Aspartame Degradation in Solution As Impacted by Buffer Type and Concentration

Leonard N. Bell* and Clinton R. Wetzel

Department of Nutrition and Food Science, Auburn University, Alabama 36849

Aspartame (APM) is a high-intensity sweetening agent incorporated into a variety of diet beverages along with some type of buffer to control the beverage pH. However, data on the effects of individual buffer types and their concentrations on APM degradation rates do not exist. This project evaluated the rate of aspartame degradation in phosphate and citrate buffer solutions at pH 3 and 7 over a concentration range of 0.01–1.0 M; experiments were performed at 25 °C. APM degradation rate constants were less at pH 3 than at pH 7 as reported previously. The reaction rates increased significantly as buffer concentration increased in both phosphate and citrate buffers. The degradation rate in phosphate buffer was significantly faster than in citrate buffer at pH 7. However, at pH 3, the difference between the rates in citrate and phosphate buffers was diminished. These results suggest that using citrate buffer rather than phosphate buffer would reduce the loss of APM in diet beverages, especially in higher pH beverages. Thus, diet beverages formulated with citrate buffer would have longer shelf lives than those formulated with phosphate buffer. In addition, this study provides general insight as to the mechanisms by which buffers may destabilize food ingredients.

Keywords: *Aspartame; citrate buffer; phosphate buffer; reaction kinetics*

INTRODUCTION

The high-intensity sweetener aspartame (α-L-aspartyl-L-phenylalanine 1-methyl ester) is being incorporated into many diverse reduced-calorie fluid products, including carbonated beverages, fruit juice beverages, and refrigerated dairy beverages. The composition of these beverages includes a buffering system of phosphate and/or citrate buffer salts. However, data indicating which buffering system enables maximum aspartame stability are unavailable and are the subject of the current research.

Many of the factors that influence the stability of aspartame have been evaluated previously. The impact of pH on aspartame degradation has been thoroughly examined in liquid and solid systems. Aspartame is most stable between pH 4 and 5 with increased acid hydrolysis at lower pH values and increased base catalysis at higher pH values (Prudel and Davidkova, 1981; Homler, 1984; Prudel et al., 1986; Ozol, 1986; Bell and Labuza, 1991a,b; Tsoubeli and Labuza, 1991; Skwierczynski and Conners, 1993). The effect of temperature on aspartame degradation has also been evaluated (Homler, 1984; Prudel et al., 1986; Ozol, 1986; Araman and Temiz, 1988; Bell and Labuza, 1991a; Tsoubeli and Labuza, 1991). Water activity also plays a significant role with respect to aspartame stability (Bell and Labuza, 1991a; Bell and Hageman, 1994).

At a given pH and temperature, a buffer solution can have two effects on aspartame stability, a concentration effect and an effect caused by the chemical composition of the buffer solution. However, many researchers have not evaluated the effect of buffer concentration and type on aspartame degradation during the course of their studies. For example, the study by Prudel and Davidkova (1981) used phosphate–citrate buffer blends at various pH values; therefore, individual buffer effects could not be distinguished. In addition, the buffer concentrations were not held constant, inhibiting the ability to differentiate between pH effects and concentration effects. Buffer concentration has been shown to influence aspartame stability; as phosphate buffer concentration increases, the rate of aspartame degradation also increases (Tsoubeli and Labuza, 1991). This study utilized only phosphate buffer over a narrow concentration range, focusing on solutions near neutrality at elevated temperatures (greater than 70 °C). Low levels of citrate and phosphate buffer added to a dairy beverage showed no significant difference in the rates of aspartame degradation (Bell and Labuza, 1994). However, the lack of a buffer effect could be due to the dairy proteins interacting with the buffer salts so that the salts were unavailable to interact with aspartame. Thus, the impact of buffer type and concentration on the rate of aspartame degradation remains to be thoroughly investigated and was the objective of the current study.

MATERIALS AND METHODS

Sample Preparation. Sodium phosphate dibasic, sodium phosphate monobasic, sodium citrate, citric acid anhydrous, and 85% phosphoric acid were obtained from Fisher Scientific (Pittsburgh, PA). Phosphate and citrate buffer solutions were prepared at various concentrations (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 M) to a pH of 7.00 ± 0.02 at 25 °C. Similarly, buffer solutions at various concentrations (0.02, 0.05, 0.1, 0.2, and 0.5 M) were prepared to a pH of 3.00 ± 0.02 at 25 °C. Aspartame (NutraSweet Co., Mt. Prospect, IL) was added to each buffer solution at a concentration of 90–180 ppm. For each solution, nine samples were stored at 25 °C and removed for analysis at regular time intervals to at least 30% loss or more frequently past the time to 1 half-life.

Samples removed from the incubator were cooled in ice and stored at 4 °C prior to analysis for a maximum of 6 h to minimize further aspartame loss. To prepare the pH 7 solutions for analysis, equal volumes of the sample solutions and a citric acid solution (0.1–0.5 M) were mixed to reduce

* Author to whom correspondence should be addressed [telephone (334) 844-3272; fax (334) 844-3268; e-mail lbell@humsci.auburn.edu].

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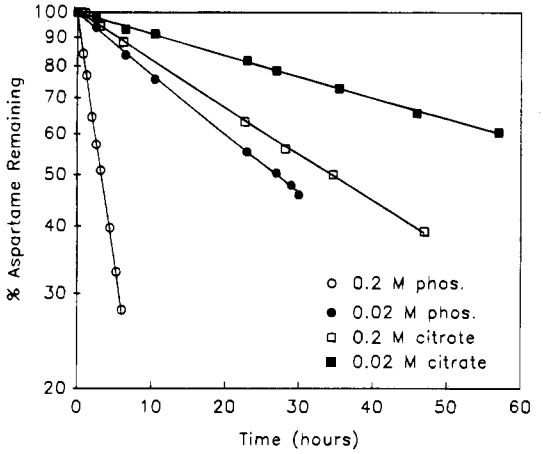

Figure 1. Pseudo-first-order kinetic plot of aspartame degradation as impacted by buffer type and concentration at pH 7 and 25 °C.

the pH to between 3.0 and 5.5 for improved aspartame stability and chromatography. The solutions at pH 3 were analyzed without further dilutions.

Sample Analysis. The HPLC method of Stamp and Labuza (1989) was modified for use in the current study. The mobile phase consisted of 80% deionized H_2O and 20% acetonitrile, along with 7 mM sodium heptanesulfonate and 5 mM sodium phosphate monobasic. The mobile phase was adjusted to a pH of 3 with 80% phosphoric acid. The separation was carried out on a 3.9 mm × 150 mm Nova-Pak C_{18} column (Millipore Corp., Milford, MA). The flow rate was set at 1.0 mL/min, and UV absorbance was read at 214 nm. Rate constants with 95% confidence limits were calculated using pseudo-first-order kinetics as done previously (Prudel and Davidkova, 1981; Tsoubeli and Labuza, 1991; Bell and Labuza, 1991a,b).

Additional Experiments. The rate of aspartame loss was also evaluated in dilute potassium hydroxide at pH 8.30, 9.17, and 9.63 as well as in dilute hydrochloric acid at pH 0.77, 1.12, and 2.41. In addition, aspartame degradation was studied in 0.1 M oxalate, malate, and succinate buffers at pH 3 and 7. These additional experiments were carried out at 25 °C.

RESULTS AND DISCUSSION

Figure 1 shows a typical pseudo-first-order kinetic plot of aspartame degradation as impacted by buffer type and concentration at pH 7 and 25 °C. The linear fit of this kinetic model was quite acceptable with correlation coefficients of greater than 0.97 in all cases. From such plots, the pseudo-first-order rate constants (i.e., the observed rate constant) for aspartame degradation with 95% confidence limits were calculated; these are presented in Table 1.

The degradation rate at pH 7 was significantly faster than at pH 3 as has been shown previously (Prudel and Davidkova, 1981; Homler, 1984; Prudel et al., 1986; Ozol, 1986; Bell and Labuza, 1991a,b). The pH dependence of the observed rate constant is indicative of an acid–base-catalyzed reaction and is indicated by the presence of hydronium and hydroxyl ion concentrations in eq 1. For the acid–base-catalyzed degradation of aspartame, the observed rate constant, k_{obs}, can be expressed in general terms as

$$k_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_{BH}[BH] + k_B[B^-] \quad (1)$$

where k_0 is the rate constant for the uncatalyzed reaction, k_H is the rate constant for hydronium-mediated catalysis, k_{OH} is the rate constant for hydroxyl-mediated catalysis, k_{BH} is the rate constant for buffer-mediated acid catalysis, and k_B is the rate constant for buffer-mediated base catalysis; $[H^+]$, $[OH^-]$, [BH], and $[B^-]$ represent the concentrations of the hydronium ions, hydroxyl ions, protonated buffer, and unprotonated buffer, respectively (Alberty, 1987). Each of the individual rate constants (k_0, k_H, k_{OH}, k_{BH}, k_B) can be subdivided further into specific components for each degradation pathway of aspartame depending upon pH. For example, at pH values below 5.2, k_H has four components representing the conversion of aspartame into diketopiperazine, α-aspartylphenylalanine, phenylalanine methyl ester, and β-aspartame. It has been hypothesized that eq 1 can be simplified into

$$k_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] \quad (2)$$

assuming the catalysis of aspartame is not significantly influenced by the buffer components (Prudel et al., 1986; Bell and Labuza, 1991b; Tsoubeli and Labuza, 1991).

As shown in Table 1, the influence of buffer concentration on the rate of aspartame degradation is not negligible. In both citrate and phosphate buffers, reaction rates increased as buffer concentration increased. At pH 7, the degradation rate increased significantly ($P < 0.05$) with increasing buffer concentration. However, in citrate buffer at pH 3, the influence of buffer concentration was less dramatic. The effect of buffer concentration on aspartame degradation is indicative of a reaction susceptible to general acid–base catalysis. This result is consistent with that found by Tsoubeli and Labuza (1991), except their results were determined at higher temperatures and over a more narrow concentration range.

Thus, eq 2 is valid only under conditions of zero buffer concentration. The observed rate constants from the experimental data were extrapolated to zero buffer concentration. At pH 7, the extrapolated rate constants were 3×10^{-5} and 8×10^{-5} min^{-1} from the phosphate and citrate data, respectively, while at pH 3, the extrapolated rate constants were 5×10^{-3} and 4×10^{-3} $days^{-1}$. As seen, there is reasonably good agreement between the extrapolated rate constants from each buffer type at a given pH.

To verify the accuracy of the extrapolated rate constants, experiments were performed in dilute hydrochloric acid (pH 0.77, 1.12, and 2.41) and dilute potassium hydroxide (pH 8.30, 9.17, and 9.63). At low pH values (in dilute hydrochloric acid), the concentration of hydroxide ions is very small and eq 2 can be simplified into

$$k_{obs} = k_0 + k_H[H^+] \quad (3)$$

while at high pH values (in dilute potassium hydroxide solutions), the concentration of hydronium ions is very small, allowing for eq 2 to be expressed as

$$k_{obs} = k_0 + k_{OH}[OH^-] \quad (4)$$

From this set of experiments and eq 3 and 4, k_H and k_{OH} were determined from plots of k_{obs} versus $[H^+]$ and $[OH^-]$ (Figures 2 and 3). The value for k_H was determined to be 1.46×10^{-3} M^{-1} min^{-1}, while k_{OH} was 79.6 M^{-1} min^{-1}. Due to one intercept being positive and the other negative, the value for k_0 could not be determined with confidence. However, on the basis of the k_{obs} at pH 2.41 being 5.8×10^{-6} min^{-1} and the $k_H[H^+]$ being $5.7 \times$

Table 1. Aspartame Degradation Rate Constants and 95% Confidence Limits at 25 °C As Influenced by Buffer Type, Concentration, and pH

	pH 7		pH 3	
concn (M)	phosphate (h^{-1})	citrate (h^{-1})	phosphate ($days^{-1}$)	citrate ($days^{-1}$)
0.01	0.0142 ± 0.0003	0.0054 ± 0.0002	ND[a]	ND
0.02	0.0255 ± 0.0006	0.0090 ± 0.0003	0.0050 ± 0.0007	0.0046 ± 0.0007
0.05	0.0555 ± 0.0030	0.0123 ± 0.0009	0.0065 ± 0.0007	0.0055 ± 0.0006
0.1	0.1167 ± 0.0069	0.0163 ± 0.0006	0.0089 ± 0.0011	0.0063 ± 0.0010
0.2	0.2111 ± 0.0028	0.0202 ± 0.0004	0.0124 ± 0.0009	0.0093 ± 0.0012
0.5	0.4950 ± 0.0087	0.0270 ± 0.0006	0.0192 ± 0.0017	0.0151 ± 0.0015
1.0	0.7309 ± 0.0168	0.0278 ± 0.0013	ND	ND

[a] ND, not determined.

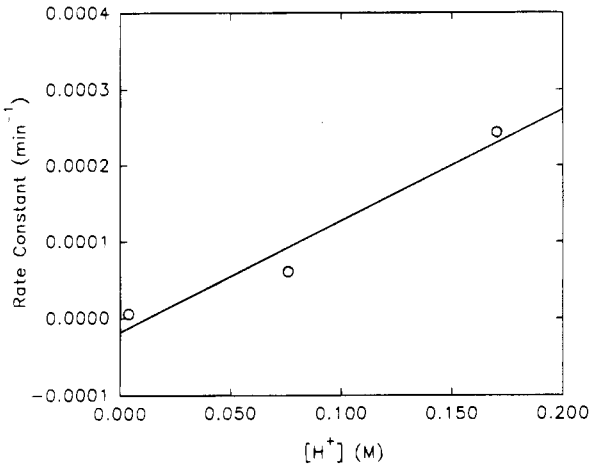

Figure 2. Aspartame degradation rate constants in HCl at 25 °C as a function of hydrogen ion concentration.

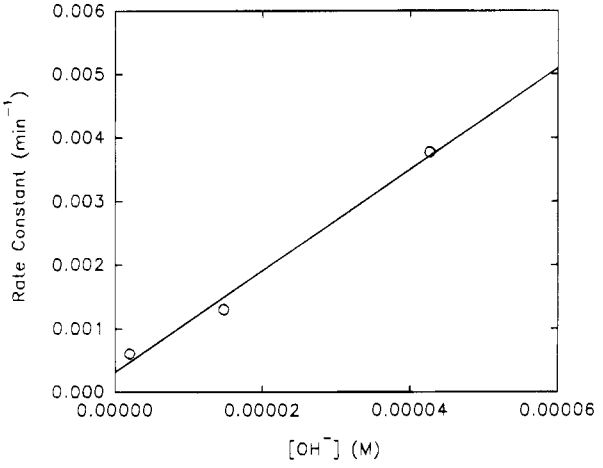

Figure 3. Aspartame degradation rate constants in KOH at 25 °C as a function of hydroxyl ion concentration.

10^{-6} min^{-1}, k_0 was estimated to be 1×10^{-7} min^{-1}. The value for the uncatalyzed degradation of aspartame is likely to be even less than that estimated above. Using these values and eq 2, the observed rate constant for aspartame degradation at any pH in the absence of buffer can be calculated. The calculated first-order rate constant at pH 7 was 0.8×10^{-5} min^{-1}, which is not too different from the extrapolated values of 3×10^{-5} and 8×10^{-5} min^{-1}. Similarly, at pH 3, the calculated first-order rate constant was 2.2×10^{-3} $days^{-1}$ which also compares favorably with the extrapolated values of 5×10^{-3} and 4×10^{-3} $days^{-1}$. At pH 7 and 0.1 M buffer concentration, catalysis by hydronium and hydroxyl ions is responsible for only 1.5% of the degradation rate constant in phosphate buffer (3×10^{-5} of 1.9×10^{-3} min^{-1}) and 11% of the rate constant in citrate buffer (3×10^{-5} of 2.7×10^{-4} min^{-1}). Thus, the buffer components are the primary culprit that enhances the degradation of aspartame. As mentioned by Tsoubeli and Labuza (1991), increased buffer concentration aids in the proton transfer required for many of the degradation pathways.

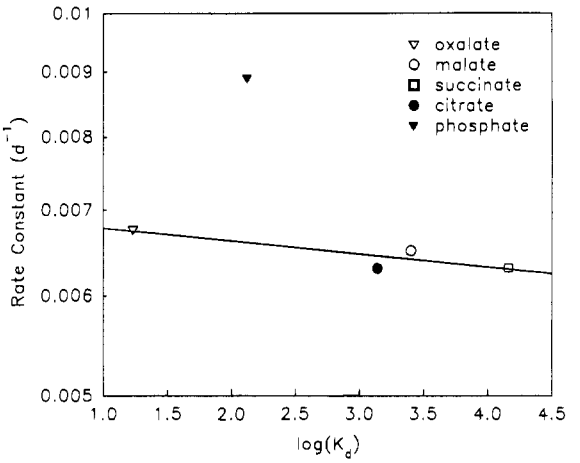

Figure 4. Log–log plot of aspartame degradation rate constants as a function of the buffer ionization constant in 0.1 M buffer at pH 3 and 25 °C.

However, the above discussion does not explain the significant difference between the aspartame degradation rates in citrate and phosphate buffers as shown in Table 1. Aspartame degradation in phosphate buffer occurred at a significantly higher rate than in citrate buffer, as shown by the 95% confidence limits in Table 1. The differences between the rates occurring in the two buffer solutions at pH 3 were not as dramatic as at pH 7.

Because the degradation of aspartame involves the transfer of protons, the strength of the acid or base (i.e., the differing ability of the buffers to accept and donate protons to the aspartame molecule) may explain the differences in reaction rates. The relation between the rate constant of an acid–base-catalyzed reaction and the strength of the buffer components is known as the Brønsted equation and is shown below, where C and z are constants and K_d is the ionization constant of the buffer salt (Alberty, 1987).

$$k_{obs} = C(K_d)^z \tag{5}$$

Taking the log of eq 5, one arrives at the working form of the Brønsted equation:

$$\log(k_{obs}) = \log(C) + (z)\log(K_d) \tag{6}$$

Figures 4 and 5 plot the rate constant (on a logarithmic scale) versus the log of the ionization constant of the buffer salt for aspartame degradation occurring in various 0.1 M buffer solutions at pH 3 and 7, respec-

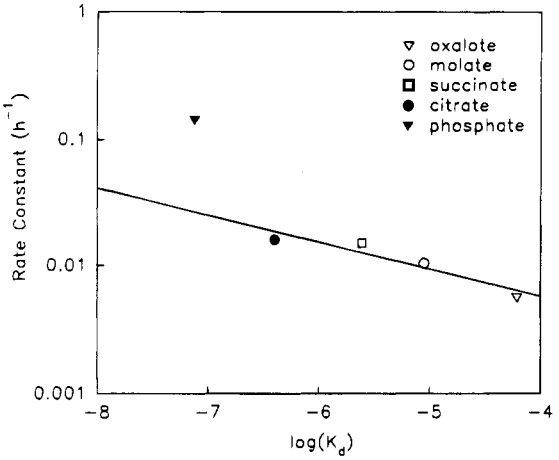

Figure 5. Log–log plot of aspartame degradation rate constants as a function of the buffer ionization constant in 0.1 M buffer at pH 7 and 25 °C.

tively. In both figures, the rate constant for degradation in phosphate buffer is at a value above the regressed line generated from the rate constants of degradation in the other buffer solutions, suggesting that the ionization constants of the buffer salts and thus their ability to accept and donate protons are not the predominant reason aspartame degradation rates differ in phosphate and citrate buffers.

Phosphate buffer ions can act as bifunctional catalysts because the ionic structure has both proton donating and accepting groups in close proximity (Jencks, 1969). The large destabilizing effect of phosphate buffer at pH 7 could therefore be attributed to the buffer acting as a bifunctional catalyst. At pH 7, a large portion of the aspartame molecules have the amine group in the unprotonated state, exposing the free pair of electrons and allowing the amine to act as a nucleophile. This nucleophilic amine attacks the carbonyl group of phenylalanine, forming a cyclic product. To complete this cyclization process, a proton must be transferred from the amine to the methyl ester oxygen. The phosphate group facilitates this transfer of protons by acting as a bifunctional catalyst, simultaneously accepting and donating a proton to the aspartame molecule. This results in the rapid release of methanol as the leaving group and the rapid formation of the diketopiperazine. The bifunctional catalytic activity of phosphate with respect to aspartame is depicted in Figure 6.

Slower aspartame degradation rates occur in citrate buffer because the citrate ions do not have the proton donating/accepting groups in the proper alignment necessary for it to act as a bifunctional catalyst. Citrate does donate and accept protons with aspartame in solution to yield reaction rates faster than those in solutions without buffer at the same pH, but the proton transfer occurs much less rapidly than the bifunctional catalytic activity of phosphate buffer.

At pH 3, the free amine of aspartame exists primarily in its protonated state. The nucleophilic ability of the free amine is thus reduced, inhibiting its interaction with the carbonyl group to rapidly form diketopiperazine. However, a small amount of aspartame exists in equilibrium as the unprotonated amine, which is susceptible to the bifunctional catalytic activity of phosphate buffer ions as mentioned previously. This results in faster degradation rates in phosphate buffer as compared to citrate buffer; however, the extent of the difference between the buffer types is much less than at pH 7.

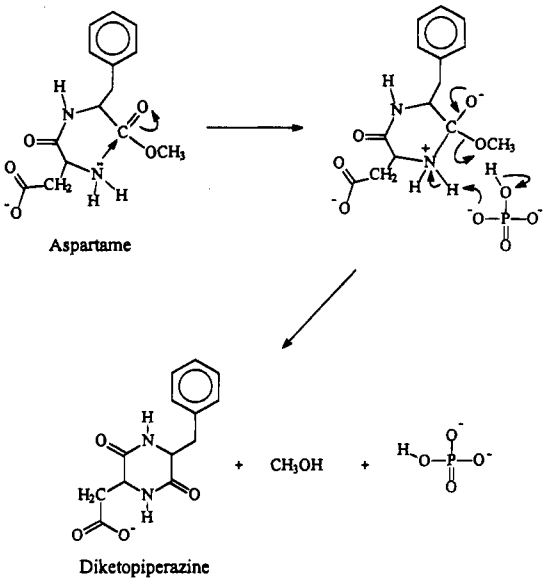

Figure 6. Bifunctional catalytic activity of phosphate buffer on aspartame degradation at pH 7.

Table 2. Percent Increase in Aspartame Degradation Reaction Rates from Smaller to Larger Buffer Concentrations

	pH 7		pH 3	
concn (M)	phosphate	citrate	phosphate	citrate
0.01–0.1	820	300	ND[a]	ND
0.02–0.2	830	230	250	200
0.05–0.5	890	220	300	270
0.1–1.0	630	170	ND	ND

[a] ND, not determined

The combination of high pH (unprotonated amine) and phosphate buffer (bifunctional catalysis) yields a system in which reaction rates increase dramatically with increasing buffer concentration as shown in Table 2. The approximately 800% (8-fold) increase in reaction rate at pH 7 per 10-fold increase in phosphate buffer concentration is similar to that found by Tsoubeli and Labuza (1991). All other systems had only a 200–300% increase in reaction rate per 10-fold increase in buffer concentration (Table 2).

TECHNICAL SUMMARY

Buffering components found in food products can participate differently in chemical reactions depending upon their structural characteristics. The rate of aspartame degradation was faster in phosphate buffer than in citrate buffer at the same pH and buffer concentration. The primary mechanism by which aspartame degrades, the formation of diketopiperazine, involves the nucleophilic attack of the carbonyl by the free amine, which requires proton transfer. The differences in the ionization constants of the buffer components may partially explain differences in reaction rates occurring in different buffer solutions but does not account for the magnitude of the rate differences observed. Phosphate buffer salts, being small with proton donating and accepting groups in close proximity, can simultaneously donate and accept protons, thus enhancing the rate of acid–base-catalyzed reactions (i.e., bifunctional catalysis). The larger structure of citrate buffer salts does not allow for bifunctional catalytic activity, so the catalytic effect due to the donation and acceptance of protons from citrate is

reduced as compared to that of phosphate buffer salts. Higher concentrations of either phosphate or citrate, due to the presence of more proton donating/accepting groups, also promoted faster acid–base catalysis of aspartame.

APPLICATIONS

The results discussed above indicate that buffer type is an important consideration in the formulation of aspartame-sweetened beverages. Diet beverages at high pH values would display increased shelf lives by incorporating citrate buffer as opposed to phosphate buffer. For diet beverages at acidic pH values, citrate buffer would only slightly increase the shelf life and delay accompanying loss of sweetness. The selection of buffering systems would also be critical in foods that contain other ingredients susceptible to acid–base catalysis.

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Received for review April 5, 1995. Revised manuscript received July 12, 1995. Accepted July 31, 1995.[⊗] This research was conducted under project ALA-13-005 of the Alabama Agricultural Experiment Station and is published as AAES Journal No. 10-954994.

JF9502010

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1995.