quantities of N-nitrosomorpholine formed between cured and uncured hams was obtained. The production of Maillard reaction volatiles in the model systems with phospholipids from the cured hams was less suppressed than by phospholipids from the uncured hams. However, there was no apparent difference in the formation of Maillard reaction products in the model systems with phospholipids from two nitrite-cured (150 and 1500 mg/kg) hams. Results indicated that nitrite prevents lipid oxidation and subsequently minimizes the extent of quenching of the Maillard reaction in the model systems. The addition of antioxidants to a model system containing cysteine, ribose and phosphatidylcholine also resulted in less suppression of the Maillard reaction. Thus, it may be concluded that the importance of nitrite in the formation of cured-meat flavor is due, in part, to the control of lipid oxidation and its possible suppression of the development of Maillard reaction volatile compounds.

The total flavor profiles of cured and uncured hams were studied. A thermal desorption system linked to GC / MS was employed to prevent the loss of trace compounds. Carbonyl compounds were predominant in the flavor profiles of both

cured and uncured hams. However, less lipid oxidation was observed in the cured hams. Two nitriles and two thiazole compounds were identified in the cured ham flavor.

It is our hypothesis that carbonyl compounds derived from lipid oxidation may suppress the formation of Maillard reaction products which contribute to basic meat aroma. Nitrite functions as an antioxidant in cured meat, thus preventing lipid oxidation and benefiting the formation of Maillard reaction volatiles. Though the quantities of the Maillard reaction volatiles isolated from the ham samples were too small for positive identification, it is still our hypothesis that the inhibition of lipid oxidation will reduce the quenching of the formation of Maillard reaction products. Model system studies confirmed this hypothesis. It remains to be proven in cured meat systems using a more sensitive analytical system.

FUTURE RESEARCH

The effect of lipids on flavor development in a Maillard reaction model system containing cysteine and ribose was studied. Results indicate that lipids influence the formation of Maillard reaction volatile compounds, and the extent of suppression is associated with the degree of unsaturation of the lipids. The amounts of Maillard reaction products formed were reduced in model system containing aldehydes. These results demonstrated that lipids suppress the formation of Maillard reaction volatile compounds. However, this study revealed other issues that require some further investigation.

1. Future studies should employ a more sensitive system for volatile compounds to ascertain whether heterocyclic compounds identified in the model systems (Chapter 2 and 3) are present in cured and uncured meats. Since relatively low concentrations of amino acids and reducing sugars are present in foods compared to those used in the model systems, a more efficient headspace collection method is necessary. A cryogenic trap linked to the injection port of GC may prevent the loss of volatile compounds from Tenax traps during desorption. Using a supercritical fluid to extract the volatile compounds from food systems may be another alternative for aroma research in the future.

2. This study focused on cured hams. Other investigations could be carried out with meat products of higher fat content such as cured and uncured pork belly. The higher fat contents would permit the attainment of higher temperatures

during cooking and might lead to the generation of greater concentrations of Maillard reaction products.

3. It is well established that the reaction between sugar, amino acids and creatinine in meats lead to the formation of carcinogenic / mutagenic heterocyclic aromatic amines. These compounds generally are formed from pyrazines and pyridines that are produced by the Maillard reaction. This study and those of Farmer and Mottram (1990a) revealed the presence of sulfur-containing heterocyclic compounds in model systems containing cysteine and ribose. It remains to be determined whether heterocyclic aromatic amines containing sulfur heteroatoms are produced in cooked meat products such as fried ground beef.

APPENDIX

A. List of authentic flavor compounds used as standards.

2-Acetyl heterocycles:

2-acetylpyrrole
2-acetylfuran
2-acetythiophene
2-acetylthiazole
2-acetylpyrridine
2-acetylpyrazine

Thizoles:

thiazole
4-methylthiazole
4,5-dimethylthiazole
2,4,5-trimethylthiazole
2-isobutylthiazole
2-ethoxythiazole

Methylated pyrazines:

2-methylpyrazine
2,3-dimethylpyrazine
2,5-dimethylpyrazine
2,6-dimethylpyrazine
2,3,5-trimethylpyrazine
2,3,5,6-tetramethylpyrazine

Miscellaneous pyrazines:

2-ethylpyrazine
2,3-diethylpyrazine
3-ethyl-2-methylpyrazine
2-methoxypyrazine
2-methoxy-3methylpyrazine
2-isobutyl-3-methoxypyrazine