Ammonia Generation during Thermal Degradation of Amino Acids

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The amounts of ammonia released from thermal degradation of amino acids were measured using the ammonia electrode method. An aqueous solution of each amino acid was adjusted to pH 8 and heated at 180 °C for 2 h in a stainless steel reactor. Among the 19 amino acids studied, asparagine, aspartic acid, cysteine, and glutamine released large amounts of ammonia under the conditions employed. The effect of temperature on the generation of ammonia was studied for asparagine, asparatic, glutamine, glutamic acid, and cysteine.

INTRODUCTION

During the thermal processing of foods, the Maillard reaction, which is the condensation of the amino group of amino acids, peptides, or proteins with the carbonyl group of reducing sugars, takes place. The Maillard reaction is the major reaction that leads to the formation of many important nitrogen-containing heterocyclic compounds such as pyrazines, pyridines, and pyrroles. The primary nitrogen source of these heterocyclic flavor compounds is the α-amino group of amino acids. Some of the amino acids such as asparagine, glutamine, lysine, arginine, histidine, and tryptophan have more than one nitrogen atom, and these additional nitrogen atoms might also contribute to the formation of nitrogen-containing heterocyclics.

Merritt and Robertson (1967) investigated the pyrolytic products of 17 amino acids and 10 peptides at temperatures up to 700 °C. Lien and Nawar (1974) also studied the decomposition products of alanine, valine, leucine, and isoleucine at temperature ranges from 180 to 270 °C. Ammonia was the primary thermal decomposition product regardless of amino acid used, which suggested the occurrence of deamination during thermal decomposition of α-amino acids.

Ammonia can also be released from the side-chain amide group of asparagine and glutamine as a result of nonenzymatic deamidation (Shih, 1991; Hamada and Marshall, 1989; Wright, 1991; Zhang et al., 1993). The ammonia released from the deamidation of asparagine and glutamine may participate in the formation of nitrogen-containing heterocyclic compounds such as pyrazines. This is evident from the paper of Izzo and Ho (1993), who observed that the amide content of gluten in the thermal reaction of gluten and glucose led to the different quantities of pyrazines formed. Recently, the contribution of amide nitrogen atoms to pyrazine formation has been confirmed (Hwang et al., 1993). When the ^{15}N isotope labeled at the amide side chain of glutamine was reacted with glucose at 180 °C in dry or aqueous systems, more than half of the nitrogen atoms in the alkylpyrazine ring consisted of ^{15}N nitrogen atoms that came from the side chain of glutamine. The above observations were also agreeable with the previous results of Bohenstengel and Baltes (1992), who found that furans were formed as major products when aspartic acid was reacted with glucose. However, when asparagine was reacted with glucose, nitrogen-containing heterocyclic compounds were generated as major products.

The present paper reports the study on the thermal stability of amino acids in terms of their ammonia generation from their available nitrogen-containing groups. We hope this information will provide the basic knowledge for predicting how nitrogen atoms incorporate into the heterocyclic flavor compounds.
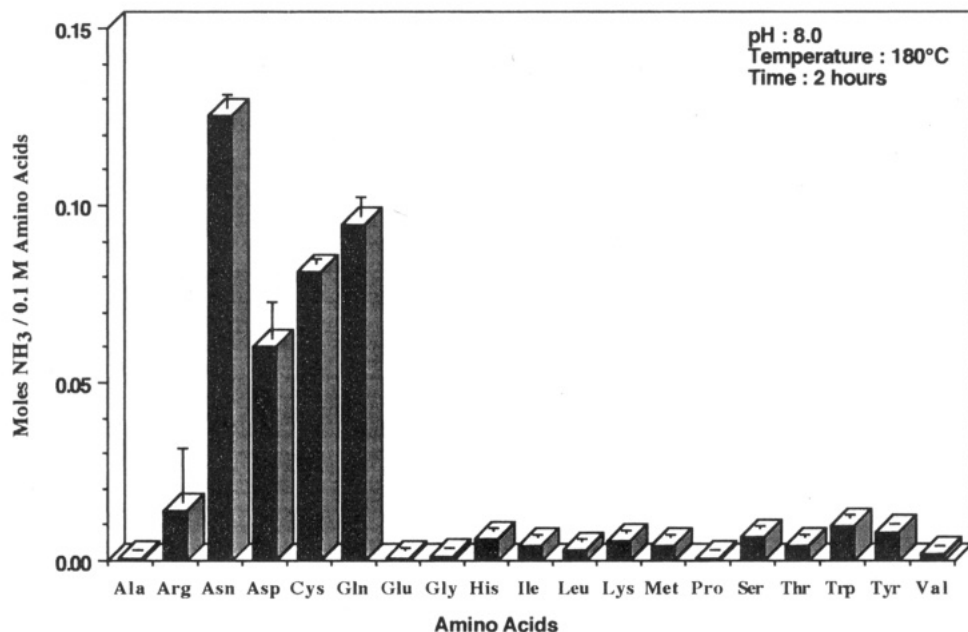
Figure 1. Mole yield of ammonia generation from amino acids. A minimum of triplicates was run, and the mean values were obtained and calculated on the basis of 0.1 M of each amino acid. Samples were reacted at 180 °C for 2 h at pH 8.

MATERIALS AND METHODS

Nineteen amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, tyrosine, and valine) were purchased from Aldrich (Milwaukee, WI) and Sigma (St. Louis, MO). Two grams of each amino acid was measured and dissolved in 250 mL of deionized distilled water freshly prepared every day. The initial pH was adjusted to 8 using 0.1–1 N sodium hydroxide solution, and samples were transferred to a 0.3 L stainless steel reactor (Hoke Co., NJ). The reactors were placed in a conventional drying oven with a preset temperature of 180 °C and reacted for 2 h. After the reaction, samples were immediately placed in an ice bath to minimize further reaction. A minimum of triplicates was run for each amino acid.

Equimolar concentrations (0.05 M) of asparagine, aspartic acid, glutamine, glutamic acid, and cysteine were also measured and reacted at three different temperatures (110, 150, 180 °C) for 2 h after the pH was adjusted to 8 using the same methods mentioned above.

The amounts of ammonia released were measured by using a gas sensing Model 95-12 ammonia electrode (Orion, MA) which measures the dissolved ammonia in aqueous solutions at alkaline conditions. The ammonia electrode offers simple and accurate detection of ammonia concentration up to 10^{-7}, and its hydrophobic membrane provides very selective detection which allows only gases to pass through. For every sample and standard, 5 mL of ionic strength adjustor (Orion, MA) was added to ensure similar ionic strength and minimize the passage of water vapor before the measurement. The stock solutions of ammonium chloride (10^{-1}–10^{-4} M) were prepared for every measurement, and a calibration curve was obtained by plotting on a semilog graph using Cricket Graph for each batch of samples.

RESULTS AND DISCUSSION

Figure 1 shows the amount and mole yield of ammonia generation from each amino acid when heated in an aqueous solution of pH 8 at 180 °C for 2 h. Amino acids that released significant amounts of ammonia were asparagine, aspartic acid, glutamine, and cysteine.

Nonpolar amino acids such as alanine, valine, leucine, isoleucine, and methionine showed less than 5% of ammonia release. Polar amino acids such as threonine and serine also released about 5–6% of ammonia from

its α-amino group with the exception of asparagine and glutamine, which might be subjected to deamidation reaction under our experimental conditions.

Amino acids containing more than one nitrogen atom such as arginine, histidine, tryptophan, and lysine showed slightly higher amounts of ammonia generation than others. The guanidino group of arginine residues released about 28% of ammonia when heated. Whether any portion of ammonia released from arginine comes from the guanidino group is not exactly known. Histidine also released 6.2% of ammonia and tryptophan released about 10% of ammonia. On the other hand, one of the most reactive amino acids, lysine, remained stable under the conditions employed. This was probably due to the basic properties of lysine with its extremely high pK_a value.

The negatively charged amino acids, aspartic acid and glutamic acid, were expected to undergo similar thermal degradation due to their similarities in chemical structures. Similarly, deamination behavior is also expected for asparagine and glutamine. However, significant differences between asparagine and aspartic acid as well as glutamine and glutamic acid were observed. Glutamine released 100% of ammonia on a molar basis when heated. Glutamic acid was shown to be quite stable and released only 1.3% of ammonia from its α-amino group. This suggested that the ammonia released from glutamine was mainly due to the deamidation of the amide group of glutamine. These phenomena, however, did not apply to aspartic acid. Both asparagine and aspartic acid released significant amounts of ammonia from their α-amino groups. Asparagine generated more than double the amount of ammonia compared to that of aspartic acid. It seems that the thermal stability and activation energy of the α-amino group of asparagine and aspartic acid are quite different from those of glutamine and glutamic acid. The additional methylene group on glutamine and glutamic acid in their side chains could be the reason for the differences in ammonia generation from those of asparagine and aspartic acid.

Under physiological conditions, it was found that the rates of deamidation of asparaginyl residues were faster
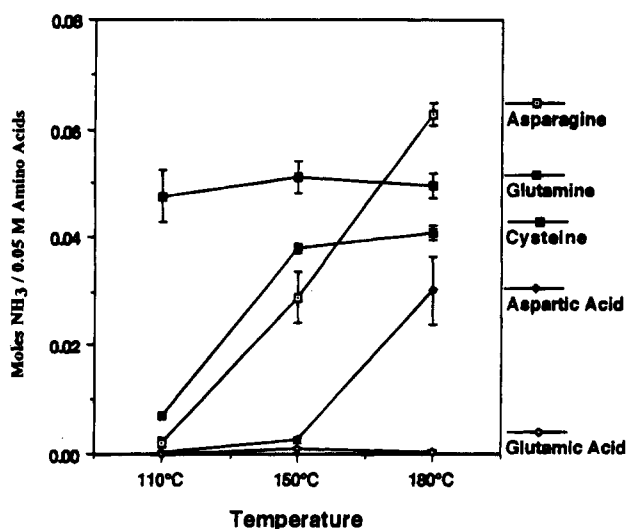
Figure 2. Mole yield of ammonia generation at three different temperatures. Amino acids (0.05 M) were reacted at 110, 150, and 180 °C for 2 h at pH 8. A minimum of triplicates was run, and the mean values were calculated and reported.

than those of glutaminyl residues in proteins and peptides. Clarke (1987) reported the formation of five-membered succinimide ring structures using HPLC in peptides. Lura and Schirch (1988) confirmed the formation of intermediate succinimide structures by ^{13}C and proton NMR. Deamidation of glutaminyl residues was found to be much slower than that of asparaginyl residues via six-membered ring structures. Wright (1991) suggested that these differences between asparagine and glutamine might be due to the size and length of the side chains.

To investigate the effects of temperature on the generation of ammonia, equimolar concentrations (0.05 M) of asparagine, aspartic acid, glutamine, glutamic acid, and cysteine were heated at three temperatures (110, 150, 180 °C) for 2 h, and the amounts of ammonia generated were measured to monitor their temperature dependency as shown in Figure 2. At the temperature of 110 °C, complete deamidation of glutamine was observed. On the other hand, glutamic acid did not release a significant amount of ammonia at the same temperature. Aspartic acid was found to be quite stable at temperature ranges between 110 and 150 °C and began to release ammonia dramatically at temperatures above 150 °C. More than 50% of its α-amino group had been decomposed when reacted at 180 °C for 2 h. From the data obtained for both asparagine and glutamine, asparagine seemed to undergo both deamidation and deamination, while glutamine seemed to undergo only deamidation. A sulfur-containing amino acid, cysteine, also released 82% of ammonia from its α-amino group, and the amounts of ammonia released increased as the temperature increased. The reactive thiol group released at low temperature might initiate nucleophilic attack on the α-carbon and promote the ammonia liberation.

Free ammonia and the amino group attached to the amino acid might undergo different reactions with the carbonyl group of sugars during the Maillard reaction. To understand the mechanisms involved during the thermal processing of foods, the thermal stability of amino acids and their reactivity in either bound or free form have to be investigated further. Systematic studies on the thermal degradation of amino acids and peptides would provide basic knowledge in optimizing the conditions required to achieved the desirable aroma quality of processed foods.

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