# Volatile Compounds Generated from the Maillard Reaction of Pro-Gly, Gly-Pro, and a Mixture of Glycine and Proline with Glucose

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A wide variety of peptides have been reported to be present in food systems and have been recognized as important flavor compounds. However, aroma generation characteristics of peptides have not been studied to an appreciable extent. The present paper reports the generation of aroma compounds from a model system consisting of dipeptides Gly-Pro, Pro-Gly, and Gly plus Pro as the amino sources and glucose as a reducing sugar. The Gly-Pro dipeptide generated a larger amount of pyrrolizine and pyridine volatile compounds than the Pro-Gly dipeptide. The pyrrolizines were generated at lower temperature than either pyrazines or pyridines. At lower temperature of 130 °C, the glycine plus proline system produced primarily pyrrolizines but no pyrazines. At elevated temperature (180 °C), the pyrrolizines and pyrazines were formed in equal abundance.

## INTRODUCTION

The Maillard reaction of proline with a variety of reducing sugars has been thoroughly investigated by Tressl et al. (1985a–d). Among the products they observed as proline-specific compounds were 2,3-dihydro-1*H*-pyrrolizines, cyclopent[*b*]azepin-8(1*H*)-ones, 2-acetyl- and 2-furylpiperidines, and pyrrolidines. These compounds possess a variety of different flavors. For instance, the pyrrolidines possess a cereal aroma, and the piperidines contribute a bitter taste to roasted malt or coffee. The pyrrolizines give a smoky or roasted aroma, and the cyclopent[*b*]azepin-8(1*H*)-ones contribute a bitter taste to roasted food.

The role of glycine in the Maillard reaction as a flavor precursor has also been elucidated by many researchers. Olson et al. (1978) gave a detailed review and illustration for the possible formation pathway of various flavor compounds generated from the reaction between glycine and glucose. Rizzi (1972) showed the possible mechanism for the formation of pyrazines from glycine and alanine with diketones.

A wide variety of peptides have been reported to be present in food systems and have been recognized as important flavor compounds. However, aroma generation characteristics of peptides have not been studied to an appreciable extent. Recently, we reported the relative reactivity of glycine, diglycine, triglycine, and tetraglycine toward the reaction of glucose (Oh et al., 1991) and the identification of novel 2(1*H*)-pyrazinones as peptide-specific aroma compounds in the Maillard reaction of Gly-Leu or Leu-Gly with glucose (Oh et al., 1992). The present paper reports the generation of aroma compounds from a model system consisting of dipeptides Gly-Pro, Pro-Gly, and Gly plus Pro as the amino sources and glucose as a reducing sugar.

## EXPERIMENTAL PROCEDURES

**Sample Preparation.** Gly-Pro, Pro-Gly, and Pro plus Gly (Sigma Chemical Co., St. Louis, MO) (0.002 mol) were separately dissolved with 0.002 mol of D-glucose in 50 mL of distilled water. Each sample mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder and heated in an oil bath at 180 °C for 2 h. Each reaction product was adjusted to pH >12 with NaOH, an internal standard was added, and the mixture was then extracted with methylene chloride in a separatory funnel by multiple extraction (5 × 50 mL). The methylene chloride extract was dried over anhydrous sodium sulfate and concentrated with nitrogen gas to a final volume of 0.2 mL.

**Volatile Separation by Gas Chromatography.** A Varian 3400 gas chromatograph equipped with an FID and a nonpolar fused silica capillary column [50 m × 0.32 mm (i.d.), 1.05-μm thickness, HP-1; Hewlett Packard] was used to analyze the volatile compounds isolated from the thermal reaction systems. For each sample, 0.2 μL was injected with a split ratio of 50:1. The GC was run with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier flow rate of 0.8 mL/min. The temperature program included an initial column temperature of 40 °C, a temperature increase of 2 °C/min from 40 to 260 °C, and a 10-min isothermal period at the final column temperature.

Quantitative determination was accomplished by addition of an internal standard. The quantity of each component was finally converted into milligrams of volatiles generated by 1 mol of Gly plus Pro or peptides. Linear retention indices for the volatile compounds were calculated using *n*-paraffin standards ($C_6$–$C_{22}$, Alltech Associates) as references (Majlat et al., 1974).

**GC/MS Analysis.** The concentrated samples were analyzed by GC/MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high-resolution mass spectrometer, using the same GC program described earlier. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and the spectra were recorded on a Finnigan MAT SS 300 data system.

## RESULTS AND DISCUSSION

Table I lists the volatile compounds generated when an equimolar solution of dipeptides, Gly-Pro and Pro-Gly, and a mixture of Gly and Pro were reacted separately with glucose at 130, 150, and 180 °C for 2 h at pH 5.3–5.5. The volatile compounds produced were analyzed by GC/MS. Spectra obtained were identified by utilizing an on-line computer library (NBS) and reference spectral data published by Tressl et al. (1985a–d). The relative concentration of the volatiles formed was quantified by comparison with an internal standard.

**Pyrrolizines.** The Maillard reaction of Gly-Pro and Pro-Gly produced two major classes of compounds, i.e.,

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**Table I. Relative Concentration of Identified Volatiles in Gly-Pro, Pro-Gly, and Gly + Pro with Glucose Model Systems**

| compound | $I_k$ | amount, ppm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Gly-Pro | | | Pro-Gly | | | Pro + Gly | | |
| | | 130 °C | 150 °C | 180 °C | 130 °C | 150 °C | 180 °C | 130 °C | 150 °C | 180 °C |
| furans | | | | | | | | | | |
| 2-methyl-5-ethylfuran | 797 | | | | | | | | | 1.7 |
| 2-acetylfuran | 883 | | | 10.1 | | | 16.1 | | | 1.7 |
| 5-methylfurfural | 937 | | | 32.7 | | | 44.8 | | | 1.4 |
| 2-acetyl-5-methylfuran | 975 | | | 1.2 | | | | | | |
| pyrroles | | | | | | | | | | |
| 2-formyl-1-methylpyrrole | 964 | | | | | | | | | 1.1 |
| pyrazines | | | | | | | | | | |
| pyrazine | 734 | 1.7 | | 1.7 | | | 11.1 | | | |
| 2-methylpyrazine | 805 | | 15.5 | 95.8 | | | 5.6 | | 3.0 | 88.5 |
| 2,3-dimethylpyrazine | 900 | | | 2.7 | | | 1.0 | | 6.0 | 16.9 |
| trimethylpyrazine | 980 | | | 5.5 | | | 2.8 | | 1.0 | 35.2 |
| tetramethylpyrazine | 1072 | | | | | | | | 26.4 | 76.8 |
| pyridines | | | | | | | | | | |
| 2,3-dimethylpyridine | 909 | | | | | | 3.1 | | | |
| 2-acetylpyridine | 1026 | | | 27.0 | | | 55.3 | | 4.6 | 44.5 |
| 2-propionylpyridine | 1165 | | | | | | 3.2 | | | |
| 2-acetyl-6-methylpyridine | 1190 | | 38.2 | 649.6 | | | 354.2 | | | |
| pyrrolidines | | | | | | | | | | |
| 1-formylpyrrolidine | 1040 | | | | | | | | 11.4 | 137.5 |
| 1-acetylpyrrolidine | 1206 | | | | | | | | | 11.1 |
| 1-furfurylpyrrolidine | 1228 | | | 29.0 | | | 7.0 | | | |
| pyrrolizines | | | | | | | | | | |
| 5-formyl-2,3-dihydro-1*H*-pyrrolizine | 1630 | 37.0 | 115.0 | 56.9 | 6.2 | 39.6 | 10.2 | 36.7 | 317.5 | 46.4 |
| 5-acetyl-2,3-dihydro-1*H*-pyrrolizine | 1687 | 800.9 | 2835.1 | 879.9 | 164.6 | 1.7 | 792.2 | 25.0 | 10.6 | 52.2 |
| 5-propionyl-2,3-dihydro-1*H*-pyrrolizine | 1840 | | 1.7 | 8.4 | 4.7 | 15.1 | 62.6 | 1.0 | 17.1 | |
| 5-acetyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine | 1950 | | 2.4 | 5.3 | 10.2 | 29.7 | 31.9 | | 11.6 | 16.1 |
| 5-propionyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine | 2165 | | | | | | | | | 4.8 |
| miscellaneous | | | | | | | | | | |
| aniline | 834 | | | | | | | | 12.3 | 46.8 |
| 2-(2-furyl)piperidine | 1800 | 1.2 | 1.6 | 6.2 | 1.2 | | 24.5 | | | |
| 2,3,6,7-tetrahydrocyclopenta[*b*]azepin-8(1*H*)-one | 1806 | | | | | | | | | 7.9 |

pyrrolizines and pyridines. The most abundant pyrrolizine was 5-acetyl-2,3-dihydro-1*H*-pyrrolizine (5-ADHP), followed by 5-formyl-2,3-dihydro-1*H*-pyrrolizine (5-FDHP). Both 5-ADHP and 5-FDHP are proline-specific Maillard products. According to the mechanism proposed by Tressl et al. (1985a), the formation of 5-ADHP and 5-FDHP requires the interaction of free proline with pyruvaldehyde, glyoxal, and α-hydroxyacetaldehyde. During the Maillard reaction, both the primary amino group of Gly-Pro and the secondary amino group of Pro-Gly are able to act as a catalyst to bring about the 1,2- and 2,3-enolizations of the glucose, leading to reactive 3- and 1-deoxyhexosones. The hexosones will then undergo retroaldol cleavage, forming reactive α-dicarbonyls and α-hydroxy carbonyls such as pyruvaldehyde, glyoxal, and α-hydroxyacetaldehyde (Tressl, 1985a). It is interesting to note that the dipeptide Gly-Pro produced more 5-ADHP and 5-FDHP than the Pro-Gly dipeptide in this model system. The Gly-Pro dipeptide produced more total volatiles, including pyrrolizines in particular, than its counterpart, the Pro-Gly dipeptide. There may be two reasons for the observed phenomena. First, the primary amino group of Gly-Pro should be more reactive in catalyzing the transformation and fragmentation of the glucose molecules. Second, the formation of Schiff base in Gly-Pro dipeptides with glucose or its degradation products is faster than in Pro-Gly dipeptides. In addition, the Schiff base formed in the Gly-Pro dipeptides may promote the hydrolysis of the dipeptide bond of Gly-Pro. The hydrolysis of the dipeptide bond in the Gly-Pro system was possibly facilitated by the formation of a neutral charge at the amino group of the "dipeptide–glucose" Schiff base compound (Figure 1). On the other hand, the formation of a positive charge at the amino group of the "diketone-Pro-Gly" Schiff base compound will resist the acid hydrolysis as the close

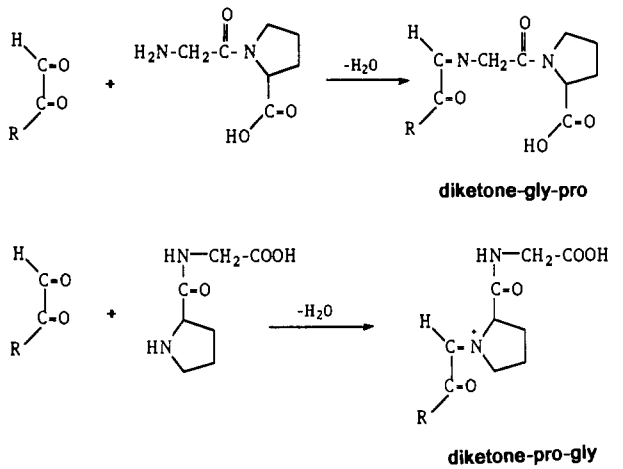


**Figure 1.** Proposed mechanism for the positively charged Schiff base diketone-Pro-Gly repels the incoming proton and retards the subsequent hydrolysis vs a neutral charged Schiff base diketone-Gly-Pro.

proximity of two positively charged van der Waals radii in the dipeptide bond is energetically unfavorable (Hill, 1965).

The rate constant for the dipeptide bond hydrolysis of a series of dipeptides has been studied (Hill, 1965). It has been shown that the Gly-Pro dipeptides ($k$ = 0.115 with 1.5 N HCl) were easier to hydrolyze compared to the Pro-Gly ($k$ = 0.189 with 1.5 N HCl) dipeptides at 100 °C in diluted acid solution. Our study showed that the Gly-Pro dipeptide produced more abundant 5-ADHP than the Pro-Gly dipeptide. These results suggested that the proline is released faster from the Gly-Pro than from the Pro-Gly dipeptide. Thus, it is possible that in the Maillard reaction the Schiff base compound formation precedes and catalyzes the hydrolysis of the peptide bond.

**Pyridines.** Another major compound worth mentioning was identified as 2-acetyl-6-methylpyridine. Alkylpyridines are found in a variety of foods such as cooked beef (MacLeod and Coppock, 1977), rice bran (Tsugita et al., 1978), the basic fraction of Chinese jasmine (Toyoda et al., 1978), fried chicken (Tang et al., 1983), roasted peanuts (Ho et al., 1982) and fried bacon (Ho et al., 1983). 2-Acetylpyridine, for example, is found in baked potatoes, roasted peanuts, and roasted beef.

The formation of larger amounts of 2-acetyl-6-methylpyridine in the Gly-Pro system than in the Pro-Gly system also could be due to the relative ease of the transamination of Gly-Pro which contains the primary amino group with the glucose or its degradation products. The relative ease of the peptide breakdown in the Gly-Pro system, as mentioned in the above discussion, will also contribute to the formation of a larger abundance of 2-acetyl-6-methylpyridine.

**Maillard Reaction of a Mixture of Proline and Glycine with Glucose at 180 °C.** The Maillard reaction of an equimolar mixture of glycine and proline with glucose produced a variety of volatile compounds derived mainly from the reaction of the individual amino acids with the glucose. The pyrrolizines and pyrazines were found in equal abundance at elevated temperatures (180 °C). At 130 °C, the glycine plus proline system produced primarily pyrrolizines but no pyrazines. The formation of pyrrolizines was faster than the formation of pyrazines at 130 °C. It is reasonable to assume that all of the pyrazines derived from glycine and the pyrrolizines from proline. This suggests that although the primary amino group of glycine may catalyze the degradation of glucose faster than the secondary amino group of proline, the free proline may be more reactive than glycine toward the reaction with $\alpha$-dicarbonyls and $\alpha$-hydroxy carbonyls derived from the degradation of glucose. The generation of the pyrrolizines was still more prominent than the formation of the pyrazines at an elevated temperature of 150 °C. As the temperature increased to 180 °C, the interaction rate of both amino acids, i.e., proline and glycine with glucose degradation products, was roughly the same. As a result, the amounts of pyrazines and the pyrrolizines were relatively similar.

In summary, dipeptides can be used to direct the formation of specific Maillard products. In this case, the Gly-Pro system can be used to produce pyrrolizines better than either Pro-Gly or a mixture of glycine plus proline. The pyrrolizines are generated at 130 °C but are best formed at 150 °C rather than 180 °C, with Gly-Pro dipeptides used as the precursor. In the mixture of glycine and proline, there probably was a competition between glycine and proline for the existing electrophiles (carbonyls) for the formation of the Schiff base compounds which led to the formation of various volatile products. This research shows that it is not always advantageous to react free amino acids to produce a specific aroma compound and that amino acids bound in peptide form may, indeed, direct reactions such that the generation of one type of aroma compound is favored.

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