Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions

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ABSTRACT: Thiamin is being incorporated into a variety of nutritionally enhanced beverages. Although thiamin degradation during thermal processing has been extensively studied, minimal data exist regarding its stability in solution during ambient storage. This study collected kinetic data for thiamin degradation in solutions as a function of pH, buffer type, and buffer concentration at 25 °C. Thiamin was incorporated into sodium phosphate and citrate buffer solutions, each prepared at 0.02 and 0.1 *M* and pH 4, 5, 6, and 7. Experimental solutions were stored in an incubator at 25 °C. Thiamin concentrations were analyzed at 9 time points over a 42-wk period. Thiamin degradation data were modeled using pseudo-1st-order kinetics. Thiamin stability generally decreased as pH and buffer concentration increased in both phosphate and citrate buffers. In 0.1 *M* phosphate buffer, the time for a 10% loss of thiamin decreased from 79 wk at pH 4 to 3 wk at pH 7. At pH 6 and 7, thiamin stability was greater in citrate buffer than in phosphate buffer. However, at pH 4 and 5, thiamin stability was greater in phosphate buffer than citrate buffer. To optimize thiamin stability in low pH beverages, phosphate buffer would be more appropriate to use than citrate buffer, whereas citrate buffer should be used for better thiamin stability in high pH beverages.

Keywords: vitamin, thiamin, buffer, pH, stability, kinetics

Introduction

Nutraceuticals and fortified food products are becoming staples in the diets of consumers. These products may contain extra vitamins, minerals, or other biologically active substances. One vitamin that has been and continues to be added to various food products is thiamin. This vitamin is incorporated as an additional ingredient into breakfast cereals, breakfast bars, meal replacement bars, and other grain products. Some beverages also contain thiamin, including meal replacement beverages, fortified sports drinks, fruit-flavored beverages, and yogurt drinks. Because thiamin is added to many foods and is quite labile, understanding factors that affect its loss is important for optimizing its stability and improving shelf life prediction.

The structure of thiamin consists of substituted pyrimidine and thiazole rings linked by a methylene bridge. Under acidic conditions, the thiamin free base becomes protonated, which is susceptible to hydrolytic cleavage to yield 2-methyl-4-amino-5-hydroxymethyl pyrimidine and 4-methyl-5β-hydroxyethyl thiazole (Windheuser and Higuchi 1962). This reaction predominates at pH values ≤ 6. In strongly acidic solutions, thiamin degrades to form oxythiamin by the replacement of the primary amino group on the pyrimidine ring by a hydroxyl group. Under alkaline conditions, thiamin is converted into the neutral pseudobase, which is further converted into the thiol form and then into a variety of sulfur-containing lower-molecular-weight compounds (Dwivedi and Arnold 1972). This reaction predominates at pH values > 7. These reactions leading to the degradation of thiamin can occur both during thermal processing and product storage.

Many researchers have studied the stability of thiamin in various foods and model systems at high temperatures (60 °C to 149 °C) in an attempt to minimize losses during thermal processing (Feliciotti and Esselen 1957; Windheuser and Higuchi 1962; Dwivedi and Arnold 1972; Mulley and others 1975a, 1975b; Mauri and others 1992; Steet and Tong 1994; Ariahu and Ogunsua 1997; Kwok and others 1998). Some of these studies also evaluated the effects of pH on thiamin stability in food products and buffer systems. In buffer solutions stored at 96 °C to 149 °C, an increase in pH accelerated the rate of thiamin loss (Farrer 1945; Windheuser and Higuchi 1962; Dwivedi and Arnold 1972; Mulley and others 1975b). Windheuser and Higuchi (1962) also showed that thiamin loss at 96.4 °C was greater in phosphate buffer than citrate buffer, and the losses increased with increasing buffer concentration. Unfortunately, it is virtually impossible to use stability data from solutions held at 96 °C to 149 °C to predict thiamin stability in beverage formulations held at typical storage conditions (20 °C to 30 °C).

Although ample data exist regarding thermal stability of thiamin at elevated temperatures, only a limited number of studies have evaluated the storage stability of thiamin in solution at ambient temperatures (20 °C to 30 °C). Thiamin degradation in parenteral nutrition (PN) solutions was enhanced by sulfites and multivitamins (Bowman and Nguyen 1983). Chen and others (1983) showed that storage of PN solutions in direct sunlight enhanced the degradation of thiamin. Baumgartner and others (1997) reported thiamin half-lives in PN solutions at 25 °C of 21 to 47 d, depending upon the solution composition; multivitamins appeared to lower the stability of thiamin compared with a PN solution without multivitamins. A PN salt solution (that is, without amino acids or carbohydrates) at pH 6.2 and 25 °C showed no measurable thiamin loss after 12.5 d (Baumgartner and others 1997).

The limited data from PN solutions provide insufficient information on which to base the development of thiamin-containing beverages. Various quantitative kinetic data are required to develop shelf-stable beverages containing thiamin, enable accurate shelf life predictions, and improve the accuracy of nutritional labeling. This type of thiamin stability data are lacking in the literature.

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The objective of this project was to collect quantitative kinetic data for thiamin degradation in buffer solutions at 25 °C that would be beneficial for the development and shelf life prediction of thiamin-fortified beverages, including evaluating the effects of buffer type, buffer concentration, and pH.

Materials and Methods

Sample preparation

Four bulk sodium phosphate buffer solutions were prepared in deionized water: 0.02 *M* and 0.1 *M* sodium phosphate monobasic monohydrate and 0.02 *M* and 0.1 *M* sodium phosphate dibasic anhydrous. Similarly, 4 bulk citrate buffer solutions were prepared: 0.02 *M* and 0.1 *M* sodium citrate tribasic dihydrate and 0.02 *M* and 0.1 *M* citric acid anhydrous. All buffering agents were obtained from Fisher Scientific (Fair Lawn, N.J., U.S.A.). Thiamin hydrochloride (Sigma, St. Louis, Mo., U.S.A.) was added to each of these 8 solutions at a concentration of approximately 20 mg/100 mL (0.59 m*M*). Although this concentration is much higher than the reference daily intake of 1.5 mg, the higher concentration facilitated thiamin analysis while reducing analytical errors. Using a higher thiamin concentration does not affect the kinetic analysis because thiamin degradation rate constants have been shown to be independent of the initial thiamin concentration (Windheuser and Higuchi 1962; Ryan-Stoneham and others 1996).

The 0.02 *M* sodium phosphate monobasic and dibasic solutions containing thiamin were mixed in various proportions to give approximately 200 mL of buffer solutions at pH 5, 6, and 7. This protocol was repeated for the 0.1 *M* phosphate buffer. To obtain a phosphate buffer solution at pH 4, 1 *M* phosphoric acid was used. This acid was required because the lowest pH achievable using the phosphate monobasic and dibasic is 4.4 (that is, 100% monobasic). The amount of acid required was 20 μL and 70 μL per 100 mL of 0.02 and 0.1 *M* phosphate monobasic, respectively. This low amount of acid changed the buffer concentration by less than 1%, which can be considered negligible. Thus, the phosphate buffer at pH 4 consisted predominately of phosphate monobasic with an insignificant amount of phosphoric acid and no phosphate dibasic. The 0.02 *M* and 0.1 *M* citrate buffer solutions (citric acid and sodium citrate) containing thiamin were mixed to yield 0.02 *M* and 0.1 *M* citrate buffers at pH 4, 5, 6, and 7. The solution pH was monitored during mixing using a pH meter with a combination electrode. The pH values of the thiamin-containing buffer solutions were to within ± 0.02 of the target value. Sixteen buffer solutions containing thiamin resulted, consisting of 2 buffer types, 2 concentrations and 4 pH values.

Because thiamin levels could be reduced by microbial growth, great care was taken to prevent sample contamination by microorganisms. Certified contaminant-free amber vials with septum-containing screw caps were used for this study (I-Chem, Chase Scientific Glass, Rockwood, Tenn., U.S.A.). An aliquot of the sample solution (5 to 10 mL) was placed in a sterile syringe, with a sterile 0.2 μm nylon filter and a sterile needle. The 5- to 10-mL aliquot was injected through the septum into the amber vial. The bottle was shaken, and the aliquot was removed using the sterile syringe and needle without the filter and discarded. Rinsing was repeated 2 additional times with fresh aliquots. At no time were the bottles opened and exposed to the external environment. The process of rinsing was done to guarantee the sterility of the bottle. Approximately 40 mL of each sample solution were then transferred into triplicate rinsed bottles using a sterilized needle, syringe, and filter.

Kinetic study

The 48 amber bottles (16 experimental solutions in triplicate) were placed in an unlit 25 °C incubator. The solutions were removed for analysis 9 times over 42 wk. For thiamin degradation in phosphate buffer, analysis occurred at 0, 3, 6, 12, 16, 20, 24, 28, and 42 d. For the citrate buffer systems, thiamin analysis occurred at 0, 3, 6, 13, 18, 24, 29, 34, and 42 d. A 2.5- to 3-mL aliquot from each bottle was removed using a sterile needle and syringe and transferred into sterile 5-mL cryogenic vials. The bottles containing the remaining experimental solutions were returned to the 25 °C incubator while the cryogenic vials were stored in the refrigerator to prevent further degradation of thiamin.

Thiamin analysis

The concentration of thiamin in the solutions was analyzed using a high-performance liquid chromatographic methodology adapted from Kamman and others (1980) and Bell and White (2000). Two C-18 columns were used in series. The 1st column was a 3.9 mm × 150 mm Waters Nova-Pak C_{18} (Millipore, Milford, Mass., U.S.A.), and the 2nd was a 4.6 mm × 150 mm Prodigy 5 ODS (3) column (Phenomenex, Torrance, Calif., U.S.A.). The mobile phase consisted of 82/18 w/w 8.6 mmol phosphate buffer/acetonitrile at pH 3. Sodium heptanesulfonate (Fisher) was added as an ion-pairing agent at a concentration of 9 mmol. The flow rate was set at 1 mL/min. Thiamin was detected at 254 nm. Thiamin eluted between 7.5 and 8 min.

Data analysis

The concentrations of thiamin at each time point were determined by a standard curve generated from freshly prepared and analyzed thiamin standard solutions. The degradation of thiamin was modeled using pseudo-1st-order kinetics, as reported previously (Farrer 1945; Mulley and others 1975a, 1975b; Kamman and others 1981; Arabshahi and Lund 1988; Steet and Tong 1994; Ryan-Stoneham and others 1996; Kwok and others 1998). The rate constants with their 95% confidence limits were determined using computerized least-squares analysis (Labuza and Kamman 1982). The regression analysis used all 27 data points for each experimental solution (that is, 9 time points in triplicate).

Results and Discussion

Kinetic data for the loss of thiamin as a function of time were collected under various conditions of pH, buffer type, and buffer concentration. As shown by the linearity of the kinetic plots in Figure 1, thiamin degradation could be modeled using pseudo-1st-order kinetics. From the slopes of the kinetic plots, the pseudo-1st-order rate constants, k_{obs}, for thiamin degradation were determined (Table 1). The thiamin degradation rate constants ranged from 3.09×10^{-4} to 3.6×10^{-1}. Baumgartner and others (1997) found that thiamin losses were insignificant in a parenteral nutrition salt solution at 25 °C and pH 6.2 after 12.5 d. In the current study, 0.2% and 0.5% of the thiamin were lost in 0.02 *M* and 0.1 *M* phosphate buffer, respectively, at 25 °C and pH 6 after 12.5 d, as calculated using the rate constants in Table 1. These losses can be considered negligible as well. The results of the previously mentioned study and the current study are therefore consistent.

As shown in Table 1, thiamin degradation rate constants generally increased as pH increased. The pattern of reactivity as a function of pH at ambient temperature storage conditions (that is, the current data) is similar to that reported by past studies conducted at the higher temperatures associated with processing. Windheuser and Higuchi (1962) studied the effects of pH on thiamin degradation in various buffers at 96.4 °C. The rate constants increased in all the buffer solutions as pH was increased from 2 to 7.5. Similarly, the extent of thiamin degradation in phosphate buffer at 120 °C to 130 °C increased as pH increased (Dwivedi and Arnold 1972;

Table 1—Rate constants (k_{obs}) with 95% confidence limits for thiamin degradation in phosphate and citrate buffers at 25 °C

Buffer type	pH	k_{obs} ± 95% CL (wk^{-1}) 0.02 *M* buffer	0.1 *M* buffer	Δk_{obs} [a]
Phosphate	4	0.000309 ± 0.00042	0.00134 ± 0.00066	0.0010
	5	0.000670 ± 0.00046	0.00373 ± 0.00093	0.0031
	6	0.00395 ± 0.00064	0.0110 ± 0.0007	0.0070
	7	0.0147 ± 0.0021	0.0360 ± 0.0012	0.0213
Citrate	4	0.00113 ± 0.00035	0.00408 ± 0.00039	0.0030
	5	0.00262 ± 0.00036	0.00695 ± 0.00051	0.0043
	6	0.00297 ± 0.00047	0.00607 ± 0.00051	0.0031
	7	0.00383 ± 0.00043	0.00778 ± 0.00046	0.0040

[a] Δk_{obs} = difference between rate constants at 0.1 *M* and 0.02 *M*.

Mulley and others 1975b). The data reported previously at elevated temperatures and the current data collected at ambient temperatures both demonstrate the rate constants for thiamin degradation are affected in part by pH, which could be attributed to the different degradation mechanisms at different pH values.

Another way to evaluate the effect of pH on reaction kinetics is by plotting the log of the rate constant as a function of pH. A slope of unity means that for each 1-unit increase in pH, the rate constant increases by a factor of 10. The implication is that hydroxyl ions are directly impacting the reaction by acid-base catalysis. The data for thiamin degradation in buffer solutions were plotted in the form of a log (k_{obs}) versus pH graph (Figure 2). As shown in this figure, the slopes of the plots were 0.58 and 0.48 for thiamin degradation in 0.02 *M* and 0.1 *M* phosphate buffer, respectively, which suggest that the effects of pH on the reaction are less dependent upon the hydroxyl ion than for an ideal acid-base catalyzed reaction, as described previously. Thiamin degradation in 0.02 *M* and 0.1 *M* citrate buffer yielded much lower slopes (0.16 and 0.078, respectively), again suggesting pH is not the major factor affecting the degradation rate constants. Plotting the data from Windheuser and Higuchi (1962) collected at high temperatures, similar results were found with larger slopes for the reaction in phosphate buffer than citrate buffer. Thus, thiamin degradation is more sensitive to pH changes in the presence of phosphate than citrate buffer. In addition to the difference in slopes observed by the current study (Figure 2), r^2 values were also lower for thiamin degradation in citrate buffer than phosphate buffer, suggesting the relationship between k_{obs} and pH is weaker in citrate buffer than phosphate buffer. The difference in slopes and r^2 values between the 2 buffer types indicates that buffer type, rather than simply pH, is another important variable affecting thiamin degradation.

A specific component of phosphate buffer present at higher pH values appears to be that primarily responsible for promoting thiamin degradation. As the pH of phosphate buffer increases, more of the phosphate dibasic anion (HPO_4^{-2}) exists. During the degradation of thiamin at high pH levels, thiamin pseudobase loses a proton to produce the thiol form of thiamin; HPO_4^{-2} appears especially effective at removing this proton. The production of the thiol form is then followed by cleavage into a variety of low-molecular-weight sulfur-containing compounds. Windheuser and Higuchi (1962) noted that both HPO_4^{-2} and PO_4^{-3} were effective catalysts of thiamin degradation at elevated temperatures. Based on buffer pK_a values, the phosphate dibasic anion is a stronger base than the citrate anion and can better facilitate the required proton removal from thiamin pseudobase. Thus, the increasing quantities of highly catalytic HPO_4^{-2} that result as pH increases may explain thiamin's greater pH sensitivity in phosphate buffer than citrate buffer.

Data in Table 1 also show that the rate constants for thiamin degradation in phosphate and citrate buffers were higher at 0.1 *M* than in 0.02 *M*. An increase in buffer concentration enhances the proton transfers required for the thiamin degradation pathways, regardless of the buffer type. In phosphate buffer, a higher buffer concentration translates into an increased concentration of the highly catalytic HPO_4^{-2}. The differences between the rate constants for thiamin degradation in 0.02 *M* and 0.1 *M* buffer (Δk_{obs}) are tabulated in Table 1. In phosphate buffer, Δk_{obs} increased as pH (and thus $[HPO_4^{-2}]$) increased. However, the Δk_{obs} values in citrate buffer gave a more parallel pattern, with k_{obs} in 0.1 *M* citrate being great-

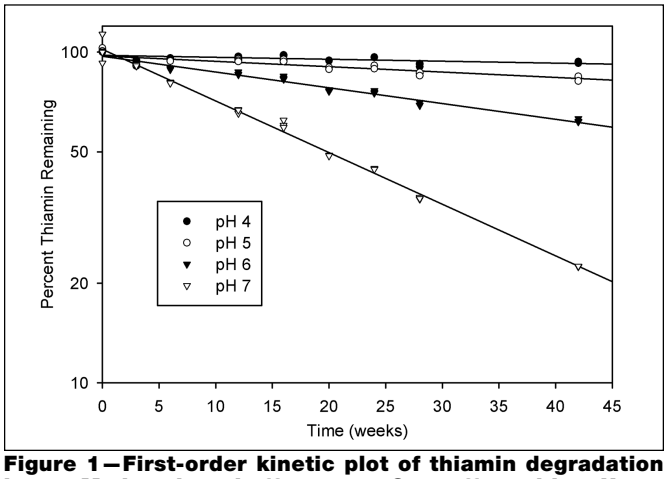

Figure 1—First-order kinetic plot of thiamin degradation in 0.1 *M* phosphate buffer at 25 °C as affected by pH.

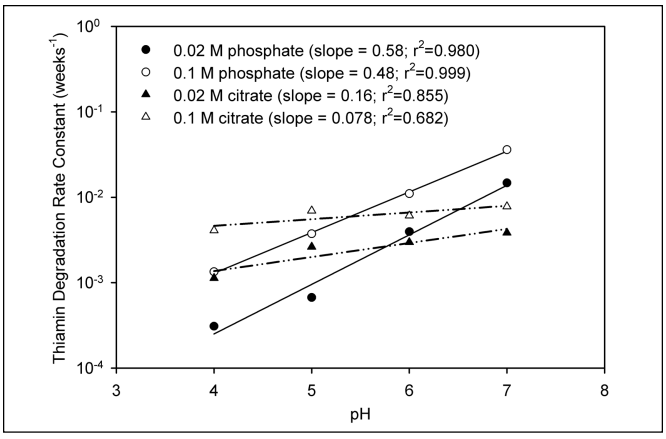

Figure 2—Logarithmic plot of the rate constants for thiamin degradation in buffer solutions at 25 °C as a function of pH as affected by buffer type and concentration.

er than k_{obs} in 0.02 *M* citrate by 0.003 to 0.004 wk^{-1}, regardless of the pH. This parallel pattern of the rate constants in 0.02 *M* and 0.1 *M* citrate buffer would indicate less catalytic ability of the citrate anions compared with the phosphate dibasic anion. As mentioned previously, the phosphate dibasic anion has a higher basicity than the citrate anion and can better facilitate proton removal at the higher pH levels. Thus, as the concentration of phosphate dibasic increased, the rate constants for thiamin degradation dramatically increased. This increasing reactivity with increasing concentration of the "basic" buffer species is an indicator of general base catalysis (Windheuser and Higuchi 1962).

The previous discussion demonstrates the complexity of the relation between pH, buffer type, and buffer concentration as factors affecting reaction kinetics. Thiamin degradation pathways depend on pH, but so too does the concentration of various buffer species that may be catalyzing the reaction. The buffer concentration affects the concentration of various individual buffer species at a given pH as well. This interrelation between the effects of pH, buffer type, and buffer concentration on reactivity can only be clarified by studies such as that currently presented.

The effects of buffer type and concentration on thiamin degradation are very similar to the buffer effects observed for other reactions. Aspartame degradation rates in phosphate buffer were significantly higher than in citrate buffer at pH 7 (Bell and Wetzel 1995). Rates were also faster at higher buffer concentrations. In a similar study by Bell (1997), the effects of buffer type (phosphate and citrate) at various concentrations on the Maillard reaction were studied at pH 7 and 25 °C. The results revealed that no glycine loss or brown pigment formation was observed in citrate buffer. However, in phosphate buffer solutions, the rates of glycine loss and browning increased with increasing phosphate buffer concentration again suggesting the effects of buffer catalysis. Although the reaction mechanisms are different, a catalytic effect of the phosphate dibasic anion on thiamin degradation remains evident.

Another way to visualize the degradation data is by comparing the times for a specified extent of loss (for example, half-lives) under the various experimental conditions. Because fortified products must maintain a high level of potency, the time for 10% thiamin loss (t_{10}) would be more appropriate to evaluate than thiamin's half-life. Based on 1st-order kinetic principles, the time for 10% loss is calculated using the equation $t_{10} = \ln(0.9)/k_{obs}$, where 0.9 represents 90% thiamin remaining (or 10% thiamin loss). Figure 3 shows the t_{10} values in solution at 25 °C as affected by pH, buffer type, and buffer concentration. A larger value indicates greater thiamin stability or less thiamin loss. The stability of thiamin was lower in citrate buffer compared with phosphate buffer at pH 4 and 5, whereas at pH 6 and 7, thiamin in phosphate buffer was less stable as compared with that in citrate buffer. The stability of thiamin was found to be least in 0.1 *M* phosphate buffer at pH 7 (t_{10} = 3 wk). Thiamin stability in both phosphate and citrate buffer decreased as the concentration increased from 0.02 *M* to 0.1 *M*. Buffer salts catalyzed the degradation as mentioned previously. The most interesting result from evaluating thiamin t_{10} values in Figure 3 is that at pH 4 and 5, stability is greatest in 0.02 *M* phosphate buffer whereas at pH 6 and 7, stability is greatest in 0.02 *M* citrate buffer. Thus, depending upon the pH of a liquid formulation, the selection of the appropriate buffer can help maximize thiamin stability.

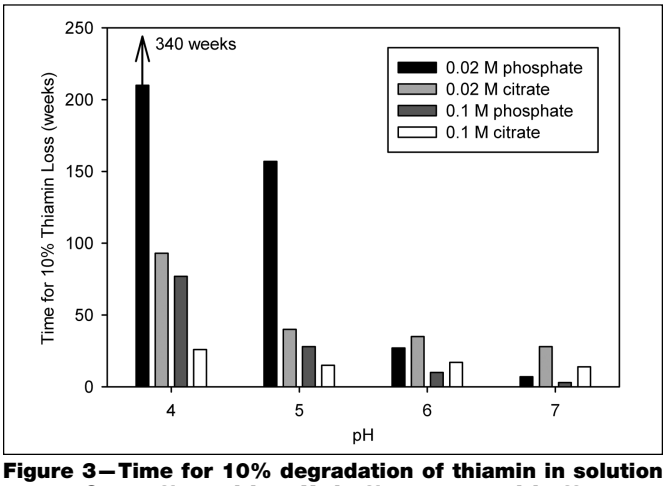

Figure 3—Time for 10% degradation of thiamin in solution at 25 °C as affected by pH, buffer type, and buffer concentration.

Conclusions

Quantitative kinetic data were collected for thiamin degradation in solutions stored under ambient conditions (25 °C). Such data have not been reported previously. As pH increased, thiamin stability decreased. Thiamin stability also decreased as the buffer salt concentration increased. In low pH solutions, stability was greater in phosphate buffer than citrate buffer. However, in higher pH solutions, thiamin stability was greater in citrate buffer than phosphate buffer. The phosphate dibasic anion appears to have a strong catalytic effect on thiamin degradation. The results from this study demonstrate how manufacturers of thiamin-fortified beverages can improve formulations to lengthen product shelf life by selecting the appropriate buffer type for the pH of their product.

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