Formation of Pentylpyridines in an Oil Medium

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The effects of an oil medium on the release of free ammonia from each of five amino acids (glycine, L-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine) and the formation of pentylpyridines were studied. Among the five amino acids, only asparagine and glutamine generated ammonia readily by deamidation of amide side chains at 180 °C under oil conditions, even though both of them produced less ammonia than under aqueous conditions. However, the difference in the relative amounts of ammonia released between asparagine and glutamine was larger under oil conditions and much more free ammonia was liberated from glutamine than from asparagine. On the other hand, when each of the five amino acids was reacted with 2,4-decadienal under oil conditions, an increased amount of 2-pentylpyridine could be produced from asparagine and glutamine compared to aqueous conditions. Specifically, >10 times the amount of 2-pentylpyridine was found in the reaction of glutamine and 2,4-decadienal under oil conditions than under aqueous conditions despite the fact that both asparagine and glutamine could liberate less ammonia in oil systems. However, only a small amount of 3-pentylpyridine was formed in the asparagine and glutamine that produced a large amount of 2-pentylpyridine under oil conditions.

Keywords: *Reaction medium; ammonia; deamidation; deamination; pentylpyridine; lipid−protein interaction*

INTRODUCTION

Pentylpyridines including 2-pentylpyridine and 3-pentylpyridine have usually been found in foods containing high fat and low water contents during roasting or frying (Buttery et al., 1977; Ho et al., 1987; Schieberle, 1993; Tang et al., 1983). 2,4-Decadienal, an autoxidation product of linoleic acid, was believed to be the precursor for the formation of 2-pentylpyridine (Henderson et al., 1980; Zhang and Ho, 1989). Recently, we reported that there was a considerable difference in the reactivity among amino acids for the formation of pentylpyridines in aqueous systems (Kim et al., 1996). The reactivity of free ammonia released from amino acids toward the formation of pentylpyridines was much higher than those of the α-amino groups bound in amino acids in the same systems. Free ammonia may also play a critical role in producing the pentylpyridines in oil systems because the formation of 2-pentylpyridine was increased after the addition of ammonium sulfate during the roasting of sesame oil (Schieberle, 1993). Therefore, quantitative data on free ammonia released from amino acids are needed in both aqueous and oil systems to understand the effects of free ammonia on the formation of alkylpyridines compared to α-amino groups in amino acids. In an earlier study, Sohn and Ho (1995) demonstrated the amounts of free ammonia liberated from amino acids at high temperatures only under aqueous conditions.

In the current study, we investigated the free ammonia released from each amino acid used in the heated oil medium. We then examined the formation of pentylpyridines from the interaction of different amino acids with 2,4-decadienal under the same condition.

EXPERIMENTAL PROCEDURES

Release and Isolation of Ammonia from Amino Acids in Oil Systems. An equimolar concentration (0.5 mol) of five different amino acids such as glycine, L-aspartic acid, L-glutamic acid, L-asparagine, and L-glutamine (Sigma Chemical Co., St. Louis, MO) was measured and added to a 250 mL round-bottom flask that contained 100 mL of medium-chain triglycerides (saturated C_8 and C_{10} acids; Stepan Food Ingredients Group, Maywood, NJ). Each sample was then heated at 180 °C for 1 h to release free ammonia. The released ammonia was purged with nitrogen (20 mL/min) and trapped in 100 mL of 2 N HCl solution cooled in an ice bath and connected to a dry ice acetone condenser during the heating period. After the ammonia was isolated from the system, these acid solutions were stored at 4 °C in sealed amber bottles until their ammonia contents were analyzed.

Ammonia Determination. Five milliliter aliquots of each acid trap were diluted in 100 mL of freshly deionized water. To keep the pH and ionic strength of each sample in the correct range for ammonia measurement, ammonia pH adjusting ionic strength adjustor (ISA; Orion Research Inc., Boston, MA) solution (2−4 mL) was added until the sample solution was turned to a blue color at alkaline range. The amount of ammonia released was determined using a gas-sensing model 95-12 ammonia electrode (Orion Research), which measures the dissolved ammonia in an aqueous alkaline solution. The ammonia electrode offers a simple and accurate detection of the ammonia concentration up to 10^{-7} M at alkaline concentrations. Ammonium chloride standard stock solutions (10^{-1}−10^{-6} M) and deionized water were freshly prepared before each measurement, and the calibration curve was obtained by plotting a semilog scale for every sample.

Volatiles from the Reactions of Amino Acids and 2,4-Decadienal in Oil Systems. Five different amino acids,

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S0021-8561(97)00719-X CCC: $15.00
Published on Web 01/23/1998

glycine, L-aspartic acid, L-asparagine, L-glutamic acid, and L-glutamine (0.005 mol), were added to a 0.3 L Hoke SS-Dot sample cylinder that contained 100 mL of medium-chain triglycerides, separately. The sample cylinders were then heated at 180 °C in an oil bath for 1 h. After the sample cylinder was cooled in ice water, 2.5 mL of 2-ethoxy-3-ethylpyrazine (1000 ppm; Pyrazine Specialties, Atlanta, GA) was added as an internal standard into each sample, the volatiles of which were purged with nitrogen at a flow rate of 400 mL/min for 6 h, keeping the sample at 70 °C and constantly agitated, and trapped in 11.7% (w/v) HCl solution (70 mL) cooled in ice water. Only basic compounds such as pyridines and pyrazines were trapped in the acid solution as salts while others passed through the acid solution. After the purge and trap step, the acid solution was extracted with methylene chloride (100 mL) three times to remove the remaining nonbasic compounds. The acid solution trapping basic compounds was then titrated with 30% NaOH solution to pH 12.5 before the basic compound was isolated with methylene chloride by multiple extractions (3 × 100 mL) in a separatory funnel. The extract was then concentrated in a Kuderna−Danish apparatus to a volume of ~5 mL and further concentrated with nitrogen gas to a final volume of 0.1 mL.

Volatile Separation by Gas Chromatography. A Varian 3400 gas chromatograph with an FID and a nonpolar fused silica capillary column [60 m × 0.25 mm (i.d.), 0.25 μm thickness, DB-1; J&W] was employed to analyze the volatile compounds produced from the thermal reactions. The GC equipment was operated with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier gas flow rate of 1.2 mL/min at 40 °C. The temperature of the GC column was increased from 40 to 280 °C at a rate of 2 °C/min, holding at 280 °C for 30 min. One microliter of the sample solution was injected with a split ratio of 50:1. Linear retention indices (RI) for the volatiles were determined by comparing their retention times with those of *n*-paraffin standard (C_6−C_{19}; PolyScience, Niles, IL), according to the method of Majlat et al. (1974).

GC/MS Analysis. The samples were analyzed by GC/MS, a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8230 high-resolution, double-focusing magnetic sector mass spectrometer (TD-GC-MS) equipped with the same column used for the gas chromatography. The operating conditions were the same as described above. Mass spectra were obtained by using an electron ionization of 70 eV and an ion source temperature of 250 °C. All mass spectra were identified by using an on-line computer library (NIST and Wiley 138.1) and published literature.

Compound Identification and Quantification. The identification of compounds was made on the basis of their retention indices and mass spectral data. Each compound was quantified by calculating its peak area relative to that of the internal standard.

RESULTS AND DISCUSSION

The amount of ammonia generated from amino acids [glycine, L-aspartic acid, L-asparagine, L-glutamic acid, or L-glutamine (0.5 mol)] was measured when each of the five amino acids was heated at 180 °C for 1 h in an oil system of medium-chain triglycerides (saturated C_8 and C_{10} acids, 100 mL). The amount of ammonia liberated from each amino acid is shown in Figure 1. Glutamine produced the largest amount of ammonia among the five amino acids. Although it has a similar amide side chain, the asparagine produced much less ammonia than glutamine when they were subjected to heating under oil conditions. At 180 °C, the amount of ammonia released from glutamine and asparagine in the oil system (7.36×10^{-3} and 8.01×10^{-4} mol of NH_3/0.1 M of glutamine and asparagine) was much less than the same reaction in aqueous system (9.48×10^{-2} and 1.25×10^{-1} mol of NH_3/0.1 M of glutamine and asparagine). As shown by Sohn and Ho (1995), in an aqueous solution at a higher temperature such as 180 °C, asparagine generated more ammonia than glutamine. This is because under these conditions the ammonia released from asparagine could come from both deamidation and deamination. However, the deamination of glutamine was found to be very insignificant.

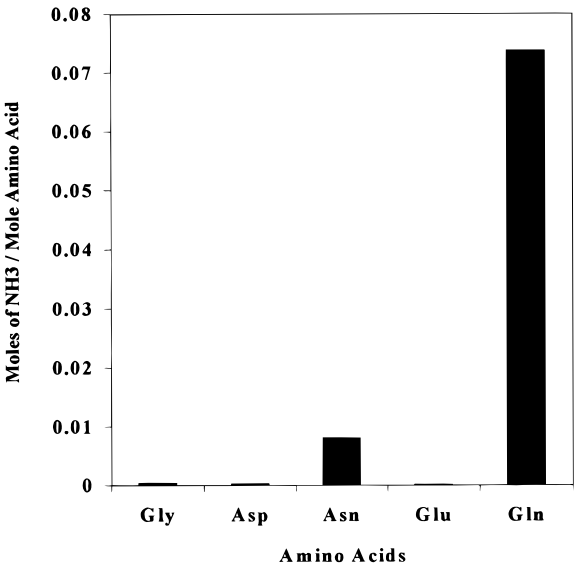

Figure 1. Amount of ammonia released from amino acids heated at 180 °C for 1 h in oil medium.

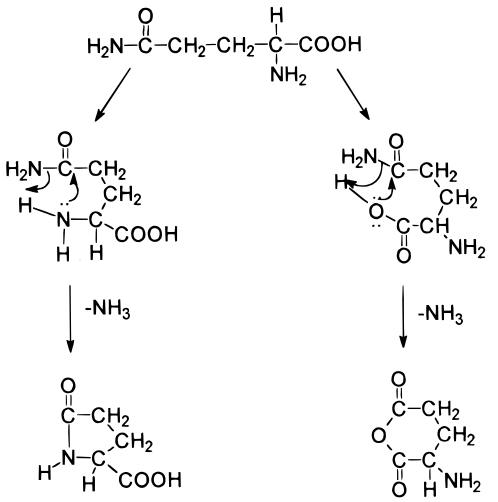

Figure 2. Proposed mechanism for the intramolecular cyclization of free glutamine to release ammonia.

Our current data indicate that the deamidation of asparagine and glutamine may follow different mechanisms in oil-containing systems. The intramolecular deamidation mechanism could be proposed to explain the difference in deamidation reactivity between glutamine and asparagine in oil systems. As shown in Figure 2, deamidation can proceed readily without water by intramolecular cyclization with its own amino group or carboxylic group in free glutamine. If the amino nitrogen attacks the amide group, the result can be a relatively stable five-membered ring structure, which can form pyrrolidonecarboxylic acid. This can be substantiated by the fact that amino-terminal glutamine readily deamidates by cyclization with its own terminal amino group in proteins (Melville, 1935). As in the ring formation by amino nitrogen, the amide group may also undergo nucleophilic attack by carboxylic oxygen, re-

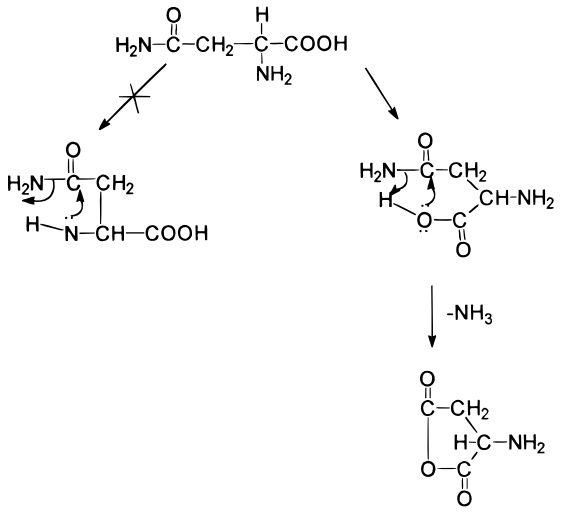

Figure 3. Proposed mechanism for the intramolecular cyclization of free asparagine to release ammonia.

sulting in a cyclic anhydride and free ammonia. This may happen due to the stability of the resulting six-membered ring structure.

On the other hand, in the case of free asparagine, deamidation may occur intramolecularly as a result of an attack of carboxylic oxygen on the amide group because it can make a relatively stable five-membered ring structure (Figure 3). The occurrence of this kind of deamidation has been shown by some researchers in proteins that have asparagine at the carboxyl-terminal position (Carpenter, 1966; Markussen et al., 1988). However the amino nitrogen will not react to form the cyclic anhydride due to steric hindrance caused by an unstable four-membered ring.

Glycine, aspartic acid, and glutamic acid released a small amount of ammonia from the amino group under the oil conditions. They could liberate ammonia only by deamination of amino groups. The cleavage of a C−N bond leads to the formation of free ammonia at high temperatures such as 180 °C (Lien and Nawar, 1974a,b).

A mixture of 2,4-decadienal and each amino acid (glycine, L-aspartic acid, L-asparagine, L-glutamic acid, and L-glutamine) was heated at 180 °C for 1 h under oil conditions; all five amino acids generated 2-pentylpyridine but not 3-pentylpyridine (Figure 4). The relative amount of 2-pentylpyridine produced from asparagine and glutamine, which generated free ammonia relatively readily, was proportional to the amount of free ammonia available in these systems under oil conditions. Since much less 2-pentylpyridine was formed from amino acids such as glycine, aspartic acid, and glutamic acid, which produced little ammonia, α-amino groups bound in amino acids might seem to be less involved in the formation of 2-pentylpyridine.

It was interesting to note that a large amount of 2-pentylpyridine was formed from the thermal interaction of asparagine and glutamine with 2,4-decadienal in oil systems. Glutamine produced >10 times the amount of 2-pentylpyridine in oil systems, with less free ammonia, than in aqueous systems. Several factors may affect the increased amount of 2-pentylpyridine formation. First of all, in an oil system, much more free ammonia can exist in the form of NH_3 instead of ammonia ion (NH_4^+). Ammonia is a stronger base than a water molecule, and the equilibrium will favor the

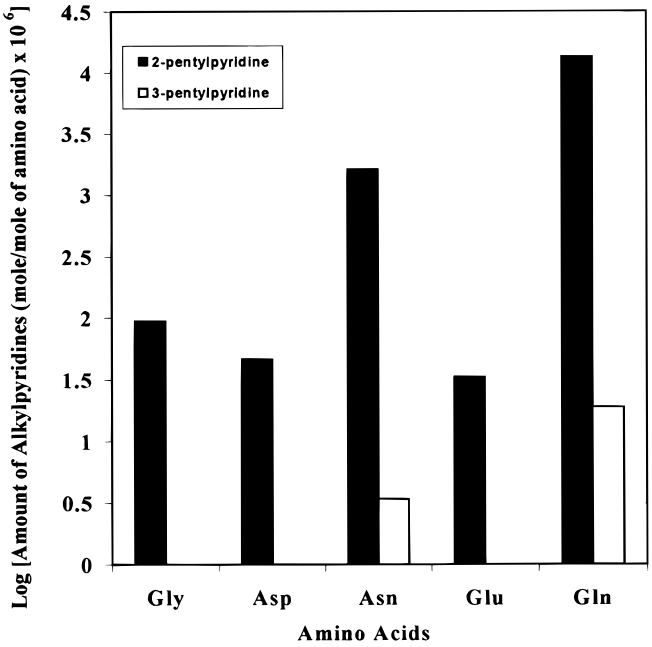

Figure 4. Amount of alkylpyridines formed from the interaction of amino acids with 2,4-decadienal at 180 °C for 1 h in oil medium.

formation of the weaker acid (NH_4^+) (Raber and Raber, 1984). Therefore, most of the ammonia generated from amino acids will become ammonium ions which cannot act as a nucleophile to attack the carbonyl center of 2,4-decadienal due to the lack of unshared electrons under aqueous conditions. Therefore, more free ammonia might be available for the formation of 2-pentylpyridine under oil conditions, even though less free ammonia is released from amino acids.

Another factor that contributes to the higher yield of 2-pentylpyridine in an oil system may be the stability of 2,4-decadienal. In an aqueous system, 2,4-decadienal could be decomposed more easily by retro-aldolization as suggested by Josephson and Lindsay (1987).

A considerable amount of 3-pentylpyridine has been found in the reaction of 2,4-decadienal and amino acids in an aqueous solution (Kim et al., 1996). Only a small amount of 3-pentylpyridine was produced from the asparagine and glutamine, which generated a large amount of 2-pentylpyridine under the oil conditions. This result indicated that other intermediates degraded from 2,4-decadienal in the presence of water were essential to the formation of 3-pentylpyridine. The exact mechanism for the formation of 3-pentylpyridine is not clear at the present time.

The present study demonstrates that the deamidation of amide-containing amino acids and the subsequent formation of nitrogen-containing heterocyclic compounds such as pyridines could be affected by the reaction medium. Not only the difference in patterns and extent of deamidation of asparagine and glutamine but also the difference in the contribution of ammonia to the formation of heterocyclic compounds under aqueous and oil conditions may result in the significant effects observed.

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Received for review August 21, 1997. Revised manuscript received November 24, 1997. Accepted December 7, 1997.

JF970719Z