Hydrogen Sulfide from Heat Degradation of Thiamine

Basant K. Dwivedi and Roy G. Arnold*

Hydrogen sulfide has been isolated and claimed to contribute to the natural flavor of a number of food products. Thiamine, on heat treatment, produces hydrogen sulfide in the low acid pH range. Experiments were conducted to study hydrogen sulfide production from thiamine under different heating conditions and in the presence of oxidizing and reducing compounds below pH 7. Results obtained in the study show that a small amount of hydrogen sulfide is produced from thiamine at the physiological pH of most foods. Reducing compounds appear to protect thiamine from heat degradation. Under oxidizing conditions, thiamine is degraded by a different mechanism which does not produce hydrogen sulfide. These observations indicate that thiamine is not a major source of hydrogen sulfide present in heated foods.

The heat sensitivity of thiamine in nonacid foods is well established (Booth, 1943; Lhoest *et al.*, 1958; Obermeyer and Chen, 1945; Sabri *et al.*, 1968). However, little published information is available concerning the chemical nature of the compounds produced as a result of heat degradation of thiamine. In the last few years a number of patents using thiamine as one of the ingredients for inducing or simulating meat and chicken flavors have been granted in the United States and Britain (Bidmead *et al.*, 1968; Giacino, 1968; International Flavors & Fragrances, Inc., 1969). Recently, Arnold *et al.* (1969) identified hydrogen sulfide, 2-methylfuran, 2-methylthiophene, and 2-methyl-4,5-dihydrothiophene from heated solutions of thiamine in phosphate buffer (pH 6.7). Hydrogen sulfide has been identified and claimed to influence the flavor of a number of food products such as chicken (Bouthilet, 1951; Minor *et al.*, 1965), beef (Hornstein *et al.*, 1960), fish (Tanakawa and Motohiro, 1959), and cheese (Grill *et al.*, 1966). Mecchi *et al.* (1964) demonstrated that cystine and cysteine are the principal sources of hydrogen sulfide in heated chicken muscle. The purpose of this investigation was to determine the potential significance of thiamine as a precursor of hydrogen sulfide in heated foods.

EXPERIMENTAL

Solutions containing 0.04 mg/ml (1.2×10^{-4} *M*) of USP grade thiamine hydrochloride were buffered to pH 3.5, 5.0, 5.5, 6.0, 6.5, and 7.0 with 0.1 *M* phosphate buffer. Thiamine solution buffered to pH 3.5 was used as control, since thiamine is stable to heat at this pH. Determinations of thiamine concentration and hydrogen sulfide production were performed in duplicate.

Nitrogen Purging System. The reflux trap apparatus shown in Figure 1 was used for heating the thiamine solutions in nitrogen atmosphere. Buffered thiamine solution (100 ml) was placed in a two-necked flask B. High purity nitrogen was passed through the thiamine solution at the rate of 4–5 ml per min. When all the air was replaced by nitrogen in the system, the thiamine solution was heated by immersing flask B in boiling water. Heating time was measured from the point that the thiamine solution reached 96° C, which occurred approximately 2 min after immersion in boiling water. Heating was continued under reflux throughout the run (5, 15, or 30 min). Hydrogen sulfide evolved during heating was carried into the zinc acetate solution by the nitrogen and was absorbed as zinc sulfide. After completion of the run, heating was discontinued and the zinc acetate solution was removed and kept in the dark to avoid loss of hydrogen sulfide by dissociation of zinc sulfide. Flask B, containing heated thiamine solution, was quickly removed from the system and cooled under tap water. Analyses for thiamine and hydrogen sulfide were carried out within 30 min of completion of the experiment.

Open-Air Heating System. Open-air heating was simulated by replacing nitrogen with air in the reflux trap system (Figure 1) and keeping the air inlet at about 1 cm above the thiamine solution in flask B. A control was simultaneously prepared by passing a known quantity of hydrogen sulfide into zinc acetate solution and estimating hydrogen sulfide. A correction factor for hydrogen sulfide lost by oxidation in the presence of air was calculated by subtracting the amount of hydrogen sulfide recovered from the amount used, and was applied to all samples.

Sealed Tube Heating System. To simulate closed heating systems, 10-ml quantities of thiamine solution were placed in screw cap test tubes sealed with Teflon-lined caps and heated for 5, 15, and 30 min in boiling water or, alternatively, autoclaved at 120° C. Previous work by Bills and Keenan (1967) had shown that volatile components were retained in these tubes when heated. Heating time was measured from the point that the solutions reached a temperature of 96° C in the water bath or 120° C in the autoclave. After heating, the tubes were refrigerated for 30 min to minimize loss of hydrogen sulfide during transfer. Hydrogen sulfide was estimated by directly opening the sealed tubes in zinc acetate solution. Controls were prepared using known amounts of hydrogen sulfide, as sodium sulfide, in hydrogen sulfide-free heated thiamine solutions (prepared by reflux heating pH 7 thiamine solutions for 1 hr with nitrogen purge and checking for residual hydrogen sulfide). The control samples indicated a 92% recovery of hydrogen sulfide. This figure was used in calculating a correction factor for samples heated in closed systems.

Oxidizing and Reducing Conditions. The effects of oxidizing and reducing conditions on thiamine destruction and hydrogen sulfide production were studied by heating buffered thiamine solutions to which small amounts of oxidizing or reducing compounds had been added. Potassium ferricyanide or potassium permanganate was used to produce oxidizing conditions, and cysteine was added to establish

Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68503

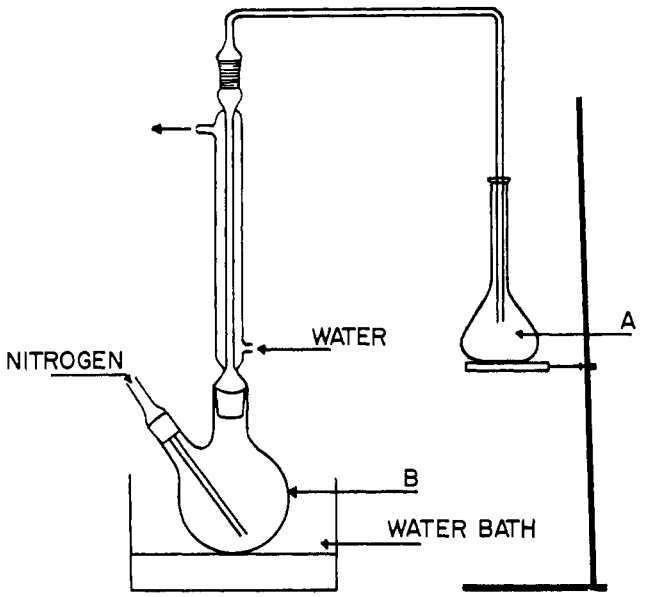

Figure 1. Reflux trap apparatus. A. Zinc acetate and sodium acetate solutions. B. Two-necked flask containing buffered thiamine solution

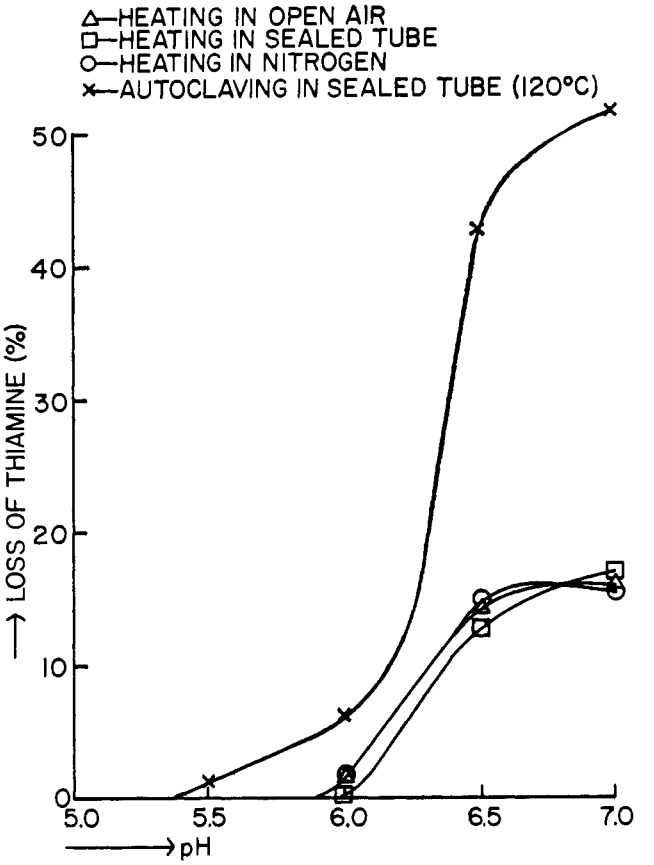

Figure 2. Heat inactivation of thiamine; effect of pH of buffered thiamine solutions on thiamine loss after 30 min of heating

reducing conditions. The concentration of the oxidizing or reducing compounds was 1 mg/ml of thiamine solution. Solutions were heated in sealed tubes.

Hydrogen Sulfide Determination. The methylene blue method of Sands *et al.* (1949), as modified by Badings and Van Der Pol (1965), was used in this study. Careful attention was given to the purity of water, cleansing of glassware, and

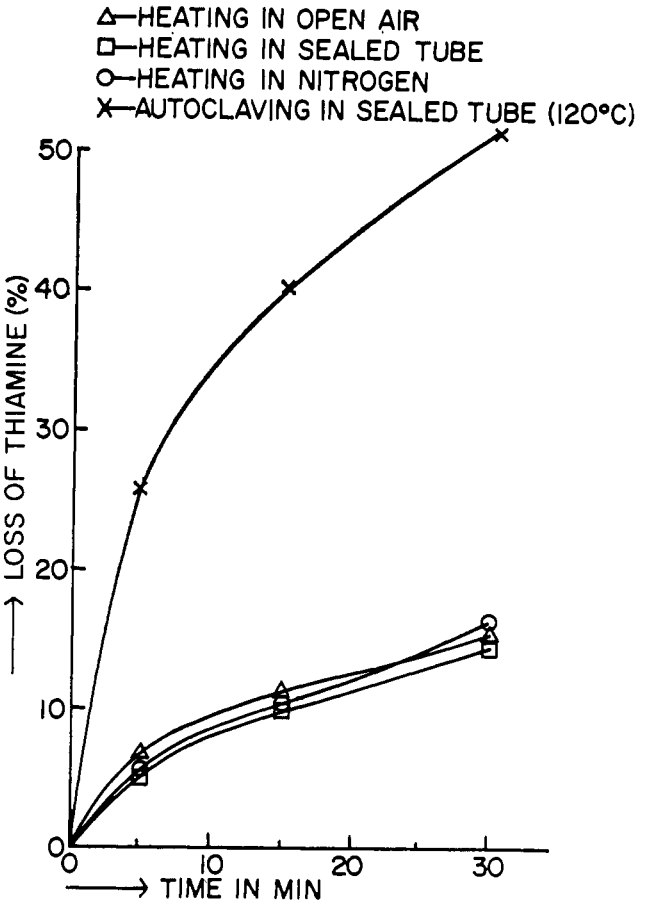

Figure 3. Heat inactivation of thiamine; relationship between time of heating of pH 7 thiamine solutions and thiamine loss

dissolution of any zinc sulfide adhering to the glass surface of the trap. Blank tests were carried out for each batch of chemical solutions. Absorbance of methylene blue color was determined on a Hitachi Perkin-Elmer Model 139 spectrophotometer at 665 nm.

Thiamine Estimations. The modified colorimetric method of Hochberg *et al.* (1945) was used in estimating thiamine losses during heating. Absorbance of the red pigment formed by the reaction of thiamine with diazotized *p*-amino acetophenone and extracted with xylene was measured at 520 nm on a Hitachi Perkin-Elmer Model 139 spectrophotometer.

RESULTS AND DISCUSSION

Loss of Thiamine during Heating. Percent thiamine destroyed during heating under varying pH and heating conditions is shown in Figure 2. Over 15% thiamine was destroyed by boiling the thiamine solutions at pH 7 for 30 min. Losses of thiamine in 30 min autoclaved samples, pH 7, were in excess of 50%. Below pH 6 losses were insignificant but increased rapidly as the pH approached 7. Figure 3 shows a nearly linear relationship between loss of thiamine and time of heat treatment at pH 7. Similar curves were obtained at pH 6 and 6.5. These findings indicate that degradation products of thiamine do not protect or induce further thiamine destruction. Other factors such as heating in open air, sealed tube, or nitrogen atmosphere were relatively insignificant (Figures 2 and 3). This is in agreement with earlier work by Williams and Spies (1938).

When thiamine solutions (pH 5–7) were heated in the presence of oxidizing agents in dilute solution, such as potassium

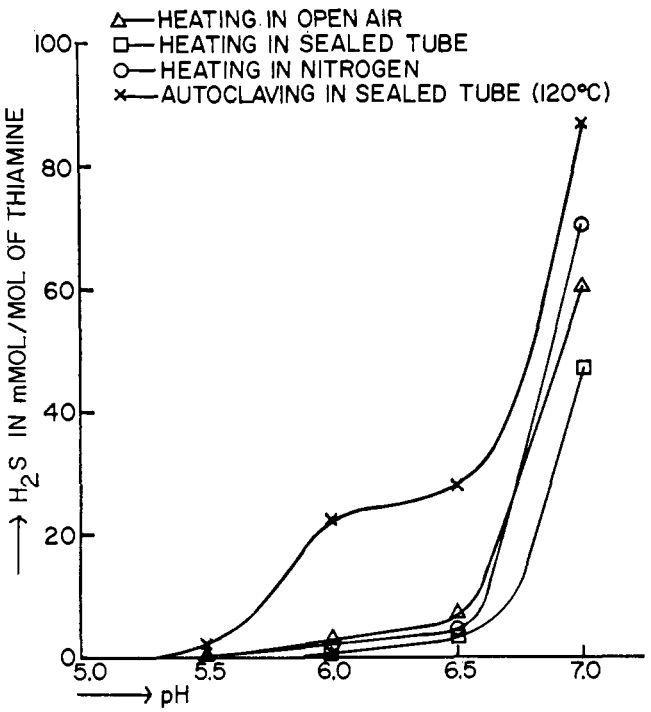

Figure 4. Effect of pH on production of hydrogen sulfide from thiamine (30 min of heating)

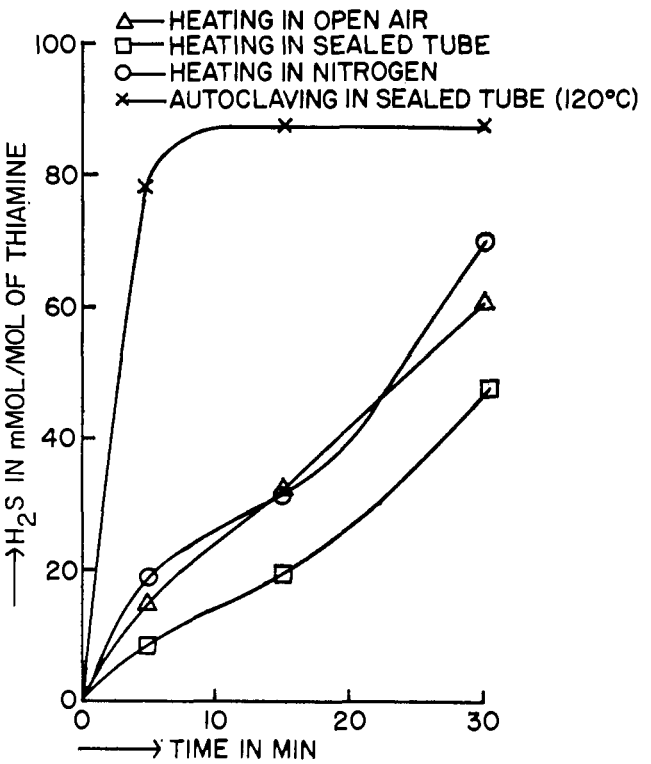

Figure 5. Relationship between time of heating of pH 7 thiamine solutions and hydrogen sulfide production

ferricyanide or potassium permanganate, thiamine was completely destroyed within 5 min of heating in boiling water. Presence of cysteine appears to protect thiamine, as no thiamine was lost when heated in boiling water for 30 min between pH 5–7 in presence of cysteine. These observations indicate that oxidizing and reducing conditions are important factors in thiamine stability in this pH range.

Hydrogen Sulfide Production. Hydrogen sulfide was noted immediately after buffered thiamine solutions were heated. Small amounts of hydrogen sulfide were produced below pH 6 (Figure 4), but hydrogen sulfide production increased rapidly as the pH was brought to 7 or above. As shown in Figure 5, hydrogen sulfide gave a nearly linear curve when plotted against time of heating in open air, sealed tube, or in nitrogen purging system, using a boiling water heating medium. When thiamine solutions were autoclaved at pH 7 hydrogen sulfide concentration increased rapidly initially and then remained static with time while thiamine destruction continued (Figures 3 and 5). At lower pH (6, 6.5), however, hydrogen sulfide production was proportional to the time of autoclaving. These observations indicate that higher concentrations of hydrogen sulfide in thiamine solutions either retard further hydrogen sulfide production on heating and promote a different mechanism for further thiamine breakdown, or react with other thiamine degradation products to produce sulfur-containing organic compounds.

In the presence of oxidizing agents (potassium ferricyanide and potassium permanganate), no hydrogen sulfide was detected by heating thiamine solutions of pH 5–7. Since thiamine was quickly destroyed under these conditions, either hydrogen sulfide is formed and instantly oxidized, or a different thiamine degradation mechanism is involved. When thiamine solutions of pH 5–7 were heated in boiling water in presence of cysteine, thiamine was protected from degradation and all hydrogen sulfide produced in the experiment was derived from cysteine.

Table I. Comparison of Maximum Theoretical Yield and Actual Yield of Hydrogen Sulfide from Heated Thiamine Solutions, pH 7

Heating system	Heating time (min)	% Thiamine destroyed	Hydrogen sulfide (μg/100 ml) Maximum theoretical yield	Hydrogen sulfide (μg/100 ml) Actual yield
Open air, boiling water	5	6.5	21.5	6.1
Open air, boiling water	10	11.5	46.9	13.1
Open air, boiling water	15	15.5	63.3	24.9
Nitrogen purge, boiling water	5	5.0	20.4	3.5
Nitrogen purge, boiling water	10	9.5	38.8	7.7
Nitrogen purge, boiling water	15	14.5	59.2	19.6

Table I compares the maximum theoretical yield, assuming that each mole of heat-degraded thiamine produces 1 mol of hydrogen sulfide, and actual yields of hydrogen sulfide obtained from heated thiamine solutions, pH 7. For the nitrogen purge and open-air heating systems, a maximum of about 40% of the sulfur theoretically available from degraded thiamine was recovered as hydrogen sulfide. With shorter heating times, less than 25% of the sulfur available from heat-degraded thiamine was recovered as hydrogen sulfide. The balance of the sulfur must therefore be present in other products of thiamine degradation, such as thiophene derivatives (Arnold *et al.*, 1969) and other unidentified compounds.

The approximate hydrogen sulfide threshold in water and chicken broth are reported to be 10 and 162 ppb, respectively (Pippen and Mecchi, 1969). If a food product contains approximately 0.1 mg of thiamine per 100 g of food, and a maximum of 40% of the sulfur present in thiamine ends up as hydrogen sulfide, only about 4 μg (40 ppb) of hydrogen sulfide

can possibly be obtained. This is much lower than the hydrogen sulfide threshold for chicken broth. This is an ideal situation, however, where all the thiamine is presumably degraded by heat and all the hydrogen sulfide produced is retained by the food product. Since thiamine degradation and hydrogen sulfide production are influenced by several factors, such as the presence of reducing compounds, oxidizing compounds, and pH, much smaller quantities of hydrogen sulfide would normally be produced. This hypothesis was confirmed by adding thiamine hydrochloride to commercial chicken broth samples and heating in the nitrogen purge system. Thiamine concentrations and heating conditions were such that significant amounts of hydrogen sulfide should have been produced. Hydrogen sulfide production was not increased measurably by the addition of thiamine, even in samples adjusted to pH 6.5 prior to heating. These observations indicate that at physiological pH of most food products, thiamine contributes little hydrogen sulfide. However, the possible contribution of other degradation products of thiamine to the flavor of certain food products cannot be ignored.

ACKNOWLEDGMENT

This investigation was supported in part by the Nutrition Foundation, Inc., research Grant No. 427. Published with the approval of the Director as Paper No. 3001, Journal Series, Nebraska Agricultural Experiment Station, Lincoln, Neb. Research reported was conducted under Project No. 16-19.

LITERATURE CITED

Arnold, R. G., Libbey, L. M., Lindsay, R. C., J. AGR. FOOD CHEM. **17,** 390 (1969).
Badings, H. T., Van Der Pol, J. J. G., *Neth. Milk Dairy J.* **19,** 283 (1965).
Bidmead, D., Giacino, C., Grossman, J., Krotz, P. (to International Flavors & Fragrances, Inc.) U.S. Patent 3,394,016 (July 23, 1968).
Bills, D. D., Keenan, T. W., *J. Dairy Sci.* **50,** 1500 (1967).
Booth, R. G., *Biochem. J.* **37,** 518 (1943).
Bouthilet, R. J., *Food Res.* **16,** 201 (1951).
Giacino, C. (to International Flavors & Fragrances, Inc.), U.S. Patent 3,394,017 (July 23, 1968).
Grill, H., Jr., Patton, S., Cone, J. F., *J. Dairy Sci.* **49,** 409 (1966).
Hochberg, M., Melnick, D., Oser, B. L., *Cereal Chem.* **22,** 83 (1945).
Hornstein, I., Crowe, P. F., Sulzbacher, W. L., J. AGR. FOOD CHEM. **8,** 65 (1960).
International Flavors & Fragrances, Inc., British Patent 1,146,337 (March 26, 1969).
Lhoest, W. J., Busse, L. W., Baumann, C. A., *J. Amer. Pharm. Ass.* **47,** 254 (1958).
Mecchi, E. P., Pippen, E. L., Lineweaver, H., *J. Food Sci.* **29,** 393 (1964).
Minor, L. J., Pearson, A. M., Dawson, L. E., Schweigert, B. S., *J. Food Sci.* **30,** 686 (1965).
Obermeyer, H. G., Chen, L., *J. Biol. Chem.* **159,** 117 (1945).
Pippen, E. L., Mecchi, E. P., *J. Food Sci.* **34,** 443 (1969).
Sabri, M. I., George, P. A., Mohan Rao, V. K., *Indian J. Technol.* **6,** 272 (1968).
Sands, A. E., Grafins, M. A., Wainswright, H. W., Wilson, H. W., *Bur. Mines Rept. Invest.* 4547 (1949).
Tanakawa, E., Motohiro, T., *Proc. Int. Congr. Refrig. 10th, Copenhagen* (1959).
Williams, R. R., Spies, T. D., "Vitamin B_1 (Thiamin) and its Use in Medicine," MacMillan, New York, N.Y., 1938.

Received for review December 4, 1970. Accepted February 24, 1971.