shunted into aspartic acid and subsequently into threonine. As a result, the threonine-U-^{14}C added in the malt fermentations will be diluted with nonradioactive threonine, causing a lower specific activity. The lower threonine specific activity will result in lower higher alcohol specific activity. This relationship of higher alcohol specific activity was demonstrated.

When threonine-U-^{14}C is added, the specific activity of *n*-propyl and *d*-amyl alcohol changed, using different types of mash. This may be due to rate differences in the transformation of threonine into these higher alcohols. Since most of the threonine, due to blockage of Pathway B by high nitrogen levels of the malt mash, is transformed *via* Pathway A into AKBA, the concentration of AKBA should be higher than in the corn fermentations, where both systems are active. This would result in a higher level of *n*-propyl alcohol in malt than in corn fermentations. Although more *n*-propyl alcohol is produced in the malt fermentations, had the malt fermentations lasted as long as the corn fermentations, this difference probably would have been greater.

Although the overflow concept accounts for much of the variation in fusel oil production in different mashes, there are some observations it does not explain. For example, in the malt fermentations, a large amino acid pool existed throughout the fermentation period, but fusel oil was formed only during the time of active ethanol production. It may well be that the level of reduced diphosphopyridine nucleotide, a necessary cofactor for the transformation of keto acids into higher alcohols (SentheShanmuganathan, 1960), becomes limiting after the cessation of ethanol production from sugar. Although large amounts of amino acid are present, there may not be enough energy for the Ehrlich mechanism to function.

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Chemistry of Thiamine Degradation in Food Products and Model Systems: A Review

Basant K. Dwivedi[1] and Roy G. Arnold*

The instability of thiamine to heat in neutral or alkaline systems has prompted extensive study of the chemistry of thiamine degradation. Literature dealing with the effect of pH and heat on the thiamine molecule is reviewed. Thermal degradation products which have been reported, such as hydrogen sulfide, elemental sulfur, 4-methyl-5-(β-hydroxyethyl)thiazole, and numerous minor products, are discussed. The extent of thermal degradation and the nature of the products formed appear to be determined by which of two proposed reaction mechanisms predominates, which is controlled by pH. Literature dealing with the effects of other factors, including oxidation–reduction systems, inorganic bases (sulfites, bisulfites), thiaminase enzymes, metal complexes, radiation, and ultrasonic waves, is also reviewed. Reactions of thiamine in model systems with proteins, amino acids, carbohydrates, other organic compounds, and certain inorganic compounds are presented. Chemical structures of thiamine degradation products reported in the literature are shown.

The sensitivity of thiamine to heat and alkali was recognized almost immediately after thiamine was discovered. Considerable information about the destruction of thiamine during cooking, processing, and storage of foods has appeared since the isolation of thiamine by Jansen and Donath (1926). However, most of this information is concerned with the loss of biological activity of thiamine during the treatment of a particular food under specified conditions. Only recently have the reaction products of thiamine in foods or in model systems been identified.

Temperature, pH, and time of heating, processing, or storage are the most important factors contributing to the loss of thiamine in food products. Rice and Beuk (1945) studied thiamine decomposition in pork at different temperatures. Farrer and Morrison (1949) studied thermal destruction of thiamine in buffered solutions and showed that the rate of destruction follows the Arrhenius equation, $\ln k = I - E/RT$, where I = constant, R = gas constant, E = energy of activation, k = rate constant, and T = temperature in degrees Absolute.

Subsequent studies showed that this equation can be successfully used in predicting thiamine retention in foods

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(Agrawal *et al.*, 1963; Bendix *et al.*, 1951; Farrer, 1950, 1953; Sabry and Tannous, 1961).

Farrer (1955), in his review on the thermal destruction of thiamine in foods, referred to the very low values for E obtained with several foods (Brenner *et al.*, 1948; Farrer, 1950, 1953; Farrer and Morrison, 1949; Feaster *et al.*, 1946; Rice *et al.*, 1944). Two possible reactions were postulated, one involving the breaking of the CH "bridge" leaving the pyrimidine and thiazole moieties, and the other involving the breakdown of the thiazole ring with the production of hydrogen sulfide. This review did not establish which of the two reactions has the lower energy of activation.

FACTORS INFLUENCING THE DESTRUCTION OF THIAMINE

The effects of various factors on thiamine destruction have been studied. Figure 1 shows the chemical structure of thiamine (1-I) and several of its degradation products referred to in subsequent discussions of factors influencing thiamine destruction.

Temperature and pH. Obermeyer and Chen (1945) reported initial cleavage of thiamine to its pyrimidine and thiazole moieties, together with some unknown degradation products, under the conditions encountered in bread baking. Matsukawa *et al.* (1951) tentatively identified 4-methyl-5-(β-hydroxyethyl)thiazole (1-VIII), formic acid, 2-methyl-4-amino-5-aminomethyl pyrimidine (1-VI), thioketone (1-XI), and 2-methyl-4-amino-5-hydroxymethyl pyrimidine (1-VII) from an aqueous thiamine solution which was refluxed for 30 hr.

Destruction of thiamine is more rapid in basic solutions. Clarke and Gurin (1935) identified hydrogen sulfide from heated alkaline thiamine solution. Hirano (1957) reported that compound 1-IV is formed by the loss of hydrogen sulfide when the sodium salt of thiamine is warmed in water. In addition to these compounds, 3-mercaptopropanol, disulfides of thiamine (1-X) and of thioketone, and thiothiazolone (1-IX) are formed (Matsukawa and Iwatsu, 1950; Watanabe and Asahi, 1957). Lhoest *et al.* (1958), using paper chromatography, studied the products resulting from the action of alkali on thiamine. These workers identified the carbinol form of thiamine (1-II), thiochrome (1-XIV), thiamine disulfide (1-X), two pyrimidine derivatives, and two unknown products. Gaudiano *et al.* (1966) studied the decomposition products of thiamine in injectable solutions by comparing R_f values of degradation products on paper chromatograms with authentic samples. These workers reported the presence of 2-methyl-4-amino-5-aminomethyl pyrimidine (1-VI), 2-methyl-4-amino-5-hydroxymethyl pyrimidine (1-VII), thiochrome (1-XIV), 4-methyl-5-(β-hydroxyethyl)thiazole (1-VIII), and several unidentified spots from autoclaved thiamine hydrochloride samples (pH 6.5). Recently, Arnold *et al.* (1969) identified hydrogen sulfide, 2-methylfuran (1-XV), 2-methylthiophene (1-XVI), and 2-methyl-4,5-dihydrothiophene (1-XVII) as volatile products of boiled thiamine solutions (pH 6.7). Other volatile compounds were isolated but not identified. On the basis of mass, infrared, and nmr spectral data, Dwivedi *et al.* (1972b) identified 4-methyl-5-(β-hydroxyethyl)thiazole as a major degradation product of heated slightly acidic or alkaline thiamine solutions. These authors also reported tentative identification of 2-methylthiophene, 4,5-dihydro-2-methylthiophene, 2-methylthio-5-methylfuran, 4,5-dimethylthiazole, 2-methyl-3-oxytetrahydrothiophene, and 2-acetyltetrahydrothiophene (Dwivedi *et al.*, 1972a). Morfee and Liska (1971, 1972) studied the distribution of thiamine degradation products in simulated milk systems heated at 121° for 50 min, and identified elemental sulfur as a major degradation product in buffered slightly acidic or basic solutions. They observed substantial bonding between products of heated thiamine-^{35}S and protein.

The mechanisms of formation of these compounds from heat degradation of thiamine have not been established. However, by studying the chemical structures of thiamine in solution at different pH levels, the formation of some of the aforementioned degradation products can be explained (Figure 1). Dwivedi and Arnold (1972) used ^{35}S-

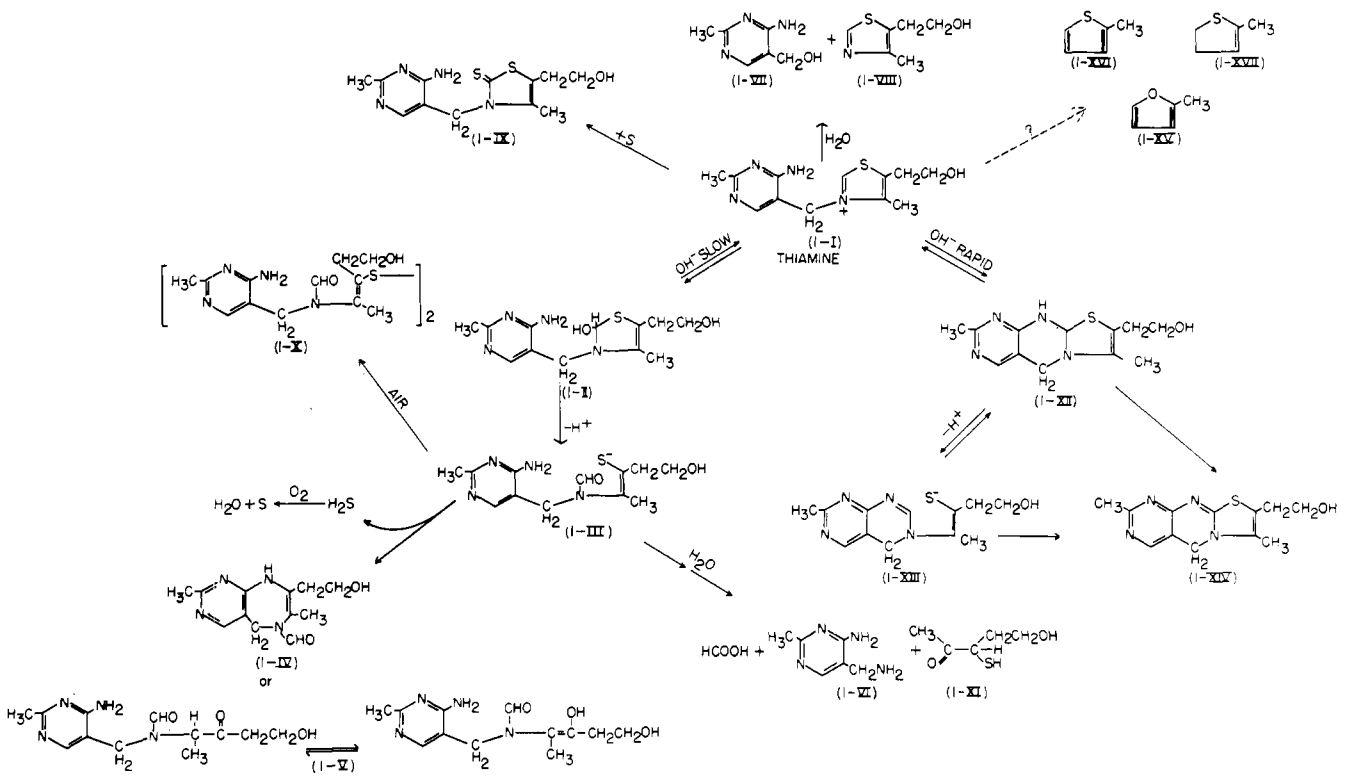
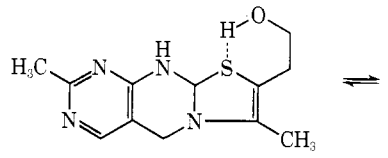


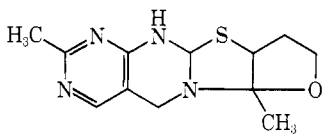
Figure 1. Thiamine degradation reactions.

labeled thiamine in studying the mechanisms of thermal breakdown of thiamine in model systems. Heating of thiamine solutions at pH 6.0 or below resulted in cleavage of thiamine at the methylene "bridge" between the thiazole and pyrimidine moieties, producing 4-methyl-5-(β-hydroxyethyl)thiazole as the principal sulfur-containing product. At pH 7.0 and above, hydrogen sulfide appeared to be a major product, along with minor amounts of numerous other sulfur-containing products. The authors concluded that the small amounts of the pseudo base and/or thiol forms of thiamine which exist along with free thiamine above pH 6.0 are responsible for these degradation products. Apparently the energy of activation for the breakdown of the thiazole ring of these alternate forms of thiamine is substantially lower than that for cleavage at the methylene "bridge."

The chemical properties of thiamine in relation to pH have been reviewed by Metzler (1960). Based on the nmr spectrum of thiamine in the presence of 2 equiv of methoxide, Risinger *et al.* (1968) recently produced evidence of a NO-acetal (2-II) which exists in equilibrium with the tricyclic structure (2-I) proposed by Maier and Metzler (1957). This may explain, in part, the formation of some of the compounds identified by Arnold *et al.* (1969) and Dwivedi *et al.* (1972a).



2-I



2-II

Oxidation-Reduction Systems. Thiamine and thiamine pyrophosphate can be oxidized by dilute hydrogen peroxide at pH 7.5 or by iodine or air in alkaline solution to form the corresponding disulfide derivative (1-X) without loss of thiamine activity (Myrback *et al.*, 1945; Zima and Williams, 1940). This conversion involves the opening of the thiazole ring (1-III). The disulfide can be reduced to thiamine by hydrogen, tin, hydrochloric acid, or thiols such as cysteine or glutathione. More vigorous oxidation of thiamine with potassium permanganate or manganese dioxide in neutral solution or with alkaline potassium ferricyanide or hydrogen peroxide produces the fluorescent compound, thiochrome (1-XIV) (Weil-Malherbe, 1940). Cigdem *et al.* (1965) reported a similar conversion using dilute alkaline solutions of ascorbic acid. Some thiamine disulfide (1-X) is also formed under these conditions. Maier and Metzler (1957) reported that thiochrome is not formed when thiamine is allowed to stand in base long enough for the yellow-colored form (1-XIII) to disappear before the oxidizing agent is added. The sequence of reactions in the formation of thiochrome and thiamine disulfide is illustrated in Figure 1.

Thiamine reacts readily as the thiol form (1-III) with such disulfides as cystine to yield mixed disulfides (3-I) (Matsukawa and Yurugi, 1953, 1954). Allicin (CH_2=$CHCH_2SSOCH_2CH$=CH_2), a constituent of onion and garlic, reacts with thiamine to yield the allyl disulfide allithiamine (R = -CHCH=CH in 3-I) (Yurugi, 1954).

$$\underset{\text{(1-III)}}{\text{Th-SH}} + \text{R-S-S-R} \rightarrow \underset{\text{(3-I)}}{\text{Th-S-S-R}} + \text{RSH}$$

On heating under nitrogen, thiamine disulfide is converted to thiothiazolone (1-IX) and dihydrothiochrome (1-XII) (Karrer and Krishna, 1952; Sykes and Todd, 1951). In the presence of oxygen, thiochrome (1-XIV) and thiothiazolone are formed (Sykes and Todd, 1951; Yurugi, 1957). Zima and Williams (1940) reported that thiamine disulfide heated with base yields hydrogen sulfide and thiamine.

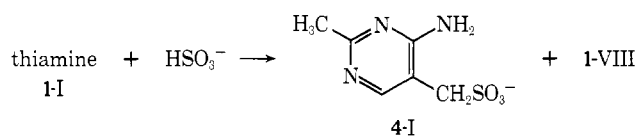
Formation of thiochrome has generally been considered an irreversible reaction. However, Risinger and Parker (1963) converted thiochrome back to thiamine in the presence of α-chloropropionic acid. Risinger *et al.* (1966) further reported that reducing systems, DPNH, lactic acid, acetaldehyde, and ascorbic acid did not significantly reduce thiochrome to thiamine. However, benzaldehyde reduced thiochrome to thiamine (57%), apparently by a Cannizzaro reaction. It is therefore possible that thiochrome present in food products may be converted back to thiamine or may form other degradation products.

Thiamine allowed to stand in alkaline solution in air is oxidized to the disulfide plus minor amounts of thiothiazolone (1-IX) (Watanabe and Asahi, 1957). The latter compound apparently arises from the reaction of thiamine with elemental sulfur (Hirano, 1957). Sulfur is likely formed by the oxidation of hydrogen sulfide, which is also produced from thiamine in alkaline solutions.

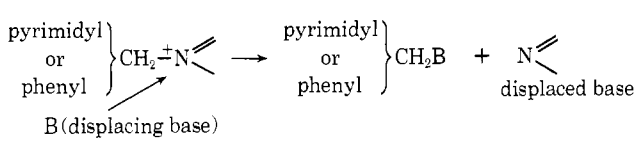
Kawasaki and Horio (1959) noted that thiamine is unstable in dilute solutions containing so-called "thermostable factors," such as rutin and hydroquinone. *p*-Aminobenzoic acid also accelerates the oxidation of thiamine by dissolved oxygen in either neutral or alkaline solutions, but the exclusion of oxygen by saturation with nitrogen or addition of alkaline ferrous ammonium sulfate and sodium tartrate prevents oxidation. Oxidation of thiamine to thiamine disulfide by dissolved oxygen in alkaline solutions has a pH optimum near 9.5 (Kawasaki and Horio, 1960). Presence of "thermostable factors" enables oxidation by dissolved oxygen in less alkaline media. If dissolved oxygen is expelled from the system, thiamine is relatively stable. In a similar study, Hosoda *et al.* (1959) found that pyrocatechol and hydroquinone induce the oxidation of thiamine to thiamine disulfide.

Bisulfites and Other Inorganic Bases. The sensitivity of thiamine to sodium sulfite has been well established. Sulfur dioxide is used as a preservative in several food systems, such as fruits and vegetables. Hermus (1969) studied the effect of storage and preparation of minced meat on sulfite-induced thiamine degradation and found that the effect of storage temperature was not significant. Thiamine losses under these conditions were linear, with sulfur dioxide concentrations up to 0.1%.

In model systems, thiamine allowed to stand in a concentrated sodium sulfite solution at pH 5–6 is cleaved into free thiazole base (1-VIII) and a pyrimidylmethane sulfuric acid (4-I). The sulfite cleavage reaction may be de-



thiamine 1-I + HSO_3^- → 4-I + 1-VIII



pyrimidyl or phenyl $CH_2{\pm}N$ → pyrimidyl or phenyl CH_2B + displaced base; B (displacing base)

scribed as a base-exchange reaction or a nucleophilic displacement on the methylene group in which sulfite is the displacing base, B (Metzler, 1960). Various quaternary benzylamines are cleaved similarly with bisulfite, sulfite, bisulfide, sulfide, thiocyanate, and thiosulfate, all acting as effective displacing bases (Snyder and Speck, 1939).

Pyridine has been reported to accelerate thiamine decomposition in sulfite-containing solutions (Matsukawa and Yurugi, 1951). These workers also discovered that incubation of a base-exchanged pyridinium salt with 4-methyl-5-(β-hydroxyethyl)thiazole in a solution containing sulfite at pH 4.6 resulted in formation of thiamine. This suggests that sulfite acts as a catalyst for base-exchange reactions.

Boissier and Tillement (1969) studied the destruction of thiamine in Tyrode's solution (a solution of sodium, potassium, and magnesium chlorides, sodium bicarbonate, disodium hydrogen phosphate, and glucose at pH 8.2) and found that at 37° thiamine was rapidly converted into 4-methyl-5-(β-hydroxyethyl)thiazole and 2-methyl-4-amino-5-hydroxymethylpyrimidine when either nitrogen or air was bubbled through the solution. The same results were obtained when sodium bicarbonate was used in place of Tyrode's solution. The function of air was not oxidative but rather to agitate the reaction mixture. Other inorganic compounds such as borate, thiosulfate, acetate, monohydrogen phosphate, and potassium dihydrogen phosphate also accelerate thiamine destruction (Watanabe and Marui, 1949). Calcium hydrogen phosphate has been reported to have a protective effect (Motoyama *et al.*, 1959).

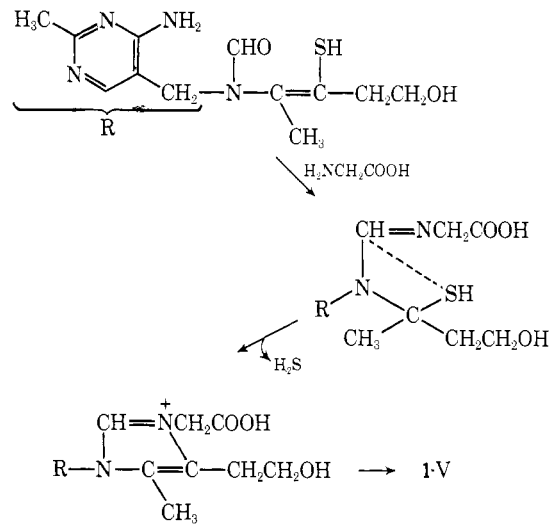
Recently, Oka *et al.* (1970) reported that thiamine and its homologs are cleaved by aromatic aldehydes. When thiamine hydrochloride was treated with 2 mol equiv of triethylamine and an excess of benzaldehyde in methanol, 4-amino-2,5-dimethylpyrimidine and 2-benzoyl-5-(β-hydroxyethyl)-4-methylthiazole were formed. The reaction was further extended to the reactions of thiamine and its homologs with a variety of aromatic aldehydes to give several new 2-acylthiazoles.

Thiaminases. Thiaminases, which are present in small concentrations in vegetable and animal food products, also degrade thiamine. Two types of thiaminases are known to exist. Thiaminase I (thiamine-base 2-methyl-4-aminopyrimidine-5-methyltransferase) catalyzes the decomposition of thiamine by base-exchange reaction involving a nucleophilic displacement of the methylene group of the pyrimidine moiety. These enzymes readily catalyze displacements by aniline and pyridine. The natural displacing bases for these thiaminases are not known, but Kupstas and Hennessy (1957) have shown that in clam tissues, hypotaurine ($H_2NCH_2CH_2SO_2H$) is apparently the cosubstrate. This reaction gives rise to 4-amino-5-(β-aminoethanesulfonyl)methyl-2-methylpyrimidine and free thiazole base, as previously illustrated for nucleophilic displacement by inorganic base such as bisulfite.

Thiaminase II (thiamine hydrolase) catalyzes simple hydrolysis of thiamine into 4-methyl-5-(β-hydroxyethyl)-thiazole (1-VIII) and 2-methyl-4-amino-5-hydroxymethylpyrimidine (1-VII) (Fujita, 1954).

Thiaminase activity has been reported in various seafood products. More recently, however, Somogyi (1966) and Kundig and Somogyi (1967) reported the isolation from carp viscera of an "antithiamine factor" that was thermostable and nonenzymatic in nature. The active factor was identified as hemin or a related compound. No reaction products were identified.

Proteins and Amino Acids. McIntire and Frost (1944) claimed that α- and β-amino acids decrease the rate of thiamine destruction. Under alkaline conditions, however, some amino acids (*i.e.*, glycine, α-alanine, β-alanine, valine, glutamic acid, etc.) have been reported to induce desulfurization of thiamine with the formation of dethiothiamine (Kurata *et al.*, 1968). In a similar study, Kawasaki *et al.* (1967) treated thiamine-HCl in a trimolar NaOH solution with equimolar glycine. Hydrogen sulfide was evolved in the reaction and a crystalline product was gradually precipitated. The following mechanism was suggested.



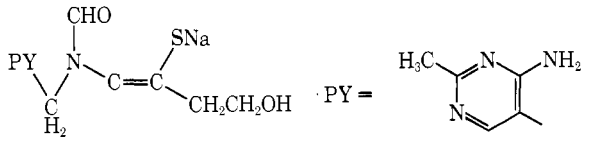
The structure of dethiothiamine (1-V) suggested by Kawasaki *et al.* (1967) is not in accord with the structure of dethiothiamine (1-IV) reported by Hirano (1957). Former workers were successful in resynthesizing thiamine from this reaction product by saturating an acetic acid solution of the product with hydrogen sulfide.

Proteins are known to protect thiamine. However, there are conflicting reports regarding the protective mechanism involved. Wada and Suzuki (1965) attempted to recover thiamine under varied conditions in the presence of egg albumin and its modified forms (NH_2 and SH groups protected), and concluded that the SH groups of albumin participate in protecting thiamine. Tada and Nakayama (1958) prepared a thiamine casein complex and claimed that it was more stable. Utsumi *et al.* (1962) prepared complexes of disulfides of thiamine with egg albumin and postulated that an interchange between SH group of protein and -SS- group of disulfide was involved. These workers, however, failed to get a complex between free thiamine and egg albumin. Leichter and Joslyn (1969), in a study of the protective action of casein-on-thiamine, found that soluble starch also protected thiamine, and proposed that some macromolecular property, *e.g.*, interfacial surface effect, was involved in the protective effect. They also observed that casein does not combine with thiamine in appreciable concentrations. Morfee and Liska (1971) observed reversible bonding between thiamine and protein in a simulated milk system. They also noticed substantial bonding between sulfur-containing breakdown products of thiamine and protein. The nature of the bonding was unknown, although disulfide bonding was considered a strong possibility.

Reactions with Aldehydes, Amines, Phosphorous Acid Esters, etc. Reactions of thiamine in alkaline conditions with aldehydes, amines, phosphorous acid esters, etc., have recently been studied. In these reactions participating groups are -CHO and -SH, which result from alkali treatment. The initial reaction takes place at the C-2 position of the thiazole moiety. The significance of these reactions in food products is difficult to visualize since the structural form of thiamine in foods will be determined by several factors in addition to the pH of the system. These factors may influence such reactions.

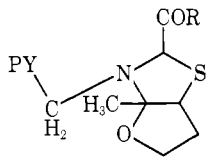
In a study of the reaction of thiamine with aldehydes

(Takamizawa *et al.*, 1967c), the carbon dioxide treated thiol form of thiamine (1-III) was allowed to react with benzaldehyde in ethanol. In this reaction, compound (5-II) was produced along with benzylphenol, thiothiazolone (1-IX), bis-2-(4-methyl-5-β-hydroxyethyl)thiazole, and 2,5-dimethyl-4-aminopyrimidine. In a similar reaction with acetaldehyde, compound (5-II) was formed along with acetoin and thiothiazolone. Similar products were obtained with other aldehydes. When compound (5-II) was treated with dilute hydrochloric acid, thiamine was formed.



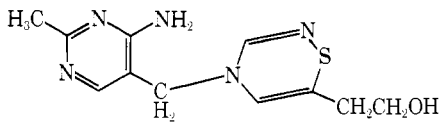
5-I

R = phenyl or methyl groups



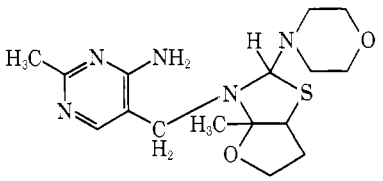
5-II

The reaction of thiamine with hydroxylamine in alkaline conditions produces diazinothiamine (6-I) as the main reaction product. Hydroxyiminothiamine and furothiazine are also produced in small amounts under these conditions (Kawasaki *et al.*, 1970; Yokoyama, 1970). Diazinothiamine is very stable in acid and alkaline media and is not converted back to thiamine or its pyrimidine moiety by reaction with oxidants or reductants.

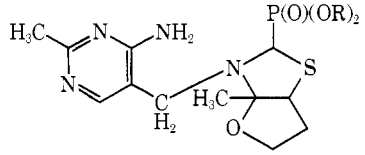


6-I

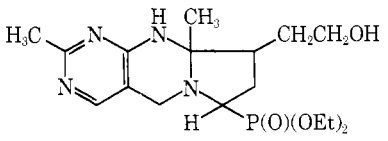
Takamizawa *et al.* (1967a) reported that thiamine at pH 7.5–8.0 in cold toluene saturated with carbon dioxide in the presence of morpholine produced 2-morpholino-3-(2-methyl-4-aminopyrimidin-5-yl)methyl - 3a - methylperhydrofuro[2,3-*d*]thiazole (7-I). The reaction of thiamine with piperidine proceeded quite similarly and produced the 2-piperidine analog of (7-I). These workers concluded that treatment of thiamine with carbon dioxide may produce pseudothiamine types which react by introducing



7-I



7-II



7-III

amines at the thiazole C-2 position of thiamine, and which may have significance in consideration of the biological behavior of thiamine. When thiamine was treated with the ethyl ester of phosphorous acid instead of morpholine under the same conditions, it gave compound (7-II). This compound, when heated in refluxing ethanol, gave the isomer (7-III) (Takamizawa *et al.*, 1967b).

Carbohydrates. Thiamine reacted quite strongly in Maillard-type reaction when a dry mixture of the vitamin and glucose was heated at 85° (Lhoest, 1957). In an extension of this work, Lhoest (1958) found that thiamine hydrochloride reacts with glucose at pH <4 to give 2-glucothiamines and other unidentified compounds. In a similar study, Van der Poel (1956) reported that thiamine, on heating with glucose solution, produced a brown discoloration and fluorescence. This behavior is analogous to the Maillard reactions of sugars and amino acids, and may be important in the loss of thiamine during processing. The studies of Ache and Ribeiro (1945) and Wai *et al.* (1962), however, show that fructose, mannitol, invertase, and inositol actually retard thiamine destruction.

Metal Complexes. Booth (1943) was the first to recognize the influence of copper ions in thiamine destruction. He reported that thiamine was destroyed more rapidly if copper was present. Farrer (1947) showed that heavy metals influenced thiamine destruction only when they were capable of forming complex anions with constituents of the medium. Spaleny (1960) reported that metal chelating agents such as disodium-calcium EDTA, 1,2-diaminocyclohexane-tetraacetic acid, ascorbic acid, and sulfites retarded thiamine decomposition in the presence of copper. Tanaka (1966a,b) successfully crystallized a copper-thiamine complex having a composition of $C_{12}H_{16-17}O_2N_4SCu$. In a further study, Tanaka (1969) reported that thiamine-copper complex shows thiamine decomposing power nearly corresponding to equimolar concentration of copper ions. He suggested that in a thiamine solution contaminated with copper, alternative formation and degradation of thiamine-copper complexes may occur and this may be the cause of gradual decomposition of thiamine by copper. Talbert and Weaver (1970) prepared solid adducts from the reactions of zinc and cobalt halides with derivatives of thiamine. They characterized the derivatives by infrared, electronic reflectance spectroscopy, and magnetic susceptibility studies, and concluded that they were complex salts without metal-S or metal-N interaction.

Radiation. Beral *et al.* (1961), in a study on pharmaceutical preparations of B vitamins, concluded that heat and light induced thiamine decomposition. Okumura (1961) and Kawasaki and Daira (1962) reported that thiamine is very sensitive to ultraviolet light. One of the main degradation products of thiamine, when irradiated with uv light, is 2-methyl-4-amino-5-aminoethylpyrimidine. Button (1968) reported a method for selective thiamine removal from culture media by ultraviolet irradiation. A 99% reduction in thiamine content was achieved by irradiating the culture medium for 5 min with a 1200-W mercury vapor lamp. Gamma irradiation, similar to ultraviolet irradiation, induces thiamine degradation (Luczak, 1966, 1968; Syunyakova and Karpova, 1966). Ueno and Fucuda (1962), in a similar study, found that loss of thiamine by irradiation is minimized by glutathione. The degradation products of thiamine irradiation with γ rays have not been identified.

Ultrasonic Waves. Matsutani *et al.* (1969) reported that the treatment of aqueous solutions of thiamine hydrochloride, thiamine naphthalene-1,5-disulfonate, dibenzothiamine, and thiamine pyrophosphate by ultrasonic waves (400–800 kHz) results in a decrease in thiochrome estimation values. They also observed that addition of compounds containing SH groups such as cysteine protected thiamine.

THIAMINE AND FOOD FLAVORS

Compounds produced by thiamine degradation, particularly those from thermal breakdown of thiamine, may be important contributors to food flavors. Hydrogen sulfide, a major thermal degradation product under neutral or alkaline conditions, has been associated with the flavor of numerous food products, particularly heated foods. Dwivedi and Arnold (1972) quantitatively determined the production of hydrogen sulfide from thiamine under various conditions of heating and concluded that thiamine would be a relatively insignificant source of hydrogen sulfide in most food systems.

Thiamine reacts readily in Maillard-type reactions (Lhoest, 1957). These reactions generally result in the formation of numerous volatile compounds of potential flavor significance. Several patents dealing with the production of meat or chicken-like flavors by heating thiamine-containing mixtures have been granted (Bidmead *et al.*, 1968; Giacino, 1968; International Flavors and Fragrances, Inc., 1969).

Certain of the volatile compounds identified as products of thiamine degradation have also been isolated and identified as volatile constituents of heated food systems. For example, 2-methylfuran has been identified as a constituent of coffee (Merritt *et al.*, 1963) and chicken meat (Nonaka *et al.*, 1967), and 2-methylthiophene has been identified as a constituent of chicken meat (Nonaka *et al.*, 1967).

Arnold *et al.* (1969) reported the production of "heated or boiled milk" and "stewed chicken" odors upon heating phosphate-buffered thiamine solutions. The odor character of 2-methylthiophene and 4,5-dihydro-2-methylthiophene, two of the compounds produced, was described as "heated onion, sulfury." Morfee (1969) reported cooked and bitter flavor defects in heated thiamine solutions of initial concentration <1 ppm. A pungent odorous compound, resembling the sensation of burning plastic, was also reported. Morfee and Liska (1972) reported the separation of an unidentified compound, with a pungent odor on thin-layer chromatograms of thiamine degradation products. These observations suggest that thiamine degradation products may contribute to off-flavors in food products. Since little quantitative data on thiamine degradation products is available, the full significance of thiamine degradation to the flavor of food products is unknown.

SUMMARY

Factors contributing to thiamine degradation, such as pH, heat, oxidation–reduction systems, inorganic bases, enzymes, metal complexes, and radiation, have been studied extensively. Numerous compounds produced by the action of these factors on thiamine, or reactions of thiamine with various inorganic or organic compounds, have been identified. Model systems have generally been employed to determine the nature of thiamine degradation reactions and products. Food systems, being complex, have received limited use in thiamine degradation studies. Lack of quantitative information on degradation products has limited our understanding of the mechanisms of thiamine breakdown. The nature of compounds produced in model systems suggests that thiamine degradation may contribute to flavor of heated food systems. Additional information with actual food systems is needed to assess the flavor and potential toxicological significance of thiamine breakdown. Radiotracer studies with ^{35}C- and ^{35}S-labeled thiamine should provide valuable information, since the structure and properties of the major thiamine degradation products are known.

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Extractability and Solubility of Leaf Protein

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A laboratory-scale extraction method was developed which maximized the extraction of leaf protein while minimizing the possibility of denaturation. The method consisted of homogenizing leaves in a micromill at 6° with 0.1 *M* Tris buffer, pH 7.4 containing 0.5 *M* sucrose, 7.5 m*M* ascorbic acid, 6.6 m*M* cysteine-HCl, and 14.2 m*M* mercaptoethanol. Protein (TCA insoluble) nitrogen equivalent to 60.8% of total leaf nitrogen was extracted from alfalfa leaves by this method. The protein content of cowpea, peanut, and soybean leaves was also investigated. The solubility of total and protein nitrogen of soybean leaf extracts was studied as a function of pH. Both the total and protein nitrogen were most soluble at pH 2.0 and 6.0 and above. Minimum solubility occurred between pH 3.2 and 3.7.

The inadequate supply of good quality protein for human consumption poses one of the major challenges of this era (Autret, 1970; Pirie, 1970). Leaf protein affords good potential as a protein supplement (Kinsella, 1970; Pirie, 1970). The quantity of leaf materials which can be ingested by humans is limited due to the presence of fiber and toxic substances. To facilitate the consumption of leaf protein it must be extracted, thoroughly washed, and concentrated. The nutritive value, high yields, and simplicity of extraction and preparation suggest that leaf protein can be an effective and feasible source of proteins for humans (Lexander *et al.*, 1970; Oelschlegel *et al.*, 1969; Stahmann, 1968). Several large scale processes have been developed for the extraction of leaf protein (Chayen *et al.*, 1961; Hollo and Koch, 1971; Knuckles *et al.*, 1971; Kohler and Bickoff, 1971; Pirie, 1971). These processes extract from 35 to 80% of the total leaf protein.

The extractability of leaf protein is influenced by a multitude of factors. Since cell walls and chloroplasts must be disrupted to extract the proteins effectively, the influence of the various factors is undoubtedly dependent upon their ability to disintegrate these cellular and subcellular membranes. A variety of the methods used to rupture cell walls have been reviewed by Stahmann (1963). Some of the factors which have been reported to influence the extraction of leaf proteins are: leaf species and stage of maturity (Boyd, 1968; Chayen *et al.*, 1961); the presence of mucilagenous material (Nazir and Shah, 1966); postharvest treatment (Huang *et al.*, 1971); pH and composition of the extractant, flotation ratios, and extraction time and temperature (Lu and Kinsella, 1972; Nazir and Shah, 1966; Poppe *et al.*, 1970). In general, higher protein yields are obtained by extracting tender leaves shortly after harvest in an alkaline medium.

The presence of endogenous proteolytic and oxidative enzymes in leaf extracts may partially impair the recovery of protein. Phenoloxidase and peroxidase catalyze the reduction of *o*-diphenols to quinones. The latter, in the presence of oxygen, polymerize and complex with the protein, thus impairing the solubility and digestibility of the protein (Horigome and Kandatsu, 1966; Loomis and Battaile, 1966; Stahmann, 1963). The formation of these complexes is hindered by extracting the leaves in the presence of reducing agents and by removing the phenolic sub-

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