1988

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Order Number 8825413

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Iowa State University, 1988

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The influence of pH, water activity, and reducing sugars on kinetics of thermal thiamin breakdown in model systems and ground pork

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Food Science

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1988

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INTRODUCTION

The implications of food processing and distribution in terms of nutrient destruction and loss have become very important in recent years. Knowledge of nutrient losses during processing is important for dietary goal recommendations and nutritional labeling regulations. Nutrient loss during preparation of foods also has been studied for decades. Foods are complex mixtures of organic acids and biological compounds; thus, heat, frozen storage, oxygen, moisture, and pH shifts can be important factors affecting nutrient retention. The nutrient content of foods at the time of consumption depends on the composition of the raw materials, on the history of the food, and in particular, on conditions of processing, storage, and preparation prior to consumption.

Labuza (1972) demonstrated the application of chemical kinetics to the study of nutrient losses in dehydrated foods. Thermodynamics is a subject in which the rate of a reaction is studied as certain parameters are varied. These parameters include the concentration of reactants, temperature, pressure, and pH. The data accumulated are then analyzed so as to give certain rate laws, and hence to yield some information about the mechanism of the reaction.

Due to the heat sensitivity of thiamin, it is used as an indicator of nutrient breakdown during processing even though the food in question may not be a significant source of the vitamin (Klein et al., 1984). Thiamin's heat stability depends on pH, temperature, ionic

strength, oxidation-reduction conditions, enzymes, metal complexes, and other reacting species (Dwivedi and Arnold, 1973). In pure thiamin solutions, destruction by heat is primarily a hydrolytic cleavage to give pyrimidine and thiazole derivatives. In most cases, thiamin destruction in both model systems and real foods follows first order kinetics. But the reaction rate is affected by pH, oxygen, trace metals, and the chemical form of the thiamin molecule. Besides these factors, the type and concentration of the reducing sugar (Doyon and Smyrl, 1983) and water activity (Kamman et al., 1981; Fox et al., 1982) are known to affect thiamin breakdown. Many investigators (Fox et al., 1982; Mulley et al., 1975b) have studied the effect of pH on thiamin retention and found that as pH increased, thiamin retention decreased. But there have not been many studies of the effect of these factors on the kinetic order or temperature dependence of thiamin breakdown.

The order of the degradation reaction of thiamin by thermal processing in the various systems under study can be ascertained graphically by plotting the logarithm of concentration against time of heating at constant temperatures. The reaction rate constants for thiamin degradation cannot be extrapolated to other temperatures unless the Arrhenius activation energy is known for the particular system. The Arrhenius equation describes the effect of time/temperature treatments on the rate and extent of nutrient destruction, and it is useful in assessing how sensitive a nutrient is to heat treatment. The Arrhenius activation energy is calculated by plotting the logarithm of

the reaction rate constant against the reciprocal of the absolute temperature at which rate constant was measured. In the present study, effect of pH, water activity, types of reducing sugars and thiamin concentration on the first order reaction rate constant and activation energy was was examined in pork and model systems held at 75, 85, and 95°C. Differences in heat resistance of thiamin in natural foods and that in aqueous and buffered solutions also were examined. There have been some reports that protein or starch in foods have an unknown protective effect on heated thiamin (Mulley et al., 1975b; Feliciotti and Esselen, 1957).

The following specific objectives were formulated.

1. To determine how much thiamin is retained in a model product under different processing conditions; temperature, heating time, pH, water activity, reducing sugars, and thiamin concentration were varied in these experiments.
2. To confirm the effect of the browning reaction between thiamin and various reducing sugars on the breakdown of thiamin.
3. To study the kinetics of the degradation of thiamin and determine the order of kinetics of thiamin breakdown.
4. To observe the temperature sensitivity of thiamin breakdown under different combinations of process parameters by testing the Arrhenius model.

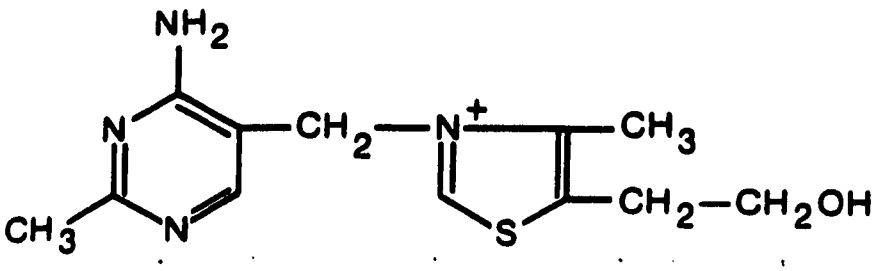
5. To determine the optimum combination of process parameters with respect to thiamin retention.
6. To apply the results from the model system to heated ground pork, comparing the kinetic parameters between the model systems and cooked meat.

REVIEW OF LITERATURE

Discovery and Synthesis of Thiamin

Thiamin, vitamin B_1, is well known as the antineuritic vitamin. Recognition of vitamin B_1 activity dates back to 1886 when the Dutch physician Eijkman observed that chickens fed a diet which consisted mainly of polished rice developed polyneuritic symptoms similar to beriberi. Additional studies showed that the paralysis resulting from feeding polished rice could be cured by adding rice polishings to the diet. From these results, Eijkman suggested that the toxic compound was contained in polished rice but could be neutralized by some protective factor in rice polishings.

In 1926, Jansen and Donath isolated a single substance that could cure beriberi patients. The isolation, structural determination and synthesis of vitamin B_1 was accomplished by Williams and Cline (1936). They demonstrated conclusively that thiamin was composed of pyrimidine and thiazole moieties and established its structure as 3-(2'-methyl-4'-amino-5'-pyrimidylmethyl)- 5-(2-hydroxyethyl)-4-methyl-thiazole (Figure 1). Thiamin has been synthesized by forming the pyrimidine and thiazole moieties separately, followed by a coupling of the two moieties. Williams and Cline used this method in their original synthesis of thiamin (Goodhart and Shils, 1980).



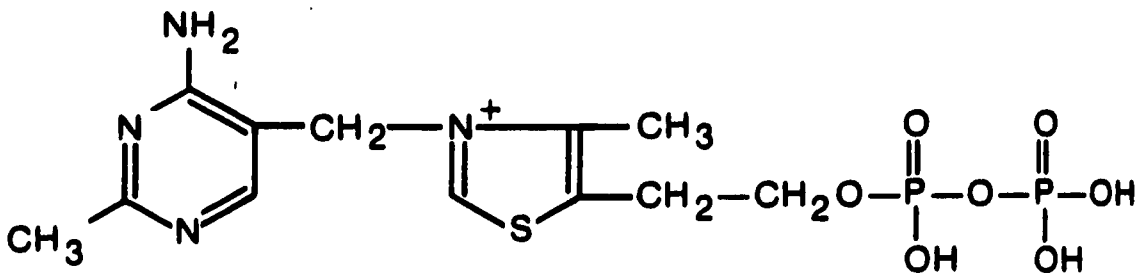


FIGURE 1. Structure of thiamin and thiamin pyrophosphate

Chemistry and Properties of Thiamin

Thiamin is a white, crystalline powder which has hygroscopic properties, a yeasty odor, and a salty and nutlike taste. The vitamin is soluble in water and alcohol, and insoluble in ethyl ether, benzene and other fat solvents; one gram of thiamin chloride hydrochoride can dissolve in 1 ml of water, and it is soluble to about 1 percent in ethanol. Thiamin melts at 246 to 250°C and has a molecular weight of 337.26. It is relatively stable toward dry heat but is destroyed by autoclaving and by sulfites, and especially readily destroyed in neutral and alkaline solution.

Thiamin is widely distributed throughout the plant and animal kingdoms, principally in cereals and cereal brans, with lesser amounts in meats and legumes. Green vegetables, fish, fruits and milk also contain useful quantities (Machlin, 1984). It plays a key role as a coenzyme in the intermediary metabolism of alpha-keto acids and carbohydrates. Thiamin can exist in foods in a number of forms, including free thiamin, the pyrophosphoric acid ester (cocarboxylase), and bound to the respective apoenzyme. In most animal products, 95-98% of the thiamin occurs in a phosphorylated form (thiamin mono-, di-, and tri-phosphates) with about 80-85% as the diphosphate, which is the active coenzyme form (Machlin, 1984).

Since thiamin contains a quaternary nitrogen, it is a strong base and will be completely ionized over the entire range of pH normally encountered in foods. In addition, the amino group on the pyrimidine

ring will be ionized, the extent being dependent upon pH (pka=4.8). The coenzyme role of thiamin is mediated through position 2 of the thiazole ring, which in its ionized form is a strong nucleophile (Tannenbaum, 1979).

An average intake for humans of about 1.5 mg/day for thiamin has been recommended (NAS/NRC, 1980). Thiamin requirements are increased when carbohydrate is ingested or consumed in large amounts, during periods of increased metabolism and during pregnancy and lactation. Deficiency symptoms in mild cases are a loss of appetite and weight. A severe deficiency of thiamin gives rise to the condition known as beriberi, and since thiamin is intimately connected with general metabolism, all types of tissue may be affected. Beriberi still occurs in some developing countries where high carbohydrate diets are common and enrichment of rice and wheat is not practiced, since thiamin plays a key role as a coenzyme in the intermediary metabolism of alpha-keto acids and carbohydrates.

Stability of Thiamin

Factors affecting thiamin breakdown

Thiamin is one of the least stable of all the vitamins. Factors that affect the stability of thiamin in the final product after handling and processing are pH, temperature, solubility, oxidation, radiation, water activity, presence of stable thiamin destroying substances, and thiaminase (Mulley et al., 1975c). Of all these

factors, temperature, pH, and duration of heating, processing, or storage are the most important factors contributing to the loss of thiamin in food products (Dwivedi and Arnold, 1973). The typical degradation reaction appears to involve a nucleophilic displacement at the methylene carbon joining the two ring systems. Therefore, strong nucleophiles such as sulfite readily cause destruction of this vitamin.

The similarity of thiamin degradation by sulfite and by alkaline pH is shown in Figure 2. Both reactions yield 5-(beta-hydroxyethyl)-4-methylthiazole and a corresponding substituted pyrimidine. With alkali, the latter compound is hydroxymethyl pyrimidine, whereas with sulfite it is 2-methyl-5-sulfomethyl pyrimidine (Leichter and Joslyn, 1969). Sulfite ion has been shown to react by a multistep mechanism with thiamin, 1'-methyl thiaminium ion and various thiamin analogues. Sulfite ion replaces the thiazole-leaving group of a thiamin and a pyridine or a phenoxy group of an analogue. As shown in Figure 2, further degradation results in such products as elemental sulfur, hydrogen sulfide, a furan, a thiophene, and a dihydrothiophene, following release of the thiazole ring. The reactions leading to these products are unclear, but extensive degradation and rearrangement of the thiazole ring must be involved.

It has long been known that thiamin is very stable at pH 3, quite stable at pH 5-6, but becomes quite unstable at pH 7, and is rapidly destroyed, largely irreversibly, above pH 8. The rate of destruction at any temperature is accelerated at higher pH. Thus the addition of

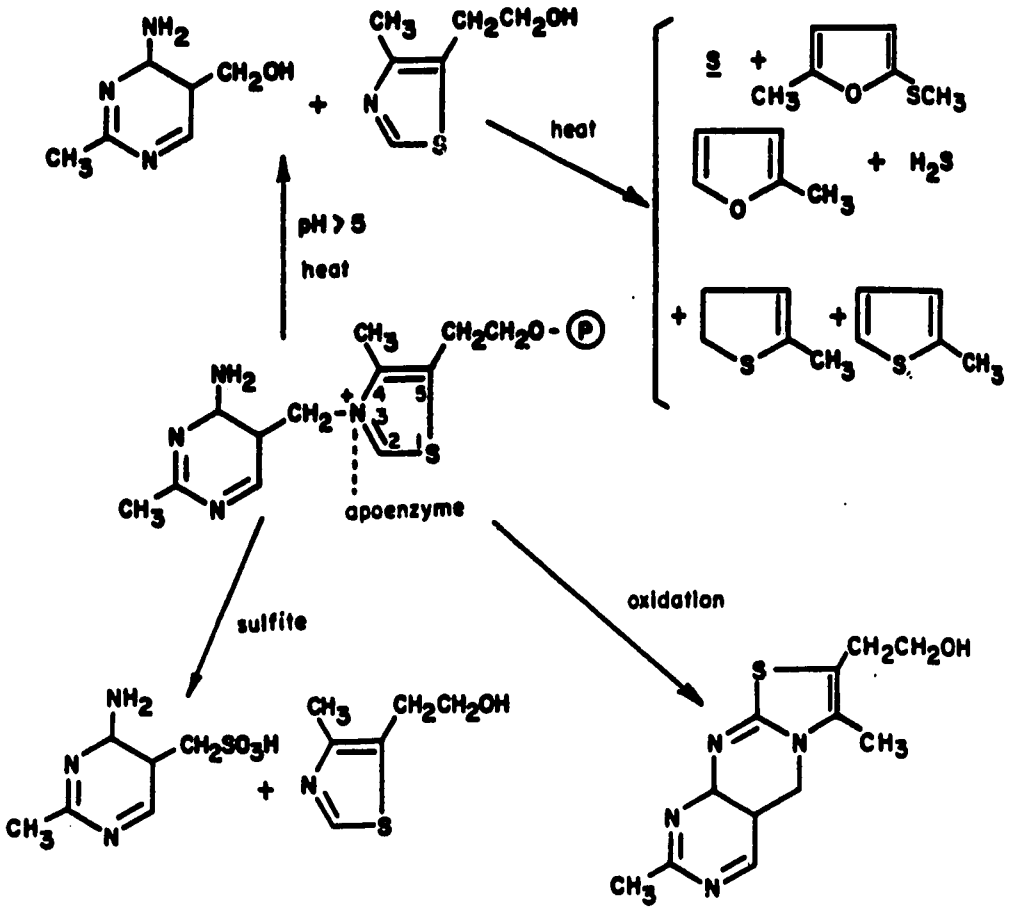


FIGURE 2. Degradation of thiamin

sodium bicarbonate to peas or green beans to better retain the green color or to dried beans to soften the skins can lead to large losses of thiamin during cooking. Similarly, in vegetables and meats that have a pH above 7, greater destruction occurs at higher temperatures than occurs in acidic vegetables and fruits (Farrer, 1955).

Destruction of thiamin by sulfite is also lessened when casein and soluble starch are present (Leichter and Joslyn, 1969). Although the mechanisms of the protective effect are still unclear, it is probable that the protective effect involves other degradative mechanisms. Proteins are known to protect thiamin from thermal degradation. Morfee and Liska (1971) observed reversible bonding between thiamin and protein in a simulated milk system. They also noticed substantial bonding between sulfur-containing breakdown products of thiamin and protein. Under alkaline conditions, some amino acids; i.e., glycine, alpha-alanine, beta-alanine, valine, glutamic acid, etc. have been reported to induce desulfurization of thiamin with the formation of dethiothiamin (Dwivedi and Arnold, 1973). Thiamin also is inactivated by nitrite, possibly via reaction with the amino group on the pyrimidine ring. However, it was noticed very early (Beadle et al., 1943) that this reaction is mitigated in meat products as compared with buffer solutions, which implies a protective effect of protein.

Since thiamin can exist in multiple forms, its stability may depend on the relative concentrations of the various forms. In the relatively few studies that have been conducted, the protein-bound

forms appear to be more stable than the free vitamin. The pH-rate profile for thiamin and cocarboxylase at elevated temperatures are shown in Figure 3. Cocarboxylase is more sensitive than thiamin, but the difference in sensitivity is a function of pH, disappearing completely when the pH is above 7.5, since both the amino group on the pyrimidine ring and the 2 position on the thiazole ring are strongly influenced by pH in the region of interest for thiamin stability, Farrer (1955) suggested that differing relative concentrations of the various forms of the vitamin may account for some discrepancies in the literature regarding thiamin stability.

Labuza and Kamman (1982) investigated contradictory reports on the stability of different salt forms of thiamin and found the explanation to lie with differences in activation energies for destruction of the mononitrate and hydrochloride forms (26.3 vs. 22.4 kcal/mole respectively). This leads to a greater stability for the hydrochloride form above 95°C, and for the mononitrate form below 95°C. Stability of both forms was lower at a water activity of 0.86 than at 0.58. The thiamin hydrochloride is much more soluble and thus generally preferred for liquid application or liquid coating systems. The thiamin mononitrate, although less soluble, is generally preferred for enrichment for dry mixtures because of its stability. Studies in dehydrated corn-soymilk samples indicated that degradation was influenced strongly by moisture content (Bookwalter et al., 1968). For example, storage at 38°C for 182 days caused no loss of thiamin when

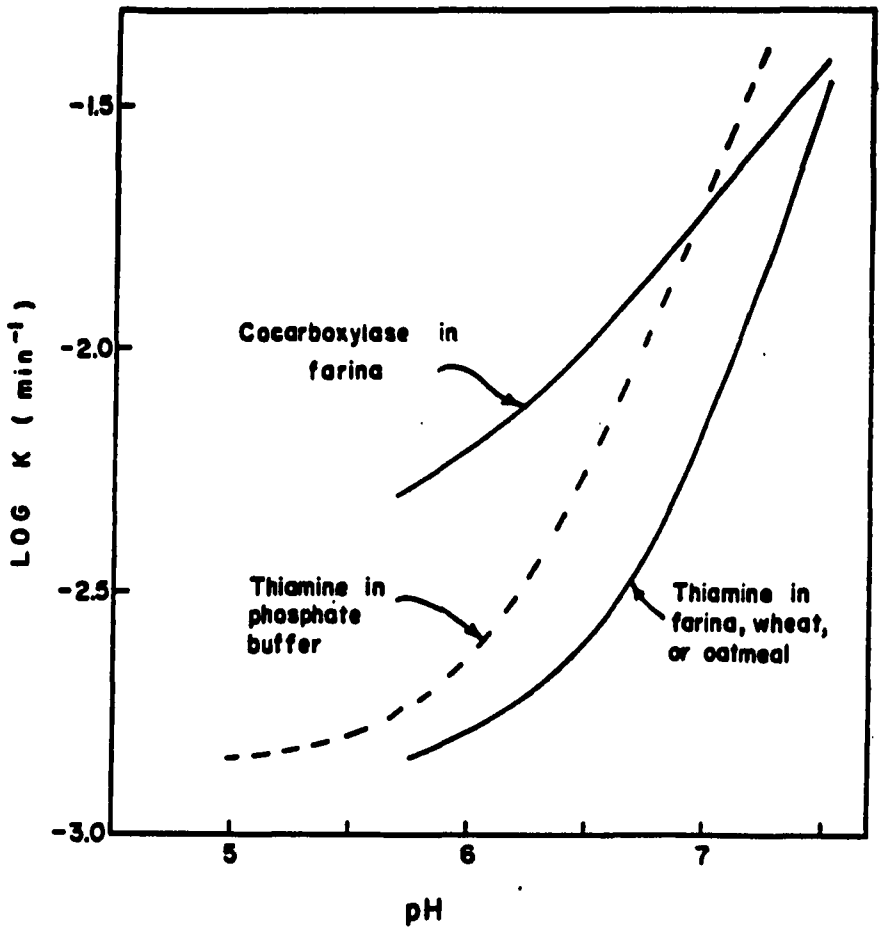


FIGURE 3. The pH stability of thiamin and cocarboxylase (Farrer, 1955)

the system was maintained below 10% moisture content, but extensive loss occurred at 13% moisture. The kind of humectant, which is used for controlling water activity, also affects thiamin loss in the system. The kinetics of thiamin degradation in a model system of pH 6.0 and water activity 0.95 (controlled with NaCl, KCl, glycerol or Na_2SO_4) has been studied by Fernandez et al. (1986). The results showed that the type of solute used to adjust water activity had a dramatic influence on the rate of degradation of thiamin. The loss of vitamin increased in the following order: NaCl > KCl > glycerol > Na_2SO_4.

Extracts from various fish and crustaceans have been found to destroy thiamin (Fujita, 1954). Thiaminase, which is present in low concentration in vegetable and animal products also degrades thiamin (Dwivedi and Arnold, 1973). Thiaminase I (thiamin-base-2-methyl-4-aminopyrimidine-5-methyltransferase) catalyzes the decomposition of thiamin by a base-exchange reaction involving a nucleophilic displacement of the methylene group of the pyrimidine moiety. Thiaminase II (thiamin hydrolase) catalyzes the simple hydrolysis of thiamin into 4-methyl-5-(hydroxyethyl)-thiazole and 2-methyl-4-amino-5-(beta-hydroxymethyl)-thiazole and 2-methyl-4-amino-5-hydroxymethyl-pyrimidine.

The presence of reducing sugars is also an important factor in the loss of thiamin through the mechanism of Maillard type browning reactions (Doyon and Smyrl, 1983).

Temperature dependence of thiamin breakdown

Temperature is the most important factor influencing thiamin stability. As the temperature is increased, thiamin retention decreases sharply. Thus in-container, high temperature-short time methods are not the best thermal processes for thiamin retention in conduction-heated foods, and moreover, each process must be individually optimized (Lund, 1977). The effects of temperature on the rate of chemical reactions were first studied by Van't Hoff in 1884, Hood in 1885, and Arrhenius in 1889. Bunker (1974) reviewed the early history of this development in chemistry, and Labuza (1980) showed some applications of it to the study of food quality losses.

Quality loss for most foods, as found by Labuza (1980), conforms to the following general equations. Decrease of desirable attribute

$$-dA/dt = k(A)^n$$

where dA/dt is the change in quantity of A with time, (A) is the measured amount of the attribute at any time, k is the rate constant in appropriate units, and n is the order of the reaction, generally 0, 1, or 2. Most shelf life data for change in a quality attribute, based on some chemical reaction or microbial growth, follows a zero-order (n=0) or first-order (n=1) pattern. For zero-order data, a linear plot is obtained by using linear coordinates, whereas for first-order data, semilogarithmic coordinates (log A) are needed to produce a linear plot. For second order data, a plot of 1/A versus time produces a linear relationship.

The thermal destruction of thiamin hydrochloride in buffered solutions follows a first order reaction rate (Farrer, 1955; Feliciotti and Esselen, 1957; Goldblith and Tannenbaum, 1966). In food products, however, deviations from first order reaction rates have been observed (Mulley et al., 1975a). In order to accurately study the effects of a heat treatment, it is desirable to obtain nearly instantaneous and uniform heating to the required temperature, a definite holding time at the required temperature, nearly instantaneous cooling to room temperature and to use small-sized containers (Farrer, 1955). But it is clear from much evidence available that the thermal destruction of vitamin B_1 in buffered and food systems can be followed by simple kinetic methods (Mulley et al., 1975c). Also the presence of up to one-third of the thiamin as cocarboxylase will not affect the thermal destruction of thiamin.

Kinetics is a subject in which the rate of a reaction is studied as certain parameters are varied; such as the concentration of reactants, temperature, pressure, pH, etc. The data accumulated is then analysed so as to give certain rate laws, and hence to yield some information about the mechanism of the reaction. The prediction of nutrient losses in foods during storage requires adequate knowledge of the reaction kinetics. Labuza (1972) has demonstrated the application of chemical kinetics to the study of nutrient losses in dehydrated foods. Recently, Kamman et al. (1981) have shown the loss of thiamin in stored pasta due to temperature effects could be predicted by a first order reaction which mathematically follows

$$\ln A_t/A_o = -kt$$

where A_t is concentration at time t, A_o is initial concentration, k is rate constant which depends upon temperature, and t is time. A common procedure for analyzing and reporting data that can be modeled by a first order reaction includes: 1. Calculation of the rate constant (k) by linear regression analysis of the logarithm of retention of concentration versus time. 2. Determination of the Arrhenius activation energy (Ea) using regression analysis of log of rate constant versus reciprocal of absolute temperature (1/T).

The reaction constants for thiamin degradation cannot be extrapolated to other temperatures unless the Arrhenius activation energy is known for the particular system. The adequacy of the Arrhenius model is an indication of an unchanged mode of thiamin degradation at normal and elevated storage temperature (Waletzko and Labuza, 1976). In phosphate buffer at pH 6.8, Goldblith and Tannenbaum (1966) found the activation energy for thiamin degradation to be 22 kcal/mole (92.4 kJ/Mole) for both conventional and microwave heating, which is similar to the value in pureed meats and vegetables found by Feliciotti and Esselen (1957). In using steady state equations, it is assumed that the Arrhenius relationship is followed at all storage temperatures. Data conforming to the Arrhenius equation yield a straight line when log k is plotted versus 1/T. It is evident that food reactions generally conform to the Arrhenius relationship over a

certain intermediate temperature range but that deviations from this relationship can occur at high or low temperature (McWeeny, 1968). In most cases, the use of the Arrhenius equation appears to be the most appropriate method, even when other approximations offer equal accuracy over a limited range of temperatures. The inconvenience of using this nonlinear relation rather than linear approximations over a limited range is readily overcome through use of electronic calculators, graphical methods, and other simple techniques. It should be noted that all equations, including the Arrhenius relation, have limited applicability and the range of validity and the influence of other factors on activation energy must be taken into consideration (Tannenbaum, 1979).

To determine the confidence interval of the rate constants as well as the Arrhenius plot, three statistical approaches have been utilized (Riboh and Labuza, 1982). First the rate constant(k) at each temperature was determined by applying the standard linear regression method on the data. Linear regression was then used on ln k versus 1/T to get the activation energy (Ea). The second method was to use the 95% confidence limits of each k at 3 temperatures and run a linear regression of the 6 points to get a measure of confidence of Ea from the ln k versus 1/T plot. This method was only used to estimate Ea. Thirdly, each data point was considered an independent experiment and extrapolating back to zero time gave a kt value. The population of kt's was used to get an average and the 95% confidence limits. This is called the point-by-point method.

The Arrhenius equation describes the effect of time and temperature treatments on the rate of nutrient destruction. In Table 1, the thermal resistance of food constituents is shown. The activation energy of nutrients and sensory factors is much lower than that of microorganisms. Activation energy is useful to optimize thermal processing conditions to maximize nutritional and quality-factor retention with maximum sterility. A reaction rate which has a higher activation energy is more temperature dependent than one that has a lower activation energy.

The equation proposed by Arrhenius was of the form:

$$d\ln k/dT = Ea/RT$$

where Ea is the activation energy (the difference between the energy of the reactants and that of the highest energy transition state), k is the rate constant, R is the gas constant (8.31 kJ/M), and T is the absolute temperature. If Ea is assumed to be independent of temperature, this equation can be integrated to give the following equation:

$$\ln (k_{T_1}/k_{T_2}) = -Ea/R\ (1/T_1 - 1/T_2)$$

or $$k = A \cdot \exp(-Ea/RT)$$

Kinetic studies indicate that the degradation of thiamin follows first order reaction kinetics, exhibiting a very high activation energy in aqueous solutions (Troller and Christian, 1978).

TABLE 1. Thermal resistances of various food constituents (Lund, 1977)

Constituent	z^a (°F)	Ea^b (kcal/mole)	$D_{121}{}^c$ (min)
Vitamins	45-55	20-30	100-1000
Color, texture, flavor	45-80	10-30	5-500
Enzymes	12-100	12-100	1-10
Vegetative cells	8-12	100-120	0.002-0.02
Spores	12-22	53-83	0.1-5.0

[a]°F temperature change needed to decrease the decimal reduction time 10 times for a known reaction.

[b]Activation energy.

[c]Time for a 90% reduction of the nutrient content at 121°C.

Heat processing of thiamin-containing foods

Compounds produced by thiamin degradation, particularly those from the thermal breakdown of thiamin, may be important contributors to food flavors. Volatile products formed during the heating at 135°C of thiamin in water and propylene glycol were examined by Hartman et al. (1984a,b). They identified carbonyls, furans, thiophens, thiazoles, dioxolanes, and other sulfur-containing compounds which have been of interest in terms of understanding the mechanisms of nutrient loss as well as aroma production. Thermal degradation in acidic conditions most likely takes place by the following reaction:

$$\text{Thiamin} + H_2O = \text{thiazole} + \text{pyrimidine}$$

Although this reaction would be first order with regard to both thiamin and water, it can be shown that the molar concentration of water is several orders of magnitude larger than the molar concentration of thiamin (Arabshahi, 1982). Thiamin reacts readily in Maillard type reactions (Lhöst, 1957). These reactions generally result in the formation of numerous volatile compounds of potential flavor significance. Dwivedi and Arnold (1973) also reported ether-soluble volatile products (hydrogen sulfide, furans, thiophens, and thiazoles) of heated thiamin solutions. Several patents dealing with the production of meat or chicken-like flavors by heating thiamin-containing mixtures have been granted (MacLeod and Seyyedain-Ardebili, 1981; Bidmead et al., 1968; Giacino, 1968).

It is appropriate to consider the action of microwave cooking on the retention of nutrients, especially thiamin, in flesh foods. Thiamin is particularly sensitive to heat and it is conceivable that the rapid heat penetration in microwave cooking might be less destructive than the slower rise in temperature in conventional ovens (Burger and Walter, 1973). However, the differences between the two methods of cooking are not particularly marked and it is pertinent that the conventional methods scored better on palatability (Bender, 1966). Nevertheless it has been reported that thiamin retention was better in frozen meals including beef, chicken, and shrimp dishes reheated in a microwave oven than in freshly prepared food held at 82°C (Kahn and Livingston, 1970).

Heating of muscle tissue brings about extensive changes in its appearance and physical properties and these changes are dependent on the time-temperature conditions imposed. Commercial heating generally has moderately detrimental effects on the vitamin content of meat. Thiamin is sensitive to heat, and it is partially destroyed during cooking or thermal processing. If the center temperature of the cooked meat is increased from 70 to 80°C, the thiamin loss will increase by 40%. This shows how important it is not to overcook the product (Skjoeldebrand et al., 1983). Certain amino acids and thiamin may interact with glucose and/or ribose of the meat (Maillard reaction), and the nutritional value can be impaired when this occurs.

Browning Reactions

Browning reactions between amino groups and reducing sugars

The term "Maillard reaction" is used to characterize a group of chemical reactions involving the amino and carbonyl functions of food components and leading to browning and flavor production. The reaction is named after the French chemist Louis Maillard, who first described the formation of brown pigments or melanoidins when heating a solution of glucose and lysine (Maillard, 1912). Maillard browning is one of the main chemical reactions causing deterioration and shortening the shelf life of intermediate moisture foods (Eichner and Karel, 1972; Flink, 1983).

The minimum reactant requirements for Maillard browning are the presence of an amino group-containing compound, usually a protein, a reducing sugar, and water. By virtue of the amino group on the pyrimidine ring of the thiamin molecule, thiamin reacts strongly in a Maillard-type browning reaction in dry or aqueous products when heated, and this reaction may be an important factor in the loss of thiamin during processing and storage (Dennison et al., 1977). Caramelization is differentiated from the amino-sugar reaction by proposing that caramel formation is due to the pyrolysis of sugars, whereas amino-sugar browning is due to the reaction of an aldehyde group with an amino group. Both reactions ultimately result in the development of brown to black color and the splitting off of water.

The effect of sugar structure on the extent of browning was noted by Maillard (1912). He found that the decrease in the extent to which common sugars brown was in the order D-xylose > L-arabinose > hexoses (D-galactose, D-mannose, D-glucose, and D-fructose) > disaccharides (maltose, lactose, and sucrose). The degree of pigment formation from a particular sugar is directly proportional to the amount of open chain (free carboxyl) sugar in the equilibrium solution (Ellis, 1959).

The Maillard reaction can be divided into 3 stages. First, simple condensation between the free reducing hemi-acetal or hemi-ketal carbonyl group and the amino group occurs; in this stage there is no overt change in product color. Secondly, there is an advanced stage that produces yellow to light brown color formation as well as some flavor compounds. At the final stage, the intermediates formed during previous stages polymerize to form unsaturated, highly colored polymers. During thermal processes, losses of free amino-group-containing-nutrients, especially lysine, frequently occur in foods. These cases are generally believed to result mainly from the formation of sugar-amino Amadori compounds during the Maillard browning reaction (Lee et al., 1984).

Reyes et al. (1982) investigated the reactivity of glucose, fructose, and sucrose with glycine under accelerated storage conditions over an extended reaction period. While fructose initially browned at a faster rate, it was later overtaken by glucose. These results showed that glucose may undergo more browning than fructose during prolonged

reaction times, and that initial reaction rates may not be predictive of eventual product formation. Sucrose was readily hydrolyzed under acidic pH and underwent Maillard browning reactions, its color and appearance being similar to the glucose solutions at the later stages of the experiment. They also showed there was no increase of absorbance in the model system solutions containing only the sugar, indicating that there was no contribution to color formation due to caramelization.

Ketose-amino browning is of a different type than that occuring in aldose-amino systems; firstly, browning is almost linear, and secondly, the rate of disappearance of amino nitrogen decreases almost to zero with time (Spark, 1969). The reaction between ketoses and amino acids was reviewed by Reynolds (1965); typically, fructose and glycine will first form a fructosylglycine, which can undergo the Heyns rearrangement (analogous to the Amadori rearrangement) to give a mixture of 2-glycyl-2-deoxy-D-glucose and 2-glycyl-2-deoxy-D-mannose.

Browning reactions in thiamin-sugar solutions and in meat

When equal weights of glucose and thiamin hydrochloride were thoroughly mixed and heated at 85°C for some days, considerable browning resulted (De Lange and Dekker, 1954). Lhöst (1957) found that thiamin hydrochloride reacts with glucose at pH < 4 to give 2-glucothiamins and other unidentified compounds. Van der Poel (1956) reported that thiamin, when heated in a 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 thiamin during processing (Dwivedi and Arnold, 1973). Influence of reducing sugars (xylose, glucose, and maltose) on the rate of thiamin destruction was studied at 95°C in aqueous solutions buffered to pH 6.75 (Doyon and Smyrl, 1983). The rate of thiamin loss was found to be dependent on both the nature of the reducing sugar and on the concentration of reducing sugar. The order of effect on thiamin breakdown was xylose > glucose > maltose.

Dennison et al. (1977) mentioned that reducing sugars are susceptible to nucleophilic attack by the thiamin molecule by virtue of the amino group on the pyrimidine ring of the thiamin molecule. The rate of condensation of an amino compound with a reducing sugar is dependent on the rate at which the cyclic sugar structure opens to form the acyclic reducible form. The order of the amount of open chain structure is pentose > hexose > reducing disaccharides.

Studies on the mechanism of browning occurring in model and meat systems have been reported (Pearson et al., 1966a). When model systems were heated, browning appeared to be due largely to the amino-sugar reaction, although some brown color development apparently occurred from caramelization of sugars. The contribution of caramelization to brownness was confirmed by blocking the amino group with acylation or the carbonyl group of sugars by addition of bisulfite or hydroxylamine. Although the data with the meat systems were not clear-cut, evidence suggested that most of the browning occurred as a result of the amino-

sugar reaction. However, a small but significant amount of browning seemed to be due to pyrolysis of the natural meat sugars. And the browning of sugars is responsible for the color development that occurs on cooking fresh pork.

Sharp (1962) investigated the influence of equilibrium relative humidity on the browning deterioration of precooked freeze-dried pork. At 37 and 50°C, the browning reaction increased as relative humidity rose to 57%, followed by a decrease as it was further increased to 70%. The influence of temperature and moisture content on the browning reaction during processing and storage has been extensively studied. However, many of these studies have not utilized a kinetic approach when evaluating the deleterious effects of the browning reaction on various food systems. By analyzing the influence of water content or water activity on the kinetic parameters of the browning reaction as demonstrated in various studies, a better understanding of the role of water in the Maillard reaction should result. An understanding of this role would be useful in controlling and predicting the shelf life of susceptible products (Labuza and Saltmarch, 1981).

Water Activity

Definition

Lewis and Randall (1961) defined the term "activity" as the ratio of fugacity, f, of a substance in a given state, and its fugacity in a standard state at the same temperature.

$$\text{Water activity } (a_w) = P/P_o = ERH/100 = N = n_1/n_1 + n_2$$

where P = partial pressure of water above the sample

P_o = vapor pressure of pure water at the same temperature

ERH = equilibrium relative humidity of the atmosphere as a percent

N = mole fraction of water

n_1 = moles of solvent

n_2 = moles of solute

The expression, $a_w=P/P_o$, is an approximation of the original activity expression of Lewis, $a_w=f/f_o$, where f is the fugacity of solvent and f_o is the fugacity of the pure solvent. At ambient pressure, the difference between f/f_o and P/P_o is so small that defining a_w in terms of P and P_o is clearly justifiable. Water activity is also related to several other terms, and these interrelations are useful. Water activity is a better indication than water content of potential for microbial growth and of stability of sensory attributes of meat and meat products. The water activity of fresh meat and several meat products is shown in Table 2. The most easily removable or least bound water molecules behave essentially like pure water ($a_w=1.0$).

Water activity determination

Water activity can be measured by several methods, (1) hygrometric method (hair hygrometer, electronic hygrometer), (2) manometric method, (3) dew point method, (4) freezing point depression method, and (5)

TABLE 2. Water activity of meats and meat products (Leistner and Rödel, 1975)

Product	a_w		
	Min.	Max.	Avg.
Fresh meat	0.98	0.99	0.99
Bologna type sausage	0.93	0.98	0.97
Liver sausage	0.95	0.97	0.96
Blood sausage	0.93	0.97	0.96
Fermented sausage	0.72	0.95	0.91
Raw ham	0.88	0.96	0.92
Dried beef	0.86	0.94	0.90

graphical interpolation of moisture adsorption and desorption method. Although numerous methods have been devised in this field, Rockland and Nishi (1980) concluded that none is totally satisfactory. The exact water activity value of saturated salt solutions is applied for developing specific water activity of samples. Greenspan (1977) of the National Bureau of Standards published a compilation of water activities for saturated solutions of salts covering relative humidity from 30 to 98% and temperatures from 0 to 110°C. Electric hygrometers are commonly used to measure water activity of food. These devices contain sensors impregnated with salts. Water adsorbs on the sensor and causes a change in electrical resistance which is measured by a Wheastone bridge.

Two basic types of electric hygrometers are used in food-related applications. The first is based on the measurement of conductivity or resistance of an hygroscopic salt in equilibrium with an ambient atmosphere. As water is adsorbed or desorbed by the salt, its ability to carry current is measurably altered. The second type has been referred to as an electrolytic hygrometer. The operation of this instrument requires that an alternating current be passed through a saturated LiCl solution suspended onto an inert carrier such as glass wool.

Humectants

Three general classes of chemical compounds are currently in use by the food industry as humectants: the polyols, sugars, and salts. The polyols are the most desirable from a moisture sorption standpoint because of their low molecular weight and in some cases the fact that they are liquids (Sloan and Labuza, 1975). Propylene glycol, glycerol, polyethylene glycol-400 are the most commonly used and probably the most desirable polyols for food use. Sodium chloride and sucrose have been the most widely used humectants to reduce the water activity of food products. On a mole-for-mole basis, NaCl is the more effective, but the taste of sucrose is more compatible with many food flavors. In a typical intermediate moisture food, pet food, sucrose is the primary solute, although small amounts of glycerol, propylene glycol, and NaCl also may be added. Sodium chloride is used mainly to reduce water activity in meat products for human consumption, such as salt cured hams, bacon, and some types of sausages. Glycerol has the advantages of being soluble, relatively stable, and nonvolatile. Propylene glycol has received limited application, because its safety is not established completely. This compound has the advantage that it possesses intrinsic antimicrobial properties in addition to its activity as a humectant. However it may be toxic at concentrations required to achieve the desired effect on a_w. Many of the above compounds produce a significant depression of food water activity only at concentrations above their flavor threshold. To overcome this difficulty, combinations of solutes may be employed.

According to Fernandez et al. (1986), the type of solute used to adjust a_w to 0.95 has a great influence on rate constants for thiamin degradation. The order was always the same; NaCl > KCl > glycerol > Na_2SO_4, i.e., NaCl has the most deleterious affect on thiamin retention. KCl, NaCl, and glycerol are considered potential solutes to control a_w in foods. However, univalent electrolytes such as NaCl and KCl greatly promoted the degradation of thiamin as compared with the nonelectrolyte glycerol.

Effect of water activity on thiamin breakdown

The effect of water on the rate of chemical reactions is important not only because of the abundance of water but also because water can perform any of the following functions: 1. Water is usually the primary solvent in foods. 2. Water may be a reactant in a hydrolytic reaction or a product of the reaction in a condensation reaction. 3. Water may influence the activities of catalysts or inhibitors (Arabshahi, 1982). Requirements in many countries for the vitamin supplementation of wheat flour have attracted much attention to the stability of thiamin in such products. Extra vitamin must often be added to compensate for losses occurring during extended storage under a variety of conditions. Hollenbeck and Obermeyer (1952) demonstrated that the type of thiamin salt strongly influences the extent of vitamin loss. In these studies, thiamin chloride hydrochloride and thiamin mononitrate were added to flour equilibrated to various moisture levels. After 4 months' storage, vitamin loss was greater at 38°C than

at 28°C. Increases in flour moisture from 9.2 to 14.5% resulted in increased loss of both forms of thiamin at 38°C, especially the hydrochloride.

Several investigators have reported that moisture content or water activity influences the thermal stability of thiamin in food (Dennison et al. 1977; Farrer, 1955). In general, an increase in a_w decreases thermal stability of thiamin in dry and dehydrated food. However, there are several instances at intermediate or high a_w, where this generalization may not be valid. Rice et al. (1944) studied stability of thiamin in dehydrated pork and canned pork, and reported that degradation of thiamin is proportional to water content in the 0 to 6% range, but canned pork (55% water) was more stable than dehydrated pork (2% water). It is also interesting to note that the addition of 5% NaCl did not stabilize the thiamin content appreciably. Labuza (1972) suggested that this phenomenon may possibly be explained by the presence of a maximum rate of degradation at some moisture level. Dennison et al. (1977) reported the rate of thiamin degradation in a model system at 45°C as a function of water activity at 0.1, 0.24, 0.4, 0.5, and 0.65. The rate appears to be maximum at a water activity between 0.4 and 0.65. Arabshahi (1982) showed that an increase in water activity from 0.65 to 0.85 resulted in a small decrease in rate of thiamin degradation. Warmbier et al. (1976) found that the water activity at which maximal browning occurs was reduced from the 0.75-0.65 a_w range to 0.59-0.40 a_w when glycerol was used to adjust the

a_w of a foodlike model system. They attributed this downward shift to the liquid, waterlike properties of glycerol. But much additional work remains before the initiation and extent of browning in foods as a function of a_w can be predicted and ultimately controlled.

Eichner and Karel (1972) and Warmbier et al.(1976) found that both liquid and solid systems containing glycerol had nonenzymatic browning rate maximas in the a_w range 0.41-0.55. They concluded that glycerol can influence the rate of browning at lower a_w values by acting as an aqueous solvent and thereby allowing reactant mobility at much lower moisture values than would be expected for water alone. However, as a_w increases, the water acts to decrease the browning rate by the mass action effect. The overall effect of glycerol or other liquid humectants on the maximum for nonenzymatic browning is to shift it to a lower a_w (Labuza and Saltmarch, 1981).

Assay of Thiamin

Available methods for analyzing thiamin can be classified as animal bioassay, microbiological methods, and chemical and physical methods. Animal assays are usually rat bioassays which are time consuming, expensive and the results vary considerably. But they are important in the determination of physiologically available thiamin. Microbiological methods are less time consuming, less expensive, and yield more reproducible results than the animal assays. The main disadvantage of microbiological methods is the tendency for the

products of thiamin degradation or other substances to respond in the same way as thiamin. There are various chemical and physical methods such as; fluorometric, colorimetric, spectrophotometric, polarographic, gravimetric, and volumetric titration methods. Among those, the thiochrome fluorometric method is most widely applicable to food products. Generally, this method consists of the following steps: (1) extraction, (2) purification, (3) conversion to thiochrome, (4) separation of thiochrome solution, (5) measurement of thiochrome, (6) preparation of blank, and (7) calculation (Assoc. of Vit. Chem. 1966).

In recent years, high performance liquid chromatography (HPLC) has been developed to increase the specificity and to shorten the time for analysis (Toma and Tabekhia, 1979; Ang and Moseley, 1980; Kamman et al., 1980). According to the results of researchers, HPLC has an advantage in reducing analysis time, and the results are not significantly different from those of the semi-automatic AOAC method.

HPLC has been used increasingly for the separation and determination of nonvolatile compounds. It has an advantage over some chemical methods in its specific resolution characteristics to differentiate some closely related chemicals. A comparative study was carried out on rice and rice products for chemical evaluation of three water soluble vitamins namely, thiamin, riboflavin and niacin by using two different methods of determination (HPLC versus wet chemistry procedures). The HPLC method proved to be rapid and accurate. Statistical data showed no significant differences between the two

methods of determination for thiamin and riboflavin (Toma and Tabekhia, 1979). A technique utilizing HPLC was developed for analysis of thiamin and riboflavin in enriched and fortified foods (Kamman et al., 1980). The vitamins were extracted from cereals and processed cereal products and simultaneously assayed by reverse-phase HPLC. The developed technique was compared to the semi-automatic modification of the AOAC method for seven cereal products. There was no statistical difference found in the values obtained from the two procedures. Augustin (1984) mentioned that the LC methods have the distinct advantages over the manual AOAC methods of eliminating the lengthy ion-exchange purification step and allowing the simultaneous determination of thiamin and riboflavin. The LC procedure, however, requires the lengthy de-esterificaton process of thiamin phosphate esters. But this system has not yet been successfully applied to food matrices, nor to the simultaneous determination of riboflavin.

An automated method for the determination of thiamin in milk and food products was described by Kirk (1974). This procedure involves the oxidation of thiamin to thiochrome, extraction into isobutanol, and fluorescence measurement. The automated procedure gave a mean recovery value of 92.7% and a standard deviation of ±0.7%, compared to 83.3±2.5% for the manual procedure. The procedure is simple, rapid, and accurate, and markedly shortens the average time for analysis.

MATERIALS AND METHODS

Preparation of Model Systems

Model systems were designed and prepared to simulate certain characteristics of pork muscle. These model systems had simple compositions to facilitate the analysis of thiamin. The model systems were prepared based on citrate-phosphate buffer solution prepared as outlined in Table 3 to represent foods ranging from neutral to a somewhat acidic pH's. Water activity was controlled by adding glycerol, approximately 38 g per 100 ml, the buffer solution to get a_w=0.90. Water activity of the model systems was checked by using Rotronic hygroscop manufactured by Rotronic. After adjusting water activity, the pH of each solution was adjusted again using 0.1 N HCl or 0.1 N NaOH to get the exact pH. In model system 1, thiamin hydrochloride stock solution (1 mg/ml) was added to obtain a final concentration of 10 μg thiamin-HCl/ml, which is similar to the level in pork.

To study the effect of sugars on thiamin retention, 4 different sugars, i.e.; glucose, sucrose, fructose, or xylose were added to give a final concentration of 2.5 mg/ml. Model system 2 is the same as model 1, except that the concentrations of thiamin and sugars were twice those of model 1.

Ten ml of each solution was tightly sealed in 50 ml screw-capped test tubes and the tubes were placed in a water bath controlled at

TABLE 3. Buffer solution

	pH5	pH6	pH7
A: 0.1M solution of citric acid (21.0g of citric acid monohydrate/ℓ)	24.3	17.9	6.5
B: 0.2M solution of dibasic sodium phosphate (28.4g of Na_2HPO_4/ℓ)	25.7	32.1	43.5
Final volume (w/ d-H_2O)	100	100	100

75±0.1°C, 85±0.1°C, or 95±0.3°C. At intervals, 2 aliquots of samples were removed and immediately cooled in tap water. Samples were kept at 4°C until thiamin analysis was done, which was always within 24 hours.

Preparation of Pork Samples

Pork psoas major muscles, purchased from a local supermarket, were prepared by removing connective tissue and external fat from the muscles, which were ground in an Oster food grinder. Immediately following grinding and mixing, glucose or fructose was incorporated to give a final sugar concentration of 2.4 g/100 g pork, and mixed thoroughly. Ten grams of prepared muscle samples were heat treated the same as the model systems.

Thiamin Determination Method

Thiamin determination followed the thiochrome assay method outlined by the Association of Vitamin Chemists, Inc. Methods of Vitamin Assay (1966) (Figure 4). This method is based on the fact that thiamin in an alkaline medium will be oxidized to thiochrome with the aid of potassium ferricyanide, which can be quantitatively determined through its strong blue fluorescence.

Size reduction of the sample before extraction is important. Since thiamin is easily decomposed in alkaline or neutral solution, it is extracted in dilute acid 0.1 N H_2SO_4. Digestion with takadiastase which is an enzyme potent in diastatic and phosphorolytic activity is a

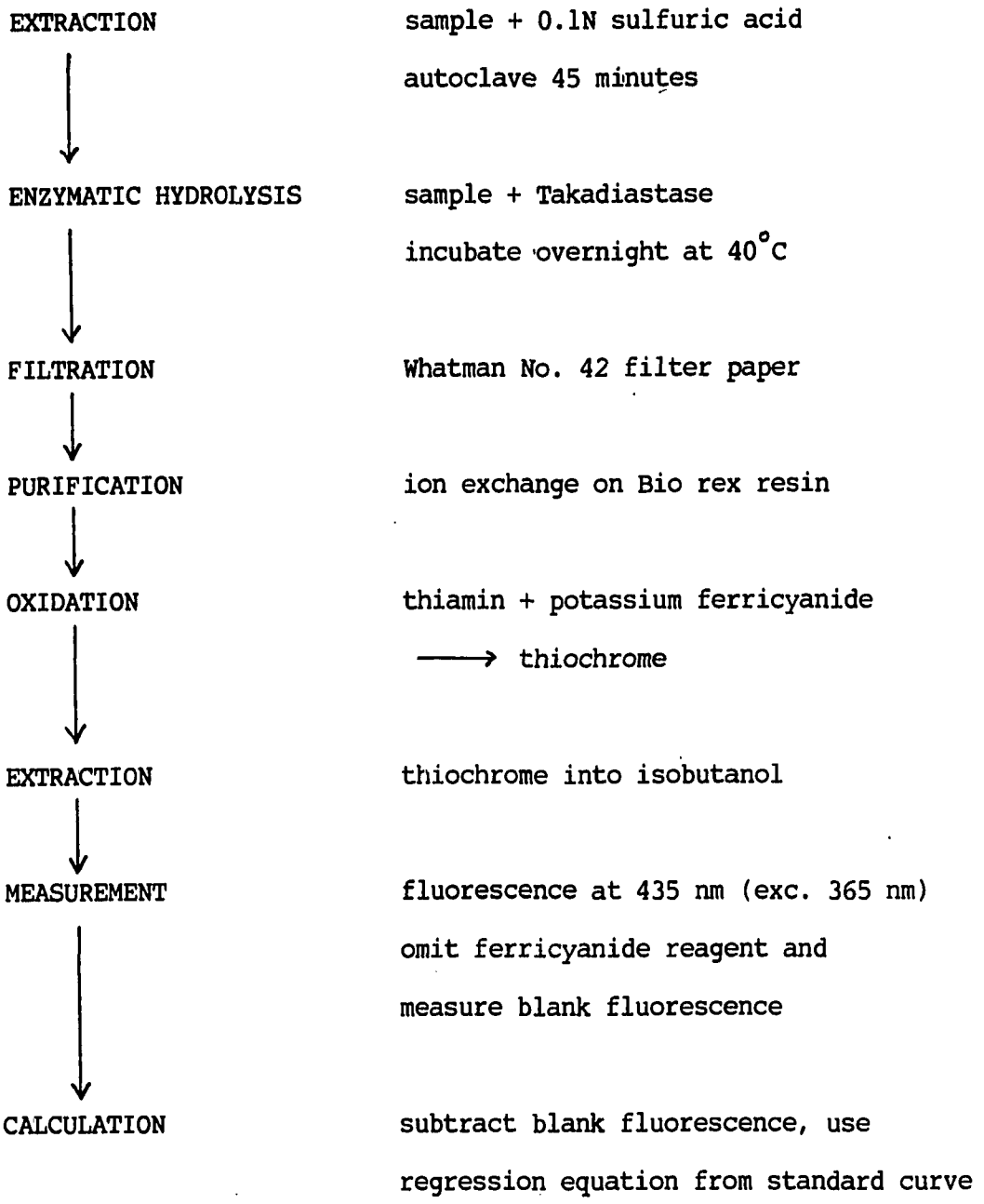


FIGURE 4. Thiamin analysis

necessary step because the thiamin phosphoric ester form is the major form of thiamin in meat and it is not available for analysis without liberating. The phosphatase hydrolyzes esters of thiamin, while the diastase facilitates the liberation of the vitamin from starchy products. After enzyme treatment, the sample extract was filtered with No. 42 Whatman filter paper and purified, using activated Bio-Rex 70 resin sodium form, 50-100 Mesh size, which was purchased from Bio-Rad Laboratories. Because thiamin is cationic, a cationic exchange resin separates undegraded thiamin from noncationic degradation products. Resins activated with 2 N hydrochloric acid and neutralized with deionized water several times can be very efficient cation exchangers and retain thiamin. After anions and other uncharged substances have passed through the column, it was washed, and thiamin was removed by exchange for potassium ions by regenerating the column with a hot, acidic potassium chloride solution. The results of column recovery tests were in the range of 95 to 97%.

To the purified thiamin solution, the oxidation solution consisting of potassium ferricyanide and sodium hydroxide was added. The oxidized reaction product, thiochrome was transferred to isobutanol, and the fluorescence was measured by using a Turner digital filter fluorometer model 112. The primary filter used was No. 7-60 with a dominant wavelength at 360 nm, and the secondary filter was a combination of No. 2-A and No. 47-B, with a dominant wavelength at 435 nm. To use the fluorometer for thiochrome measurement, it first must

be adjusted to be zero percent transmission with a black blanking rod, and samples then are read against the sample blank in the cuvets.

To govern the reproducibility of the fluorometer measurements, we occasionally checked the fluorescence of 1.0 μg/ml quinine sulfate solution dissolved in 0.1 N H_2SO_4. The standard curve equation of thiamin solution is shown in Table 4. To get this standard curve equation, linear regression was used as a statistical method for finding a straight line that best fits the sets of data pairs, thus providing a relationship between two variables: fluorescence and thiamin concentration. After a group of data pairs had been accumulated, we also calculated the correlation coefficients (r) from the linear equation $y = Ax + B$ by using the least squares method. The correlation coefficient of this linear equation was 0.9944.

For a test of thiamin recovery, about 10 μg thiamin hydrochloride/g pork was added to a meat sample, and the recovery ratio was found to be 91.4±1.9% (Table 5).

Determination of Reducing Sugars in Pork Muscle

(AOAC, 1984, pp. 24.075-24.077)

Finely ground and thoroughly mixed pork muscle (10g) was weighed into centrifuge bottles, and fat was extracted with petroleum ether after filtering. We added zinc acetate solution and potassium ferricyanide solution as precipitating agents and added hot 1.5 N HCl to melt adhering fat and to free starch of extracted solution, then

TABLE 4. Standard curve equation of thiochrome fluorescence

Thiamin conc.	(10 × sensitivity)
Blank	0.5 ± 0.1
2.5 μg/ml	25.9 ± 1.2
5.0 μg/ml	37.6 ± 2.1
7.5 μg/ml	64.1 ± 4.0
10.0 μg/ml	87.4 ± 3.7

$$y = 0.1166\,x - 0.0267$$

$$r = 0.9945$$

x: fluorescence

y: thiamin concentration

r: correlation coefficient value

TABLE 5. Recovery of added thiamin from ground pork

Amount of thiamin (μg/g)			Recovery[a]
Average in pork	Added	Total found	(%)
9.66±0.57	10.0	18.8	91.4
	10.0	19.0	93.4
	10.1	18.7	89.5
			Avg. 91.4

[a]% Recovery = {(total found - average amount) / amount added} × 100.

hydrolyzed for 1.5 hour in a boiling water bath. After cooling and filtering the above solution, we added Fehling's solution, which is made of copper sulfate solution and alkaline Rochelle salt; boiled for 2 minutes and added potassium iodide solution and titrated reducing sugars with $Na_2S_2O_3$ solution in the presence of starch indicator. This method is based on the stoichiometric reaction in which two equivalents of S_2O_3 correspond to one equivalent of I_2.

Moisture and Lipid Analysis of Pork Muscle

(AOAC, 1984, pp. 24.002 and 24.005)

Moisture was determined as the mean weight loss of 5 g pork samples after vacuum oven drying for 4 hours at 80°C. Fat content was determined as the weight loss of separate dried samples after extraction with petroleum ether for 4 hours in a Goldfisch apparatus. The temperature was adjusted to allow 3 to 4 drops of ether per second to drip through the sample.

Kinetics

At specified intervals during heat treatment, the remaining thiamin concentrations were measured. Semilog plots of thiamin retention against heating time were linear, thus indicating first order kinetics for the degradation of thiamin. Statistical analyses were performed by using the Statistical Analysis System (SAS). Rate constants were determined after application of linear regression to

determine the best-fit regression lines based on a first order rate equation:

$$k = (2.303/t) \log (A_o/A_t)$$

where k: rate constant

t: heating time

A_o: beginning thiamin concentration

A_t: remaining thiamin concentration at time t

The standard error in the four replications and also the coefficient of determination were calculated at each reaction rate constant (k) in every case. From the k value, half life ($t_{1/2}$) was calculated according to the equation:

$$t_{1/2} = 0.693/k.$$

Temperature dependence of the thiamin retention was evaluated by Arrhenius activation energy, which was calculated by plotting the logarithm of the reaction rate constant (k) against the reciprocal of the absolute temperature (1/T) at which the reaction rate constant had been measured. Linear regression analysis was applied to this plot to determine the best-fit regression line. The 95% confidence interval of activation energy also was calculated. The activation energy describes the effect of time/temperature treatments on the rate and extent of nutrient destruction and assesses the sensitivity of the nutrient to heat treatment. The Arrhenius equation follows:

$$\ln (k_2/k_1) = Ea/R \ (1/T_1 - 1/T_2)$$

where ln: natural logarithm

k_1: rate constant at absolute temperature T_1

k_2: rate constant at absolute temperature T_2

Ea: activation energy (J/M)

R: ideal gas constant (8.31 J/M°K)

RESULTS AND DISCUSSION

Composition of Model Systems

Model system parameters of pH, thiamin and reducing sugar concentrations were chosen to approximate values in raw pork muscle. The pork muscle used in the kinetic studies was the psoas major muscle. This muscle was chosen because it could be readily identified and was easily obtained, free of bone and external fat. According to Agriculture Handbook 8-10 (USDA, 1983) pork tenderloin (psoas major) muscle contains 0.974 mg thiamin per 100 g. The number of tenderloin samples analyzed by the USDA was not specified. A mean value of 0.856 mg per 100 g was reported for 52 samples of raw top loin muscle. A sample of psoas major muscle obtained from a local supermarket was found to contain nearly the same quantity of thiamin reported in Handbook 8-10 for this muscle (Table 6). Thus the level of thiamin in a system designated model 1 was set at 10 μg/ml. A system designated model 2 contained twice this level of thiamin.

Pearson et al. (1966b) analyzed pork muscle for reducing sugar content and used meat slurries and model systems to study the effect of sugar content on browning during heating. Meat slurries contained 277 mg% of reducing sugar, expressed as glucose. The meat sample analyzed in this study contained 2.41 mg reducing sugar per gram. Reducing sugar level in model 1 was set at 2.5 mg/g and in model 2, at 5.0 mg/g.

TABLE 6. Analysis of ground pork psoas major muscle

Moisture	73.60 ± 0.46 (%)[a]
Crude lipid	3.77 ± 0.34 (%)[b]
Reducing sugar	2.41 ± 0.66 (mg/g)[c]
Thiamin	9.66 ± 0.57 (μg/g)[d]

[a]Vacuum oven method: AOAC 24.002.

[b]Goldfish extraction: AOAC 24.005.

[c]Starch in meat - titrimetric method: AOAC 24.075 - 24.077.

[d]Fluorometric method: AOAC 43.024 - 43.030.

The ultimate pH of pork muscle can vary from about 5.3 to 6.8, but a pH of about 5.5-5.7 is typical of normal pork after 24 hours postmortem (Forrest et al., 1975). Model 1 samples were adjusted to pH 5, 6 and 7 to cover the range of pH values that might be expected in fresh pork. Model 2 was studied only at pH 5 and 7.

Water activity of raw meat is 0.98 to 0.99 (Leistner and Rödel, 1975). Samples at pH 5 and 7 were studied both at an unadjusted water activity (1.0) and at a_w=0.9. Model 1 at pH 6 was studied only at a_w=1.0.

Thiamin Retention during Thermal Processing

Kinetic analysis of thiamin retention

In recent years, there has been increased interest in the application of kinetics to the loss of quality in foods during storage and processing. The time-dependence for the majority of losses in foods appears to be described by zero or first order models (Lenz and Lund, 1980). By using a kinetic model, the rate constant can be calculated for each of several processing temperatures. Then these rate constants are generally used in the Arrhenius equation to calculate an activation energy for the reaction. The important facet in kinetic analysis of nutrient changes in foods is confirmation of the kinetic model best describing the change.

In our study, a semilog plot of thiamin retention versus heating time (hours) at 95, 85, and 75°C, for model systems and ground pork,

gave straight lines in all cases investigated, thus indicating first order kinetics for degradation of thiamin (Figure 5-Figure 11). Also, the presence of glycerol (which was used to reduce water activity to 0.9) and reducing sugars or sucrose did not affect the kinetic order of thermal thiamin destruction. Tables 7 and 8 show the reaction rate constant (k), half-life ($t_{1/2}$), decimal reduction time (D), and coefficient of determination(r^2) for thiamin loss in systems subjected to different combinations of temperature, pH, water activity, thiamin concentration, and sugar content. We can see the dramatic temperature effect on reaction rate in every system, i.e., as the temperature is increased, thiamin retention decreases sharply.

The high coefficients of determination (r^2=0.95-0.99) in Tables 7 and 8 show that the thermal destruction of thiamin in phosphate buffer systems and ground pork follows a first order kinetic model quite well. First order reaction behavior also has been reported for thiamin and other vitamin degradation in foods and model systems under different conditions by a number of workers (Goldblith and Tannenbaum, 1966; Mulley et al., 1975a, b, c; and Arabshahi, 1982). One of the reasons for very high coefficients of determination is that we used raw data instead of relative retention or percent retention in calculating the reaction rate constants. Because there can be variability in the initial concentration, it is not appropriate to divide all observations by the average value of the measured initial concentration. According to Mulley et al. (1975a), deviations from first order reactions have

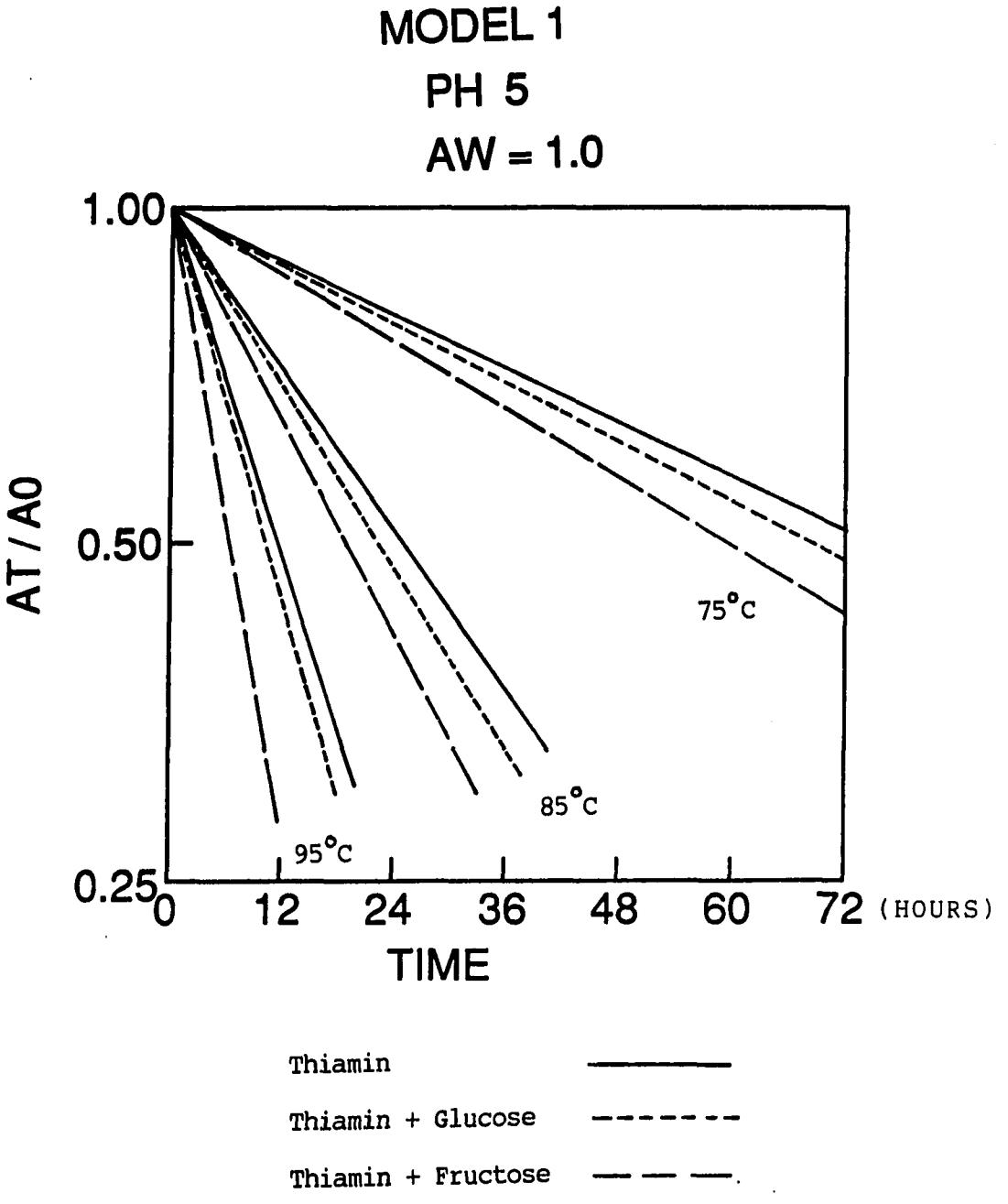


FIGURE 5. Effect of heating temperature and reducing sugar on thiamin retention (model 1, pH 5, and a_w 1.0)

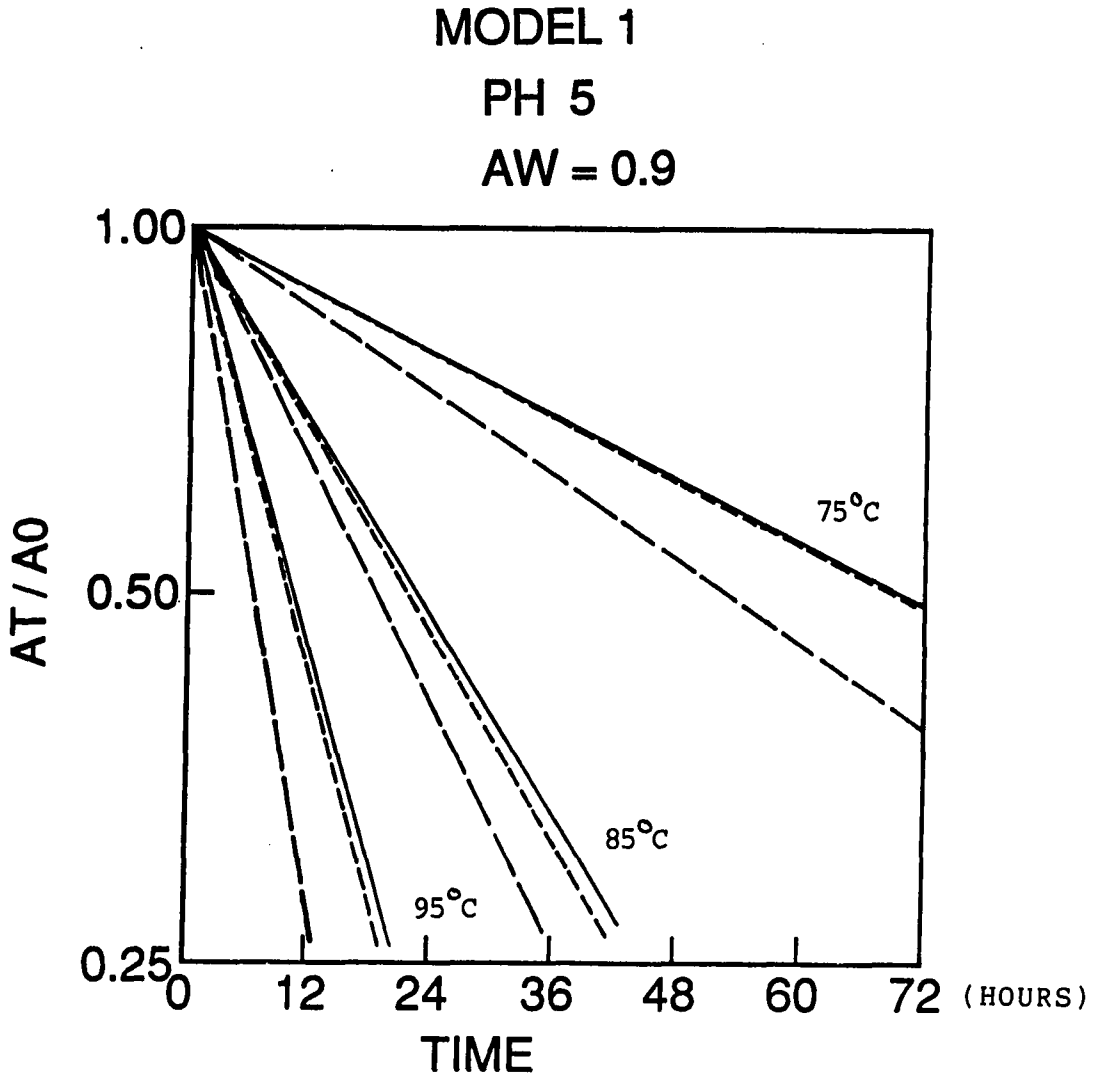


FIGURE 6. Effect of heating temperature and reducing sugar on thiamin retention (model 1, pH 5, and a_w 0.9)

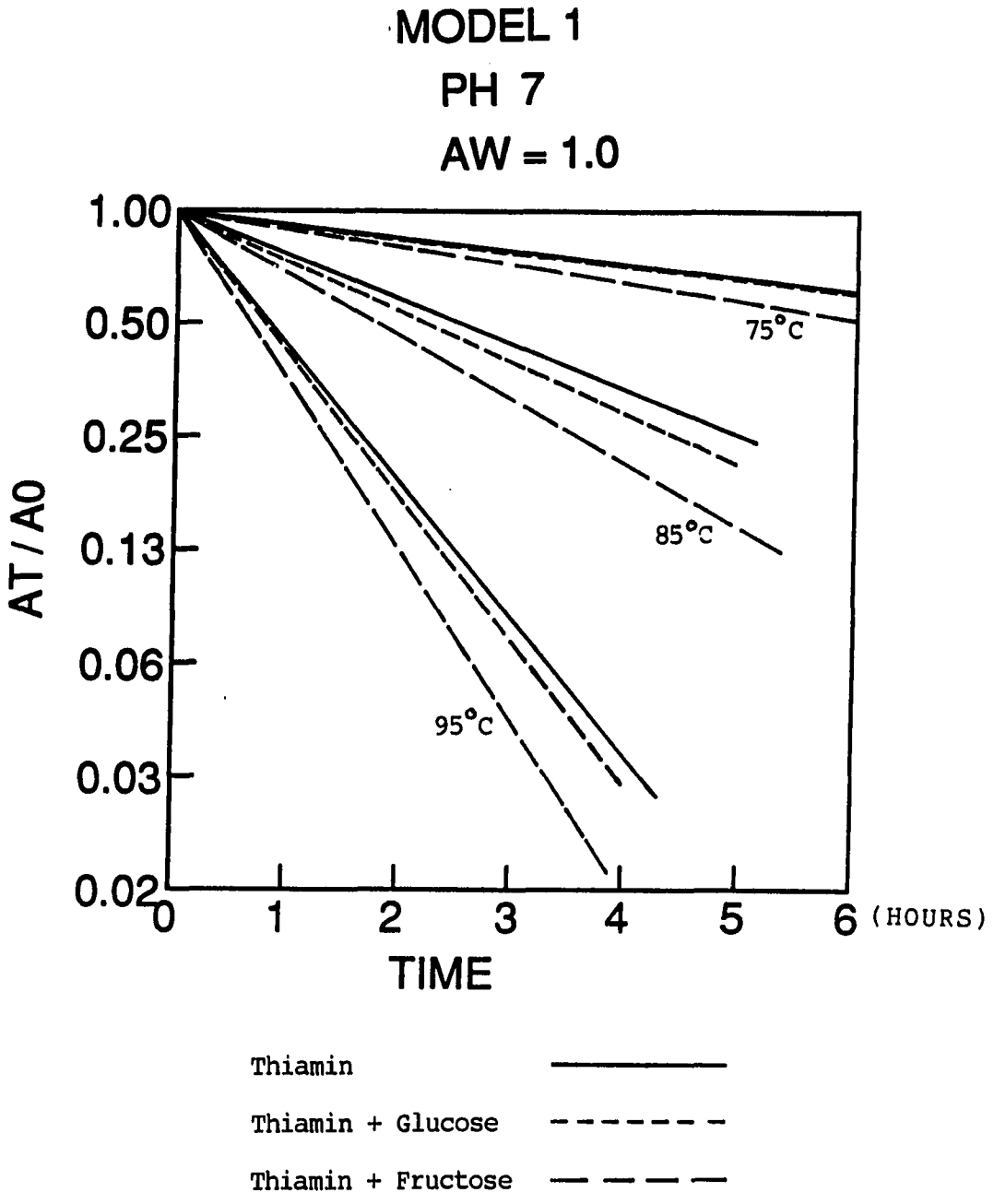


FIGURE 7. Effect of heating temperature and reducing sugar on thiamin retention (model 1, pH 7, and a_w 1.0)

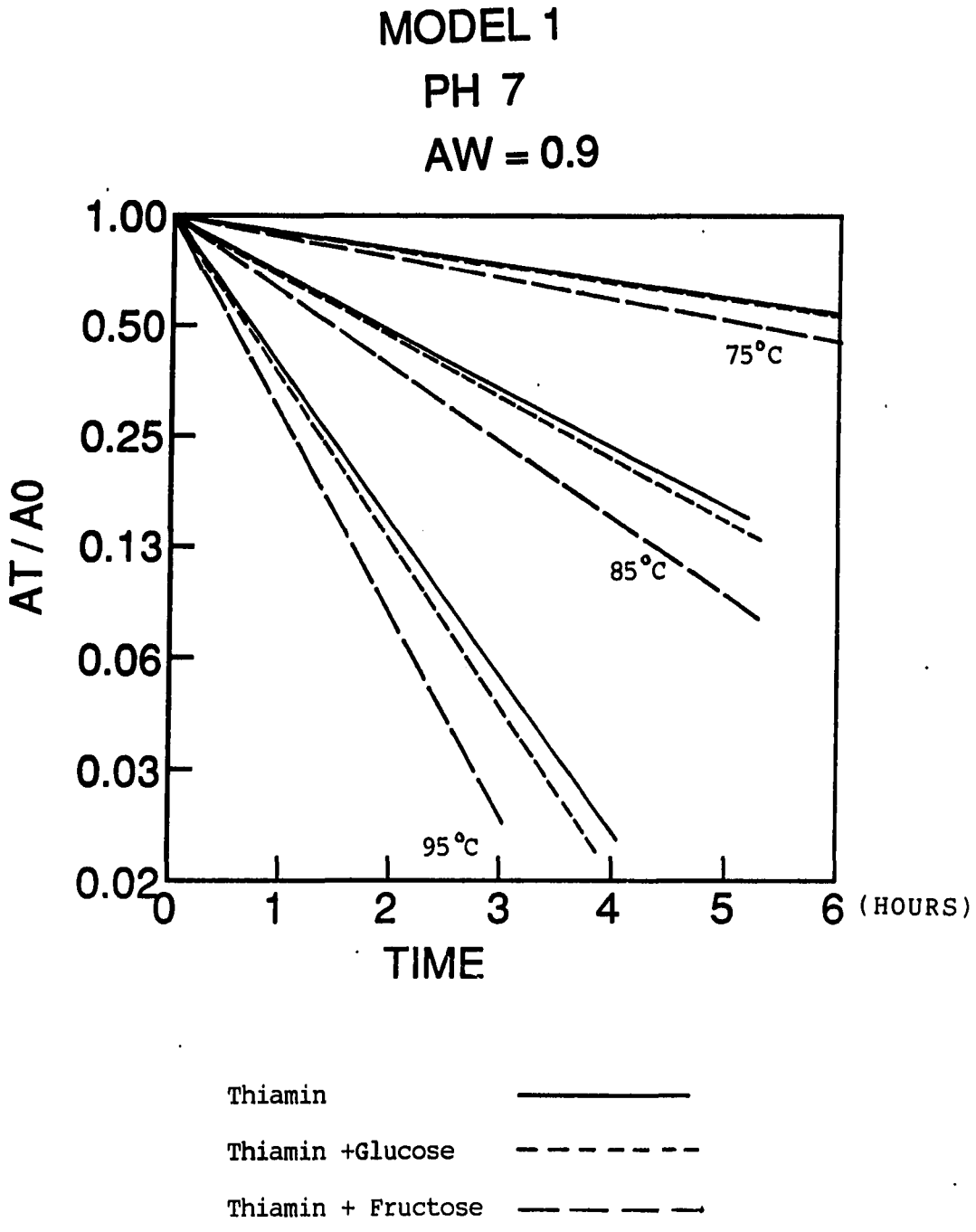


FIGURE 8. Effect of heating temperature and reducing sugar on thiamin retention (model 1, pH 7, and a_w 0.9)

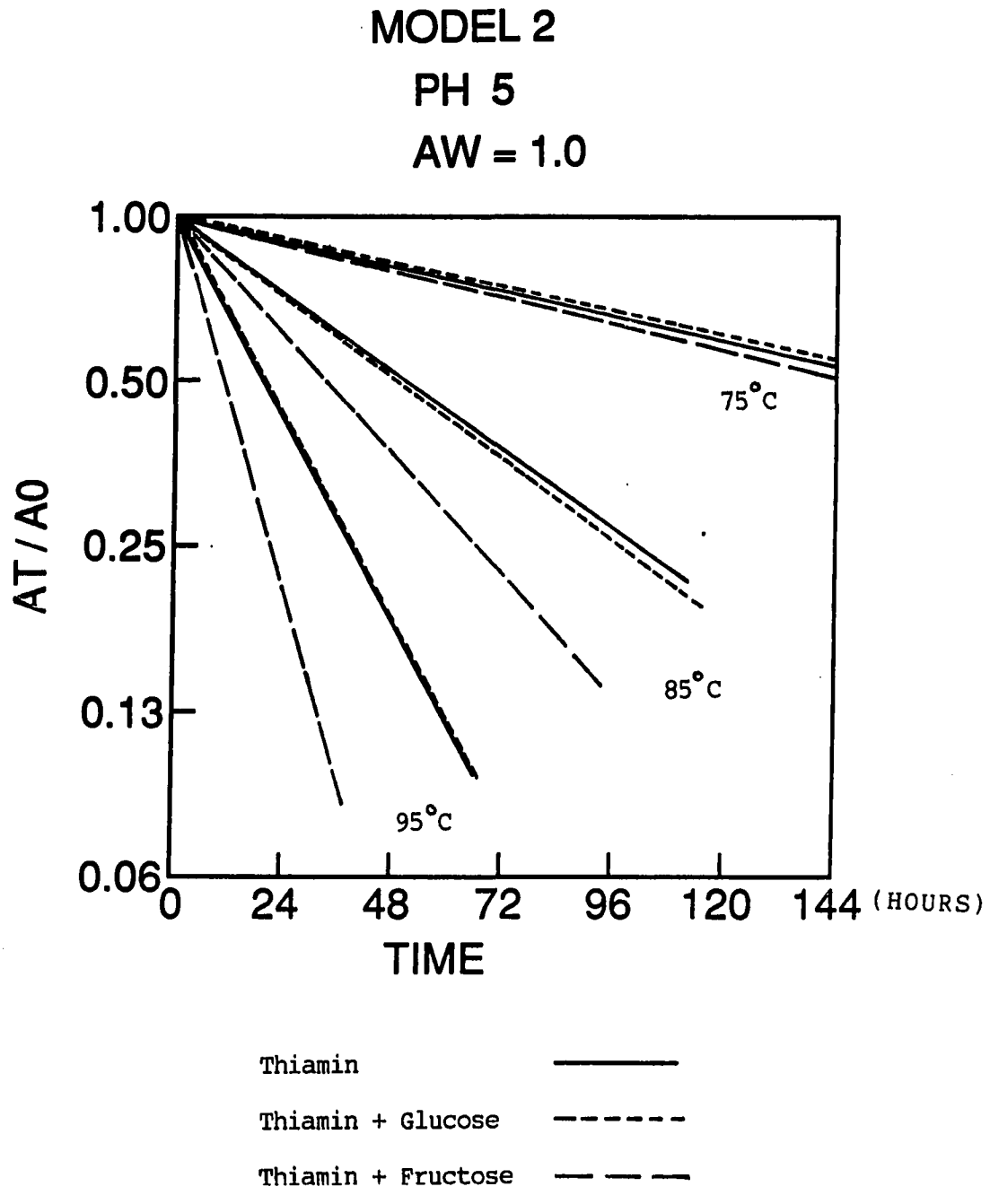


FIGURE 9. Effect of heating temperature and reducing sugar on thiamin retention (model 2, pH 5, and a_w 1.0)

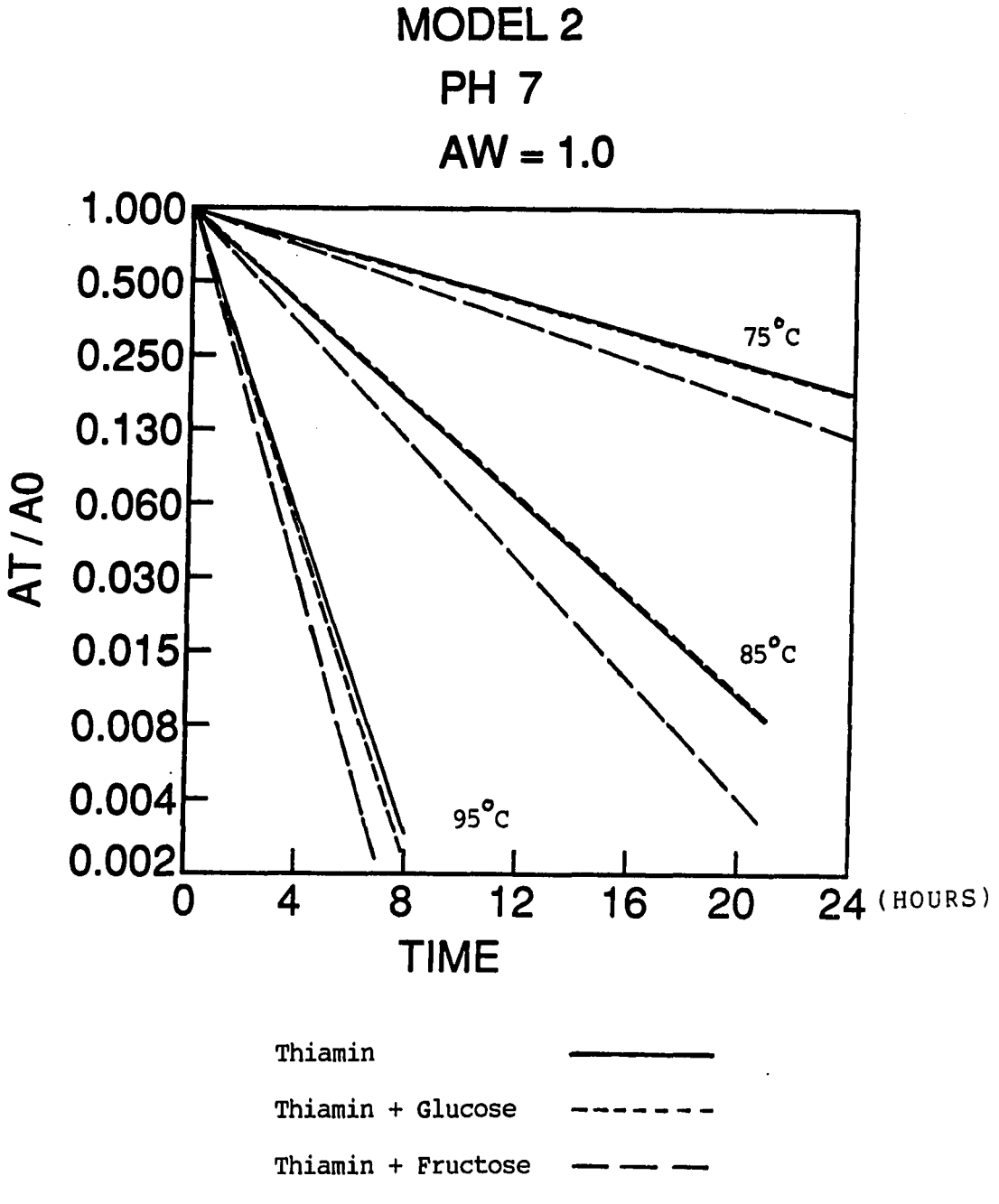


FIGURE 10. Effect of heating temperature and reducing sugar on thiamin retention (model 2, pH 7, and a_w 1.0)

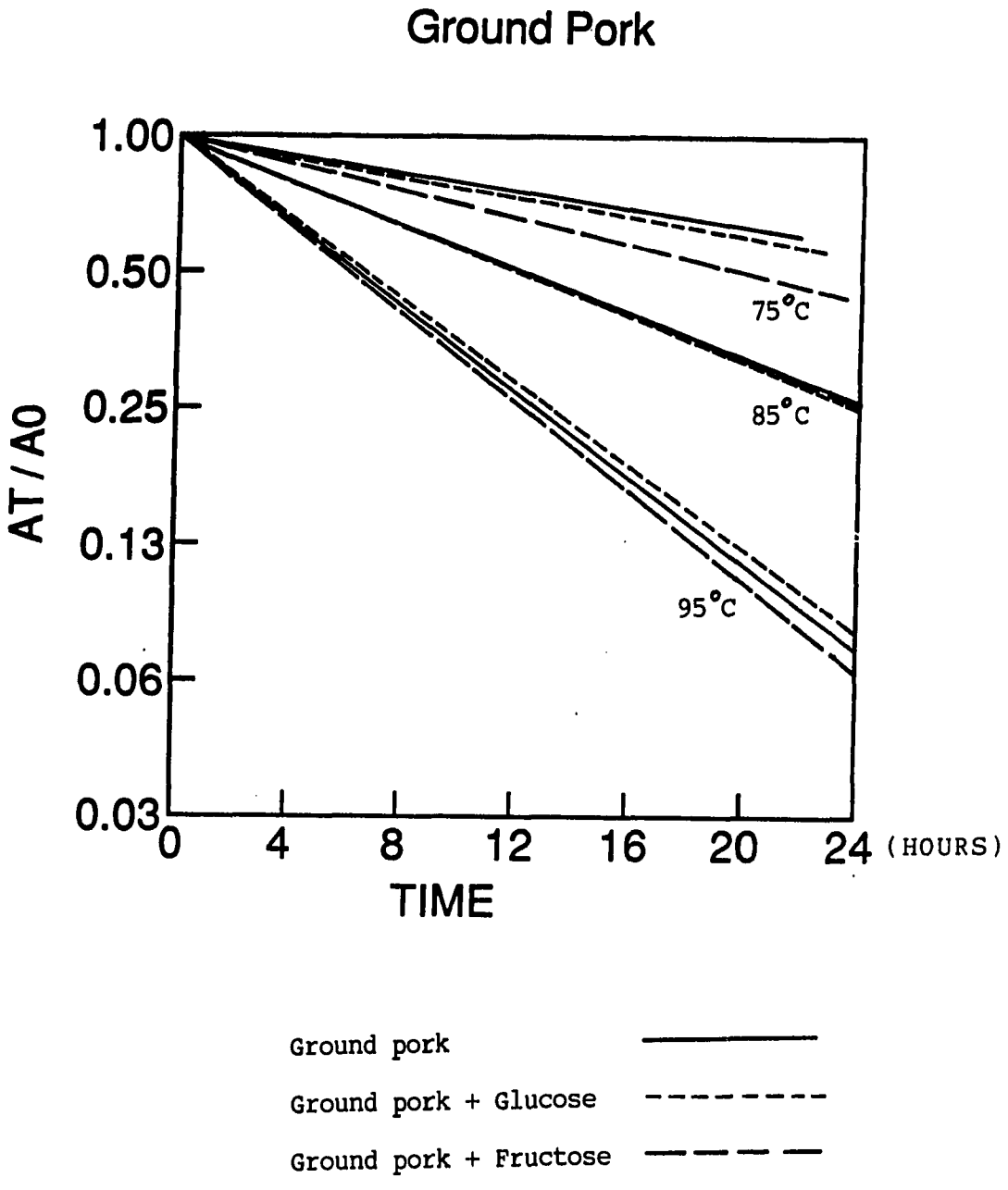


FIGURE 11. Effect of heating temperature and reducing sugar on thiamin retention (ground pork)

TABLE 7. Kinetic parameters of thiamin breakdown in model 1 system (thiamin 10 μg/ml + reducing sugar 2.5 mg/ml)

		pH=5 & a_w=1.0		
		75°C	85°C	95°C
Control	$k \times 10^{-4}(hr^{-1})$	91.59 ± 3.51	268.6 ± 3.1	585.8 ± 8.6
	$t_{1/2}$(hr)	75.67	25.80	11.83
	D(hr)	251.45	85.74	39.31
	r^2	0.9868	0.9984	0.9974
+Glucose	$k \times 10^{-4}(hr^{-1})$	100.3 ± 3.5	305.3 ± 4.4	648.6 ± 11.4
	$t_{1/2}$(hr)	69.09	22.70	10.69
	D(hr)	229.60	75.44	35.53
	r^2	0.9858	0.9975	0.9963
+Sucrose	$k \times 10^{-4}(hr^{-1})$	96.46 ± 3.23	281.7 ± 5.4	603.8 ± 11.2
	$t_{1/2}$(hr)	71.86	24.61	11.48
	D(hr)	238.81	81.78	38.15
	r^2	0.9867	0.9957	0.9959
+Fructose	$k \times 10^{-4}(hr^{-1})$	112.0 ± 3.3	346.9 ± 9.5	1011 ± 22
	$t_{1/2}$(hr)	61.87	19.98	6.85
	D(hr)	205.61	66.40	22.76
	r^2	0.9894	0.9912	0.9943
+Xylose	$k \times 10^{-4}(hr^{-1})$	107.4 ± 3.2	333.0 ± 7.3	929.2 ± 21.0
	$t_{1/2}$(hr)	64.52	20.82	7.46
	D(hr)	214.41	69.19	24.79
	r^2	0.9898	0.9943	0.9939

TABLE 7. (Continued)

		pH=5 & a_w=0.9		
		75°C	85°C	95°C
Control	$k\times10^{-4}(hr^{-1})$	99.07 ± 2.06	298.5 ± 3.3	630.4 ± 8.5
	$t_{1/2}$(hr)	69.97	23.22	10.99
	D(hr)	232.53	77.17	36.52
	r^2	0.9949	0.9985	0.9978
+Glucose	$k\times10^{-4}(hr^{-1})$	100.3 ± 3.0	316.0 ± 5.7	682.3 ± 10.2
	$t_{1/2}$(hr)	69.12	21.95	10.16
	D(hr)	229.70	72.94	33.76
	r^2	0.9891	0.9962	0.9973
+Sucrose	$k\times10^{-4}(hr^{-1})$	98.81 ± 3.09	306.3 ± 3.5	628.0 ± 15.1
	$t_{1/2}$(hr)	70.14	22.63	11.04
	D(hr)	233.09	75.20	36.69
	r^2	0.9884	0.9985	0.9931
+Fructose	$k\times10^{-4}(hr^{-1})$	125.3 ± 1.8	354.6 ± 10.2	1043 ± 21
	$t_{1/2}$(hr)	55.30	19.54	6.64
	D(hr)	183.77	64.94	22.07
	r^2	0.9976	0.9902	0.9952
+Xylose	$k\times10^{-4}(hr^{-1})$	121.9 ± 1.8	361.0 ± 8.6	925.4 ± 13.2
	$t_{1/2}$(hr)	56.84	19.21	7.49
	D(hr)	188.89	63.84	24.89
	r^2	0.9975	0.9933	0.9976

TABLE 7. (Continued)

		pH=7 & a_w=1.0		
		75°C	85°C	95°C
Control	$k \times 10^{-3}(hr^{-1})$	74.40 ± 1.84	262.9 ± 4.7	809.1 ± 11.9
	$t_{1/2}$(hr)	9.31	1.90	0.86
	D(hr)	30.94	6.31	2.86
	r^2	0.9927	0.9963	0.9974
+Glucose	$k \times 10^{-3}(hr^{-1})$	78.86 ± 2.38	294.9 ± 5.5	877.1 ± 13.9
	$t_{1/2}$(hr)	8.79	2.35	0.79
	D(hr)	29.21	7.81	2.63
	r^2	0.9892	0.9959	0.9970
+Sucrose	$k \times 10^{-3}(hr^{-1})$	76.47 ± 1.10	274.3 ± 6.6	888.4 ± 21.5
	$t_{1/2}$(hr)	9.06	2.53	0.78
	D(hr)	30.11	8.41	2.59
	r^2	0.9975	0.9930	0.9930
+Fructose	$k \times 10^{-3}(hr^{-1})$	109.0 ± 1.7	366.9 ± 8.0	1023 ± 21
	$t_{1/2}$(hr)	6.36	1.89	0.68
	D(hr)	21.14	6.28	2.26
	r^2	0.9970	0.9943	0.9947
+Xylose	$k \times 10^{-3}(hr^{-1})$	113.4 ± 2.3	374.0 ± 6.2	1113 ± 11
	$t_{1/2}$(hr)	6.11	1.85	0.62
	D(hr)	20.30	6.15	2.06
	r^2	0.9949	0.9967	0.9988

TABLE 7. (Continued)

		pH=7 & a_w=0.9		
		75°C	85°C	95°C
Control	$k \times 10^{-3}(hr^{-1})$	101.1 ± 1.6	344.7 ± 4.5	955.7 ± 13.2
	$t_{1/2}$(hr)	6.86	2.01	0.73
	D(hr)	22.80	6.68	2.43
	r^2	0.9968	0.9980	0.9977
+Glucose	$k \times 10^{-3}(hr^{-1})$	100.5 ± 1.1	365.4 ± 7.0	1010 ± 19
	$t_{1/2}$(hr)	6.89	1.90	0.69
	D(hr)	22.90	6.31	2.29
	r^2	0.9987	0.9956	0.9959
+Sucrose	$k \times 10^{-3}(hr^{-1})$	96.06 ± 1.55	347.3 ± 6.4	930.1 ± 17.6
	$t_{1/2}$(hr)	7.21	2.00	0.75
	D(hr)	23.96	6.65	2.49
	r^2	0.9969	0.9959	0.9957
+Fructose	$k \times 10^{-3}(hr^{-1})$	129.9 ± 2.4	474.6 ± 11.6	1232 ± 25
	$t_{1/2}$(hr)	5.33	1.46	0.56
	D(hr)	17.71	4.85	1.86
	r^2	0.9958	0.9928	0.9953
+Xylose	$k \times 10^{-3}(hr^{-1})$	125.4 ± 3.3	490.0 ± 6.2	1254 ± 28
	$t_{1/2}$(hr)	5.53	1.41	0.55
	D(hr)	18.38	4.69	1.83
	r^2	0.9919	0.9981	0.9939

TABLE 8. Kinetic parameters of thiamin breakdown in model 2 system (thiamin 20 μg/ml + reducing sugar 5 mg/ml)

		pH=5 & a_w=1.0		
		75°C	85°C	95°C
Control	$k\times10^{-4}(hr^{-1})$	42.08 ± 1.49	129.6 ± 3.6	336.8 ± 11.9
	$t_{1/2}$(hr)	164.65	53.45	20.58
	D(hr)	547.17	177.63	68.39
	r^2	0.9926	0.9960	0.9926
+Glucose	$k\times10^{-4}(hr^{-1})$	40.63 ± 1.05	134.4 ± 5.2	342.4 ± 12.9
	$t_{1/2}$(hr)	170.55	51.58	20.24
	D(hr)	566.78	171.41	67.26
	r^2	0.9960	0.9910	0.9915
+Sucrose	$k\times10^{-4}(hr^{-1})$	41.22 ± 1.22	133.5 ± 2.8	370.0 ± 14.8
	$t_{1/2}$(hr)	168.10	51.90	18.73
	D(hr)	558.64	172.48	62.24
	r^2	0.9948	0.9973	0.9905
+Fructose	$k\times10^{-4}(hr^{-1})$	47.83 ± 0.72	207.8 ± 6.7	647.8 ± 59.2
	$t_{1/2}$(hr)	144.87	33.35	10.70
	D(hr)	481.44	110.83	35.56
	r^2	0.9986	0.9938	0.9518
+Xylose	$k\times10^{-4}(hr^{-1})$	45.85 ± 0.83	240.9 ± 15.0	643.4 ± 54.5
	$t_{1/2}$(hr)	151.16	28.76	10.77
	D(hr)	502.34	95.58	35.79
	r^2	0.9980	0.9771	0.9585

TABLE 8. (Continued)

		pH=7 & a_w=1.0		
		75°C	85°C	95°C
Control	$k \times 10^{-3}(hr^{-1})$	70.47 ± 1.17	230.4 ± 7.6	722.6 ± 29.6
	$t_{1/2}$(hr)	9.83	3.01	0.96
	D(hr)	32.67	10.00	3.19
	r^2	0.9983	0.9935	0.9917
+Glucose	$k \times 10^{-3}(hr^{-1})$	70.70 ± 1.27	226.7 ± 4.7	755.9 ± 36.0
	$t_{1/2}$(hr)	9.80	3.06	0.92
	D(hr)	32.57	10.17	3.06
	r^2	0.9981	0.9975	0.9886
+Sucrose	$k \times 10^{-3}(hr^{-1})$	69.03 ± 1.15	222.2 ± 5.2	778.0 ± 31.7
	$t_{1/2}$(hr)	10.04	3.12	0.89
	D(hr)	33.37	10.37	2.96
	r^2	0.9984	0.9967	0.9901
+Fructose	$k \times 10^{-3}(hr^{-1})$	89.43 ± 1.26	275.9 ± 3.8	853.0 ± 4.9
	$t_{1/2}$(hr)	7.75	2.51	0.81
	D(hr)	25.76	8.34	2.69
	r^2	0.9988	0.9989	0.9998
+Xylose	$k \times 10^{-3}(hr^{-1})$	97.25 ± 4.71	284.9 ± 3.3	869.9 ± 12.7
	$t_{1/2}$(hr)	7.13	2.43	0.80
	D(hr)	23.69	8.08	2.66
	r^2	0.9861	0.9992	0.9989

been observed in the case of food products. But our ground pork study (Table 9) shows quite high coefficients of determination (r^2=0.96-0.99) for thermal thiamin breakdown in the temperature range studied.

The reaction rate constant (k) in pH 7 buffer system was about ten times that in pH 5 buffer system; these results are similar to those reported in an earlier study of Farrer (1955). The factors affecting thiamin retention will be discussed later individually.

Statistical analyses of reaction rate constants

Statistical analyses of thiamin breakdown rate constants in model system and pork were performed using the SAS analysis of variance (Tables 10 and 11). The analysis of variance was introduced by Sir Ronald A. Fisher and is essentially an arithmetic process for partitioning a total sum of squares into components associated with recognized sources of variation. It has been used to advantage in all fields of research where data are measured quantitatively. The F value is obtained by dividing the treatment mean square by the error mean square. A significant F implies that the evidence is sufficiently strong to indicate that all the treatments do not belong to populations with a common mean.

As shown in Table 10, temperature, pH, a_w, and addition of sugars had significant effects ($p<0.001$) on thiamin breakdown rate in model 1. In the ground pork study, the effects of temperature and addition of sugars were significant at $p<0.001$ (Table 11).

TABLE 9. Kinetic parameters of thiamin breakdown in ground pork (reducing sugar 2.4 g/100 g pork)

		75°C	85°C	95°C
Control	$k \times 10^{-3}(hr^{-1})$	18.32 ± 0.62[a]	55.45 ± 0.91	106.8 ± 3.4
	$t_{1/2}$(hr)	37.82	12.50	6.49
	D(hr)	125.68	41.54	21.57
	r^2	0.9899	0.9960	0.9823
+Glucose	$k \times 10^{-3}(hr^{-1})$	20.69 ± 1.15	58.20 ± 2.78	103.7 ± 4.7
	$t_{1/2}$(hr)	33.49	11.91	6.68
	D(hr)	111.30	39.58	22.20
	r^2	0.9729	0.9669	0.9640
+Fructose	$k \times 10^{-3}(hr^{-1})$	30.31 ± 1.52	58.15 ± 2.24	162.8 ± 8.4
	$t_{1/2}$(hr)	22.87	11.92	4.26
	D(hr)	76.00	39.61	14.16
	r^2	0.9778	0.9782	0.9686

k: reaction rate constant

$t_{1/2}$: half life

D: time for a 90% reduction of the nutrient content

r^2: coefficient of determination

[a]Values given are k ± S.E. for quadruplicate determinations.

TABLE 10. SAS analysis of variance for thiamin breakdown rate constants (k) in model 1 system

Source	df[a]	Sum of squares	Mean square	F value	Pr[b]>F
Model	59	15.4066	0.2611	497.91	0.0001
Error	60	0.0315	0.0005		
Corrected total	119	15.4381			
Reducing sugar	4	0.1524	0.0381	72.67	0.0001
pH	1	6.0733	6.0733	11580.16	0.0001
a_w	1	0.0527	0.0527	100.41	0.0001
Temp	2	5.0375	2.5188	4802.65	0.0001

[a]df: degree of freedom.
[b]Pr: probability.

TABLE 11. SAS analysis of variance for thiamin breakdown rate constants (k) in ground pork

Source	df[a]	Sum of squares	Mean square	F value	Pr[b]>F
Model	8	0.0547	0.0068	648.11	0.0001
Error	18	0.0002	0.00001		
Corrected total	26	0.0549			
Reducing sugar	2	0.0032	0.0016	153.23	0.0001
Temp	2	0.0478	0.0239	2266.24	0.0001

[a]df: degree of freedom.
[b]Pr: probability.

The least significant difference (lsd) test was done to compare means when treatment effects were significant. The results will be mentioned in a later chapter.

Temperature dependence of thiamin retention

The activation energy is defined as the minimum energy state of molecules participating in a reaction, and it also provides a measure of the temperature dependence of the reaction (Labuza, 1980). For calculating the Arrhenius activation energy (Ea), concentration is regressed on time at a constant temperature to determine the reaction rate constant, k, and ln k is regressed on reciprocal temperature to determine Ea. Tables 12, 13, and 14 list the activation energies (Ea) calculated from the slopes of the Arrhenius plot (ln k versus 1/T). The coefficients of determination (r^2) for Ea values are quite high (0.84-0.99).

Constant activation energies are an indication of an unchanged mode of thiamin degradation over the temperatures studied regardless of changing pH, water activity, thiamin concentration and the presence or absence of reducing sugars. The Arrhenius model was tested to describe the dependence of the reaction rate on temperature. The results indicate that the Arrhenius model adequately describes the temperature dependence of the rate constant for thiamin degradation.

An early study by Farrer (1955) and later studies (Goldblith and Tannenbaum, 1966; Mulley et al., 1975b,c; Fox et al., 1982) showed that the loss of thiamin due to heating could be readily predicted by a

TABLE 12. Arrhenius activation energies (Ea) of thiamin breakdown for model 1 system

	Ea ± 95 % C.I. ($KJ \cdot mol^{-1}$)			
	pH=5 & a_w=1.0	pH=5 & a_w=0.9	pH=7 & a_w=1.0	pH=7 & a_w=0.9
Control	98.865±22.816 r^2=0.9336	98.625±28.360 r^2=0.9054	127.020±6.702 r^2=0.9929	119.628±13.528 r^2=0.9785
+Glucose	99.473±28.700 r^2=0.9049	102.197±30.206 r^2=0.9011	128.260±15.092 r^2=0.9771	122.822±19.625 r^2=0.9631
+Sucrose	97.728±24.087 r^2=0.9261	98.580±33.998 r^2=0.8753	130.518±3.115 r^2=0.9974	120.921±22.102 r^2=0.9543
+Fructose	117.067±0.108 r^2=0.9999	112.704±9.126 r^2=0.9999	119.183±11.837 r^2=0.9823	119.832±26.061 r^2=0.9396
+Xylose	114.814±4.208 r^2=0.9956	107.886±8.092 r^2=0.9883	121.501±3.715 r^2=0.9965	122.887±33.036 r^2=0.9150

Ea: Arrhenius activation energy

C.I.: confidence interval

r^2: coefficient of determination

TABLE 13. Arrhenius activation energies (Ea) of thiamin breakdown for model 2 system

	Ea ± 95 % C.I. ($KJ \cdot mol^{-1}$)	
	pH=5 & a_w=1.0	pH=7 & a_w=1.0
Control	110.721 ± 10.494 r^2= 0.9835	123.847 ± 2.185 r^2= 0.9999
+Glucose	113.515 ± 18.941 r^2= 0.9611	126.031 ± 9.862 r^2= 0.9998
+Sucrose	116.813 ± 8.857 r^2= 0.9881	128.816 ± 14.194 r^2= 0.9975
+Fructose	138.788 ± 24.181 r^2= 0.9577	119.972 ± 6.059 r^2= 0.9999
+Xylose	140.857 ± 56.366 r^2= 0.8449	116.536 ± 9.556 r^2= 0.9998

TABLE 14. Arrhenius activation energies (Ea) of thiamin breakdown for ground pork

	Ea ± 95 % C.I. ($KJ \cdot mol^{-1}$)
Control	94.020 ± 37.605 r^2= 0.8411
+Glucose	85.955 ± 38.465 r^2= 0.8117
+Fructose	89.231 ± 39.662 r^2= 0.8666

first order reaction. The activation energies derived from the Arrhenius relationship for loss of thiamin in those systems were generally about 80-130 kJ/mole. Ea values in our model systems and ground pork (86-140 kJ/mole) are similar to the range reported by these studies, even with added reducing sugars and glycerol. We can see that generally the Ea values at pH 7 are slightly higher than those at pH 5, and also phosphate buffer model systems also had higher Ea's than ground pork. The other parameters; i.e., a_w, reducing sugars, and thiamin concentration do not show any consistent effect on Ea value.

The constant Ea over the investigated temperature range (75-95°C) is an indication that the degradation pathway remained the same over the temperature range. The Arrhenius equation says that a plot of ln k versus the reciprocal of absolute temperature gives a straight line, the slope of which is the activation energy divided by the gas constant R. Thus, by studying a reaction and measuring k at two or three temperatures, we can extrapolate with a straight line to a lower temperature and predict the rate of the reaction. Having established the kinetic basis of each thermal process, the process can be optimized for retention of nutrients by examining the reaction rates for thermal destruction and, more importantly, considering the temperature dependence of the reaction rate constant.

It should be noted that it is quite common in the food science literature to derive an Ea value from three temperatures, which generally results in an r^2 of approximately 1. However, the

statistical 95% confidence levels of Ea obtained from such a plot are generally as large as the Ea because of the large t value. But, this does not mean the data are wrong. In these cases, the average Ea is probably an adequate measure of the temperature dependence of the reaction.

Effect of Processing Parameters on Thiamin Retention

Effect of sugars on thiamin retention

The effects of 4 different sugars on thiamin loss were studied: the sugars were glucose, sucrose, fructose, and xylose. Due to the amino group on the pyrimidine ring of the thiamin molecule, thiamin is known to react strongly in a Maillard type browning reaction with reducing sugars in dry or aqueous systems when heated (Dennison et al., 1977). Therefore, the type of sugar can be an important factor in the loss of thiamin during processing and storage.

According to the lsd test, all the investigated reducing sugars (glucose, fructose, and xylose) had significantly ($p<0.05$) different effects on reaction rates of thiamin breakdown compared with the control. Thiamin loss in systems containing fructose or xylose was significantly higher than in systems with glucose and sucrose. But there was no significant difference between fructose and xylose, or between glucose and sucrose. In model system 2, where the thiamin and reducing sugar concentrations were double the concentrations in model system 1, the half-life ($t_{1/2}$) of thiamin retention was greatly

increased, for example at 95°C, pH=5, a_w=1.0, and in the presence of xylose, the half-life in model system 2 became almost 150% of that in model system 1. This result suggests that doubling the thiamin concentration had an advantageous effect on thiamin retention, i.e., the increased thiamin concentration retarded the thiamin degradation rate, even though the reducing sugar concentration also was doubled. When glucose and fructose were added to ground pork, there was a significant increase in the rate constant with added fructose, compared with the control or samples with glucose. At the 5% level of probablity (lsd test) only fructose was significantly different from the control.

These results support a previous study (Doyon and Smyrl, 1983) which examined the influence of reducing sugars; i.e., xylose, glucose, and maltose, on the rate of thiamin destruction at 95°C in aqueous solutions buffered at pH 6.75. The order of effect of reducing sugar on thiamin breakdown was xylose > glucose > maltose. Results from both studies match the general findings in studies of the browning reaction; i.e., the rate of condensation of an amino compound with a reducing sugar is dependent on the rate at which the cyclic sugar structure opens to form the acyclic reducible form.

The order of the amount of open chain structure is pentose > hexose > reducing disaccharides. Aldopentoses are more reactive than the aldohexoses and the reducing disaccharides are still less active (Spark, 1969). Sucrose, a nonreducing sugar, should not participate in

Maillard reactions as such, but only after hydrolysis of the glycosidic bond, releasing the constituent monosaccharide. Much more drastic conditions are therefore necessary to obtain a reaction with sucrose. Since low pH values and relatively high moisture and high temperature treatment favor the hydrolysis of sucrose, sucrose can participate in reactions with amino groups in such conditions.

In our study, the presence of sucrose did not show a significant effect on the thiamin reaction rate or extent of browning. Our results showed that xylose or fructose is significantly ($p<0.05$) more reactive with thiamin than is glucose or sucrose.

Reyes et al. (1982) investigated the reactivity of glucose, fructose, and sucrose with glycine in Maillard browning reactions over a 280-hour reaction period. Whereas fructose browned at a faster rate initially, it was overtaken by glucose after 80 hours at 60°C. These results show that glucose undergoes more browning than fructose during prolonged reaction times. But in our study, the longest period was 72 hours at 75°C in pH 5 buffer, and glucose did not exceed fructose in its reaction rate. And our results also showed that thiamin destruction in the presence of any reducing sugar followed first order kinetics, with high correlation coefficients being obtained. The kinetic study of thiamin losses in the presence of reducing sugars has not been investigated. Previous studies have demonstrated that the loss of amine in the Maillard reaction follows first order kinetics, at least during the initial stages of the reaction. Warmbier et al.

(1976) studied the loss of available lysine in an intermediate moisture food system containing casein, glucose, and glycerol and found that the initial loss rate of available lysine followed first order kinetics.

Watanabe and Sakaki (1944) studied the effects of sucrose, lactose, and glucose on thiamin destruction at 110°C and reported a slight accelerating effect due to sugars. Lhöst (1957) found that thiamin hydrochloride reacts with glucose at pH values less than 4 to give 2-glucothiamins and other unidentified compounds. Van der Poel (1956) reported that thiamin, when heated in a glucose solution, showed brown discoloration and fluorescence. This reaction is analogous to the Maillard reactions of sugars and amino acids. The minimum reactant requirements for Maillard browning are the presence of a compound, usually a protein, which has a free amino group, a reducing sugar, and some water.

In our study, obvious brown color development was observed when samples were heated for a long time in the presence of fructose or xylose with thiamin solution. But we could not see any significant accelerating effect of glucose or sucrose on thiamin loss and brown color development under our experimental condition.

Pearson et al. (1966a) studied the browning reaction in heated pork and mentioned that the amount of brown color development was related to the level of reducing sugars in the tissues. The extremely high relationship between free sugar content and degree of brownness suggested that sugar was, at least in part, responsible for the brown color developed upon heating.

Effect of water activity on thiamin retention

It is now generally accepted that water activity (a_w) is more closely related to the physical, chemical, and biological properties of foods than is the total moisture content (Troller and Christian, 1978). Several authors have studied the rates of thiamin destruction in food or model systems of varied a_w (Kamman et al., 1981; Labuza and Kamman, 1982; Fox et al., 1982). Generally, an increase in water activity decreases thermal stability of thiamin in dry and dehydrated foods. However, several studies have shown that an increase in water activity at intermediate or high range decreased the rate of thiamin degradation. In our model study, we controlled a_w to 0.90 by using glycerol and we observed a significant ($p<0.05$) accelerating effect on the thiamin breakdown during thermal processing.

In general, in the high water activity range, reducing water activity by adding glycerol does not significantly contribute to reducing the thiamin breakdown rate. On the contrary, at the higher moisture content, the concentration of reactant is decreased, so the reaction rate is reduced. Fox et al. (1982) showed that four different a_w values (0.90, 0.93, 0.96, 1.0) had little effect on the stability of thiamin in food during thermal processing, and they concluded that within the a_w range of water-rich foods the effect of water activity is negligible. Arabshahi (1982) showed that an increase in water activity from 0.65 to 0.85 resulted in a small decrease in the rate of thiamin degradation. This effect with increasing a_w at intermediate or high a_w

range can be explained in the following way. Higher moisture content results in a more polar medium and therefore decreases the rate of reaction (Arabshahi, 1982). Arabshahi's experiments (1982) support the idea that the increased dielectric constant at increased moisture contents could be responsible for the drop in the rate of thiamin degradation when a_w is increased above an intermediate range of a_w. According to his experiments, thiamin in the model system containing PEG-400, which has a low dielectric constant, i.e., is less polar, showed a significantly faster degradation than the rate in the model system containing only glycerol. However, the effect of polarity on reaction rate can be masked by effects of other factors such as viscosity and pH of the reaction medium.

On the other hand, increasing moisture content decreases the viscosity of the liquid phase of the model systems, which tends to increase the rate of the reaction. At low water contents, an increase in water concentration has a drastic effect on the viscosity of the solution, but at higher water concentration, the magnitude of change in viscosity becomes very small. Thus at low a_w the effect of viscosity predominates and an increase in water content accelerates the reaction, while above the intermediate range of a_w, polarity has the greater effect causing the rate of reaction to decrease. So the slightly increased reaction rate that resulted from the reduction of a_w to 0.9 in the current study may be explained by reduced polarity.

In Figure 12, Fennema and Carpenter (1984) showed the tendency of the thiamin curve to turn downward in terms of reaction rate above an a_w of about 0.6. This phenomenon is believed to occur because above an a_w of 0.6 water is present in sufficient quantity to solubilize and mobilize constituents: additional water simply dilutes the reactants. Warmbier et al. (1976) found that the water activity at which maximum browning occurs was reduced from the 0.75-0.65 a_w range to 0.59-0.40 a_w when glycerol was used to adjust the a_w of a foodlike model system. They attributed this downward shift to the liquid, waterlike properties of glycerol and increased reactant mobility and solubility at a_w's below which most water soluble reactions occur very slowly.

Effect of temperature and pH on thiamin retention

Temperature and pH are known to be the most important factors influencing thiamin stability. Thiamin breakdown reaction rates differed significantly at the three different heating temperatures, 75, 85, or 95°C (lsd test, $p<0.05$) Also there were significant differences in thiamin breakdown reaction rate constants at pH 5 and at pH 7. Thus, in food products that have a pH above 7, greater destruction occurs at higher temperatures than occurs in acidic vegetables and fruits (Farrer, 1955). Thiamin is more heat sensitive in neutral and alkaline foods such as cake mixes than in more acidic foods such as bread which has been leavened with yeast. Table 15 shows the pH effects (pH 5, 6, and 7) on thiamin breakdown rate.

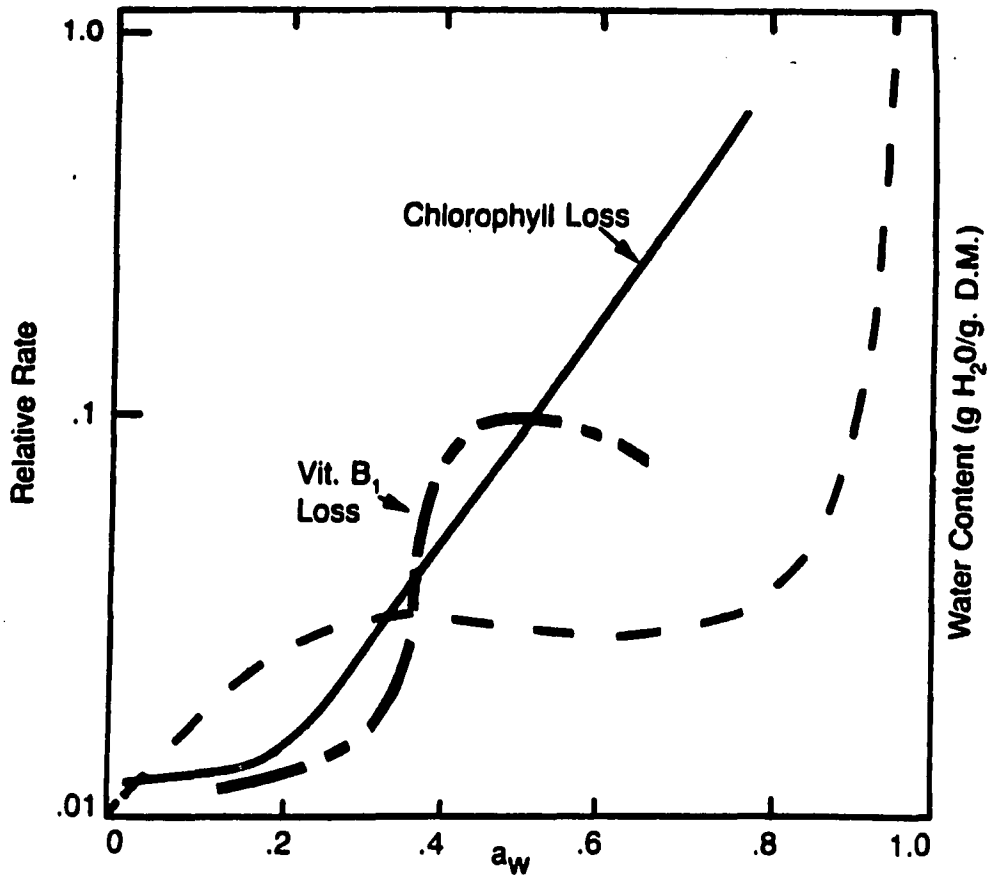


FIGURE 12. Effect of water activity on losses of chlorophyll and vitamin B_1 (Fennema and Carpenter, 1984)

TABLE 15. Comparison of thiamin breakdown at three pH's (thiamin 10 μg/ml + reducing sugar 2.5 mg/ml)

			pH = 5	pH = 6	pH = 7
Control	$k \times 10^{-4}$ (hr^{-1})	75°C	91.59 ± 3.51	178.6 ± 5.3	744.0 ± 18.4
		85°C	268.6 ± 3.1	432.9 ± 7.6	2629 ± 46
		95°C	585.8 ± 8.6	1023 ± 34	8091 ± 119
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		98.865±22.816	92.910± 4.986	127.020± 6.702
+Glucose	$k \times 10^{-4}$ (hr^{-1})	75°C	100.3 ± 3.5	192.3 ± 3.9	788.6 ± 23.8
		85°C	305.3 ± 4.4	468.5 ± 23.6	2949 ± 55
		95°C	648.6 ± 11.4	1096 ± 29	8771 ± 139
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		99.473±28.700	92.593±10.321	128.260±15.092
+Fructose	$k \times 10^{-4}$ (hr^{-1})	75°C	112.0 ± 3.3	235.3 ± 7.3	1090 ± 17
		85°C	346.9 ± 9.5	578.7 ± 20.8	3669 ± 80
		95°C	1011 ± 22	1586 ± 50	10230 ± 215
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		117.067± 8.108	101.699±8.612	119.183±11.837

Dwivedi and Arnold (1973) identified 4-methyl-5-(beta-hydroxyethyl)- thiazole as a major degradation product of heated slightly acidic or alkaline thiamin solutions. Heating of thiamin solutions resulted in cleavage of thiamin at the methylene bridge between the thiazole and pyrimidine moieties. Morfee and Liska (1971) studied the distribution of thiamin degradation products in a simulated milk system heated at 121°C for 50 minutes, and identified elemental sulfur as a major degradation product in buffered slightly acidic or basic solutions.

Comparison of Thiamin Loss in Phosphate Buffer Model and in Pork

In this study we tried to see whether there is any relation between the thiamin breakdown reaction rate constant for pork and the model system which had a similar pH, water activity, and reducing sugar content to that of pork. We compared the kinetic parameters of pork with that in pH 6 phosphate buffer system, to determine whether some protective effect on thiamin loss might be noted in pork compared with that in phosphate buffer. But no consistent difference in the reaction rate constant, k was apparent (Table 16). In the control model system, without reducing sugars, the rate constant, k, in pork was higher than that in pH 6 phosphate buffer, but in the presence of 25 mg/ml of fructose which accelerates thiamin loss due to the browning reaction, the k value was higher in the buffer system. There was no difference in activation energy of the control sample between pork and the

buffered model systems, but the presence of glucose or fructose increased activation energy in the phosphate buffer system over that in pork. Apparently, the browning reaction induces the difference in temperature dependence between two systems.

Our results show that the thiamin breakdown parameters in pork are not much different from those in the pH 6 buffer system, thus pH may be the most important of the factors studied in affecting thiamin retention, besides temperature. These results do not support Mulley et al.'s (1975a) report that thiamin is destroyed more rapidly in phosphate buffer than in the food systems. In their study, the thermal destruction of thiamin in phosphate buffer and selected low acid foods was studied over the temperature range 121.1 to 137.8°C. A similar finding in their study and ours is that the reaction involved in the thermal degradation of the thiamin molecule is a first order type, as evidenced by linear destruction rate curves at constant temperature. The activation energies observed in our studies agreed with those reported in former studies. In phosphate buffer at pH 6.8, Goldblith and Tannenbaum (1966) found the activation energy for thiamin degradation to be 22 kcal/mole (92.4 kJ/mole) for both conventional and microwave heating, which is similar to the value in pureed meats and vegetables found by Feliciotti and Esselen (1957). Our study also showed an activation energy of 94.02 kJ/mole (22.4 kcal/mole) for pork. As was shown in many other studies of thiamin retention in foods, there are wider variations in activation energy in foods due to the different

TABLE 16. Comparison of thiamin breakdown between pork and phosphate buffer (reducing sugar 25 mg/ml)

			pork(pH 5.98±0.65)	pH = 6 buffer
Control	$k \times 10^{-3}$ (hr^{-1})	75°C	18.32 ± 0.62	17.86 ± 0.53
		85°C	55.45 ± 0.91	43.29 ± 0.76
		95°C	106.8 ± 3.4	102.3 ± 3.4
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		94.020 ± 37.605	92.910 ± 4.986
+Glucose	$k \times 10^{-3}$ (hr^{-1})	75°C	20.69 ± 1.15	20.31 ± 1.02
		85°C	58.20 ± 2.78	49.36 ± 1.98
		95°C	103.7 ± 4.7	132.6 ± 7.2
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		85.955 ± 38.465	99.986 ± 8.126
+Fructose	$k \times 10^{-3}$ (hr^{-1})	75°C	30.31 ± 1.52	29.36 ± 2.35
		85°C	58.15 ± 2.24	63.78 ± 4.25
		95°C	162.8 ± 8.8	192.7 ± 9.3
	Ea ± 95% C.I. ($KJ \cdot mol^{-1}$)		89.231 ± 39.662	100.495 ± 20.370

thermal gradients compared with a controlled model system. According to Labuza (1972), for pork, activation energy is 18.5 kcal/mole. Other foods show values such as: cheese, 20 kcal/mole; dehydrated pork, 26 kcal/mole; orange juice, 16 kcal/mole; and yeast extract, 23.5 kcal/mole.

General Observations from Thiamin Retention Studies

Thiamin is probably the most heat sensitive of the B vitamins, especially in nonacid foods, and its stability depends on pH, temperature and many other factors (Dwivedi and Arnold, 1973). Many studies have shown that increasing the severity of a heat treatment accelerates thiamin destruction. Mulley et al. (1975b) and Morfee and Liska (1971) studied thiamin retention as a function of pH and found that, as pH increased, thiamin stability decreased. Other factors which have been implicated in thiamin destruction in foods include; sulfite treatment (Leichter and Joslyn, 1969), reducing sugars (Doyon and Smyrl, 1983), and water activity (Dennison et al., 1977; Fox et al., 1982). Farrer (1955) reviewed the losses of thiamin found in cereals and bread. Losses varied between as little as 3% and up to 30%, with an average of 20%, in the making of bread. He also reviewed the losses in meat and vegetable processing and found wide variations. Dwivedi and Arnold (1973) studied the mechanism of the degradation of thiamin. The data when analyzed kinetically show that the rates were very rapid in aqueous solution, with a high activation energy. Our

results are similar, i.e., the Ea's for pork are lower than the Ea's for a phosphate buffer system. Rice et al. (1944) systematically studied thiamin decomposition in foods at different temperatures. They reported that the rate of loss varied with the type of product. For example, after 21 days of storage at 49°C, skim milk had lost no thiamin, wheat flour lost 15%, dry eggs lost 65%, and pork lost 90% of the thiamin. This variation would complicate the use of solution studies in predicting the loss during storage of various foods. Farrer (1955) has shown that there is no deviation from the Arrhenius equation for thiamin destruction in buffered solutions at temperatures between 50 and 110°C.

Thiamin has long been considered to be the most thermally unstable of the vitamins used in the enrichment of cereal products. Commercially, the vitamin is available in either the hydrochloride or mononitrate salt form. The observed difference in stability of the two salts can be explained by the higher activation energy (26.3 vs. 22.4 kJ/mole) for the mononitrate which results in a crossover point at about 95°C in an Arrhenius plot. Temperature is an important factor influencing thiamin stability during storage of food stuffs. Data from Freed et al. (1949) show differences in thiamin retention among various foods held at two different storage temperatures. Rate constants for thiamin degradation in various foods including peas, carrots, cabbage, potatoes, and pork, ranged from 0.0020 to 0.0027 per minute at 100°C. In several studies on cooking pork, Farrer (1955) was able to calculate

rate constants from the data at several temperatures, for instance, the rate constant at 89°C was 0.15 $hour^{-1}$. In the current study, the rate constant at 95°C was 0.107 $hour^{-1}$.

CONCLUSION

The objective of this study was to determine how much thiamin remains in a system containing a known initial quantity of thiamin after the system had been processed by using different sets of parameters; i.e., temperature, time, pH, water activity, and reducing sugars, and to study the kinetics of thiamin degradation as a function of the above parameters. Based on analyses of pork, we designed an aqueous buffered model system to simulate thiamin retention studies in cooked meat.

In our study, a semilog plot of thiamin retention versus heating time (hours) at 75, 85, and 95°C gave straight lines with high coefficients of determination (r^2=0.95-0.99) in most cases investigated. This observation indicates that the rates of thiamin degradation in aqueous buffer systems and ground pork are adequately described by a first order reaction model. After calculating rate constant values (k), a regression analysis of the ln k on the reciprocal temperature was made to determine the Arrhenius activation energy. Activation energy values (Ea) were in the range of 86 to 140 kJ/Mole in both model systems and ground pork; these values are within the range reported by other researchers (Goldblith and Tannenbaum, 1966; Mulley et al., 1975b; and Fox et al., 1982). Our values indicated that thiamin in pork (85-93 kJ/Mole) was less temperature dependent than that in aqueous buffered model systems (100-138 kJ/Mole). The Ea values at pH=7 were slightly higher than those at

pH=5. This fact may suggest that the more adverse conditions result in more temperature dependent reaction rates. The other factors; i.e., water activity, reducing sugars, and thiamin concentration did not show any consistent differences in activation energy values. The constant Ea over the investigated temperature range (75-95°C), which was validated by high coefficients of determination (0.84-0.99), is an indication that the degradation pathway remained the same over the temperature range.

Among the various factors investigated, temperature was the most important factor influencing thiamin stability. The pH also strongly influenced thiamin retention in these systems. A pH of 7 was drastically adverse for thiamin retention compared with a pH of 5 or 6. At high temperatures in alkaline pH, thiamin was very unstable and easily destroyed during thermal processing. When we compared model system 1 and 2, the results showed that increasing thiamin concentration twofold had an advantageous effect on thiamin retention, i.e., contributed to a retarded thiamin degradation rate.

One of the main interests in this research was to confirm that the browning reaction between thiamin and reducing sugars affected the rate of the breakdown of thiamin. Due to the amino group on the pyrimidine ring of thiamin molecules, thiamin is known to react in a Maillard type browning reaction, therefore we studied the effects of 4 different sugars on thiamin loss. Xylose and fructose affected thiamin breakdown to a greater extent than did glucose and sucrose. These results match

the general findings in browning reactions between amino acids and reducing sugars; i.e., the rate of condensation of an amino compound with a reducing sugar is dependent on the rate at which the cyclic sugar structure opens to form the acyclic reducible form. Our results showed that xylose or fructose was much more reactive than glucose or sucrose with thiamin, and especially, sucrose did not show any effect on thiamin reaction rate or browning. Brown color development was apparent when thiamin solutions were heated for a long time in the presence of fructose or xylose. When we reduced the water activity of the model system to 0.90 by adding glycerol as a humectant, there was an adverse effect on thiamin retention. The higher moisture content causes higher polarity and may therefore have decreased the rate of reaction at the high water activity range.

In this study, kinetic parameters of thiamin breakdown were compared between pork and the model system which had a similar pH, water activity, and reducing sugar content. Our results showed that the thiamin breakdown rates in pork were not much different from those in this buffer system. There was not much difference in heat resistance of thiamin in pork and in aqueous and buffered solutions when the environmental and compositional factors were similar. But the activation energy in pork was slightly lower than that in the buffer system.

In conclusion, thiamin breakdown follows the first order kinetic model very well whether in both aqueous model systems and in pork, and

a simple Arrhenius-based equation was found adequate to predict thiamin loss at the investigated temperatures. Thiamin is very sensitive to high temperature and alkaline to neutral pH. Reducing the water activity of high moisture food to around a_w=0.9 did not show any advantage for thiamin retention; actually there was a deleterious effect. We also found that the different kinds of reducing sugars had different effects on thiamin retention. The presence of fructose or xylose significantly enhanced thiamin breakdown in every case investigated.

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ACKNOWLEDGMENTS

I would first like to express my heartfelt gratitude to my major professor, Dr. Jane A. Love, for her support and guidance throughout my study at I. S. U. Her judgement and encouragement were invaluable for my work.

Special thanks are extended to Dr. Jacqueline Dupont, Dr. Pamela White, Dr. Patricia Murphy, and Dr. David Cox for giving suggestions and guidance as my graduate committee.

Much appreciation also goes to the wonderful families in Ames who shared the brightness and the gloominess in life.

I wish to thank my parents for their endless love and support. Their confidence in me at all times has inspired me to accomplish the highest degree.

Finally, I am most grateful to my family; my husband Chong Ho for his continued faith and encouragement, little David for being a constant source of happiness and liveliness.