Aspartame Degradation in Solutions at Various pH Conditions

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ABSTRACT: This study of aspartame degradation in phosphate-citrate buffer solutions of pH 2, 4, 6, 7, 8, 10, and 12 using reverse-phase high-performance liquid chromatography (HPLC) showed different major degradation products for different pH ranges. The major degradation product found at pH 2 to 6 was L-phenylalanine methyl ester (PME). The major degradation product found at pH 7 to 10 was 3,6-dioxo-5-phenylmethylpiperazine acetic acid (diketopiperazine, DKP). L-aspartyl-phenylalanine (Asp-Phe) was the major product detected at pH 12.

Keywords: aspartame, L-aspartyl-L-phenylalanine methyl ester, diketopiperazine, degradation

Introduction

IT IS NOW WELL KNOWN THAT L-ASPARTYL-L-PHENYLALANINE methyl ester (aspartame, APM), a low-calorie sweetener, exhibits a limited stability in aqueous systems. Its degradation, which is an acid-base-catalyzed reaction, depends on pH, buffer concentration, type of buffer, and temperature (Homler 1984; Prudel and others 1986; Bell and Wetzel 1995). Various decomposition products have been identified, including L-aspartic acid (Asp), L-aspartylphenylalanine (Asp-Phe), L-phenylalanine methyl ester (PME), L-phenylalanine (Phe), 3,6-dioxo-5-phenylmethylpiperazine acetic acid (diketopiperazine, DKP), as well as its isomerized product, β-aspartame (Furda and others 1975; Tsang and others 1985; Prodolliet and Bruelhart 1993). Although many studies have been done on the determination of aspartame stability and its degradation products, most reports covered only limited pH range. This paper presents a study of aspartame degradation in phosphate-citrate buffer solutions covering the pH range of 2 to 12. Type of degradation product formed at each pH was identified, and pH-dependent degradation pathways of this compound are proposed.

Materials and Methods

Materials

Aspartame, aspartic acid and phenylalanine methyl ester were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Aspartyl phenylalanine and 5-benzyl-3,6-dioxo-2-piperazineacetic acid (3-carboxymethyl-6-benzyl-2,5-diketopiperazine) were purchased from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.). Phenylalanine, potassium dihydrogen phosphate, disodium phosphate and citric acid were purchased from Fluka Chemie AG (Buchs, Switzerland). Methanol, acetonitrile and sodium hydroxide were obtained from J.T. Baker Inc. (Phillipsburg, N.J., U.S.A.). Phosphoric acid was obtained from Unilab Ajax Chemical Ltd. (Auburn, Australia).

High-performance liquid chromatography

The HPLC method of Prodolliet and Bruelhart (1993) was modified for this study. The mobile phase used was KH_2PO_4 (pH 3.5) : acetonitrile 85:15 (V/V) at a flow rate of 0.8 mL/min. The HPLC system consisted of a Model 662 pump by Waters (Milford, Conn., U.S.A.); a manual injector by Rheodyne (Rohnert Park, Calif., U.S.A.) with a 10 μL injection loop; a Model 486 MS variable-wavelength UV detector (Waters), and a Model 600 S controller (Waters). The column used was μBondapak-C18, particle size 10 μm, 300 × 3.9 mm I.D (Waters). Chromatograms and peak areas were processed using PC 800, Version 2.0 software®. The UV detector was set at 214 nm.

Degradation study

Phosphate-citrate buffer solutions of pH 2, 4, 6, 7, 8, 10, and 12 were prepared according to the method of Snell and Biffen (1964), using deionized, sterile water. Aspartame solutions of various pHs were then prepared to a final concentration of 1000 ppm. These solutions were kept at 4 °C and were withdrawn for HPLC analyses at the appropriate time. Besides using retention time, confirmation of each peak in the chromatogram was done by spiking each standard into the test sample before re-injection. Relative abundance of each species was calculated from peak integration data and absorptivity of each species. Absorptivity of each species was first obtained by HPLC analyses of solutions containing exact concentrations of the standards.

Results and Discussion

FIGURE 1 SHOWS THE DEGRADATION OF ASPARTAME SOLUTION at pH 2, 4, 6, 7, 8, 10, and 12. It is obvious that between pH 2 to 12, aspartame degraded much more slowly under acidic pH than under neutral or basic pH. In this study, it was found that aspartame solution at pH 2 degraded a little more slowly than the solution at pH 4. This result disagreed with previous findings which indicated that aspartame is most stable at pH 2.5 to 5.0 (Prudel and others 1986). From the graphs, major degradation products could be grouped into 3 categories; namely, product found at acidic pH (pH 2 to 6), product found at neutral to basic pH (pH 7 to 10) and product found at the very basic pH (pH 12). It should be noted here that in this study, Asp and methanol could not be detected by using a UV detector.

Figure 2 summarizes the degradation pathways of aspartame at various pHs. The major degradation product found at pH 2 to 6 was PME. This finding was quite interesting because, in aqueous solution, the 3 major decomposition products of aspartame previously reported were DKP, Asp-Phe, and Phe (Prodolliet and Bruelhart 1993), with DKP and Asp-

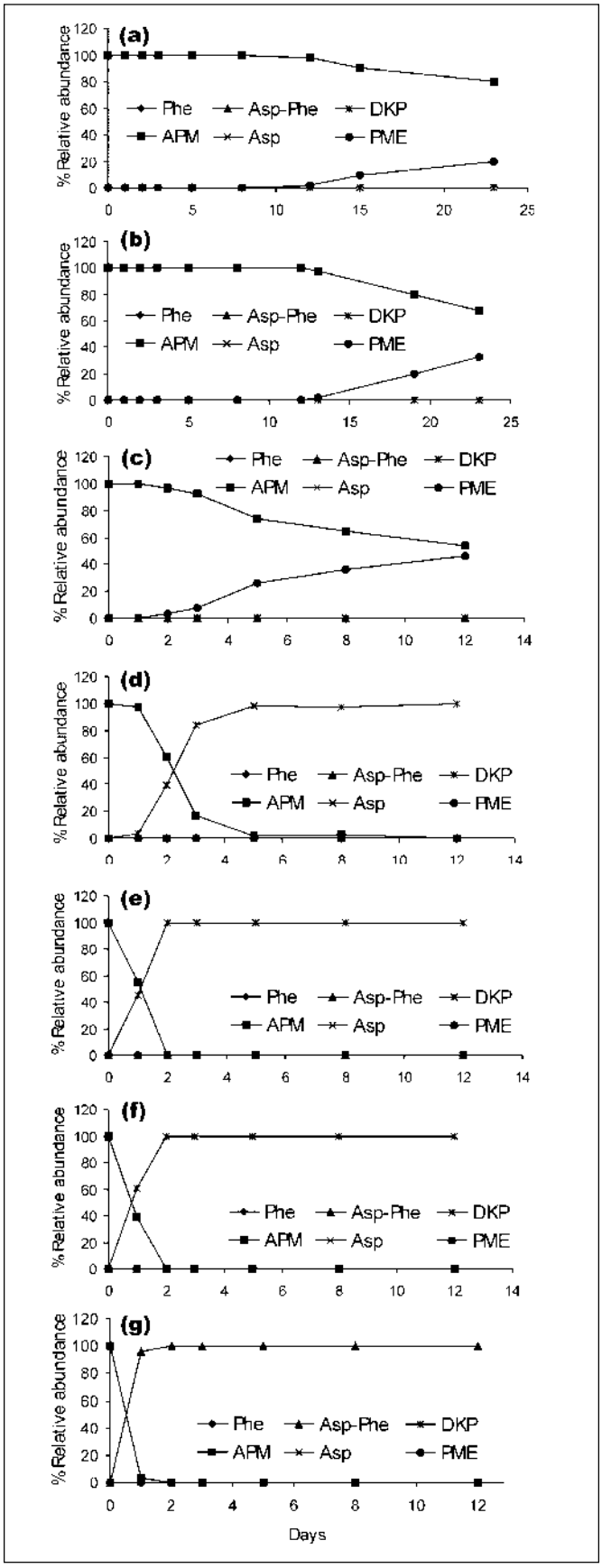

Figure 1—Degradation of aspartame in phosphate-citrate buffer solutions of pH 2 (a), 4 (b), 6 (c), 7 (d), 8 (e),10 (f) and 12 (g), at 4 °C.

Phe reported to be the major products found under acidic pH (Prudel and others 1986). We also re-performed this experiment at 25 °C and still found that PME was the only major degradation product. The most pronounced degradation product found at pH 7 to 10 was DKP. Asp-Phe was the major product detected at pH 12.

Proposed degradation mechanisms yielding the 3 products are as following:

Under acidic condition, amine groups in the APM molecule are likely to be protonated. Therefore, the most prominent reaction would be the attack of the lone pair of electrons on the oxygen of the hydroxy group of the aspartic moiety, on the carbonyl carbon at the amide linkage, yielding PME and Asp. When the pH of the solution is around 7 to 10, the lone pair of electrons on the amino group of the aspartic moiety can easily attack the carbonyl carbon at the phenylalanine methyl ester. The 6-membered ring cyclization product, DKP, can then be formed with the release of methanol. In a very basic environment (pH 12), there would be many hydroxyl ions, and therefore, the attack of the hydroxyl ion on the carbonyl carbon at the methyl ester, yielding Asp-Phe and methanol, would be the dominant reaction.

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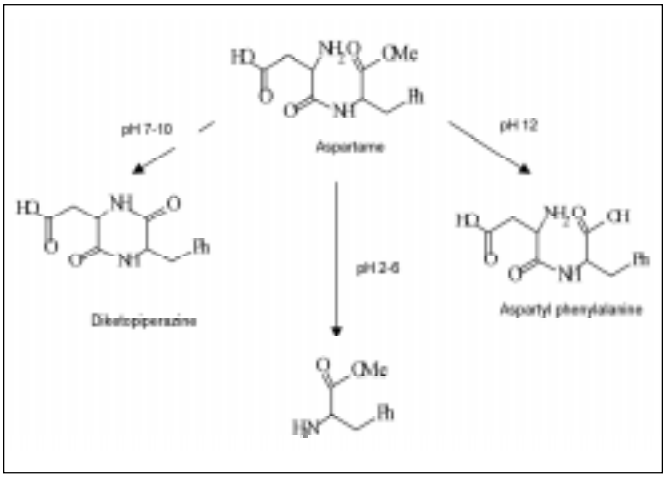

Figure 2—Degradation pathways of aspartame in aqueous solutions at various pH values