Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

SECOND EDITION

FLAVOR CHEMISTRY and TECHNOLOGY

GARY REINECCIUS

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University of Wisconsin - Madison
728 State Street
Madison, WI 53706-1494
U.S.A.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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CRC Press is an imprint of Taylor & Francis Group

No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 1-56676-933-7 (Hardcover)
International Standard Book Number-13: 978-1-56676-933-4 (Hardcover)
Library of Congress Card Number 2005041778

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Library of Congress Cataloging-in-Publication Data

Reineccius, Gary.
Flavor chemistry and technology – 2nd ed. / Gary Reineccius.
p. cm.
Includes bibliographical references and index.
ISBN 1-56676-933-7
1. Flavor. 2. Flavoring essences. I. Title.

TP372.5.R374 2005
664¢.5—dc22 2005041778

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5 Changes in Food Flavor Due to Processing

5.1 INTRODUCTION

The characteristic flavor of some food products develops during processing or home preparation. Examples of foods where this occurs include sauerkraut, yogurt, meats, chocolate, coffee, baked goods, and deep fat fried foods. The primary routes for flavor formation in these foods are nonenzymatic browning, fermentation, and thermal oxidations of fats (recognizing there are a host of other minor reactions that can significantly influence the flavor of a food, e.g., thiamin degradation in meats). This chapter will present an overview of these key pathways leading to food flavor during processing.

5.2 THE MAILLARD REACTION

The Maillard reaction is one pathway falling under the umbrella of nonenzymatic browning (caramelization, Maillard reaction, and ascorbic acid browning). Of these pathways, the Maillard reaction plays the major key role in flavor development. This reaction is responsible for some of the most pleasant flavors enjoyed by man. There is no question that freshly baked bread, a steak, a freshly brewed cup of coffee, or a piece of chocolate is appreciated by the consumer. Yet none of the characterizing flavors existed in the product until the food processor (or cook) heated the product to develop the flavor. While the following will present an overview of this reaction as a generator of flavor, greater detail may be found in several books including those edited/written by Schieberle and Engel [1], Nagodawithana [2], Parliment et al. [3], Tressl and Rewicki [4], and Kerler and Winkel [5]. In the scientific literature, one should search on "C.-T. Ho" who has made major contributions in this field (in the U.S.).

It should be mentioned that today the medical field has become the major discipline researching the Maillard reaction since it plays a role in aging as well as several diseases. The dominance of the medical discipline at the last few international symposia on the Maillard reaction illustrates this occurrence [6–9].

5.2.1 General Overview of the Maillard Reaction

Hodge [10] was the first to present an outline of the overall reactions involved in the Maillard reaction. Generally speaking, the Maillard reaction is a reaction between carbonyls and amines. The carbonyls in foods most often are reducing sugars, while the amines come from either amino acids or proteins. In the flavor industry, the

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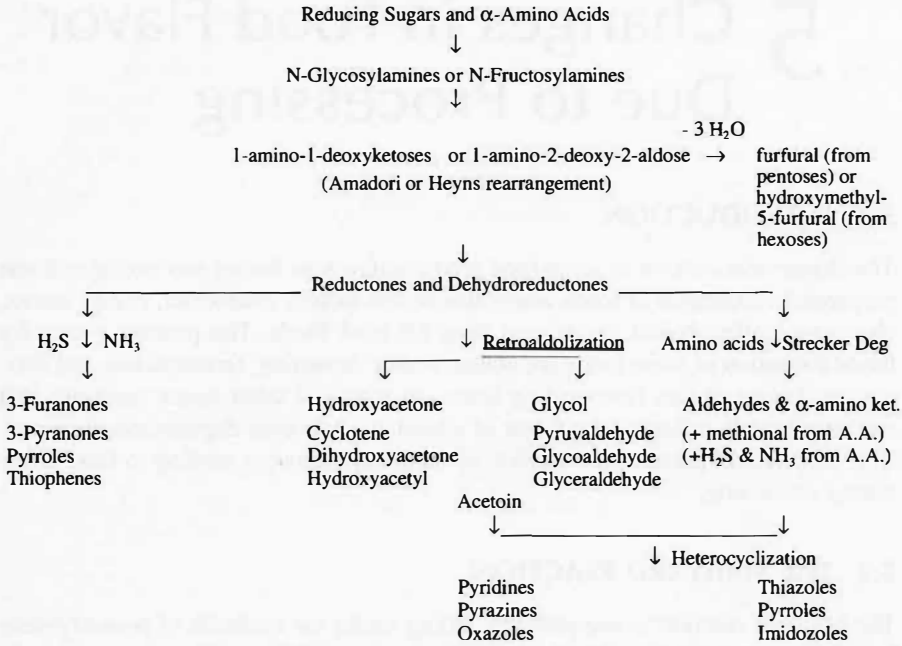

FIGURE 5.1 Formation of flavor compounds via the Maillard reaction. (From Vernin, G., C. Parkanyi, *Chem. Heterocycl. Comp. Flavours Aromas*, p. 151, 1982. With permission.)

carbonyl may be a pure compound (e.g., diacetyl) and the amine, simply ammonia, or an amine. The major end products of the Maillard reaction are melanoidins and other nonvolatile compounds. However, in excess of 3,500 volatile compounds have been attributed to this reaction with numerous having very low sensory thresholds making them important to food aroma (Figure 5.1). While these volatiles comprise a very minor portion (in mass) of the overall reaction products, they are major contributors to the flavor of these foods. For example, Reineccius et al. [11] have shown that while 1.3 g (per 100 g beans) of reducing sugars and amino acids (total) were lost during the roasting of cocoa beans, only 0.9 mg of pyrazines (characterizing volatiles of chocolate) were formed. Thus, about 0.07% of the reactants were transformed into pyrazines while the remainder of the reactants went into other products. Yet these pyrazines are exceptionally important to the flavor of chocolate. It is typically these minor products that make the largest contribution to flavor.

5.2.2 Pathways for Flavor Formation via the Maillard Reaction

As was mentioned earlier, more than 3,500 volatile compounds have been identified as Maillard products. This number documents the large number of pathways potentially active in this reaction. A simplified view of this process begins with the reaction between amino acids and reducing sugars to form imines (Figure 5.2). These imines rearrange to form unstable Amadori (aldose precursor) or Heyns (ketose precursor)

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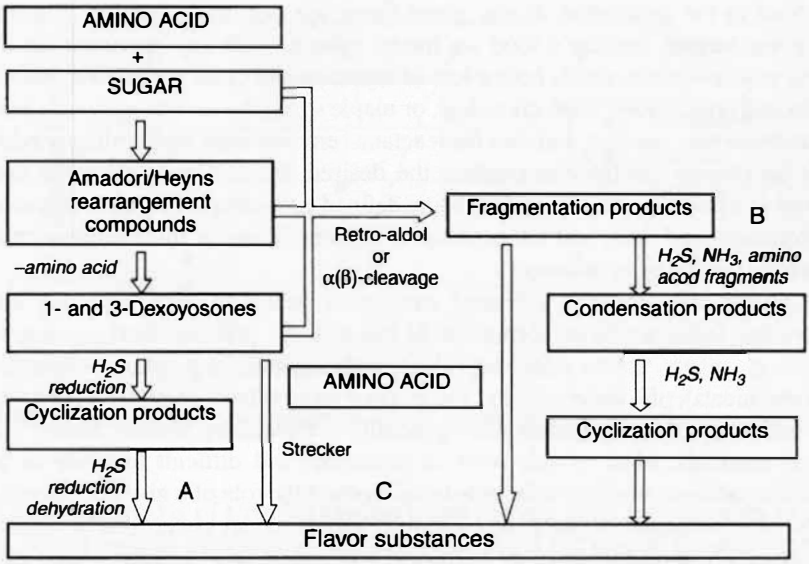

FIGURE 5.2 Major pathways for the formation of flavor substances during the Maillard reaction. (From Kerler, J., C.Winkel, *Food Flavour Technology*, A.J. Taylor, Ed., Sheffield Publ., Sheffield, U.K., 2002, p. 302. With permission.)

products. Then, they may lose an amino acid to form 1- and 3-deoxyosones, which will undergo numerous other steps to form heterocyclic aroma compounds (Scheme A). Alternatively, the Amadori/Heyns products will fragment through retro-aldol condensation or α-, or β-cleavage. These fragments will readily undergo aldol condensations or fragment/amino acid reaction ultimately resulting in the formation of heterocyclic aroma compounds (Scheme B). The remaining pathway is the Strecker degradation (Scheme C). In this pathway, dicarbonyls or hydroxycarbonyl intermediates deaminate and decarboxylate amino acids to yield the corresponding Strecker aldehydes. Tressl and Rewicki [4] have provided a detailed discussion of these pathways.

A substantial amount of work has been done to determine the pathways for the formation of specific classes or individual aroma compounds. These pathways generally appear in original research articles where a given compound is found since the author will often propose a mechanism of formation.

5.2.3 Factors Influencing the Maillard Reaction

Most food science students learn about the Maillard reaction in one of their food chemistry courses. They typically learn about this reaction in the context of either nutritional losses (e.g., the loss of essential amino acids or loss of digestibility) or color formation. It should be recognized that nutritional loss and color formation are generic in the sense that the heating of foods under nearly any conditions will result in the loss of nutritional quality and in color formation. However, the reactions

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that lead to the generation of any given flavor are extremely specific. Illustrating this point further, heating a food (or model system) with any precursor set under nearly any conditions yields both a loss of reactants and color production, but it will not form a typical roast beef, chocolate, or maple syrup flavor. The pathways leading to flavor are very specific, and thus the reactants, environment, and heating conditions must be chosen carefully to produce the desired flavor. Flavor character can be viewed as a point in space, the space being defined by a complex balance of reactants, environment, and time, and temperature of heating. If any of these components are incorrect, the flavor is incorrect.

Unfortunately, there is a limited amount of published work reporting on the factors that influence flavor formation. In much of the previous work, system reactants (e.g., sugars, amino acids, peptides, proteins, lipids [e.g., phospholipids], and pure chemicals), pH, water activity, and thermal process have commonly been varied. The outcomes of these studies were generally evaluated by sensory and/or instrumental methods. Most of this work is piecemeal and difficult to relate to other literature. Also, in an effort to be able to understand the role of a given compositional factor, environmental or processing variable, simple model systems have been used. Unfortunately, the addition of an additional component (e.g., another amino acid or buffering component) or small change in environment or process can greatly change the reaction rate and direction. Thus, it is extremely difficult to translate model system data to real food data. As a result, our knowledge is incomplete, especially in terms of understanding this reaction in complex food systems.

5.2.3.1 Heating Time/Temperature

One of the most important parameters influencing flavor formation via the Maillard reaction is processing temperature. This effect is obvious if one considers, for example, the sensory quality of roasted vs. stewed meats. Stewed meat lacks flavor notes characteristic of the roasted product. This is primarily because the stewed product has a water activity of approximately 1.0 and never exceeds a temperature of ca. 100°C. The roasted meat, however, dries on the surface so water activity is substantially less than 1.0. Also, since the surface dries, surface temperature may exceed 100°C. The lower water activity and higher surface temperatures favor the production of flavor compounds giving the meat roasted notes from the same basic reactants rather than stewed notes.

The influence of temperature on flavor formation may be understood better by recalling that each particular pathway of flavor formation has its own activation energy. To illustrate this influence, consider the plot shown in Figure 5.3 (note that this plot does not contain valid data but is only for illustrating an idea). In this plot, furanthiol has the highest activation energy, followed by pyrazine and furfural. (Activation energy is equal to the slope of the line divided by R [natural gas constant]). This plot shows that furanthiol would have the highest rate constant (or highest rate of production) at high temperatures followed by pyrazine and then furfural. As temperature is decreased to typical storage temperatures, the furfural would be produced at the highest rate and now furanthiol would have the lowest

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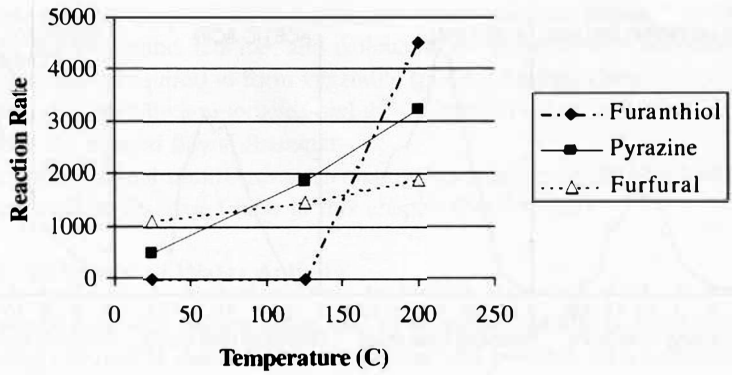

FIGURE 5.3 Influence of temperature on rate of formation of various aroma compounds.

rate. Since each class of volatiles products, and perhaps even each member of each class, would have its own activation energy, one could put several hundred lines on this figure and realize how important temperature is in influencing the formation of flavor compounds via the Maillard reaction.

There is substantial information on the influence of temperature on pyrazine formation in model systems. While Koehler and Odell [13] reported that essentially no pyrazines were formed at temperatures less than 100°C, Shibamoto and Bernhard [14] and Leahy [15] both reported pyrazine production at temperatures as low as 70°C. In all cases, pyrazine production increased rapidly with temperature. A kinetic study by Leahy (1985) reported an activation energy of approximately 35 kcal/mole for pyrazine and 2-methyl pyrazine, and about 43 kcal/mole for the dimethyl pyrazines. These high activation energies indicate a very strong temperature dependence of pyrazine formation and explain why most pyrazines (roasted, nutty, toasted notes) are not formed during storage but only during high temperature treatment. This statement must be qualified in that some pyrazines can be formed at room temperature if the proper reactants are present. Several investigators [16–19] have found pyrazines to be formed in various cheeses from a reaction of microbial metabolic products. Rizzi [20] reported on the ability to form pyrazines under very mild conditions in model systems if the proper pH and reactants were present. It must be noted that Rizzi's systems were not typical Maillard systems, i.e., reducing sugar and protein/amino acid systems. In these systems select (very reactive) pyrazine precursors were combined to form pyrazines. These precursors typically have to be generated via other pathways of the Maillard reaction.

Processing time is also critical in determining flavor character. While in many situations, one method of getting more of some product is to increase the reaction time. Increasing the reaction time of a Maillard reaction does not necessarily increase flavor intensity but changes the final balance of flavor compounds and thereby changes the flavor character. Work by Yamanishi [21] on the roasting of coffee beans (Figure 5.4) illustrates this point, as one can see that the amount of each component

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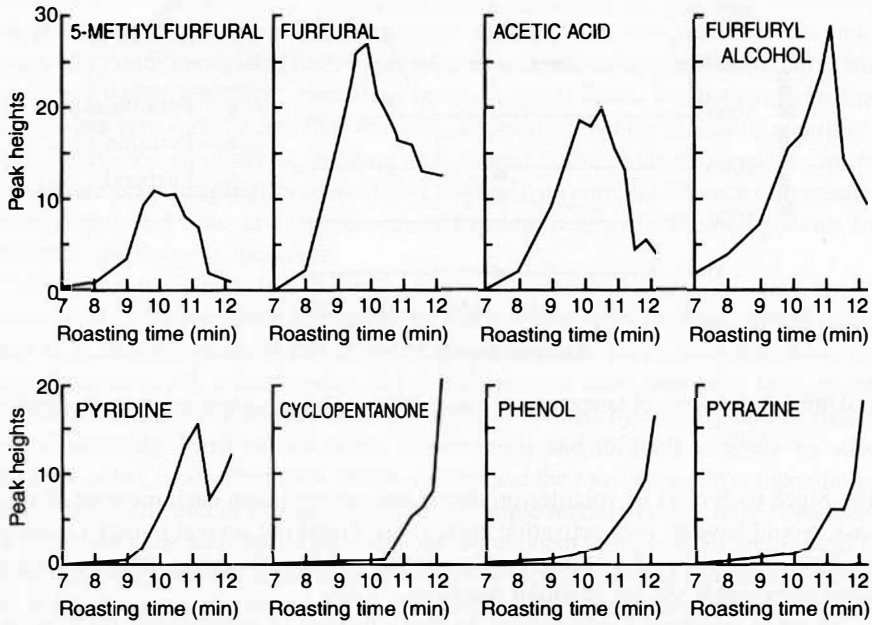

FIGURE 5.4 The influence of roasting time on the amount of some aroma compounds in coffee beans. (From Yamanishi, T., *Flavor Research: Recent Advances*, R. Teranishi, R.A. Flath, H. Sugisawa, Eds., Marcel Dekker, New York, 1981, p. 231. With permission.)

in the coffee beans changes with roasting time and thus the balance of components, i.e., sensory character, changes with heating time.

5.2.3.2 Influence of System Composition

Numerous classical studies have been conducted evaluating the effect of reactant choice (and concentration) on the rate of the Maillard reaction (loss of reactant and/or color formation). One finds that amino acid and sugar type both influence the rate of this reaction. In general, the rate of the reaction is influenced by sugar composition as follows: pentoses (xylose or arabinose) > hexoses (glucose or fructose) > disaccharides (lactose or maltose) > trisaccharides > corn syrup solids > maltodextrins > starches. The reaction rate is also dependent upon the amino acid(s) present with glycine being amongst the most reactive.

If we consider this reaction in terms of flavor formation, the type of carbohydrate tends to play a larger role in determining the rate of the reaction than influencing the flavor character. The rate of reaction largely follows that of the loss of reactants and/or color formation. Sugar type may have some influence on flavor character but the amino acid selection generally plays a much greater role in this respect.

This observation is obvious to those who create process flavors (Chapter 9). When one makes a process flavor, the choice of sugar type is of minimal importance in determining flavor character while the choice of amino acid is all important. As

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is discussed in this chapter, sulfur-containing amino acids are required to form meat or coffee flavors; valine, leucine, and isoleucine are required for chocolate flavors; and methionine is required to form vegetable (potato) flavors. These flavors demand the presence of specific amino acids and the absence of others in a reaction mixture to produce the desired flavor character.

There has been substantial research on how lipids influence the Maillard reaction. This topic will be discussed later in this chapter (Section 5.3).

5.2.3.3 Influence of Water Activity

Water availability will influence the rate of numerous Maillard pathways thereby influencing the rate of overall flavor formation and possibly flavor character. This is anticipated since some chemical reactions produce water as a byproduct (reaction pathway is inhibited by water) while other chemical reactions consume/require water (reaction pathway is promoted by water).

Leahy and Reineccius [22] found the influence of water activity on the rate of formation of alkyl pyrazines in a model system to parallel that of classical Maillard reaction. Briefly, the accumulation of pyrazines reached a maximum when heated at an Aw of ca. 0.75 and decreased with either increasing or decreasing Aw. This observation suggests that the rate of pyrazine formation is controlled by the initial stages of the Maillard reaction as opposed to an effect on specific pathways of pyrazine formation.

The accumulation of thiazoles during the heating of a cysteine:Dimethylhydroxy-furanone (DMHF) model system decreased with increasing water content while the accumulation of thiophenones and 3,5-dimethyl-1,2,4-trithiolane had an optimum moisture content (ca. 80%) and 2,4-hexanedione and 3-hydroxy-2-pentanone increased with moisture content [23.24]. There is little question that the Aw (or moisture content) influences both the rate of the Maillard reaction and influences the sensory properties of a system on heating.

5.2.3.4 Influence of pH

pH may also influence the rate of specific Maillard pathways thereby changing the balance of volatiles formed. For example, pH has been found to have a very strong influence on pyrazine production [13–15,25]. Leahy [15] reported nearly a 500-fold increase in total pyrazine production between a model system heated at pH 9.0 vs. the same system at pH 5.0 (13 ppm vs. 24 ppb). Mottram and Whitfield [26] demonstrated the effect of pH on the formation of several classes of volatiles during the heating of a model system (Table 5.1). They showed that there may be an optimum pH for the formation of some volatiles, or volatile production may be enhanced or retarded at increasing pH. The situation is analogous to that of water activity effects on the formation of specific volatiles: some chemical pathways are enhanced, others retarded, while others are unaffected by pH of the system. The take home message is that pH may strongly influence the balance of aroma compounds present in a heated food/model system, and thus small changes in pH of a food may significantly change its aroma profile after heating.

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TABLE 5.1
The Effect of pH on the Formation of Volatiles During the Heating of a HMF/Cysteine Model System

	pH	2.4	4.7	7.0
Flavor Compound		—	mg Yield	—
3-Hydroxy-2-pentanone		16.5	27.2	8.2
2,4-Hexanedione		64.3	31.9	4.3
2-Acetylthiazole		7.5	14.1	24.7
3,5-Dimethyl-1,2,4-trithiolanes		33.7	50.7	33.9
Thiophenones		101	104	23.7

Source: Mottram, D.S., F.B. Whitfield, *Thermally Generated Flavors*, T.H. Parliment, R.J. McGorrin, C.-T. Ho, Eds., Amer. Chem. Soc., Washington, D.C., 1994, p. 180. With permission.

5.2.3.5 Influence of Buffer/Salts

The salt (buffer) type and concentration may also influence reaction rate. While buffers vary in their effect on the Maillard reaction, it is generally accepted that phosphate is the best catalyst [27]. The effect of phosphate on reaction rate is pH dependent with it having the greatest catalytic effect at pHs between 5–7. Potman and van Wijk [27] found the Maillard reaction rate in a phosphate buffered model system increased from 10- to 15-fold compared to a phosphate free reaction system.

In some unpublished work, we chose to thermally process (extrude) low and normal salt (NaCl) lots of a cooked cereal base. We then analyzed the volatile profile of the two extruded products by gas chromatography. We found that the low salt formulation contained substantially less volatiles (quantitatively) than the normal salt product. It appears that the salt levels used in extruded cereal products influenced the rate of the Maillard reaction. This observation is important in efforts to manufacture thermally processed low salt foods. It appears that taking the salt out of a food may influence both *aroma* and taste (saltiness).

5.2.3.6 Influence of Oxidation/Reduction State

Oxidation/reduction effects on the Maillard reaction were investigated by Shibamoto and Bernhard [14]. They studied the effects of metal ions, oxygen, antioxidants, and sodium hydroxide on the formation of pyrazines in glucose-ammonia systems. Their results indicated that oxygen does not catalyze pyrazine formation. Propyl gallate significantly inhibited the formation of pyrazines for short reaction times (2 hr) at 100°C, but not for long reaction times (18 hr) as compared to the control. BHT inhibited production slightly with long reaction times. However, reaction under an oxygen blanket gave slightly depressed yields. They concluded that antioxidants may reduce yields for reasons other than reducing the amount of oxygen available to the system. Adding $CuCl_2$ and $ZnCl_2$ also decreased yields of pyrazines while increasing browning, as was noted visually.

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5.2.4 Kinetics of the Maillard Reaction and Flavor

There is very little work published on the kinetics of aroma formation via the Maillard reaction. However, what work has been done has indicated that volatile formation is very sensitive to both compositional and processing changes. One can generate kinetic data for volatile formation in a simple model system, include another precursor that should have no direct effect on the kinetics of interest, and find major changes in reaction kinetics.

No organized experiments were performed to obtain kinetic data, i.e., studies where the researcher chose to heat a model system at several temperatures over sufficient times to gather reliable kinetic data, until the late 80s. The available literature will be presented.

5.2.4.1 Pyrazines

Perhaps it is not surprising that much of the kinetic data in the literature is on the formation of pyrazines. There has been a long-term interest in pyrazines due to their impact on the flavor of many thermally processed foods [28,29]. In terms of kinetics, Leahy and Reineccius [22,30] found the formation of the pyrazines during the heating of model systems to be zero order. Later work by Huang et al. [31] on aqueous systems (pH 10 at heating temperatures ranging from 120 to 140°C) and Huang et al. [32] in a study of high-pressure effects on pyrazine formation (aqueous and nonaqueous solvents) also noted a linear formation of pyrazines during heating. Thus, they also reported pyrazine formation as being a zero order reaction in both of these studies. The most recent kinetic work [33] carried the reaction further and used a fractional conversion data analysis technique. In this study they found that 2,5-dimethylpyrazine and 2-methylpyrazine formation was initially linear but formation leveled off at later stages of heating. Since zero order kinetics cannot account for the leveling off in formation, they chose to use first order reaction kinetics for these data. They noted that the reactants (glucose and lysine) were consumed well before the pyrazine concentration plateaued, and thus proposed that there is an intermediate rate limiting step in pyrazine formation. Their data treatment yielded r^2 values in excess of 0.96, demonstrating the excellent quality of prediction.

In terms of kinetic constants (activation energy [Ea]) for pyrazine formation, some variability exists in the literature. Leahy and Reineccius [22,30] found the Ea for pyrazine formation to range from about 33.4 to 44.8 Kcal/mole. Huang et al. [31] found substantially lower activation energies for similar pyrazines (19.5 to 29.0 Kcal/mole); however, they used arginine as the amino acid source while Leahy [22,30] used lysine. Also, Leahy's work was done at pH's ranging from 5 to 9 while Huang et al. [31] used pH 10. pH is known to greatly influence the rate of pyrazine formation via the Maillard reaction [15]. Huang et al. [32] reported Eas for the formation of pyrazines from intermediate precursors (3-hydroxy-2-butanone/ammonium acetate) in the same range in water (ca. 18.9 Kcal/mole), but Ea decreased to 13.1 Kcal/mole when ethanol was used as the solvent.

The most recent work by Jusino et al. [32] was done at lower moisture contents with glucose/lysine as the reactants. They reported an Ea 13.5 Kcal/mole for 2,5-dimethylpyrazine and 2-methylpyrazine under these conditions (note this is a first

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order Ea). When they applied pseudo zero order kinetics to the initial linear portion of the formation curves, they calculated Eas of 36.9 and 43.2 Kcal/mole for 2,5-dimethylpyrazine and 2-methylpyrazine, respectively. These values are very close to those calculated by Leahy [15] for her model systems and nonfat dry milk.

Leahy [15] studied the influence of Aw and pH on the formation of pyrazines. She found that pyrazine formation increased linearly with increasing Aw up to an Aw of about 0.75 and then either decreased or was unchanged at an Aw of 0.84, depending upon the pyrazine. A maximum in reaction rate at an Aw around 0.75 is consistent with Maillard browning as measured by the consumption of reactants or the formation of pigments. Leahy [15] reported that pyrazine formation rate increased by an average of 1.37-fold for each increase in Aw of 0.1 units. Similarly, a linear relationship was found between the rate of pyrazine and 2-methylpyrazine formation and pH (within the range of pH 5–9).

5.2.4.2 Oxygen-Containing Heterocyclic Compounds

There has been very little kinetic work done on other aroma compounds. Schirle-Keller and Reineccius [34] have published a study on the formation of oxygen-containing heterocyclic compounds during the heating (80–150°C) of aqueous model systems (cysteine and glucose). A summary of their results is presented in Table 5.2 (top). Briefly, they found a linear relationship between time of heating and the formation of volatiles, and thus applied zero order kinetic treatment to the data. The r^2 values for first order data treatment were substantially poorer than for the zero order treatment. The Eas are of the same order of magnitude as those for pyrazines reported by Leahy [15].

TABLE 5.2
Kinetic Data for the Formation of Volatiles During the Heating of Model Systems

Compound	Ea (Kcal/mole)	Compound	Ea (Kcal/mole)
Furfural	35.2[a]	2-Acetylfuran	36.2[a]
5-Methylfurfural	37.0[a]	Di(H)di(OH)-6-methylpyranone	30.7[a] (16)[b]
Hydroxymethylfurfural	28.1[a]		
Isovaleraldehyde	19[b]	2-Acetyl-1-pyrroline	14[b] (12.4 and 16.5)[c]
Phenylacetaldehyde	22[b]	5-Me-2-phenyl-2-hexenal	
2-Acetylfuran	18[b] (34.2)[a]		

[a] From Schirle-Keller, J.P., G.A. Reineccius, *Flavor Precursors: Thermal and Enzymatic Conversion*, R. Teranishi, G. Takeoka, M. Guntert, Eds., Amer. Chem. Soc., Washington, D.C., 1993, p. 244. With permission.

[b] From Chan, F., G.A. Reineccius, *Maillard Reactions in Chemistry, Food, and Health*, T.P. Labuza, G.A. Reineccius, V. Monnier, J.O. O'Brien, J.W. Baines, Eds., Royal Chem. Soc. London, London, 1994, p. 131. With permission.

[c] Unpublished data.

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5.2.4.3 Sulfur-Containing Compounds

Chan and Reineccius [36] have reported on the kinetics of the formation of sulfur-containing aroma compounds during the heating (75 to 115°C) of more complex, buffered (0.1 M phosphate buffer), aqueous model systems (glucose with leucine, methionine, phenylalanine, and proline). They generally found that the amount of sulfur-containing volatiles increased with time or temperature of heating. However, a plateau in concentration was reached for methional and dimethyl disulfide at longer heating times and higher temperatures. This plateau was likely due to the exhaustion of reactants. However, the plateau was not achieved at the same concentration for different pHs, suggesting that the plateau concentration was dependent on secondary degradation reactions (pH dependent).

The activation rates for the sulfur compounds measured ranged from 15 to 33 Kcal/mole. 2-Acetylthiophene had the highest Ea ranging from 22 to 33 Kcal/mole while methional and dimethyl disulfide Eas ranged from 15 to 27 Kcal/mole. The values for the latter compounds are consistent with the Eas for the Maillard reaction in general.

Zheng and Ho [37] reported on the kinetics of hydrogen sulfide formation during the heating (80 to 110°C) of aqueous solutions (pH 3, 5, 7, 9) of glutathione and cysteine. They found that H_2S was released from both precursors following first order reaction kinetics (r^2 values ranged from 0.955 to 0.999) and that rate of release increased with pH. Zheng and Ho [37] found activation energies for H_2S to range from 29.4 to 31.8 Kcal/mole for cysteine and 18.8 to 30.8 for glutathione. These data support earlier literature noting that H_2S is more rapidly released from glutathione than cysteine.

5.2.4.4 Miscellaneous Compounds

Chan and Reineccius [35] also have published some kinetic work on other aroma compounds (isovaleraldehyde, phenylacetaldehyde, 2-acetyl-l-pyrroline, 2-acetylfuran, and di(H)di(OH)-6-methyl pyranone). All of these volatiles followed pseudo zero order reaction kinetics in their early stages of reaction (Table 5.2). The fact that the concentrations of isovaleraldehyde, phenylacetaldehyde, and the methylpyranone reached plateaus late in heating suggests that a first order fit, as proposed by Jusino et al. [32], might be more appropriate.

The activation energies of the volatiles presented in Table 5.2 (bottom) are consistent with those found by Ho's group and the Maillard reaction in general. The values found for 2-acetyl-1-pyrroline are very consistent across systems and researchers (values in parentheses). However, the values found for 2-acetylfuran and the methyl pyranone were lower than we have previously observed [34]. This discrepancy in values may be due to the differences in model systems used between the two studies. Chan [38] used a complex amino acid/glucose/phosphate buffered model system while Schirle-Keller and Reineccius [34] used a simple unbuffered glucose/cysteine system. The additional amino acids may serve as good catalysts for the breakdown of glucose into the oxygen-containing volatiles noted, but apparently have little effect on the formation of 2-acetyl-1-pyrroline. This discrepancy in values suggests that the data one obtains from a simple model system may not be

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valid in complex food systems and care should be exercised in data interpretation based on model systems. This is not a new concern but is reinforced here.

The very low activation energy for 2-acetyl-l-pyrroline may help explain why this volatile is found by sniffing methods in nearly every food studied. It appears that this compound is very readily formed even under mild heating conditions. This fact, coupled with its extremely low sensory threshold, make its detection in foods likely. The Strecker aldehydes, isovaleraldehyde and phenylacetaldehyde, followed similar kinetics during heating. This is due to the similar mechanism of formation and the fact that they are both consumed through reaction to form 5-methyl-2-phenyl-2-hexenal [39].

5.2.4.5 Summary

There has been limited work done studying the kinetics of flavor formation via the Maillard reaction. Of this work, the majority has been on the formation of pyrazines in simple model systems. There is an initial linear period for flavor formation that can be accurately modeled using pseudo zero order reaction kinetics. For longer heating times, flavor concentration in the system will generally plateau (or decrease) and this phenomenon is better modeled using first order kinetics. The activation energies for flavor formation have ranged from about 12 to 45 Kcal/mole. The higher values are for pyrazine formation in simple model systems and represent the linear portion of the formation period. Values for many other volatiles are closer to 20 Kcal/mole, which are more in agreement with the Maillard reaction as characterized by the consumption of reactants or pigment formation.

The work to date has demonstrated that the formation of flavors is very system dependent. For some volatiles, more complex model systems may have quite different reaction kinetics than a simple model system. This suggests caution when one predicts the formation of flavors in real foods based on model system work. Other volatiles, e.g., 2-acetyl-l-pyrroline, appear to be relatively unaffected by the model system complexity and model predictions can be quite accurate.

Reaction rates are generally influenced by pH and Aw of the model system. The effect of pH is variable as one might expect. Some volatiles are formed more slowly at higher pHs while others are formed more quickly. Also, secondary reactions may consume some volatiles, and thus their final concentrations in a system are dependent upon the secondary reaction kinetics as well. Data to date (very limited!) on pH effects on pyrazine formation demonstrates that the rate of formation increases with increasing pH. Data on other volatiles depends upon the volatile compound being considered. At this stage of knowledge, it is impossible to make generalizations in this respect. Overall, we have come to accept that flavor formation/concentration is a dynamic situation. Flavor compounds are formed and reacted to give a blend that we recognize as a given flavor. This balance can be very sensitive to the chemical environment and thermal processing.

5.2.5 Flavor Formation via the Maillard Reaction

The most abundant flavor compounds formed via the Maillard reaction are aliphatic aldehydes, ketones, diketones, and lower fatty acids. However, heterocyclic compounds

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containing oxygen, nitrogen, sulfur, or combinations of these atoms are much more numerous and significant to the flavor of thermally processed foods [40]. The remainder of this section will provide examples of flavor compounds formed via the Maillard reaction, some mechanisms of formation, as well as information on sensory properties. Quite obviously, this section cannot be comprehensive but only a sampling of the diverse number of compounds formed via the Maillard reaction.

5.2.5.1 Carbonyl Compounds

The major pathway leading to the formation of carbonyls is the Strecker degradation. This reaction occurs between dicarbonyls and free amino acids. The dicarbonyls involved have vicinal carbonyls (carbonyl groups separated by one double bond) or conjugated double bonds [41]. While these carbonyls typically are intermediates in the Maillard reaction, they may also be normal constituents of the food (e.g., ascorbic acid), be end products of enzymatic browning (e.g., quinones), or be products of lipid oxidation[42].

The end products of the Strecker degradation are CO_2, an amine, and the corresponding aldehyde of each deaminated and decarboxylated amino acid. At one time these aldehydes were considered to be quite important to the flavor of heated food products. This was primarily because they are the most abundant volatiles formed via the Maillard reaction (and thus assumed to be important). It is now realized that the heterocyclic volatiles are more important. The Strecker aldehydes are often monitored in foods since they are present in quantity and can serve as indicators of the Maillard reaction [43].

Strecker aldehydes may also be formed via free radical mechanisms [44]. The oxidation of amino acids by hydrogen peroxide or lipid peroxides yields CO_2, ammonia, and the corresponding Strecker aldehydes. While this is a viable pathway for the production of Strecker aldehydes, the above-mentioned pathway typically predominates.

The aldehydes formed in this reaction may undergo additional reactions. Pokorny [45] has outlined aldol condensations leading to dimeric unsaturated aldehydes, and eventually, high molecular weight polymers. Takken et al. [46] have demonstrated the reaction of aldehydes with α-dicarbonyls, ammonia, and hydrogen sulfide. A host of thiazoles, dithiolanes, thiazolines, and hydroxythiazolines were found as products of these reactants. An additional fate of the Strecker aldehydes is that they may end up being bound onto various food constituents (e.g., protein). The binding may be sufficiently strong that the aldehydes may not have adequate vapor pressure to contribute to food odor.

5.2.5.2 Nitrogen-Containing Heterocyclic Compounds

More than 100 different pyrazines have been identified in various food products. The sensory properties of the pyrazines are quite diverse. The alkyl pyrazines (Figure 5.5a) generally possess roasted, nut-like notes while methoxypyrazines (Figure 5.5b) often possess earthy, vegetable notes [47]. The 2-isobutyl-3-methoxy pyrazine has a freshly cut green pepper flavor with a sensory threshold of 0.002 ppb in water. The acetyl pyrazines typically have a popcorn character, and 2-acetonyl pyrazine has a

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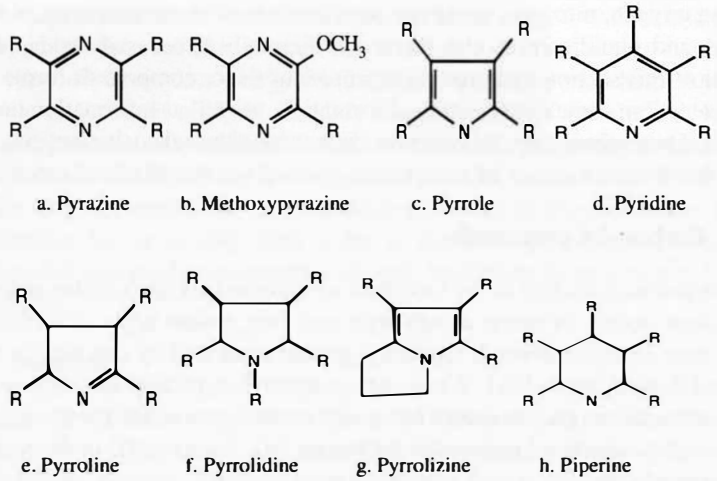

FIGURE 5.5 Classes of nitrogen-containing heterocyclic volatiles formed via the Maillard reaction (a. pyrazine, b. methoxypyrazine, c. pyrrole, d. pyridine, e. pyrroline, f. pyrrolidine, g. pyrrolizine, and h. piperine).

toasted or burned note. Several bicyclic pyrazines have been identified in foods. The sensory properties of these pyrazines have been described as being burnt, roasted, grilled, and/or animal in character. Several mechanisms have been proposed for the formation of various pyrazines [12]. The alkyl pyrazines are probably formed from the reaction of α-diketones with amino acids to form α-amino ketones (Strecker degradation). These α-amino ketones may condense with other α-amino ketones to yield a heterocyclic compound. This heterocyclic may undergo oxidation to form the tri-unsaturated pyrazines. The alkyl substitution is dictated by the dicarbonyl fragment: the amino acid contributes only the amine to the pyrazine [48].

Pyrroles are nitrogen-containing heterocyclic compounds (Figure 5.5c). 2-formyl pyrrole and 2-acetyl pyrrole are the two most abundant and widely occurring pyrroles in foods [47]. 2-formyl pyrrole has a sweet corn-like odor while the 2-acetyl pyrrole has a caramel-like odor. 1-acetonyl pyrrole has been found to have a cookie or mushroom aroma while the pyrrole lactones have a spicy peppery flavor.

Hodge et al. [49] originally proposed a mechanism for the formation of pyrroles, which is also supported by Tressl et al. [50]. This basically is the participation of proline and hydroxy proline in the Strecker degradation to yield pyrroles. If the food system does not contain either proline or hydroxy proline, then a sugar of at least five or more carbons is required for pyrrole formation [51].

Rizzi [52] proposed an alternate pathway for the formation of acylalkyl pyrroles involving the reaction of an amino acid with the corresponding furan. The amino acid reacts with the number 5 carbon on the furan ring to open the furan ring. The ring closes via dehydration with N in the ring structure. Depending upon the amino acid involved and subsequent rearrangements, a variety of pyrroles may arise.

Pyridine compounds (Figure 5.5d) appear to be less widely distributed in browned foods than the pyrroles and pyrazines [53]. While they possess a wide

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range in odor properties, green notes are quite common [54]. 3-methyl pyridine has a green odor, as do many of the singly substituted pyrazines. 3-methyl-4-ethyl pyridine has been characterized as being sweet and nutty. Pyridine is judged as being pungent and offensive while 2-acetyl-pyridine is tobacco-like in odor. The contribution of the pyridines to browning flavor is dependent on the individual pyridine formed and its concentration in the food. At low concentrations, the pyridines typically contribute very pleasant notes. However, they generally become harsh and offensive at higher concentrations.

The mechanisms of pyridine formation are poorly characterized. Vernin and Parkanyi [12] have proposed three mechanisms. The first two mechanisms are dependent upon aldol condensations to yield unsaturated aldehydes with side groups. Reaction then with ammonia or an amino acid and ring closure would yield a nitrogen-containing heterocyclic. The oxidation of this heterocyclic would result in the formation of a pyridine. The third pathway involves the reaction of dialdehydes with ammonia followed by dehydration to produce pyridines.

Tressl et al. [50,55,56] have identified an additional 59 nitrogen-containing heterocyclic compounds which, like the pyrroles, are believed to arise from the reaction of proline and hydroxy proline with dicarbonyls via the Strecker degradation. These compounds fall into the classes of pyrrolines, pyrrolidines, pyrrolizines, and piperidines (Figure 5.5e–h, respectively). Odors associated with these compounds are most often cereal or roasted in nature. For example, 2-acetyl-l-pyrroline is characterized as being popcorn-like [50]. l-acetonyl pyrrolidine has a cereal aroma while the furan-substituted pyrrolidines are considered to have sesame-like notes [56]. While the piperidines [e.g., 2-acetyl piperidine] have been characterized as having weak woody odor and very bitter taste, they are readily transformed into the corresponding tetrahydropyridines which have bready, cracker-like aromas [55]. 2-acetyl pyrrolizine has a smoky roasted aroma. Azepine derivatives generally are exceptionally bitter with little odor [50].

5.2.5.3 Oxygen-Containing Heterocyclic Compounds

The furanones and pyranones are oxygen-containing heterocyclic compounds associated with both caramelized and Maillard flavors [57]. The odor characters most common to this group of compounds would be caramel-like, sweet, fruity, butterscotch, nutty, or burnt. They predominate both in proportion and absolute amount in condensates of carbohydrates that are subjected to browning reactions [47].

The caramel character is associated with a planar, contiguous C-alkyl-enol carbonyl group (alkyl-C=C(OH)-C:O) in the molecule [58]. Maltol (Figure 5.6a) was one of the first compounds in this class to be identified in foods. Ethyl maltol (2-ethyl-3-hydroxy-4(4H)-pyranone) also has a caramel odor but is approximately four to six times stronger in flavor strength than maltol. Furaneol (4-hydroxy-2,5-dimethyl-3(2H)furanone, Figure 5.6b) is the trade name of a compound used extensively in the flavor industry and, like maltol and ethyl maltol, is a flavor enhancer for sweet products. Furaneol itself has a burnt pineapple odor.

The five carbon analogs of maltol and furaneol possess odor properties similar to their oxygen-containing counterparts. Cyclotene (trade name for compound,

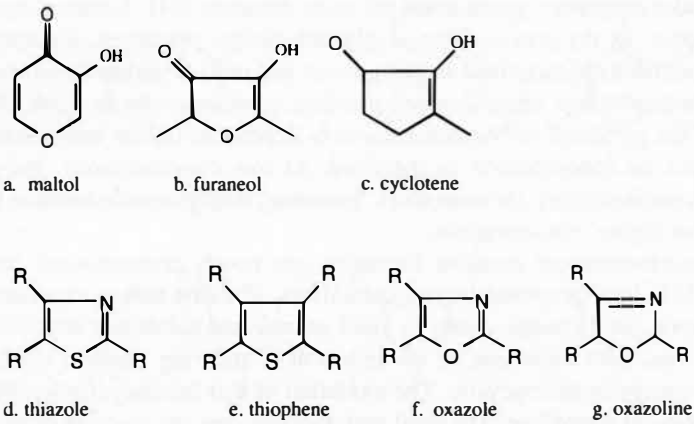

FIGURE 5.6 Oxygenated and sulfur-containing compounds formed via the Maillard reaction.

Figure 5.6c) has a very characteristic sweet maple character. The related compound, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, also finds extensive use in the flavor industry for similar applications (caramel, nut, maple, and butterscotch flavorings).

Compounds of this class that do not meet the structural requirements for being caramel in character may have quite different sensory properties. For example, 4-methoxy-2,5-dimethyl-3(2H)-furanone has an odor similar to sherry. The n-butyl ether version of this furanone has a jasmine-like odor.

Since this group of compounds does not contain nitrogen, the mechanism of formation generally involves the cyclization of non nitrogen-containing browning intermediates. These intermediates may be products of major browning pathways involving sugar dehydration or the Strecker degradation [59,60].

5.2.5.4 Sulfur-Containing Heterocyclic Compounds

Numerous different types of heterocyclic compounds containing sulfur are produced via the Maillard reaction [61,62]. These include thiophenes, dithioles, dithianes, dithiins, trithiolanes, trithanes, tetrathianes, thiazoles, thiazolines, and thiazolidines. The major heterocyclic sulfur-containing compounds produced via the Maillard reaction are thiazoles and thiophenes (Figure 5.6d and Figure 5.6e, respectively).

Thiazoles and pyrazines have somewhat similar sensory properties. Pittet and Hruza [54] and Ho and Jim [63] have reported that the alkylthiazoles give green, nutty, roasted, vegetable, or meaty notes. Trimethyl thiazole is reported to have a cocoa, nutty character. 2-isobutyl thiazole is one of the best known thiazoles and has a strong, green odor of tomato leaf. This compound is considered to be important to tomato flavor. 2,4-dimethyl-5-vinyl thiazole has a nut-like odor. 2-acetyl thiazole is characterized as having a nutty, cereal, and popcorn flavor [47].

With the exception of cranberries, thiophenes have only been found in cooked or roasted food products [47]. Thiophene has a pungent character while 2,4-dimethyl thiophene is well known for its importance to the flavor of fried onions. 2-acetyl-3-methyl thiophene has a honey-like sensory quality at 0.25 g/100 L concentration but

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is nutty and starchy at 0.11 g/100 L concentration. 5-methyl thiophene-2-carboxaldehyde has a burnt coffee note [51].

These compounds, like the thiazoles and thiophenes, typically are formed via the reaction of sulfur-containing amino acids with intermediates of the Maillard reaction. An alternative mechanism involves the initial formation of H_2S from the sulfur-containing amino acids and then the reaction of the H_2S with browning intermediates [64].

5.2.5.5 Oxygen-Containing Compounds

With the exception of the report by Stoffelsma and Pypker [65], oxazoles and oxazolines (Figure 5.6f and Figure 5.6g, respectively) have been found only in food systems that have undergone Maillard reaction[66,67]. The oxazoles typically have green, sweet, floral, or vegetable-like aromas [68,69].

As an example, 4-methyl-5-propyl oxazole has been characterized as having a green vegetable aroma. However, oxazoles which have a 4 or 5 carbon length chain on the oxazole ring and no alkyl group on carbon-2 or 4 have distinct bacon-fatty notes (e.g., 5-butyl oxazole). When a methyl or ethyl group is substituted on carbon-2 (e.g., 2-ethyl-5-butyl oxazole), the fatty aroma is reduced and sweet-floral aromas become more characteristic. The sweet-floral character is further enhanced by additional methyl or ethyl substitution on carbon-4 [69]. The oxazolines tend to have a wide variety of sensory properties. 2-isopropyl-4,5,5-trimethyl-3-oxazoline has a rum-like note while 2-isopropyl-4,5-diethyl-3-oxazoline has a typical cocoa aroma.

5.3 FLAVORS FROM LIPIDS

Lipids may undergo changes during the processing of foods that make a flavor contribution. Later in this text we shall discuss lipid oxidation and lipolysis as undesirable flavors imparted by changes in the lipid fraction of a food (Chapter 7). This chapter discusses lipid changes that impart desirable flavors.

5.3.1 Deep Fat Fried Flavor

Deep fat fried foods, e.g., French fried potatoes, doughnuts, and snacks, are generally well liked by consumers. While their popularity may partially lie in the physical properties imparted to the food by fats, e.g., lubricity, richness, and texture, the characteristic fried flavor is very unique and desirable. This flavor comes from both thermally induced changes in the food (Maillard reaction) and flavor developed in the frying oil [70]. It is well known that a deep fat fried product does not obtain its characteristic flavor until the oil has been used for a period of time, i.e., fresh frying oil does not give a good flavored product. The mechanism of flavor development in heated oils is essentially that of lipid oxidation. As Ohnishi and Shibamoto [71] and later, Shibamoto and Yeo [72] have outlined (Figure 5.7), thermally induced oxidation involves hydrogen radical abstraction, the addition of molecular oxygen to form the peroxide radical, formation of the hydroperoxide and then decomposition to form volatile flavor compounds. The products of thermally induced oxidations differ from

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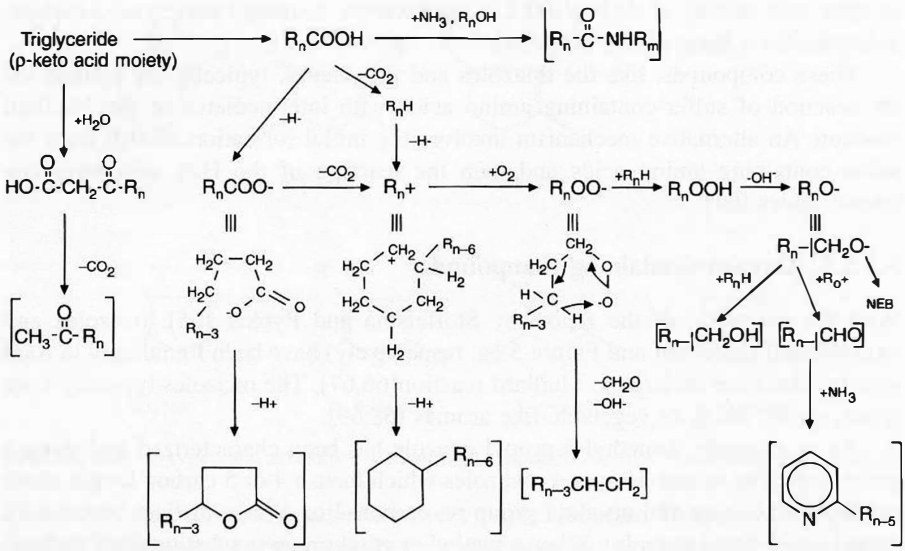

FIGURE 5.7 Proposed mechanism for the formation of volatiles from heated animal fats. (From Ohnishi, S., T. Shibamoto, *J. Agric. Food Chem.*, 32(5), p. 987, 1984. With permission.)

typical lipid oxidation products formed at room temperature. The reason for these differences goes back to kinetic considerations as was illustrated in the previous section on the Maillard reaction. Each chemical reaction has its own unique activation energy, and therefore, rate at a given process temperature. Thus, the reactions occurring in the frying oil and the volatiles formed are dependent on the temperature of processing.

A second distinguishing factor of thermal oxidations is their more random nature than typical room temperature oxidations. Very high temperatures make more sites available on the fatty acid for oxidation to occur. Thus a wider range in end products (volatile flavor components) will occur. So even though the same chemical mechanisms are involved in flavor formation in deep fat fried foods, the flavor developed is unique to this process.

Chang et al. [73] and Nawar et al. [74] have identified many of the volatiles formed during deep fat frying. They have found numerous acids, alcohols, aldehydes, hydrocarbons, ketones, lactones, esters, aromatics, and a few miscellaneous compounds (e.g., pentylfuran and 1,4-dioxane) as products of deep fat frying. More recently, Wagner and Grosch [75] have studied the key contributors to French fry aroma. The list of key aroma compounds in French fries includes: 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 3-isobutyl-2-methoxypyrazine, (E,Z), (E,E)-2,4-decadienal, trans-4,5-epoxy-(E)-2-decenal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, methylpropanal, 2- and 3-methylbutanal, and methanethiol. If one examines this list, it is obvious that the Maillard reaction (pyrazines, branched chain aldeydes, furanones, and methional), and lipid oxidation (unsaturated aldehydes) are the primary sources of this characteristic aroma.

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Chang et al. [76], Nawar et al. [74] and Wagner and Grosch [75] have studied the volatiles produced by different types of oil upon heating. The study reported by Chang et al. [76] reported on qualitative differences while Nawar et al. [74] focused on quantitative differences. Wagner and Grosch [75] considered the influence of oil type on key aroma compounds. The qualitative study noted numerous differences between the volatiles produced by heated corn oil vs. hydrogenated cottonseed oil [76]. This is expected since the oils differ greatly in fatty acid composition. The quantitative study indicated that corn and soybean oils produced similar volatiles but coconut oil was quite different [74]. The coconut oil produced less of the decadienals and unsaturated aldehydes and more saturated aldehydes relative to the corn and soybean oils. Methyl ketones, and γ-lactones were also present in greater quantities in the coconut oil. Wagner and Grosch [75] found that frying potatoes in coconut oil resulted in the presence of γ-octalactone as a key aroma contributor. This finding supports the work of Chang et al. [73] who reported that the γ-lactones with unsaturation at the 2 or 3 position are of particular significance to deep fat fried flavor.

The quantitative effects of heating time and introduction of moisture during deep fat frying were also reported by Nawar et al. [74]. In general, the production of individual volatile components increased with heating time up to 48 h of heating. The introduction of moisture during heating of the oil resulted in a large reduction in volatiles in the oil. This apparent protective effect of water may be due to the steam stripping of volatiles during frying, the displacement of oxygen by the steam, or some combination of both [74].

5.3.2 Lactones

Lactones may be formed in foods via microbial action, extensive lipid oxidation (ambient or thermal), or heating [77]. The formation of lactones in heated dairy products is relevant to this chapter. It appears that as a result of inborn errors in lipid metabolism, hydroxy acids may be synthesized and incorporated into the bovine triglycerides [78]. These hydroxy acids are relatively unstable and are readily hydrolyzed from the triglyceride in the presence of water with heat. Hydrolysis of the hydroxy acid results in its cyclization to a lactone. Since the hydroxy acid has no flavor as long as it is bound to the triglyceride, thermal processing frees the acid and a flavorful lactone is produced. This conversion is readily observed if one takes butter and heats it. On heating, the flavor changes to a very pleasant sweet, caramelized, character largely due to the formation of lactones [79,80]. Whitfield [42] has noted that lactones can be formed from other lipids (not hydroxy fatty acids) as well through heat-induced oxidations.

5.3.3 Secondary Reactions

Lipids may contribute to food flavor formation through participation in other chemical pathways, most notably, the Maillard reaction. Whitfield [42] has provided a very comprehensive review of how lipids and their degradation products may participate in the Maillard reaction. He lists the primary means of interaction as:

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1. The reaction of lipid degradation products with ammonia from the Strecker degradation or the amino groups of cysteine. Thermally induced lipid oxidation provides an abundant source of reactive carbonyls, e.g., aldehydes and ketones. These carbonyls will readily react with free ammonia or amines provided by the Maillard reaction (Figure 5.7). Lipids provide unique carbonyl reactants in that they may have long carbon skeleton chains. The heterocyclic aroma compounds found in heated foods that have R groups of 4 of more carbons are assumed to be derived from lipid sources. This has particular relevance to the snack food industry. One of the issues plaguing this industry is the development of baked snacks with a similar flavor profile as fried snacks. If we look at some of the flavor components identified in fried foods (e.g., 2-pentyl-3,5,6-trimethylpyrazine, 5-heptyl-2-methylpyridine, 5-pentyl-2-ethylthiophene, 2-octyl-4,5-dimethylthiazole and 4-methyl-2-ethyloxazole), we find that these aroma compounds are cyclic and have relatively long alkyl side chains, i.e., they are derived from lipid sources. It then becomes understandably problematic to form these long chain aroma compounds in foods that contain little fat (baked vs. fried foods).
2. The reaction of the NH_2 group of phosphatidylethanolamine with sugar derived carbonyls. Lipids may also contribute amine groups for the Maillard reaction (e.g., phosphatidylethanolamine). This would increase the amount of free NH_2 for the reaction. An additional comment can be made regarding the participation of phospholipids in the Maillard reaction. There is significant evidence that the presence of phospholipids decreases the amounts of typical heterocyclic compounds formed on heating a food (or model system). It is hypothesized that phospholipid degradation products compete for the available NH_2 thereby reducing the heterocyclic compounds normally produced via the Maillard reaction. This would change the distribution of aroma compounds found in the heated food and therefore, its aroma.
3. The participation of free radicals from oxidizing lipids in the Maillard reaction.
4. The reaction of hydroxy or carbonyl lipid degradation products with free hydrogen sulfide from the Maillard reaction. This is essentially analogous to the participation of lipid derived oxidation products with NH_2 from the Maillard reaction. One finds many S-containing heterocyclics in heated foods that must have been derived from lipid sources (long R groups).

This interdependency of reactions has been most studied in meats, or model meat reaction systems [42,72,81]. Wasserman [82] was amongst the first to find that the lean portion of the meat supplied the meaty, brothy character and the fat provided the species character much of which is due to lipid/Maillard interactions. This knowledge has long been used in the manufacture of process products (meat flavors). Meat process flavors contain approximately the same sugars and amino acids for the basic meat flavor but contain different fats to give the unique pork, beef, or chicken notes.

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5.4 FLAVORS FORMED VIA FERMENTATION

Fermentation is a process that provides us with a variety of unique flavors to enjoy. We daily come in contact with a variety of food products produced via fermentation. Some examples include soy sauce, cheese, yogurt, bread, beer, wine, fermented fish products, and sausages. The flavor of these products may be developed from the primary metabolism of the fermentation microorganisms or from residual enzymatic activity once the microbial cell has lysed. Primary metabolism is responsible for much of the flavor of alcoholic beverages while residual enzymatic activity is essential for the development of aged cheese flavor.

Microorganisms can produce a large number of different classes of flavor compounds. Tressl et al. [83] have summarized some of the reactions that microorganisms may accomplish (Table 5.3). This section will present some metabolic pathways leading to the development of flavor in fermented food products. The book by Margalith [84] is dated but still a very good reference for greater detail.

5.4.1 Esters

Esters are quite important to the flavor of both natural foods (e.g., fresh fruit) and fermented foods. Of the fermented foods, esters probably are most important to the flavor of some of the alcoholic beverages. The TNO-CIVO [85] compilation of volatiles in foods lists 94 esters that had been identified in beer. Most of the esters found in beer are formed via primary fermentation. They are produced intracellularly in yeast by enzymatic action [86]. Lipid metabolism by the yeast provides a large number of acids and alcohols that may undergo esterification to yield a variety of esters. While pure chemical reactions can lead to ester formation, this reaction is

TABLE 5.3
Reactions Catalyzed by Microbes

Oxidation	Acylation
Oxidation of CH or C=C	Transglycosidation
Dehydrogenation of CHOH	Methylation
Dehydrogenation of CH-CH	Condensation
Reduction	Cleavage of C-C
Hydrogenation C=O	Decarboxylation
Hydrogenation C=C	Dehydration
	Amination/Deamination
Isomerization	Halogenation
Esterification/Hydrolysis	Phosphorylation

Source: From Tressl, R., M.Apetz, R. Arrieta, K.G. Grunewald, *Flavor of Foods and Beverages*, G. Charalambous, G.I. Inglett, Eds., Academ. Press, New York, 1978, p. 145. With permission.

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too slow to account for the esters in most foods. Cristiani and Monnet [87] have provided a comprehensive review on the formation of esters in fermented foods.

5.4.2 Acids

Acid production via microorganisms is important to the flavor of many fermented foods. The acid of greatest importance to the flavor of fermented dairy products is lactic acid [88]. Lactic acid is an optically active acid existing as the D, L, or optically inactive mixture, depending upon the microorganism involved in its synthesis. There appears to be no difference in flavor between the optical isomers. Lactic acid is characterized as being odorless and having a sour milk taste.

The organisms most commonly associated with lactic acid formation are classified as being either homofermentative (e.g., *Lactobacillus bulgaricus,* which produces primarily lactic acid, >85% of the metabolic end products) or heterofermentative (e.g., *Leuconostoc* sp., which produces lactic acid, acetic acid, ethanol, carbon dioxide, and other metabolites). The homofermentative organisms employ the Embden-Meyerhof pathway for lactose fermentation while the heterofermentation organisms metabolize lactose via the hexose-monophosphate pathway [84]. Lactic acid will reach concentrations of up to 2% or greater in cultures of L. *bulgaricus* or L. *acidophilus* (Figure 5.8).

Lactic acid is also formed in wines during fermentation from L-malic acid. Malic acid is a very tart acid present in grapes, which needs to be converted to lactic acid

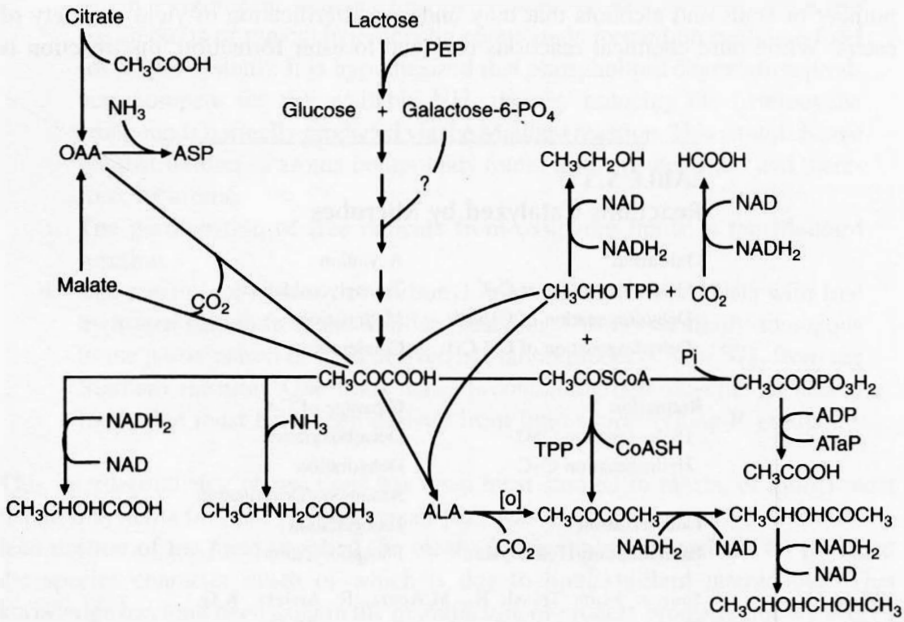

FIGURE 5.8 Formation of various flavor compounds via fermentation of lactose and citrate. (From Sandine, W.E., P.R. Elliker, *J. Agric. Food Chem.*, 18(4), p. 557, 1970. With permission.)

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to smooth out the harsh acidity. This malo-lactic fermentation is the result of bacterial (most commonly *Leuconostoc* sp.) action during the last stages of fermentation.

Numerous other organic and aliphatic acids are produced during fermentation. Most microorganisms have active lipase systems that attack triglycerides to yield glycerol, monoglycerides, diglycerides, and free fatty acids. These free fatty acids can make a substantial contribution to the flavor of various cheeses and sausages. Secondary reactions of the free fatty acids (e.g., oxidation) can again yield a host of new flavor compounds. Acids may also come from the deamination of amino acids. The products are various aliphatic (linear and branched chain) and aromatic acids.

5.4.3 Carbonyls

Carbonyl compounds make a particularly significant contribution to the flavor of fermented dairy products. Diacetyl, characterized as having a buttery, nut-like aroma, is one of the most important carbonyls to the flavor of these products. Diacetyl is produced via the fermentation of citrate. The most important citric acid fermenters are *Leuconostoc citrovorum*, L. *creamoris*, L. *dextranicum*, *Streptococcus lactis* subspecies *diacetylactis*, S. *Thermophilus*, and certain strains of *Proprionibacterium shermani* [90,91]. The metabolic pathway leading to the synthesis of diacetyl involves the degradation of citrate to acetate and oxaloacetate, and the oxaloacetate is then decarboxylated to form pyruvate (Figure 5.9) [92]. Pyruvate plus acetaldehyde forms α-acetolactate, and ultimately, diacetyl. Diacetyl is relatively nontoxic to the bacteria cell so excess pyruvate is channeled into diacetyl.

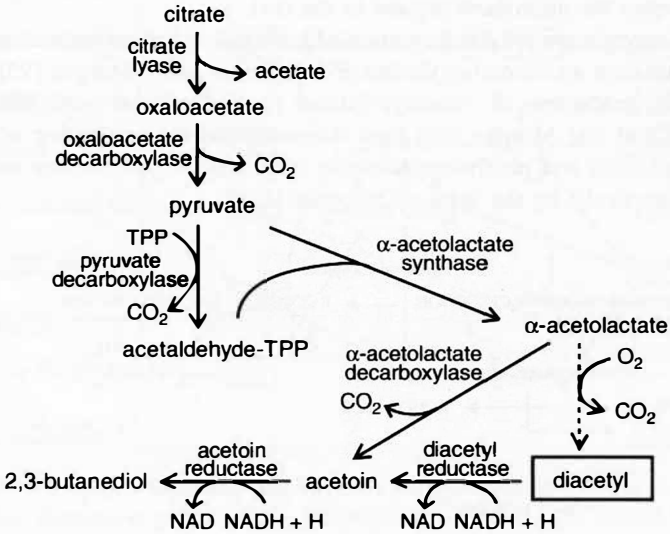

FIGURE 5.9 Formation of diacetyl during fermentation in dairy cultures. (From Hutkins, R.W., *Applied Dairy Microbiology*, E.L. Marth, J.L. Steele, Eds., Marcel Dekker, New York, 1993, p. 207. With permission.)

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Unfortunately diacetyl is not stable in most cultured food products. The microorganisms that synthesize diacetyl also contain diacetyl reductase that reduce diacetyl to acetoin and 2,3-butanediol (Figure 5.9). Thus fermented products such as buttermilk, which depend on diacetyl for flavor have an optimum or peak flavor. As the product is stored, the diacetyl is reduced and flavor strength and quality decrease.

A second dairy product where carbonyls are considered as flavor impact compounds is yogurt. Here acetaldehyde is credited with providing the characteristic pungent, green character. Acetaldehyde is a metabolic end product of L. *bulgaricus* and/or S. *thermophilus* during the fermentation of milk to yogurt [84].

Carbonyls are quite important to the flavor of cheeses. The TNO-CIVO compilation of volatiles in foods lists 29 carbonyls as having been identified in Cheddar cheese at that time. Carbonyls (methyl ketones) may arise in fermented products initially via lipase activity of the starter culture. Dairy products contain a significant quantity of α-keto acids which are readily hydrolyzed from the triglyceride by microbial lipases and then decarboxylated to form odd carbon number methyl ketones.

Methyl ketones and aldehydes may also be formed via microbially induced lipid oxidations [93]. The oxidation may be initiated by microbial lipases, hydrogen peroxide produced by microorganisms and/or lipoxidase-like activity.

Alford et al. [94] have reported on the action of various microorganisms on lard oxidation. They found 10 of the 28 microorganisms studied destroyed the low levels of peroxide in fresh lard. Fourteen of the 28 had no effect on peroxides in the lard, while five strains of streptomyces increased peroxides by threefold. *Pseudomonas ovalis* increased peroxides by 8-fold, and *Micrococcus freudenreichii* increased peroxides 14-fold. The extent of oxidation observed in the lard was found to be very dependent upon the microflora present in the lard.

A final mechanism for the formation of carbonys via microorganisms involves the transamination and decarboxylation of free amino acids. Morgan [95] has demonstrated the production of 3-methyl butanal by S. *lactis* var. *maltigenes* (Figure 5.10). MacLeod and Morgan [96] have demonstrated the production of 2-methyl butanal, methional and phenylacetaldehyde from leucine, methionine and phenylalanine, respectively, by the same microorganism.

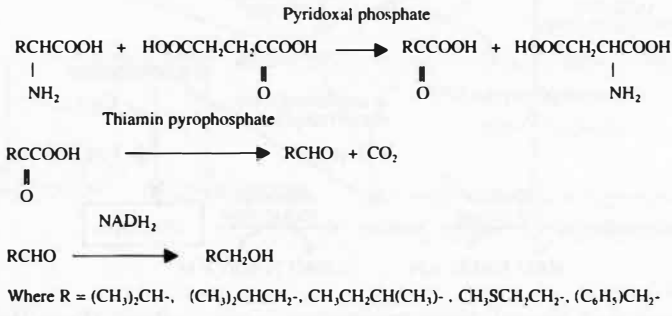

FIGURE 5.10 Metabolic pathway leading to the formation of aldehydes and alcohols. (From Morgan, M.E., *Biotechnol. Bioeng.*, 18(7), p. 953, 1976. With permission.)

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Carbonyl compounds typically are significant to the flavor of most fermented food products. As has been shown, these carbonyls may arise due to carbohydrate or citrate metabolism, lipid oxidation or amino acid degradation.

5.4.4 Alcohols

Alcohols typically make a minor contribution to flavor unless present in relatively high concentrations (ppm) or they are unsaturated (e.g., 1-octen-3-ol). Alcohols may arise via primary metabolic activity of a microorganism (e.g., ethanol) or by reduction of a carbonyl to the corresponding alcohol.

Reazin et al. [97] have outlined metabolic pathways leading to the major congeners during grain fermentation (Figure 5.11). This figure illustrates that the fusel alcohols (alcohols larger in size than ethanol) can be formed from either carbohydrate or amino acid metabolism. Alcohol production from amino acids may occur either by transamination, decarboxylation, and reduction, or by oxidative deamination followed by decarboxylation and reduction. In either pathway, the product is always the amino acid minus the amine group and one carbon atom.

The mechanism of fusel oil production from carbohydrates follows the typical Embden-Meyerhof-Parnas pathway to pyruvic acid. Pyruvic acid may be reacted with a second pyruvic acid to yield acetolactic acid. Acetolactic acid enters the valine-isoleucine biosynthesis pathway and is converted to α-keto isovaleric acid. The α-keto isovaleric acid may then be reduced to isobutyl alcohol or isobutyric

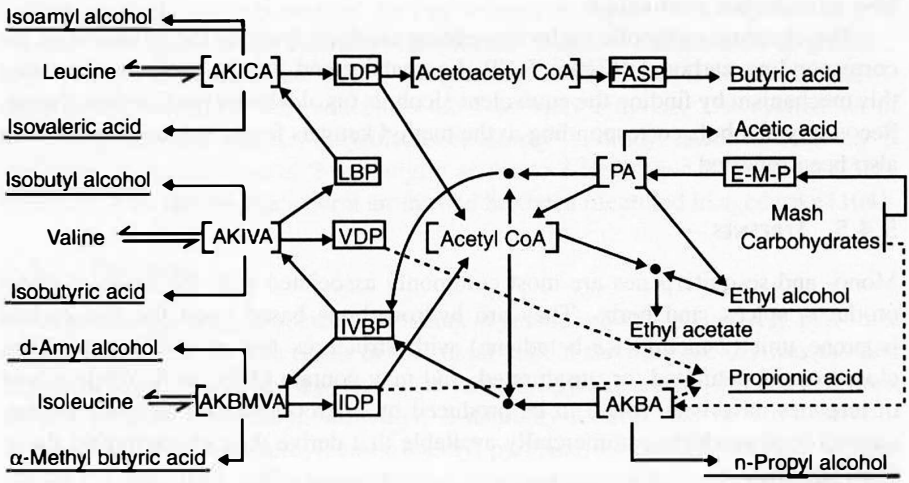

FIGURE 5.11 Proposed metabolic pathways for the synthesis of whiskey congeners: —, synthetic and degradative pathways; ---, hypothetical pathways; LBP, leucine biosynthesis; IVBP, isoleucine-valine biosynthesis; FASP, fatty acid biosynthesis; LOP, leucine degradation; VDP, valine degradation; IOP, isoleucine degradation; E-M-P, Embden-Meyerhof-Parnas fermentation pathway. (From Reazin, G., H. Scales, A. Andreasen, *J. Agric. Food Chem.*, 18, p. 585, 1970. With permission.)

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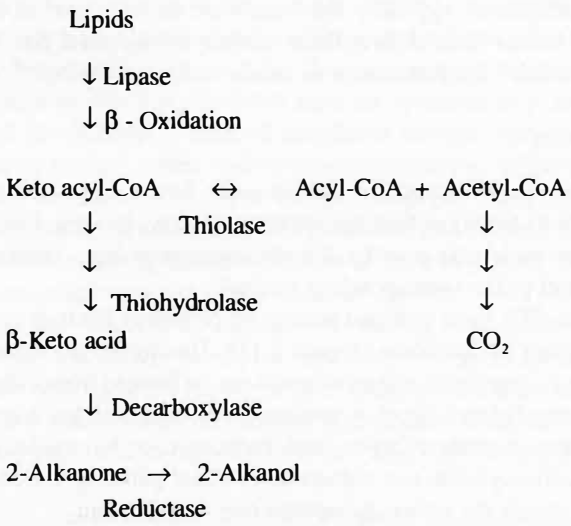

FIGURE 5.12 Formation of methyl ketones and the corresponding alcohols. (From Lamparsky, D., I. Klimes, *Flavour '81*, P. Schreier, Ed., deGruyter, Berlin, 1981, p. 557. With permission.)

acid. The interconnection of pathways as shown in Figure 5.11 results in the formation of numerous alcohols. Note that Acetyl CoA may also enter into these pathways. Acetyl CoA may come from fatty acid metabolism, which ties fatty acids also into alcohol production.

The alternate metabolic pathway yielding alcohols involves the reduction of the corresponding carbonyl (Figure 5.12). Lamparsky and Klimes [98] demonstrated this mechanism by finding the equivalent alcohols (as aldehydes) in Cheddar cheese. Secondary alcohols corresponding to the methyl ketones found in blue cheeses have also been reported.

5.4.5 Terpenes

Mono- and sesquiterpenes are most commonly associated with the flavor of citrus products, spices, and herbs. They are hydrocarbons based upon the five carbon isoprene unit (2-methyl-1,3-butadiene) with structures that may be open chain, closed chain, saturated, or unsaturated, and may contain O, N, or S. While a host of terpenes have been found to be produced by microorganisms, there are no fermented food products commercially available that derive their characteristic flavor from terpenes.

The primary interest in terpene metabolism by microorganisms is due to the potential for the commercial production of specific terpenes via fermentation [99]. In many cases, microorganisms are used to accomplish specific terpene transformations that convert abundant low cost terpenes to high value products. The literature abounds with references to this effort [100–103].

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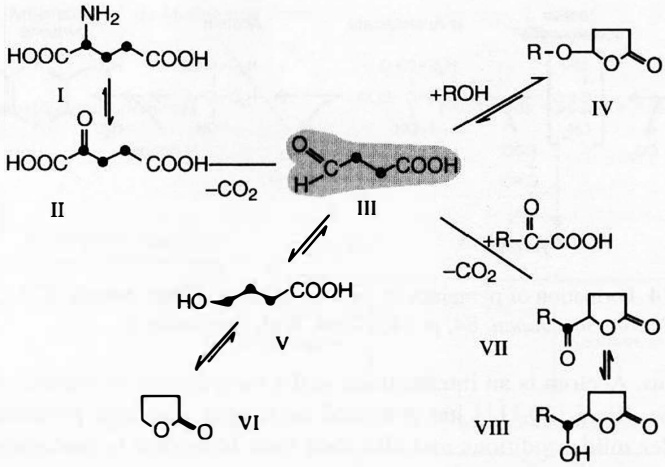

FIGURE 5.13 Formation of lactones by microorganisms. (From Tressl, R., M.Apetz, R. Arrieta, K.G. Grunewald, *Flavor of Foods and Beverages*, G. Charalambous, G.I. Inglett, Eds., Academ. Press, New York, 1978, p. 145. With permission.)

5.4.6 Lactones

Lactones make a significant contribution to the flavor of several fermented food products, particularly dairy products and alcoholic beverages. Tressl et al. [83] have summarized some of the previous literature on lactone synthesis by microorganisms in fermented alcoholic beverages. As can be seen in Figure 5.13, lactones may be formed from amino acids. Pathway A shows the oxidative deamination of glutamic acid to yield 2-oxoglutarate. The 2-oxoglutarate is decarboxylated to produce 4-oxobutyrate, which is then reduced to the 4-hydroxy butyrate. The cyclization of 4-hydroxy butyrate yields γ-butyrolactone, alkoxy- and acyl-lactones. Pathway B illustrates the conversion of 2-oxobutyric acid into 3-hydroxy-4,5-dimethyl-2(5H)-furanone. This lactone has a burnt aroma and has been identified in aged sake [104].

5.4.7 Pyrazines

Pyrazines are generally associated with nonenzymatic browning. However, pyrazines have also been found as a result of microbial action. *Bacillus subtilis* was the first organism found to produce a pyrazine (tetramethyl pyrazine [105]). Since that time, several pyrazines (2-acetyl, dimethyl, 2-methoxy-3-ethyl, 2,5 or 2,6-diethyl-3-methyl, trimethyl, tetramethyl, and trimethyl pyrazine) have been identified in aged cheese [16,106–109]. While some of these pyrazines may be formed via the Maillard reaction during aging of the cheese, it is likely that they were formed via microbial action.

Adachi et al. [110] was the first to postulate a mechanism for the formation of a pyrazine by B. *subtilis* (Figure 5.14). He proposed that this pyrazine could be formed from 2 moles of acetoin and 2 moles of ammonia, both metabolic products

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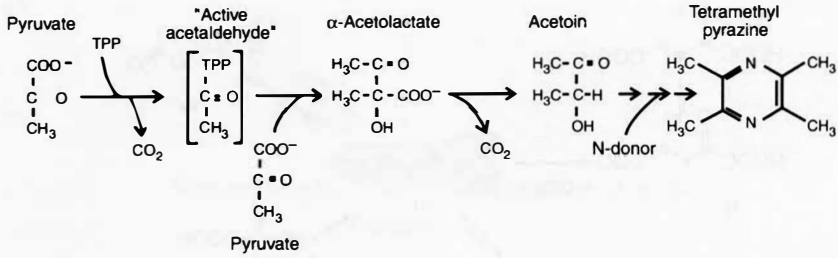

FIGURE 5.14 Formation of pyrazines by microorganisms. (From Adachi, T., H. Kamiya, T. Kosuge, *J. Pharm. Soc. Japan*, 84, p. 545, 1964. With permission.)

of B. *subtilus*. Acetoin is an intermediate in the biosynthesis of leucine, valine, and pantothenate. Rizzi [20,111] has provided an insight into how pyrazines may be formed under mild conditions and also then their formation in biological systems. The key is the biological production of very reactive pyrazine precursors that can chemically react at low temperatures to form these compounds.

5.4.8 Sulfur Compounds

The origin of the majority of sulfur-containing aroma compounds formed by microorganisms is sulfate, which is initially incorporated into the sulfur amino acids (L-methionine and L-cysteine) and the peptide, glutathione [112]. These sulfur-containing precursors are metabolized to a variety of aroma compounds of sensory significance. Spinnler et al. [112] have provided a good discussion of this process.

Cysteine may react with carbonyls to yield flavor compounds (e.g., trithiolanes) or be decarboxylated to give cysteamine, deaminated to provide α-keto-3-thiopropionic acid or degraded to free H_2S. While each of these pathways may yield significant aroma compounds, the formation of free H_2S is particularly important. H_2S is a flavor compound in its own right and also is very reactive with carbonyls and free radicals to form very potent aroma compounds (e.g., ethyl sulfide, diethyl disulfide, amyl mercaptan, and 3-methyl-2-butenethiol).

Methionine may be degraded to yield numerous sulfur-containing aroma compounds, the most important of which is methional. Methional has a very low sensory threshold, and thus is found to contribute to the flavor of many foods. Methionine may also be degraded by microbial lyases to yield methanethiol. Methanethiol may make a contribution to flavor itself or through secondary reactions that yield various sulfides, disulfides, tetrasulfides, and thioesters (Figure 5.15).

5.4.9 Conclusions

Some of the mechanisms leading to the formation of several classes of flavor compounds via microbial activity are outlined in this chapter. At this point, two additional ideas should be mentioned. First, we have only considered the formation of flavor via metabolic activity of the living microorganism. Microorganisms could

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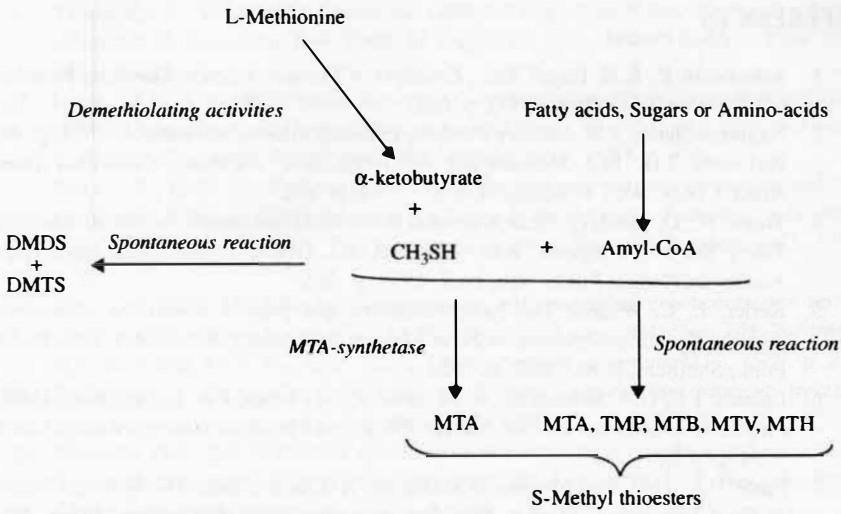

FIGURE 5.15 Conversion of methionine and Acyl-CoA to thioesters. (From Spinnler, H.E., N. Martin, P. Bonnarme, *Heteroatomic Aroma Compounds*, G.A. Reineccius, T.A. Reineccius, Eds., Amer. Chem. Soc., Washington, D.C., 2002, p. 54. With permission.)

be viewed as a small packet of enzymes, which upon death and lysis of the cell, may continue to function. A good example of the significance of bacterial enzymes to flavor is in aged cheeses. The primary metabolic activity of the microflora in the cheese occurs during the early stages of cheese making and aging. Yet there is little characteristic flavor at this point. Only during aging do we see protein degradation via microbial proteases to yield the soft texture, free amino acids (meaty, brothy flavor background), peptides (bitterness) and free sulfur compounds so important to the flavor of aged cheeses. Changes in lipids during aging of the cheese produce free fatty acids, oxidized fatty acids (e.g., methyl ketones) and a host of other flavor compounds. These changes during aging are occurring when microbial activity is decreasing and enzymes from lysed cells are increasing. One must remember that microbial growth in a food product can have a pronounced effect both on the development of desirable flavor and off-flavor even though no viable bacterial cells exist in the food.

The second idea that should be brought out in closing this section of the text is that the flavor system in a fermenting product is very dynamic [93,113]. A microorganism may produce a metabolite that initially accumulates in the product and may make a significant contribution to flavor. A second organism may then reach significant metabolic activity (population) and start metabolizing this first metabolite. Therefore, the flavor of the product will change. Microorganisms may also alter flavor compounds produced in the product via other mechanisms, e.g., lipid oxidation or nonenzymatic browning. Thus lipid oxidation or the Maillard reaction may result in a different flavor in a fermented product than in a nonfermented product.

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