Benzaldehyde Formation from Aspartame in the Presence of Ascorbic Acid and Transition Metal Catalyst

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Benzaldehyde was produced from aspartame in aqueous acidic solutions containing ascorbic acid and Cu(II) or Fe(III) ion. Benzaldehyde was identified in the system by GC−MS. The yield of benzaldehyde decreases dramatically as the pH of the medium increases above 2.0. EDTA and DTPA completely inhibited benzaldehyde production, while desferrioxamine inhibited only the Fe(III)-catalyzed reaction. Benzaldehyde is not produced under anaerobic conditions unless H_2O_2 is added to reaction mixtures. H_2O_2 is produced by reduction of atmospheric oxygen under aerobic conditions. Benzaldehyde production was dependent on ascorbic acid concentration, but the yield of benzaldehyde decreased as the concentration of ascorbic acid exceeded that of aspartame. Addition of ethanol to the reaction mixture had little or no effect on benzaldehyde production, suggesting a mechanism that may not involve free hydroxyl radical. A mechanism is proposed for the reaction.

Keywords: *Aspartame; benzaldehyde; ascorbic acid; free radical degradation products; copper(II)*

INTRODUCTION

The artificial sweetener aspartame (*N*-L-α-aspartyl-L-phenylalanine methyl ester) has been thoroughly studied for safety and stability (Stegink and Filer, 1984), and several of its hydrolytic decomposition products have been identified (Homler, 1984). Because it is a dipeptide of naturally occurring amino acids (aspartic acid and phenylalanine), its hydrolysis products or metabolites, other than methanol, are nontoxic in the amounts that would be consumed by normal use of this sweetener. However, little is known regarding its interaction with other food components, such as natural nutrients or food additives.

Mazur (1976) has reported the pH stability profile for aspartame in aqueous solution, identifying among the hydrolysis products 3-benzyl-2,5-piperazinedione-6-acetic acid, the diketopiperazine derivative of aspartame. These products were subsequently identified in several soft drinks that were stored for 6 or 36 months (Tsang et al., 1985). Stamp and Labuza (1989) showed that β-aspartylphenylalanine, β-aspartame, and phenylalanine methyl ester are products of aspartame degradation in dilute acid solution. Hussein et al. (1984) found that several food-flavoring aldehydes, such as benzaldehyde, cinnamaldehyde, and vanillin, will react with the free amino group of aspartame in absolute alcohol to produce the corresponding Schiff base.

Ascorbic acid (vitamin C) is a natural component of many foods and is often added to foods and beverages as a vitamin supplement and antioxidant. Several studies have addressed the stability of ascorbic acid in aqueous solution in the presence of other food components, including aspartame (Ho et al., 1994; Hsieh and Harris, 1987, 1991). Earlier work in this laboratory (Gardner and Lawrence, 1993) showed aerobic solutions of ascorbic acid could produce benzene from the widely used food preservative sodium benzoate under conditions that are commonly found in beverages. These studies have been extended in an attempt to identify possible products of ascorbic acid dependent free radical degradation of aspartame.

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EXPERIMENTAL PROCEDURES

Reagents. Aqueous stock solutions of the following reagents were prepared from analytical reagent grade chemicals using distilled deionized water and stored in the refrigerator: 1−50 mM aspartame (L-aspartyl-L-phenylalanine methyl ester; Sigma) in 0.05 N HCl; 52 mM phenylalanine (Sigma) in 0.05 N HCl; 9.3 mM aspartylphenylalanine (Asp-Phe; Sigma) in 0.10 M phosphate buffer, pH 2.0; 1.6 mM copper(II) sulfate pentahydrate (G.F. Smith); 150 mM hydrogen peroxide (Fluka); 8 mM benzyl alcohol (Sigma); 8 mM benzaldehyde (Sigma); 4 mM sodium benzoate (Sigma); 100 mM ascorbic acid (Fluka) in 0.10 N HCl; 0.10 M monochloroacetic acid buffer, pH 2.1−3.3; and 0.10 M sodium dihydrogen phosphate buffer, pH 1.6−7.7, adjusted to desired pH with NaOH. Aspartame stock solutions were prepared fresh every few days. Hydrogen peroxide concentration in stock solutions and reaction mixtures was determined colorimetrically with saturated $TiOSO_4$ solution in 2 M H_2SO_4 (ϵ = 717 M^{-1} cm^{-1} at 410 nm; Ellis and Sykes, 1973).

Reaction Conditions. A typical reaction mixture (1.0 mL) contained 50 mM buffer (buffer components and pH varied), 5.0 mM aspartame, 0.16 mM $CuSO_4$, 8 mM H_2O_2, and 5.0 mM ascorbic acid. The pH was checked at the end of a reaction because the presence of HCl in the aspartame and ascorbic acid stock solutions caused some shift in pH of the buffer when these were added to the reaction mixtures. The reaction mixtures were placed in a 40 °C water bath (except where indicated) and analyzed by direct injection of an aliquot into the liquid chromatograph 15 min after the reaction was initiated (except where indicated). The reaction conditions were varied to study the effects of pH and concentration of reagents. In some cases $Fe_2(SO_4)_3$ was substituted for $CuSO_4$, and oxygen was purged from some reaction mixtures by bubbling with water saturated, prepurified N_2.

The metal-chelating agents disodium ethylenediaminetetraacetic acid (Na_2EDTA), diethylenetriaminepentaacetic acid (DTPA), glycine, and desferrioxamine (desferal mesylate; a gift of Ciba Pharmaceutical Co.) were added to some reaction mixtures to determine their effect on the metal catalysts. Ethanol was added as a competitive hydroxyl radical scavenger to determine whether "free" hydroxyl radical is involved in these reactions.

The concentration of ascorbic acid was followed during the progress of the reaction by diluting a 20 μL aliquot of the

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reaction mixture in 1.98 mL of 0.10 M phosphoric acid at a given time and absorbance measured at 245 nm ($\epsilon = 7943\ M^{-1}\ cm^{-1}$; Graselli, 1973). Other reactants gave little or no contribution to this absorbance (aspartame $\epsilon_{245} = 102\ M^{-1}\ cm^{-1}$; this study). Benzaldehyde produced in the reaction has a strong absorbance at 245 nm ($\epsilon_{247} = 11\,482\ M^{-1}\ cm^{-1}$; Graselli, 1973), and its contribution at any time in the reaction could be calculated from the chromatographic quantitation. Other products of the reaction, such as aromatic hydroxylation products, would absorb at 245 nm, but their contribution is probably not significant at low pH (judging from chromatographic results). The concentration of hydrogen peroxide was monitored colorimetrically during the progress of the reaction as described above.

Liquid Chromatography. The LC system consisted of a Knauer model 64 pump, Rheodyne 7125 injection valve with 20 μL injection loop, and Knauer variable wavelength UV detector at 255 nm with response recorded on either a Kipp and Zonen BD41 strip chart recorder or a Hewlett-Packard 3396B integrator. A Hewlett-Packard model 1050 liquid chromatograph with diode array detector became available at the end of this study (gift of the Hewlett-Packard Educational Grants Program) and was used for some analyses. The analytical column for analysis of aspartame and benzaldehyde was a 4.6 × 100 mm Spheri-5 (5 μm) cyano column, with 4.6 × 30 mm guard cartridge (Brownlee). The mobile phase was 0.05 M sodium dihydrogen phosphate, 50 μM DTPA containing 8% (v/v) acetonitrile. The mobile phase was filtered through 0.2 μm membrane filters prior to use and pumped at a flow rate of 1.3 mL/min. Retention times were aspartame, 1.9 min; benzaldehyde, 2.8 min. These analytes were quantified by comparison of peak height with known standards. A product of aspartame degradation was not well resolved from the aspartame peak and interfered with quantitation by peak area; consequently peak height was used for quantitation of both aspartame and benzaldehyde.

The chromatographic system for analysis of benzyl alcohol, benzoic acid, and benzaldehyde in the presence of aspartame consisted of a Spheri-5 C8 (octylsilane) column (4.6 × 100 mm) with C8 guard cartridge (Brownlee) and mobile phase containing 0.05 M sodium dihydrogen phosphate, 0.01% (w/v) sodium octylsulfate, and 8% (v/v) methanol adjusted to pH 3.9 with phosphoric acid solution and pumped at a flow rate of 3.0 mL/min. Retention times were benzyl alcohol, 2.0 min; benzoic acid, 3.1 min; benzaldehyde, 4.3 min; aspartame, 6.3 min.

Gas Chromatography−Mass Spectrometry. A Hewlett-Packard 5890/5971 GC−MS (gift of the Hewlett-Packard Educational Grants Program) was equipped with a 30 m HP-1 (nonpolar) capillary column. GC parameters as follows: initial temperature, 100 °C; initial time, 2.0 min; rate, 10 °C/min; final temperature, 160 °C; final time, 2.0 min; injector temperature, 200 °C. MS parameters as follows: low mass, 50; high mass, 200; threshold, 150; sampling rate, 2/s. A high yield of a major product observed in the liquid chromatographic analysis was obtained by placing the standard reaction mixture in a distillation apparatus and collecting the distillate at 85 °C. This product could be extracted into organic solvents, such as cyclohexane, but attempts to concentrate it by evaporation of the solvent resulted in loss of product as well. A small (0.5 μL) aliquot of the distillate was injected (split ratio 60:1) into the GC−MS for analysis. The major gas chromatographic peak ($m/z > 50$) was identified as benzaldehyde.

Solid Phase Extraction (SPE). Once benzaldehyde was identified as a product of aspartame oxidation in the hydroxyl radical-generating system, attempts were made to isolate and identify related benzyl derivatives (benzyl alcohol and benzoic acid). A systematic investigation of SPE adsorbents was undertaken, using octadecyl (C18), octyl (C8), cyano (CN), and phenyl packings in 3 mL cartridges (Supelco or Baker). Reversed-phase packings (C18, C8, phenyl, and cyano) were rinsed sequentially with water, 50% aqueous methanol, 100% methanol, 50% methanol, and then water prior to addition of 2 mL of reaction mixture. After the reaction mixture was loaded, the cartridges were rinsed sequentially with 2 mL of water, with 2 mL of 5% methanol, and several times with 2 mL of 50% methanol. Each 2 mL eluent was analyzed by HPLC for benzyl alcohol, benzaldehyde, benzoic acid, and aspartame, using the C8 analytical column. The bulk of the benzyl derivatives was eluted in the first 50% methanol fraction.

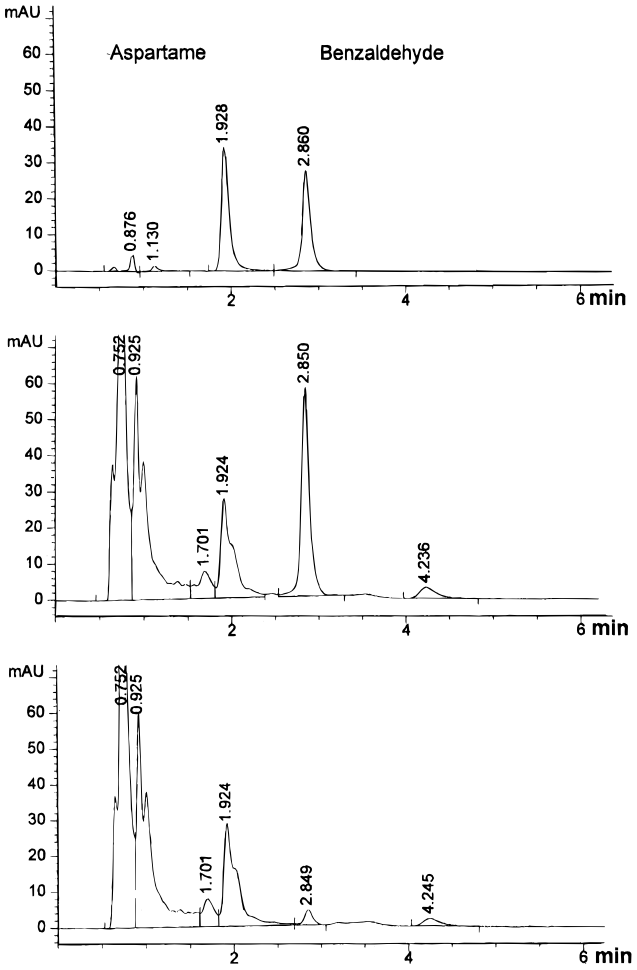

Figure 1. Liquid chromatograms of (top) aspartame (5.1 mM) and benzaldehyde (45 μM) standard, (middle) reaction mixture containing 0.05 M phosphate buffer, pH 2.0, 5.1 mM aspartame, 0.16 mM $CuSO_4$, 8.4 mM H_2O_2 and 5.0 mM ascorbic acid incubated for 30 min at room temperature, and (bottom) reaction mixture after extraction twice with 0.5 mL of cyclohexane/mL of reaction mixture. Chromatographic conditions on the Hewlett-Packard 1050 LC as described under Experimental Procedures, detector response at 255 nm.

RESULTS

Identifying Benzaldehyde as a Product of Aspartame Degradation. Preliminary studies of the ascorbic acid−hydrogen peroxide−Cu(II) free radical-generating system in the presence of aspartame revealed many unidentified peaks in the liquid chromatograms of the reaction mixtures. There was a noticeably large peak under acidic reaction conditions with a retention time close to that for aspartame that disappeared almost completely upon extraction of the reaction mixture with nonpolar organic solvents (Figure 1). An attempt to evaporate the organic solvent and dissolve the extracted material in mobile phase resulted in loss of the unidentified product, suggesting it was a volatile compound. Even partial evaporation of organic solvent resulted in a nearly linear loss of the product. Consequently, solid phase extraction with octadecyl or octyl SPE cartridges showed that a very nonpolar product

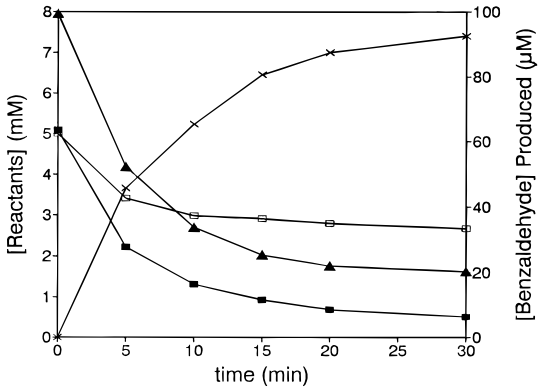

Figure 2. Rate of disappearance of reactants and formation of benzaldehyde in reaction mixtures containing 0.05 M phosphate buffer, pH 2.6, 5.0 mM aspartame, 0.16 mM $CuSO_4$, 8.4 mM H_2O_2, and 5.0 mM ascorbic acid, incubated at 40 °C. Ascorbic acid (■) and H_2O_2 (▲) were measured as described in the Experimental Section; aspartame (□) and benzaldehyde (×) were determined chromatographically.

could be partially purified but always contained some detectable impurities in the eluent containing this product.

Since this product was found to be volatile, attempts to collect it in concentrated form by distillation of the aqueous reaction mixture were successful. A highly concentrated solution of the unknown product of interest was obtained in the 85 °C distillate, devoid of noticeable impurities. This distillate was analyzed by GC−MS, and the single peak (m/z > 50) gave a mass spectrum that closely matched that of benzaldehyde. A standard solution of benzaldehyde was found to give a peak in the liquid chromatogram with the same retention time as that of the unknown peak of interest in the free radical reaction mixture containing aspartame. A standard addition of benzaldehyde to the reaction mixture further indicated the unknown peak of interest was benzaldehyde. The UV absorbance spectrum of the peak, using a diode array detector, indicated benzaldehyde was the product.

Optimum Conditions for Benzaldehyde Production. A time dependence study for the reaction (Figure 2) indicates there is a rapid phase for benzaldehyde production at pH 2.6 that is complete in about 30 min at room temperature or within 15 min at 40 °C. There is a slower continuous production of benzaldehyde and consequent disappearance of aspartame over several hours, although this latter phase accounts for a relatively small amount of the overall reaction. Subsequently, reaction mixtures stood for 15 min at 40 °C or for 30 min at room temperature for analysis of products as reaction conditions varied. The initial rapid phase of the reaction is complete before the concentrations of ascorbic acid and hydrogen peroxide are completely diminished. The H_2O_2 and ascorbic acid remaining in the reaction mixture continue to decrease at a relatively slow rate (Figure 2). This may be due to chelation of the Cu(II) ion by one of the products (see Discussion).

The production of benzaldehyde from aspartame in the free radical-generating system showed a strong dependence on pH and buffer composition. There was a sharp decrease in yield of benzaldehyde as pH of the reaction mixture increased from 2 to 3 (see Figure 3). Only trace amounts of benzaldehyde were produced in the neutral pH range, with a broad, late eluting peak (unidentified) appearing in the liquid chromatograms in the higher pH range.

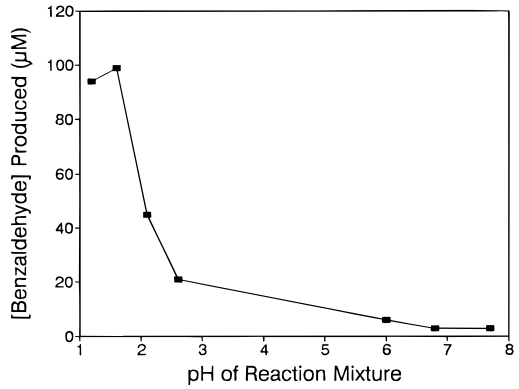

Figure 3. Dependence of benzaldehyde production on pH of the medium. Each reaction mixture contained 0.05 M phosphate buffer at pH indicated; other conditions were as in Figure 1, middle, except reaction mixtures were incubated for 15 min at 40 °C.

Table 1. Benzaldehyde Production from Aspartame under Varying Reaction Conditions

additions to reaction mixture	[benzaldehyde] produced (μM)
complete reaction mixture	110
anaerobic conditions	111
+ 32 mM ethanol	103
+ 500 μM DTPA	nd
+ 200 μM Na_2EDTA	nd
+ 500 μM glycine	120
in 0.05 M chloroacetate buffer, pH 2.3[a]	66
in 0.05 M glycine buffer, pH 2.1[a]	30
with 4.6 mM Asp-Phe instead of aspartame[b]	166
with 5.2 mM Phe instead of aspartame[b]	61
−H_2O_2, aerobic[c]	18
−H_2O_2, anaerobic[c]	nd
no metals added[d]	15
+ 880 μM desferrioxamine	nd
+ 500 μM DTPA	nd
+ 500 μM Na_2EDTA	nd
+ 500 μM Glycine	6
with varying [Cu(II)] added[e]	
+ 16 μM Cu(II)	52
+ 48 μM Cu(II)	82
+ 96 μM Cu(II)	98
+ 316 μM Cu(II)	120
with added Fe(III) in place of Cu(II)[d]	
67.5 μM $Fe_2(SO_4)_3$; [Fe(III)] = 135 μM	18
+ 200 μM Na_2EDTA	nd
+ 32 mM ethanol	16
in 0.05 M chloroacetate buffer, pH 2.3[a]	9

[a] Each reaction mixture contained 0.05 M phosphate buffer, pH 2.3 (except those with footnote a), 5.0 mM aspartame (except those with footnote b), 8 mM H_2O_2 (except those with footnote *c*), 168 μM $CuSO_4$ (except those with footnotes *d* and *e*), and 5 mM ascorbic acid. Reaction mixtures were incubated in a sealed vial for 15 min at 40 °C prior to removal of an aliquot for direct injection in the liquid chromatograph. All reactions performed in duplicate or triplicate. nd = not detectable.

Under acidic conditions, there was a much greater yield of benzaldehyde when phosphoric acid/phosphate buffer was used compared to chloroacetic acid or glycine buffers (Table 1). The latter buffers may act as radical scavengers or bind the Cu(II) ion, inactivating or changing the relative reactivity of this catalyst for free radical production. Phosphate and glycine buffers were prepared with constant ionic strength (I = 0.10 M with added $NaClO_4$) in the acidic pH range and found to give the same yield of benzaldehyde as those without added $NaClO_4$ at any given pH (data not shown), indicating the decrease in yield of this product with increasing pH was not due to a nonspecific ion effect.

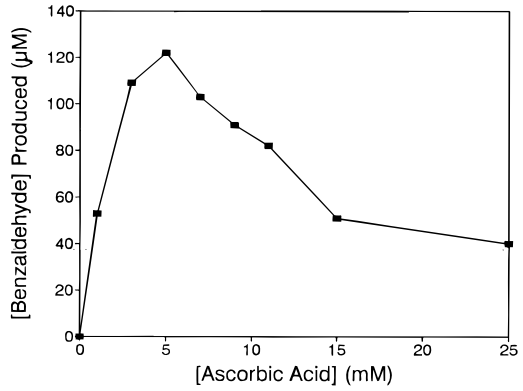

Figure 4. Dependence of benzaldehyde production on initial ascorbic acid concentration. Conditions were the same as in Figure 1, middle, except concentration of ascorbic acid was varied. Reaction mixtures were incubated for 15 min at 40 °C.

There is a nonlinear dependence on initial hydrogen peroxide concentration in the reaction mixture that may be partially due to the limiting amount of ascorbic acid present or to binding of the Cu(II) catalyst to products. A significant amount of benzaldehyde was produced when hydrogen peroxide was omitted (see Table 1). This production of benzaldehyde in the absence of hydrogen peroxide could be eliminated by deaerating the reaction mixture with nitrogen. This indicates hydrogen peroxide could be formed from ascorbic acid dependent reduction of ambient oxygen in the solution. Purging the complete reaction mixture with nitrogen (i.e., when hydrogen peroxide was present) had little or no effect on benzaldehyde production (see Table 1).

There is a complex dependence of the reaction on ascorbic acid concentration (Figure 4). The yield of benzaldehyde increases with increasing concentration of ascorbic acid in the reaction mixture up to 5 mM but then decreases with increasing concentration of ascorbic acid in excess of 5 mM. This can be explained by the fact that the concentration of aspartame in the reaction mixture was 5 mM, and ascorbic acid can compete with aspartame as a free radical scavenger in this system. It should be noted that there was not detectable benzaldehyde production when ascorbic acid was absent from the reaction mixture, and hydrogen peroxide was present, even after 24 h at room temperature. Although Cu(II) catalyzes the disproportionation of H_2O_2, this rate is extremely slow in acidic media. There was no detectable loss of H_2O_2 in 3 h in 0.05 M phosphate buffer at pH 2.6, in either the presence or absence of aspartame.

When Cu(II) ion was omitted from the reaction mixture, there was a dramatic decrease in benzaldehyde production, but it was still measurable (Table 1). This background production of benzaldehyde in the absence of any added metal ion catalyst was likely due to the presence of trace amounts of iron in deionized, distilled water and reagents. When desferrioxamine, a strong Fe(III) ion chelator, was added to the Cu(II)-deficient reaction mixture, benzaldehyde was not detectable. Addition of EDTA or DTPA, two other metal ion chelators, also completely inhibited benzaldehyde production, whether Cu(II) was added or omitted (Table 1). This indicates these latter chelating agents are very effective at inhibiting both Fe(III) and Cu(II) ions from catalyzing the title reaction. Addition of 500 μM glycine to reaction mixtures containing 168 μM $CuSO_4$ had no significant effect on benzaldehyde production. However, when 50 mM glycine was used to buffer the reaction mixture, there was a significant decrease (−76%) in benzaldehyde production (Table 1).

The addition of 32 mM ethanol to the reaction mixture had no effect on benzaldehyde production. Ethanol is a competitive $OH^\bullet$ scavenger that would be expected to diminish products of $OH^\bullet$ attack at this concentration in the reaction mixture. The lack of inhibition of benzaldehyde production by ethanol suggests a mechanism that may involve an aspartame−Cu ion complex that undergoes an intramolecular, site-specific attack on aspartame.

DISCUSSION

The present study shows that benzaldehyde is among the products of aspartame degradation in the presence of ascorbic acid autoxidation, catalyzed by Cu(II) or Fe(III) under acidic conditions. Addition of hydrogen peroxide to the system augments the production of benzaldehyde, whereas metal ion-chelating agents strongly inhibit the reaction. We have not found any report of benzaldehyde as a decomposition product of aspartame in the literature, and its identification as a major product under these conditions was quite unexpected.

Buettner (1986) found complete oxidation of ascorbic acid (0.1 mM) in 15 min in air-saturated solutions at pH 7. All chelating agents used in that study resulted in inhibition of the copper ion-catalyzed autoxidation of ascorbic acid, although EDTA augmented the iron ion-catalyzed reaction. Hsieh and Harris (1991) found that aspartame would augment the copper ion-catalyzed autoxidation of ascorbic acid, although they did not report measuring any aspartame decomposition products. The present study has identified a new decomposition product of aspartame in solutions containing ascorbic acid under conditions that might prevail in foods.

When ascorbic acid was omitted from reaction mixtures, there was no detectable formation of benzaldehyde from aspartame, even when hydrogen peroxide and Cu(II) were present. Cu(II) is an effective catalyst for the disproportionation of H_2O_2 by the following scheme (Gutteridge and Wilkins, 1983):

$$Cu(II) + H_2O_2 \rightarrow Cu(I) + HO_2^\bullet + H^+ \quad (1)$$

$$HO_2^\bullet \rightarrow H^+ + O_2^- \quad (2)$$

$$Cu(II) + O_2^- \rightarrow Cu(I) + O_2 \quad (3)$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH^\bullet + OH^- \quad (4)$$

However, in this study it was found that there was no detectable decomposition of H_2O_2 by Cu(II) ion in acidic media over several hours. There was no detectable production of benzaldehyde even after 24 h in the absence of ascorbic acid.

The presence of ascorbic acid in the reaction mixture results in reduction of Cu(II) to Cu(I) in the system (eq 5), which would facilitate reduction of H_2O_2 to $OH^\bullet$ (or $Cu(OH)^{2+}$ or CuO^+, *vide infra*) by a copper-catalyzed Fenton reaction (eq 4).

$$Cu(II) + H_2Asc \rightarrow Cu(I) + HAsc^\bullet \quad (5)$$

It is likely that the Cu(II) present in the reaction mixtures is coordinated to aspartame, which may alter

the rates and specificities of these reactions relative to the aquated Cu(II) ion. A Cu(II)−aspartame complex has been used for resolution of amino acid enantiomers by capillary electrophoresis (Gozel et al., 1986) and HPLC (Gilon et al., 1979). Cu(II) readily forms 1:1 and 1:2 complexes with aspartame, with the 1:1 complex predominating below pH 4 (Aihara et al., 1992). It appears that the coordination of Cu(II) results in a ligand-directed, site-specific free radical attack on the aspartame, since high concentrations of ethanol, a noncoordinating $OH^{\bullet}$ scavenger, had no effect on benzaldehyde production. Furthermore, the effect of varying buffer ions (phosphate, chloroacetate, and glycine, Table 1) shows that stronger coordinating ligands inhibit benzaldehyde production.

Gutteridge and Wilkins (1983) showed that proteins, in general, inhibit Cu(II)-mediated (but not Fe(III)-mediated) $OH^{\bullet}$ generation from H_2O_2 disproportionation and subsequent degradation of deoxyribose. However, the Cu(II) ions appeared to bind to the proteins and generate $OH^{\bullet}$ in a site-specific attack on the protein (sparing the deoxyribose). Czapski et al. (1983) have shown a similar ascorbic acid (or superoxide) dependent, Cu(II)-catalyzed site-specific damage to proteins in the presence of H_2O_2, which is not inhibited by hydroxyl radical scavengers. The nature of the oxidizing radical species in these systems has been proposed to be a Cu(II)·$OH^{\bullet}$ species rather than free $OH^{\bullet}$ for reactions involving Cu(I) ion with H_2O_2. The pK_a for the probable $Cu(OH)^{2+}$ has been estimated to be less than 3.5, from the product yield observed for the Cu(II)−H_2O_2 reaction with methanol (Johnson et al., 1985). There is a similar decrease in benzaldehyde yield in the pH range of 2−3.5, indicating a pK_a near 2.5 for one of the reactive species (possibly a Cu−aspartame−active oxygen intermediate).

Snook and Hamilton (1974) have shown that benzaldehyde is the major product of $OH^{\bullet}$ attack on 1-phenylalkanols (PhCHOHR) when R was isopropyl or *tert*-butyl, indicating the radical-stabilizing effect of the substituent alkyl groups favored cleavage, whereas a methyl group favored oxidation to acetophenone. $OH^{\bullet}$ attack on dipeptides results in $H^{\bullet}$ abstraction from the C−H bond adjacent to the amide nitrogen rather than near the protonated amino terminal (Taniguchi et al., 1970).

We propose a similar mechanism, whereby a Cu(II)−OH complex attack on aspartame results in $H^{\bullet}$ abstraction from the α-CH of the phenylalanyl moiety followed by scission to yield the benzyl radical and an oxidized peptide backbone (aspartyldehydroglycine methyl ester, ADGME), which is highly stabilized by a conjugated π-system (Figure 5). There is a slow, continual formation of benzaldehyde over several hours, with consequent decrease in the three major reactants after the initial rapid burst of product. It was initially suspected that ascorbic acid was completely diminished after the initial burst of product, and excess hydrogen peroxide was accounting for the slow phase of the reaction. However, lack of any benzaldehyde production in the absence of ascorbic acid suggested this was not so. Measurement of ascorbic acid in the reaction mixture indicated there was still a significant amount of ascorbic acid left when the initial rapid phase of the reaction was over. Consequently, chelation of Cu(II) ion by one of the products of the reaction (ADGME?) is proposed to be the cause of this slowing of the reaction.

When phenylalanine replaced aspartame as the scavenger, the yield of benzaldehyde was only about 55% of

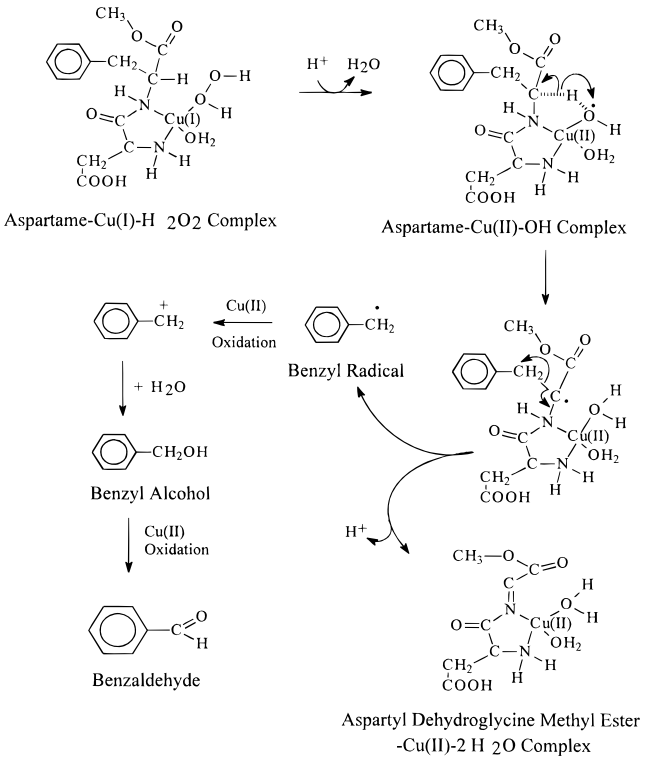

Figure 5. Proposed reaction scheme.

the yield with aspartame. This may be due to less π-stabilization of the nonaromatic product in the phenylalanine reaction. Asp-Phe augmented the production of benzaldehyde (+51%); the nonaromatic product of this scavenger would have greater π-stabilization than aspartame due to increased resonance in the carboxylate group. Addition of H_2O to the benzyl radical yields benzyl alcohol, which would be rapidly oxidized by Cu(II) to benzaldehyde under the reaction conditions. A concerted mechanism for scission and oxidation of the benzyl radical may be implied, since coordination of the Cu(II) catalyst by the nonaromatic product has been invoked. Benzaldehyde is not readily oxidized to benzoic acid by Cu(II) (March, 1968).

Walling (1975) has proposed a phenyl (ring-centered) radical cation in equilibrium with the hydroxycyclohexadienyl radical in acidic media, in the case of $OH^{\bullet}$ attack on phenylacetic acid. It was suggested the phenyl radical cation intermediate can undergo decarboxylation to yield benzyl radical, which is oxidized to benzyl alcohol and ultimately benzaldehyde in the presence of Cu(II) ion. However, the hydroxycyclohexadienyl radical intermediate can undergo oxidation to yield the corresponding phenol in the presence of Cu(II) and O_2. Phenolic products from $OH^{\bullet}$ attack on aspartame have been identified under neutral conditions (unpublished results). The pH of the medium strongly influences the distribution of products in this reaction, i.e., benzaldehyde vs phenolic products. There appears to be a decrease in yield of phenolic products below pH 3 (in conjunction with the increased benzaldehyde yield).

Attempts to measure benzyl alcohol and benzoic acid in the reaction mixtures showed there was little if any of these products formed. There was no benzyl alcohol detected in the GC−MS analysis of the distilled reaction mixture. The octyl (C8) column described in the Experimental Section gave good resolution of these benzyl derivatives and aspartame, but chromatograms of the

reaction mixtures monitored at 220 nm contained many peaks, and trace amounts of benzyl alcohol and benzoic acid could have been obscured by other peaks. It was estimated by standard addition that as little as 30 μM benzyl alcohol or benzoic acid could be detected in these reaction mixtures, but this level was not observed. It should be noted that UV detection for benzaldehyde is possible at 254 nm with a limit of detection near 1 μM, whereas benzyl alcohol has no significant absorption at wavelengths > 225 nm, and benzoic acid absorbs only weakly above 230 nm.

This study shows that aspartame, in the presence of ascorbic acid and a transition metal catalyst, such as Cu(II) or Fe(III), under aerobic conditions can produce benzaldehyde via a free radical attack on the aspartame. Although benzaldehyde is a commonly used flavoring agent (almond flavoring), and the title reaction would have little or no significant impact on public health, these results show that ascorbic acid, in the presence of trace amounts of metal catalysts, can initiate some very interesting chemical reactions in commonly used food additives.

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