

Generation of Swiss Cheese Flavor Components by the Reaction of Amino Acids with Carbonyl Compounds¹

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ABSTRACT

Previous work has shown that carbonyl-amino acid complexes are important in generating flavors in aqueous extracts of cheeses and *Lactobacillus bulgaricus* cultures. Glyoxal, methylglyoxal, dihydroxyacetone, and ethanal were prominent among the carbonyls in such extracts, so the ability of these carbonyls to generate flavors from various amino acids at room temperature was evaluated. Gas chromatography and mass spectrometry of ether extracts of these mixtures were used to identify the particular compounds responsible for the aromas. The amino acids important in the flavor-generating reactions were valine, leucine, isoleucine, methionine, cysteine, phenylalanine, proline, and lysine. Isovaleraldehyde, 2-methylbutanal, isobutanal, phenylacetaldehyde, and methional, respectively, were formed from leucine, isoleucine, valine, phenylalanine, and methionine via the Strecker degradation. Important flavors in addition to the aldehydes produced by the Strecker degradation were benzaldehyde and acetophenone from phenylalanine, dimethyldisulfide and dimethyltrisulfide from methionine, 2-acetylthiazole from cysteine and methylglyoxal, alkylpyrazines from lysine and dihydroxyacetone, 2-acetyl-1-pyrroline from reaction of certain carbonyls with proline and lysine, 2,5-dimethyl-4-OH-3(2H)-furanone from methylglyoxal, and 2-methylbenzaldehyde from proline and ethanal. Other

volatiles identified include isomers of acetyl-methyl-2,3-dihydropyrrolizine from the proline-methylglyoxal and proline-dihydroxyacetone complexes and δ -valerolactam from the lysine-carbonyl combinations. The reaction products accounted for most of the flavors previously noted in aqueous cheese extracts and cultures of cheese microorganisms.

INTRODUCTION

The chemistry of cheese flavor has been the subject of several reviews (1, 2, 3, 13, 14, 15, 17, 19). Numerous compounds have been reported, but despite extensive research on Swiss cheese flavor, its sweet, nutty flavor still is not completely understood.

Biede and Hammond (5, 6) conducted a partial flavor analysis of Swiss cheese by fractionating it into three phases (oil, water-soluble, and water-soluble-nonvolatile). They were unable to identify and quantify many of the flavor notes they found in the oil and water-soluble-nonvolatile fraction. Vangtal and Hammond (28) improved on the techniques of Biede and Hammond and reported quantitative data for free fatty acids in Swiss cheese and cheese oil. They were able to correlate many of the cheese flavor notes with chemical parameters.

The work of Kowalewska et al. (11) revealed that many of the flavors of the water-soluble-nonvolatile fraction of cheese were generated from carbonyl-amino acid complexes and identified a number of these flavors. Reys et al. (21) identified the carbonyls in this complex and showed that these carbonyls could be produced by *Lactobacillus bulgaricus*. A number of mono- and dicarbonyls were identified.

The purposes of this investigation were to react the carbonyls produced by *L. bulgaricus* in significant amounts with various amino acids at room temperature under cheeselike condi-

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tions, to note and identify the flavors that are produced, and to account for the compounds found by Kowalewska et al. (11) in the water-soluble-nonvolatile fraction of Swiss cheese.

MATERIALS AND METHODS

Amino acids and carbonyls were obtained commercially. Diethyl ether was redistilled from lithium aluminum hydride to remove butylated hydroxytoluene (BHT), acids, and carbonyls. 2-Acetyl-1,4,5,6-tetrahydropyridine and 2-acetyl-1-pyrroline were synthesized by the method of Buttery et al. (8). 2,5-Dimethyl-4-OH-3(2H)-furanone and 5-methyl furfural were synthesized according to Shaw and Berry (24).

Mixtures of .01 mol of each amino acid and .002 mol of each carbonyl were prepared by mixing the amino acid with a 30% aqueous solution of the carbonyl in 5-ml test tubes. The tubes were sealed with disPo plugs (American Scientific Products, McGaw Park, IL) and stored overnight at ambient temperature (25°C). The aromas generated by each mixture were evaluated. The differences evaluated were quite obvious, and there was complete agreement by three observers about the ranking of odor strength and consensus about the odor descriptions.

Amino acid-carbonyl mixtures that generated distinct aromas were reacted as follows. Glass wool and approximately .3 g of Celite 545 saturated with 15 drops of 20% sodium carbonate solution were packed in the end of a glass column (30-cm by 11 mm i.d.) fitted with a Teflon stopcock. The amino acid (.02 mol) was mixed with an equal weight of Celite 545 in a 50-ml beaker, .004 mol of 30% aqueous carbonyl were added, and the mixture was added to the column. After overnight incubation, the columns were eluted with 10 ml of ether, and the eluates were dried over anhydrous sodium sulfate and concentrated to .2 ml by either leaving them uncapped overnight or by a slow heating method. The slow heating method entailed heating the eluate in a water bath tempered around 40°C (approximately the boiling point of the solvent). The ether level was kept above the water level to avoid volatile loss.

DL-Ornithine hydrochloride (.01 mol) was adjusted to pH 10 and refluxed with .002 mol

of 30% dihydroxyacetone solution in 50 ml water for 2 h. The reaction mixture was extracted with three 10-ml portions of ether, dried over anhydrous sodium sulfate, and concentrated to .2 ml.

Separations were performed with a Model 3700 Varian (Varian Associates, Inc., Palo Alto, CA) gas chromatograph (GC) fitted with a flame ionization detector, a linear temperature programmer, and a SPB1-DB nonpolar capillary column (30 m). Injector and detector temperatures were 250°C, and the column temperature was increased from 40 to 190°C at 8°C/min after a 3-min delay. The final temperature was held for 1 min. A 10- μ l syringe was used to inject 1 μ l of the sample for GC analysis. Aromagrams were run under the same GC conditions. The flame detector head was removed, and the effluent was smelled at the flame tip by at least two observers at various concentrations. The odorous regions were identified and their relative intensities were noted. There was agreement by the observers about the relative importance of the various aromas.

After chromatograms and aroma profiles of sample were obtained, ether concentrates were investigated further by using a Hewlett Packard 5970 Series Mass Spectrometer (Hewlett Packard Company, Palo Alto, CA) coupled with a Varian Aerograph Series 1520 Gas Chromatograph. Because the mass spectrometer was less sensitive than the hydrogen flame detector, samples were concentrated to .1 ml, and 1 μ l was injected. The GLC parameters were similar to those used previously except that a SE-30 nonpolar glass capillary column was used. The mass range was set between 40 and 350 mass units.

RESULTS AND DISCUSSION

Screening Test

The major carbonyls (methylglyoxal, glyoxal, ethanal, and dihydroxyacetone) reported in *L. bulgaricus* by Repts et al. (21) generated odors with certain amino acids at ambient temperature as shown in Table 1. The odors were generated immediately. The dicarbonyls and dihydroxyacetone generally generated more intense odors than ethanal. Amino acids other than those listed in Table 1 gave no odors with these carbonyls under the conditions that we

TABLE 1. Odors and their intensities generated by mixtures of carbonyls with certain amino acids at ambient temperature.

| Amino acids | Carbonyls ¹ | | | |
|---------------|-----------------------------|------------------------|--------------------------|----------------------|
| | Methylglyoxal | Glyoxal | DHA ² | Ethanal |
| Cysteine | Roasted meat ⁺⁺⁺ | Putrid ⁺ | Sulfur ⁺ | Sulfur ⁺ |
| Isoleucine | Malt ⁺⁺ | Malt ⁺⁺ | | Malt ⁺⁺ |
| Leucine | Malt ⁺⁺ | Malt ⁺⁺ | | Malt ⁺⁺ |
| Valine | Malt ⁺⁺ | Malt ⁺ | | Malt ⁺⁺ |
| Methionine | Cabbage ⁺⁺⁺ | Cabbage ⁺⁺⁺ | Cabbage ⁺⁺⁺ | Cabbage ⁺ |
| Proline | Cereal ⁺⁺⁺ | Cracker ⁺⁺⁺ | Cereal ⁺⁺⁺ | Floral ⁺ |
| Phenylalanine | Rose ⁺⁺ | Rose ⁺⁺ | Rose ⁺⁺ | Floral ⁺⁺ |
| Lysine | | Cracker ⁺⁺⁺ | Ammoniacal ⁺⁺ | |
| Glycine | | | Sweet ⁺ | |
| Histidine | | | Vitamins ⁺ | |

¹ Weak (+), moderate (++) , and strong (+++) odors.

² Dihydroxyacetone.

used. Some of the odors were essentially the same no matter which carbonyl compound was used and obviously were produced from particular amino acids.

Methylglyoxal

Several unknown ether-soluble volatile products were produced from methylglyoxal by itself, although all the odors generated from such methylglyoxal controls were very weak. Even the largest of these peaks represented only a 1% yield, but the number and size of these peaks were large relative to those of the flavor compounds that were sought, and the presence of these methylglyoxal-generated peaks obscured and complicated the identification of the flavor compounds. The other carbonyls used in this study did not generate any peaks by themselves. Aromagrams were used to identify the significant odor compounds that would account for the aroma of the reaction mixtures, and our efforts were focused on the identification of those compounds.

When methylglyoxal was reacted with ammonia or any of the amino acids, a burned, caramel aroma was produced. Kowalewska et al. (11) reported that the burned flavor found both in Swiss cheese and in *L. bulgaricus* fermentations was caused by 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and this compound

had the same odor and retention index as the methylglyoxal reaction mixtures; however, we were unsuccessful in producing enough of this compound to yield a mass spectrum. A balanced equation for the condensation of methylglyoxal to form this furanone requires a reduction step. When the reducing agent, stannous chloride, was added to the methylglyoxal reaction mixtures, the strength of the burned odor was enhanced, but no peak or mass spectrum was detected that corresponded to the aroma.

Table 2 shows that, on the basis of GC and mass spectrometry, valine, leucine, isoleucine, methionine, and phenylalanine generated with methylglyoxal isobutanol, isovaleraldehyde, 2-methylbutanol, methional, and phenylacetaldehyde, respectively. These are produced via the well-known Strecker degradation. Some of the carbonyl compounds generated in this way have rather intense flavors. The aldehydes produced from the branched aliphatic amino acids, valine, leucine, and isoleucine all have more or less malty aromas, and these compounds accounted for the aroma produced when these amino acids were reacted with carbonyls. Isovaleraldehyde has been reported in low concentrations in ripe Gruyere cheese (7).

Phenylacetaldehyde is floral and smells like roses (Table 2). In addition to expected Strecker degradation product, phenylalanine and methylglyoxal also produced a good deal of benzaldehyde, which has an almond or cherry-like

TABLE 2. Flavor volatile products produced by reaction of methylglyoxal with various amino acids. The aromas, retention indices, and mass spectra were identical with authentic compound.

| Amino acids | Products | Aroma ¹ |
|---------------|--------------------|----------------------------|
| Valine | Isobutanal | Malt ⁺⁺ |
| Leucine | Isovaleraldehyde | Malt ⁺⁺ |
| Isoleucine | 2-Methylbutanal | Malt ⁺⁺ |
| Phenylalanine | Phenylacetaldehyde | Rose ⁺⁺⁺ |
| | Benzaldehyde | Cherry ⁺ |
| | Acetophenone | Floral ⁺ |
| Methionine | Methional | Cheese ⁺⁺ |
| | Dimethyldisulfide | Sulfur ⁺⁺⁺ |
| | Dimethyltrisulfide | Cabbage ⁺⁺⁺ |
| Cysteine | 2-Acetylthiazole | Roasted nuts ⁺⁺ |

¹ Weak (+), moderate (++), and intense (+++) aromas.

flavor, and a trace of acetophenone, which had a mild floral odor. It is possible that benzaldehyde results from a Strecker intermediate that undergoes oxidation. The mechanism of acetophenone formation is unclear. These three flavor compounds accounted for the aroma of the phenylalanine-methylglyoxal reaction mixture, and they have been reported in cheese (11, 12, 20).

Several sulfur compounds were produced from the reaction of methylglyoxal with methionine. When methionine undergoes Strecker degradation, the main product is methional; to many observers, methional has a cheese-like aroma (10). In addition to methional, methionine yields dimethyldisulfide and dimethyltrisulfide, which smell like cabbage or like a freshly struck match. Other sulfurous aromas with short retention times were produced from methional, but the technique used in this study did not make identification of those compounds possible because they were lost in the solvent peak. These compounds may be methanethiol and dimethyl sulfide. Methanethiol, which has been reported as a breakdown product of methional (4), can oxidize and form dimethyldisulfide (Figure 1). Thus, methional and its degradation products accounted for the aroma of the methionine-methylglyoxal reaction mixture. Dimethyldisulfide, dimethyltrisulfide, and methional have been reported as contributors of cheese flavor (18, 20).

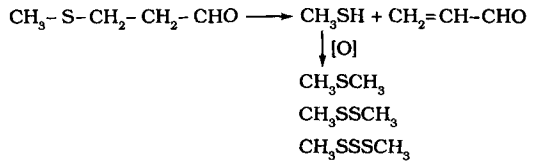


Figure 1. A possible route for the conversion of methional to methanethiol, methyl sulfide, methyl disulfide, and methyl trisulfide.

Methylglyoxal and cysteine produced a very strong aroma caused by 2-acetylthiazole, which smelled like roasted nuts or roasted meat. This compound has been reported previously in roasted meats by Tressl et al. (26). They proposed that cysteinamine, the decarboxylation product of cysteine, is the precursor. We found that 2-acetylthiazole was produced only by methylglyoxal. A mechanism for its formation is proposed in Figure 2. With other carbonyls (ethanal, glyoxal, dihydroxyacetone) and cysteine, a sulfury odor was noted in the solvent peak but could not be further identified by mass spectrometry for the reasons given earlier. This sulfury odor may have been hydrogen sulfide. This volatile sulfurous odor was responsible for the odor of the reaction mixtures of cysteine with glyoxal, dihydroxyacetone, and ethanal.

Phenylalanine generated some of the same flavor volatiles with ethanal as with methylglyoxal (Table 3). These compounds accounted for the aroma of the phenylalanine-ethanal reaction mixture.

A flower-like odor was generated from ethanal and proline. This was identified as 2-

TABLE 3. Flavor volatiles produced by ethanal with phenylalanine and proline. The aromas, retention indices, and mass spectra were identical with authentic compounds.

| Amino acids | Products | Aroma ¹ |
|---------------|---------------------------------|-----------------------|
| Phenylalanine | Benzaldehyde | Cherry ⁺⁺⁺ |
| | Phenylacetaldehyde ² | Rose ⁺⁺⁺ |
| Proline | 5-Methylbenzaldehyde | Flower ⁺⁺ |

¹ Moderate (++) and intense (+++) aromas.

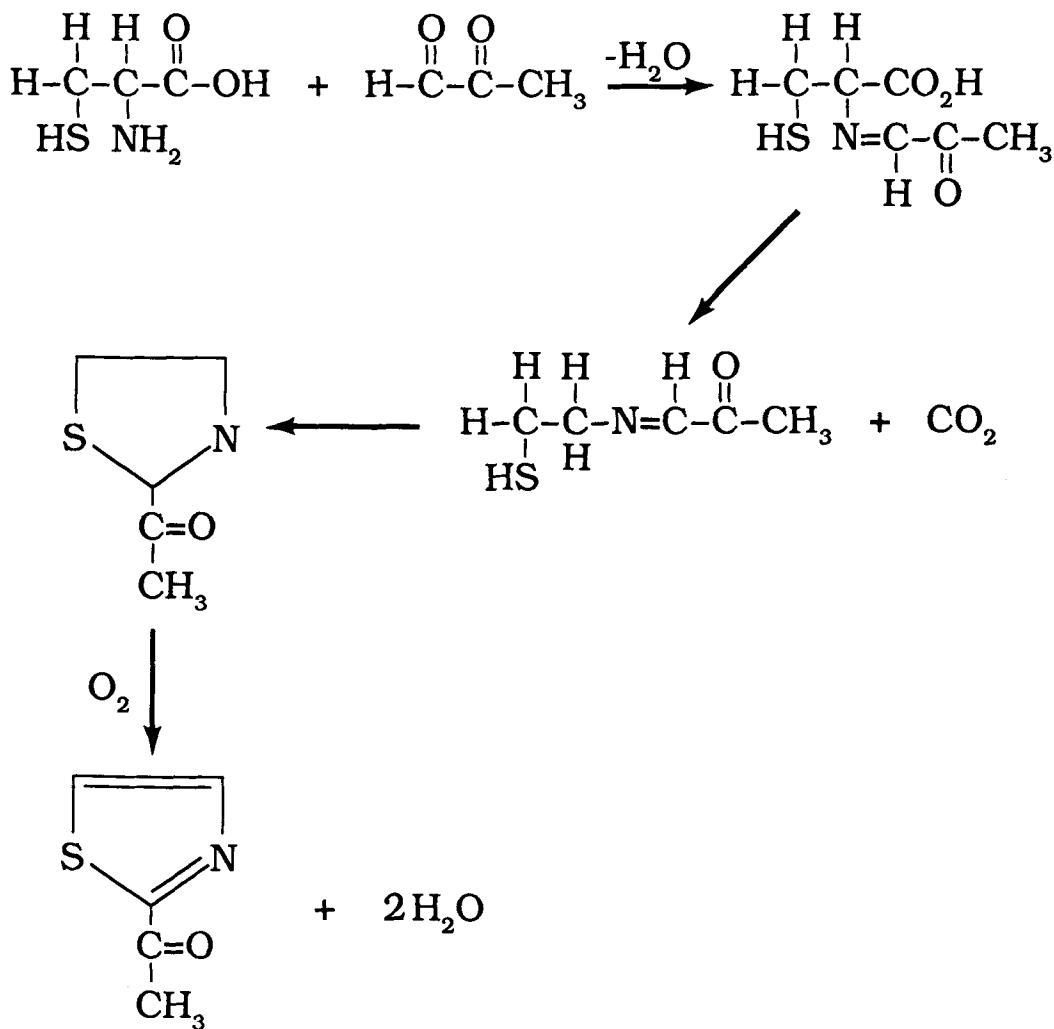
² A very small amount of phenylacetaldehyde was present compared with that formed from the methylglyoxal and phenylalanine complex.

methylbenzaldehyde. A possible pathway for the formation of 2-methylbenzaldehyde from the polymerization of four molecules of ethanal is given in Figure 3. In addition, the ethanal-proline complex produced a cereal-like aroma with a Kovat's index of 927 but associated with no detectable peak. This aroma also was produced from proline and lysine by other carbonyls, and they are discussed in the next section. The cereal and floral aromas accounted

for the aroma of the proline-ethanal reaction mixture.

Flavors Produced from Carbonyl-Lysine and Carbonyl-Proline Complexes

Several proline-carbonyl and lysine-carbonyl combinations generated strong ammonia-like and cracker-like odors with similar retention times. Table 4 lists compounds identified from



2-acetylthiazole

Figure 2. Proposed formation of 2-acetylthiazole from cysteine and methylglyoxal.

the complexes. Pyrrolidine, which has an ammoniacal odor, was formed by several of the mixtures. Pyrrolidine could be produced from proline by decarboxylation via the Strecker degradation. Pyrrolidine's route of formation from lysine is not known but may involve cyclization of lysine's side chain.

Potent cracker-like aromas were produced from several complexes (Table 4). In some of the mixtures, two distinct crackery aromas were generated. No mass spectra that could be attributable to these aromas could be detected,

so the reaction conditions were modified in an attempt to increase yields. The carbonyl-amino acid mixtures were heated to 50°C and 82°C, and various additives were tried (stannous chloride, dichromate, ferrous sulfate, and copper sulfate), but these measures usually produced relatively less cracker odor. Finally, the reaction mixtures were scaled up 10-fold. A mass spectrum for 2-acetylpyrroline was obtained from the glyoxal-lysine complex. This compound has been reported in wheat bread crust and rice (8, 22, 23). Authentic 2-acetyl-

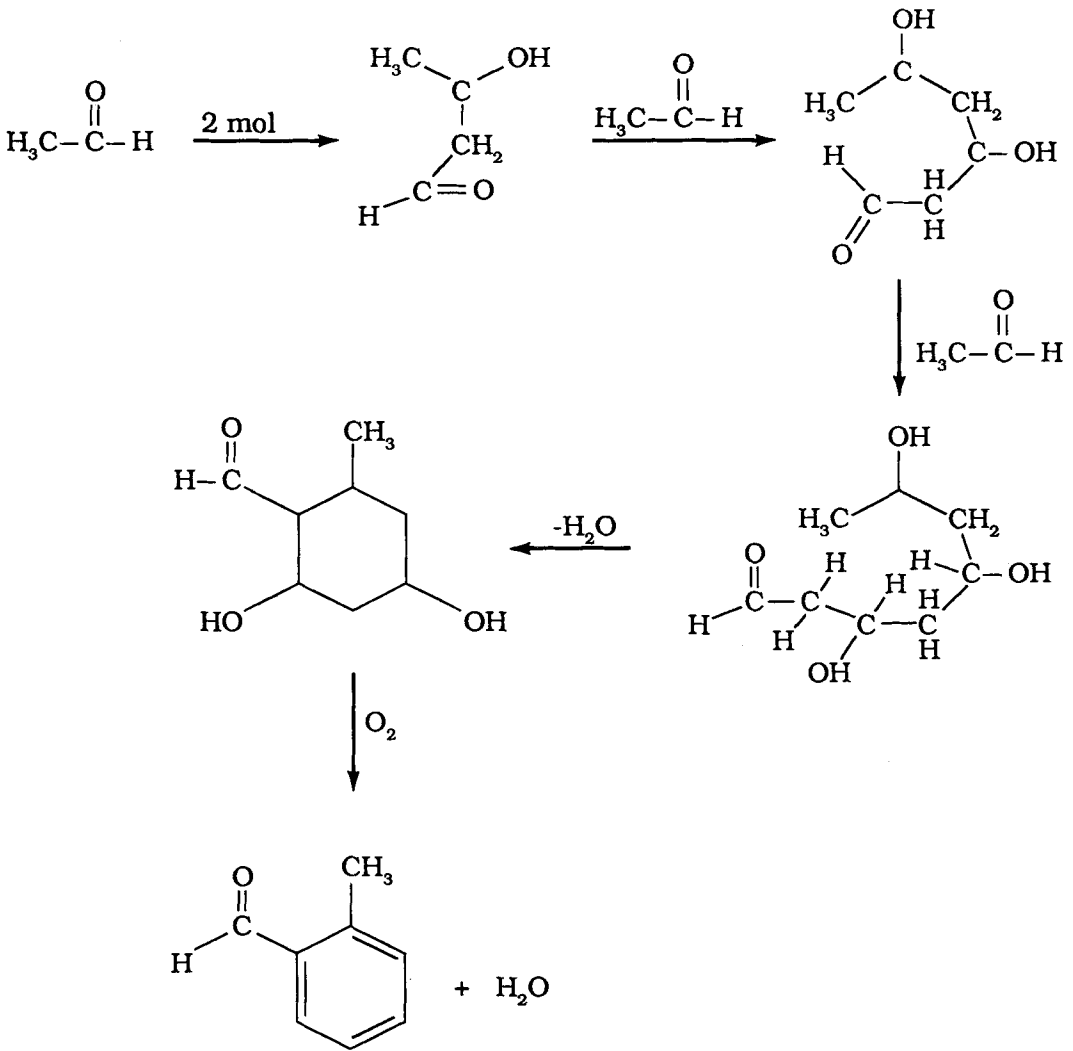


Figure 3. A proposed polymerization of ethanal to 2-methylbenzaldehyde.

TABLE 4. Some volatiles produced from the interaction of lysine and proline with various carbonyls.

| Complex(es) | Reaction product(s) | Aroma ¹ |
|-----------------------------------------------------------------------------------------------------------|--------------------------------------------------|-----------------------------|
| Methylglyoxal-proline Dihydroxyacetone-proline Glyoxal-proline Glyoxal-lysine | Pyrrolidine ² | Ammoniacal ⁺⁺ |
| Methylglyoxal-proline Ethanal-proline Glyoxal-proline Dihydroxyacetone-proline Glyoxal-lysine | 2-Acetylpyrroline ² | Cracker-like ⁺⁺⁺ |
| Glyoxal-proline Dihydroxyacetone-proline Glyoxal-lysine | 2-Acetyltetrahydropyridine ³ | Cracker-like ⁺⁺⁺ |
| Glyoxal-lysine Dihydroxyacetone-lysine | δ -Valerolactam ² | Musty |
| Methylglyoxal-proline Dihydroxyacetone-proline | 2,3-Dihydro-4-1-pyrrolizine isomers ⁴ | |

¹ Weak (+), moderate (++) , strong (+++) aroma.

² Identity confirmed by odor, retention index, and mass spectrum.

³ Identity confirmed by odor, and retention index.

⁴ Identity confirmed by mass spectrum only.

pyrroline matched the retention time (Kovat's index 927) aroma and mass spectra of that from the glyoxal-lysine sample.

We did not succeed in obtaining a mass spectrum for the second cracker-like aroma (Kovat's index 1010). 2-Acetyl-1,4,5,6-tetrahydropyridine has been reported as having a cracker-like aroma in rye bread crust (8, 9, 22, 23) as has 2-acetylpyrazine. Both these compounds had the same odor and retention index as the second cracker-like aroma. One of them probably is responsible for this aroma.

A relatively large amount of δ -valerolactam (2-piperidinone) was produced from the glyoxal-lysine and dihydroxyacetone-lysine complexes. This compound had a very weak musty odor.

In the methylglyoxal-proline reaction, three isomers of 2,3-dihydro-1-H-pyrrolizine were found (Table 4). The two isomers of this compound eluting at higher temperatures also were produced in the dihydroxyacetone-proline mixture. The mass spectra data agreed with those reported by Tressl et al. (27), who produced these pyrrolizine derivatives by heating proline with various sugars. These compounds had little aroma significance.

In addition to the compounds shown in Table 4, the glyoxal-proline and glyoxal-lysine complexes produced volatiles with Kovat's indices between 1029 and 1030 whose mass spectra was rich in fragments with mass 85 and 127. Dihydroxyacetone-proline and glyoxal-lysine complexes contained volatiles with 85 and 113 fragments with retention indices 1120 to 1150 and 1150 to 1210. Compounds pro-

TABLE 5. Some volatiles produced by the reaction of dihydroxyacetone with lysine. The aromas, retention indices, and mass spectra were confirmed with authentic compounds.

| Product(s) | Aroma ¹ | Relative ² peak size |
|------------------------|-----------------------|------------------------------------|
| Pyrrolidine | NH ₃ -like | ++++ |
| Methylpyrazine | Green | +++ |
| 2,5-Dimethylpyrazine | Peanuts | ++++ |
| Trimethylpyrazine | Musty | +++ |
| Ethyl dimethylpyrazine | Musty | ++ |
| | Sweet nuts | |
| δ -Valerolactam | Musty | ++++ |

¹ All aromas were judged moderately strong.

² Small (++) , medium (+++), and large (++++) peaks

TABLE 6. Ether-soluble volatiles from acetoin-lysine, acetol-lysine, and dihydroxyacetone-ornithine complexes.

| Carbonyl | Product | Relative ¹ peak size | Aroma ² |
|----------------------------|------------------------|------------------------------------|-----------------------|
| Acetoin-lysine | Tetramethylpyrazine | ++++ | Musty ⁺ |
| Acetol-lysine | 1,2-Dimethylpyrazine | ++ | Peanuts ⁺⁺ |
| | Ethylidimethylpyrazine | ++ | Musty ⁺⁺ |
| Dihydroxyacetone-ornithine | 2,5-Dimethylpyrazine | ++++ | Peanuts ⁺⁺ |
| | 5-Methylfurfural | ++ | Burned ⁺ |
| | Trimethylpyrazine | ++++ | Musty ⁺⁺ |
| | Ethylidimethylpyrazine | + | Musty ⁺⁺ |
| | Tetramethylpyrazine | + | Musty ⁺ |

¹Very small (+), small (++), medium (+++), and large (++++) peak by hydrogen flame detector.

²Weak (+) and moderate (++) aromas.

ducing such fragments were identified by Kowalewska et al. (11) in cheese and cultures and attributed to either alkylpiperidines or alkylpyrrolidines. These experiments suggest that probably they are some sort of glyoxal addition product to pyrrolidine.

Various pyrazines were produced from the dihydroxyacetone-lysine mixture as shown in Table 5. The pyrazines are well-known flavor compounds and are responsible for the musty, earthy flavors noted in the mixture. Although

not as strong as the cracker-like aroma, at least when the reaction is run at room temperature, the pyrazines are important aroma compounds in the mixture.

In an attempt to understand the mechanism of pyrazine formation, lysine-acetoin, lysine-acetol, and ornithine-dihydroxyacetone complexes were formed, and the results are in Table 6. When lysine was reacted with acetol, pyrazines similar to those produced with dihydroxyacetone were formed. When lysine was

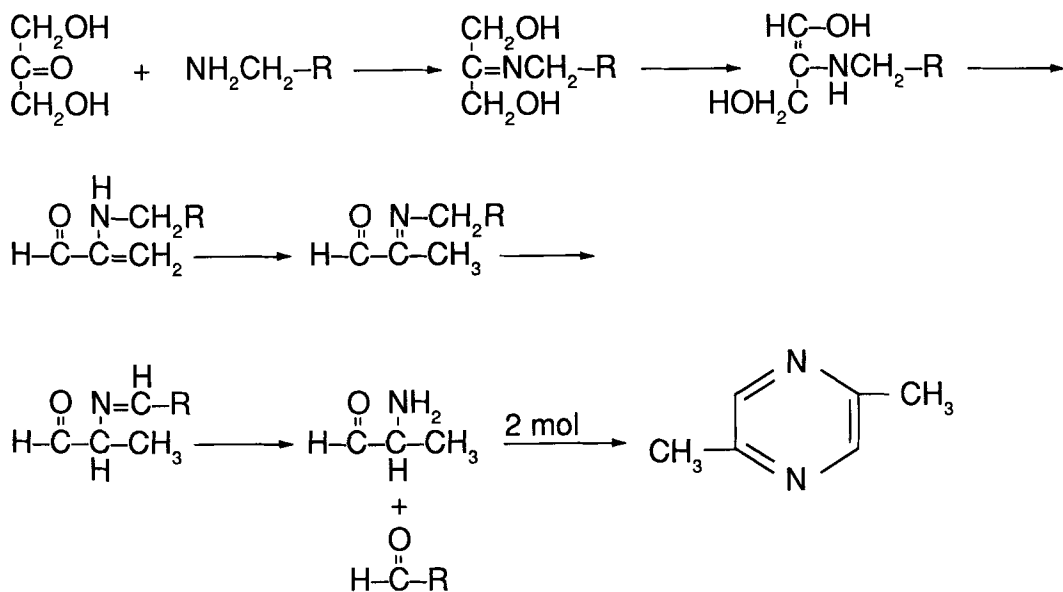


Figure 4. Possible mode of formation of dimethylpyrazine from dihydroxyacetone and lysine.

reacted with acetoin, only tetramethylpyrazine was found. Ornithine, which has a chain one carbon shorter than lysine, reacted with dihydroxyacetone very similarly to the lysine. Lysine reacted with dihydroxyacetone (Table 5) and acetoin (Table 6) to yield δ -valerolactam, but a compound with a different mass spectrum was formed [97(100), 99(90), 43(76), 41(14), 98(6), 69(5), 57(5)] from the acetol-lysine reaction (Table 6). Ornithine with dihydroxyacetone formed 5-methylfurfural in addition to the pyrazines. Figure 4 shows a possible route for the formation of dimethylpyrazine. Various alkylpyrazines such as the ones just mentioned have been identified in cheeses (3, 5, 6, 16, 25).

Significance to Cheese Flavor Research

The reaction conditions used in these studies gave pH and water activities similar to those in cheese, but the flavors were generated in the presence of oxygen, whereas the interior of cheese is strongly reducing. The mechanism of formation of several of the compounds identified seem to require oxidation or reduction. Other flavors might have been generated under reducing conditions, and the relative importance of some of the flavors found might have been increased or diminished.

This study has revealed that glyoxal, methylglyoxal, dihydroxyacetone, and acetoin can react with certain amino acids at room temperature to produce a number of significant flavor compounds. This reaction accounts for many of the flavor compounds previously found to be generated by the water-soluble-nonvolatile fraction of cheeses and cultures of *L. bulgaricus*. It now seems possible to account for all the significant flavors of Swiss cheese noted several years ago by Biede and Hammond (5, 6). These results suggest that the final stages of flavor formation in cheese are dominated by chemical reactions rather than enzymatic or microbiological processes. The key roles of methionine, proline, lysine, and cysteine in flavor formation have been accentuated. These results suggest a number of ways that flavor development in cheese might be enhanced and accelerated. These results also suggest that synthetic Swiss cheese flavors of reasonable fidelity now should be possible.

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