THERMAL DEGRADATION OF THIAMINE IN BREAD

by

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ABSTRACT

Thiamine is an important nutrient found in significant amounts in wheat flours. This vitamin is heat labile thus destruction occurs during bread baking. Using a kinetic approach, the effect of heat and pH on thiamine degradation in a model system were studied. In order to compare the stability of thiamine from natural (whole wheat) and synthetic (enriched white) sources, thermal destruction of thiamine in the two breads was investigated.

Destruction rates of thiamine hydrochloride in phosphate buffer at pH 6.0 and temperatures between 80 and 120°C were measured. The breakdown reaction could be described by first order kinetics. An energy of activation of 34.2 kcal/mole was obtained. Destruction rates of thiamine hydrochloride in phosphate buffer at 120°C were measured for pH values between 4.0 and 7.0. The reaction rate increased as the system was made more alkaline, with greater destruction at pH 6.0 and above.

Thiamine losses in an enriched white flour system baked at a nominal temperature of 246°C (475°F) for 60, 75 and 90 min were found to be 2.4, 27.9 and 29.2%, respectively. Two experiments were carried out with 450 g (1 lb) enriched white loaves baked at 221°C (430°F). Baking times were 30

min for the first experiment, and 15, 37 and 60 min for the second experiment. No appreciable thiamine destruction were found in either experiment.

The main investigation was with a semi-model system of 12g bread loaves made from enriched white and whole wheat flours. Four different nominal oven temperatures of 177, 221, 246 and 288°C (350, 425, 475 and 550°F) were used with four different baking times for each run. The pH of the dough and baked bread were determined. Oven, crust and loaf center temperatures were monitored. The mass average temperature data of the bread during baking showed a changing rate of temperature rise, and because of this, it was not possible to obtain kinetic data. However, a linear relationship was obtained when the logarithm of the percent thiamine retention was plotted against time. This experiment showed a lower thiamine stability with higher oven temperature. Thiamine was less stable in whole wheat bread than in enriched white bread. This might be explained by higher pH and ash content in whole wheat bread. Thiamine losses during normal baking of whole wheat and enriched white bread were found to be in the range of 28.3 to 47.8%.

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ACKNOWLEGMENTS

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I

INTRODUCTION

Thiamine is an important nutrient. The wheat kernel contains significant amounts of thiamine and therefore baked goods made from wheat flour are potentially a good source of this nutrient. However, this vitamin is heat labile and some destruction occurs during the baking process. It is desirable therefore to determine the stability of this nutrient in these foods during the production procedures, particularly the baking period.

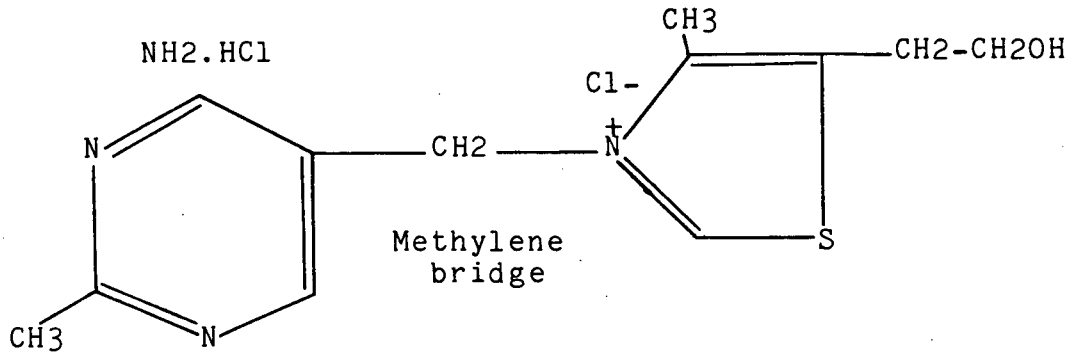
Knowledge about the mechanisms of thiamine degradation can be obtained by studying the behaviour of thiamine under different conditions of temperature, time and pH in bread baking as well as in model systems. Such studies can lead to the definition of optimum conditions for thiamine retention. It would also be interesting to know whether different bread systems, that is, breads made from whole grain flours or enriched white flours, have any effect on the retention of thiamine. The most reliable and satisfactory approach to obtaining insight into this is through simple reaction kinetics.

II

LITERATURE REVIEW

2.1 NOMENCLATURE AND FUNCTION

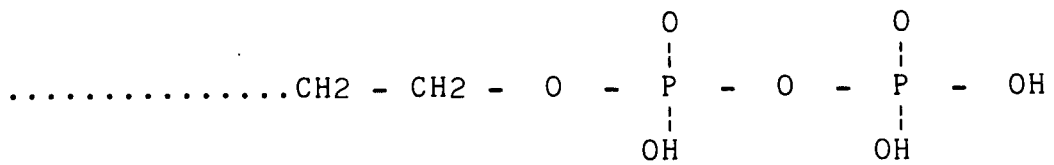
Vitamin B_1 activity was recognized in 1890 by the Dutch physician Eijkman (Neal and Sauberlich, 1973). Prof B.C.P. Jansen of Amsterdam with W.F. Donath first isolated the substance from a natural source in 1926 (Williams, 1938; Dwivedi and Arnold, 1973; Neal and Sauberlich, 1973). Jansen suggested the trivial name Aneurine, which has come into extensive use in Europe. However, because of therapeutic implications, this name, aneurine, was eventually replaced by thiamine (thiamin) (Williams, 1938; Neal and Sauberlich. 1973). It was only in 1936 that R.R. Williams and his colleagues isolated sufficiant quantities of the vitamin to fully identify its structure. They established it as being composed of a pyrimidine and a thiazole moiety and as 3-(2 -methyl -4'-amino -5'-pyrimidylmethyl) -5-(2 -hydroxyethyl)-4 - methylthiazole (Williams, 1938; Harper, 1973; Neal and Sauberlich, 1973). The formula below shows the thiamine hydrochloride form :



Pyrimidine moiety

Thiazole moiety

The rest of this section is documented from Holvey (1972), Harper (1973) and Neal and Sauberlich (1973). Thiamine is readily absorbed from the small intestine with no appreciable storage in the tissues. All excess intake is eliminated in the urine. The active form of the thiamine is TPP (thiamine pyrophosphate), diphospho thiamine or cocarboxylase:



Phosphorylation, which is reversible, occurs in the intestinal mucosa, kidney and liver (Harper, 1973; Neal and Sauberlich, 1973).

TPP participates as a coenzyme in the oxidative decarboxylation of alpha keto acids to aldehydes. These reactions play a major role in energy production (ATP) in plants and animals. In yeasts, TPP promotes alcoholic fermentation.

TPP is also the coenzyme of transketolase that participates in the direct oxidation of pentoses.

Some of the first signs of deficiency are loss of appetite (anorexia), weight loss, loss of muscular tone and depression. Beri-beri, the classical syndrome of thiamine deficiency, is characterized by peripheral neurological changes and cardiovascular troubles with oedema (wet beri-beri) or without (dry beri-beri). Accumulation of pyruvic acid and lactic acid, as well as pentoses, in the tissues is probably responsible for these troubles. Beri-beri is mainly found in the countries where polished rice is the principal food. In the occidental world, a deficiency is observed with alcoholism, particularly where the food intake is very low. This leads to the Wernicke-Korsakoff syndrome (mental confusion, psychosis) when the deficiency is severe and chronic. Thiamine deficiency can be detected by depletion of thiamine in urine and blood, reduction of the activity of erythrocyte transketolase, a thiamine pyrophosphate-requiring enzyme, and abnormal elevation of pyruvic acid and alpha ketoglutarate in blood and urine.

The requirements for thiamine in human nutrition are usually based on caloric intake. They are 0.23 to 0.50 mg per 1000 kilocalories. For added safety, the Food and Nutrition Board (1980) recommends 0.5 mg of thiamine for each 1000 kilocalories in the diet. Therefore the recommended average daily intake for men is 1.2 to 1.4 mg and 1.0 mg for women.

Lipids seem to have a saving effect on thiamine requirements while carbohydrates have an opposite effect.

In the different regions of the world, thiamine is provided by different foods. In North America, cereals provide 35% of the thiamine intake; meat (especially pork), 30%; fruits and vegetables, 15% and dairy products, 10%.

2.2 THERMAL DESTRUCTION OF THIAMINE

Many researchers have worked on the thermal degradation of thiamine for several years. At first, the results were not very accurate because of the poor methods of thiamine determination, for example, rat growth and diazotation methods. The thiochrome procedure is now the most widely used method. Only recently has the kinetic approach been used to determine the degradation of thiamine in several food systems. (Farrer, 1955).

Some of the degradation reaction products in food or in model systems have just been identified (Dwivedi and Arnold, 1973). The main mechanism involves hydrolytic cleavage of the C-N bond of the methyl bridge between thiazole and pyrimidine moieties of thiamine, leading to a pyrimidine derivative, probably 2- methyl- 4- amino- 5- hydroxymethyl- pyrimidine, and 4- methyl- 5- (B hydroxyethyl) thiazole (Williams, 1938; Dwivedi and Arnold, 1972, 1973). A second reaction involves the breakdown of the thiazole ring with hydrogen sulfide as the major degradation product and other volatile products (Dwivedi and Arnold, 1972, 1973).

Many different factors affect the thermal degradation of thiamine, and among them, temperature, time of heating and pH are the most important ones.

According to a number of different authors, as reviewed by Farrer (1955),[1] Feliciotti and Esselen (1957) and Mulley et al. (1975), the degradation reaction for thiamine can be described as following first order kinetics and adhering to the Arrhenius equation. The rate of a first order reaction is directly proportional to the concentration of the reactant. The rate expression which describes a first-order reaction is:

$$-\frac{dc}{dt} = k\,c$$

where c = concentration of reactant

t = time

k = reaction rate constant or velocity coefficient (expressed in units of time $^{-1}$)

Upon rearranging:

$$-\frac{dc}{c} = k\,dt$$

and integrating, this becomes:

$$-\ln c = k\,t + \text{constant}$$

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[1] This author as well as Coppock et al. (1956) have written excellent reviews and much of the information obtained prior to 1955 is in these reviews.

Integrating this equation between limits of concentration c_1 at t_1 and c_2 at a later time t_2, gives:

$$-\int_{c_1}^{c_2} \frac{dc}{c} = k \int_{t_1}^{t_2} dt$$

$$-\ln c_2 - (-\ln c_1) = k\,(t_2 - t_1)$$

$$k = \frac{2.303}{t_2 - t_1} \log \frac{c_1}{c_2}$$

This can be modified to:

$$k = \frac{2.303}{t} \log \frac{c_o}{c} \qquad (1)$$

where c_o = concentration at the beginning (when t is 0)

c = concentration after time t

This equation written in exponential form is:

$$c = c_o\, e^{-kt}$$

The period of half-life, τ 1/2, which is the time necessary for half a given quantity of material to decompose, can be calculated by the substitution of the appropriate numerical values into equation (1):

$$k = \frac{2 \cdot .303}{\tau\, 1/2} \log \frac{1}{1/2}$$

$$\tau\ 1/2 = \frac{0.693}{k}$$

τ 1/2 is usually independent of the concentration of the initial substance.

The rate constant generally depends on the absolute temperature following the law first proposed by Arrhenius in 1889:

$$k = A\, e^{-Ea/RT}$$

where k = reaction rate constant

A = frequency factor

Ea = energy of activation (kcal/mole)

R = gas constant (1.987 kcal/mole K^{o})

T = absolute temperature (K^{o})

(Daniel and Alberty, 1958; Boudart, 1968; Capellos and Bielski, 1972; Adamson, 1979)

A review of the kinetics of thiamine in buffer systems and in foodstuffs is given in Table 1.

Many researchers reported the well known fact that thiamine is rapidly destroyed in neutral and alkaline medium. Farrer (1941) plotted log k against the corresponding pH and showed an abrupt increase in the slope of the curve, forming two distinct straight lines. He concluded that the reaction rate constant was inversely proportional to the hydrogen ion concentration. Lincoln et al. (1944) studied the loss of thiamine during cooking (at 100°C) of flour enriched with thiamine hydrochloride and cocarboxylase at five different pH values ranging from 5.8 to 7.5. Cooking losses increased with pH. They also looked at the same two forms of thiamine in phosphate buffer solutions at pH values of 3.5 to 7.0 at

Table 1

Review of kinetic studies for the thermal degradatation of thiamine

Reference	Medium	pH	Temperature range	Ea Kcal/mole	range (min^{-1})
Watanabe (1939)[1]	aqueous solution		248°F		
Greenwood et al. (1944)	pork (lunch meat)		210-250°F		
Rice and Beuk (1945)	pork (lean)		49-121°C		
Farrer and Morrison (1949)	phosphate buffer		120-230°F		
Bendix et al. (1951)	whole peas	natural pH	220-270°F	21.2	0.0058-0.0351
Farrer (1953)	potatoes	6.0	212°F		0.0026
	carrots	5.7	212°F		0.0022
	peas	6.5	212°F		0.0021
	cabbage	5.5	212°F		0.0027
Garrett (1956)[2]	B_1-HCl liquid multivitamin prep.	3.2	39-158°F	26	0.00118[a]
Feliciotti and Esselen (1957)	aqueous solution	3.5	228-300°F		0.0026-0.0944
	phosphate buffer	4.5-7.0	228-300°F	28.8	0.0024-2.092
	carrot purée	6.1	228-300°F	27.0	
	green bean purée	5.8	228-300°F	27.0	
	pea purée	6.8	228-300°F	27.0	
	spinach purée	6.7	228-300°F	27.0	0.0049-0.2326
	beef heart purée	6.1	228-300°F	27.0	
	beef liver purée	6.1	228-300°F	27.0	
	lamb purée	6.2	228-300°F	27.0	
	pork purée	6.2	228-300°F	27.0	

Table 1 (continued)

Reference	Medium	pH	Temperature range	Ea Kcal/mole	range (min^{-1})
Gillepsy (1962)[2]				20.0	
Mulley et al. (1975a)	phosphate buffer	6.0	250-280°F	29.4	0.015-0.067a
	pea purée	natural	250-280°F	27.5	0.0093-0.038a
	beef purée	pH	250-280°F	27.4	0.0091-0.037a
	peas-in-brine purée	"	250-280°F	27.0	0.010-0.039a

[a]Calculated from the D values (k=2.303/D).

[1]Referenced in Farrer (1955).

[2]Referenced in Lund (1975).

120°C. Losses increased with pH, with greater losses between pH 6.25 and 7.0. Pace and Whitacre (1952) studied the pH effect in corn bread. At some pH value between 6.2 and 6.6 for the batter (corresponding to pH 7.2 to 8.9 for the resultant breads) there appeared to be a critical point at which thiamine was rapidly destroyed during baking. With increasing pH from 4.5 to 7.0 in a phosphate buffer at 228, 246, 264 and 282°F (109, 119, 129 and 139°C), Feliciotti and Esselen (1957) observed an increase in the rate of thiamine destruction. The most pronounced change was between pH 6.0 and 6.5, as indicated by a change in the slope of the log k versus pH curves (similar to Farrer, 1941). The most recent study on the pH effect in a phosphate buffer solution is by Mulley et al. (1975b). The rates of destruction curves at 265°F (129°C) were determined for thiamine hydrochloride, cocarboxylase and mixtures of the two at pH 4.5, 5.0, 6.0 and 6.5. Log D values (time for 90% destruction) were plotted against pH. The D values for pH 4.0 and 5.0 were similar for each system individually. However, when the pH exceeded 6.0, the D values decreased sharply showing a decreased stability of the thiamine molecule. In the case of cocarboxylase, the change in the slope of the curve occurred at a lower pH than it did for the thiamine hydrochloride. Dwivedi and Arnold (1972) suggested that thiamine would be more stable at pH 3.5 than pH 5.0 or 6.0 because the protonated form of thiamine, which predominates at pH 3.5, is less prone to thermal destruction.

It is well recognized that thiamine can occur in three forms: free thiamine, pyrophosphate ester (cocarboxylase) and protein-bound thiamine (Booth, 1943; Greenwood et al., 1943; Lincoln et al., 1944; Farrer, 1955). Greenwood et al. (1943) showed that cocarboxylase was slightly more resistant than thiamine, however other workers (Booth, 1943; Lincoln et al., 1944; Farrer, 1945; Mulley et al., 1975b) found the opposite to be true, i.e. cocarboxylase was much more susceptible to degradation whether in buffer solutions or in foodstuffs. Mulley et al. (1975b) also mentioned that concentrations of less than 35% of the cocarboxylase form (which is the amount normally present in foods) did not affect the kinetics of the thermal destruction of this vitamin. The protein-bound thiamine has a greater thermal stability, according to Farrer (1955). However, Feliciotti and Esselen (1957) mentioned that the combined form (probably the protein-bound form) was found to be less stable, at a given pH, than the free form. These authors suggested that thiamine degradation in foods may be dependent on the interrelationship of pH and the proportion of the free and the combined form of the vitamin.

The work of these different researchers has demonstrated that thiamine in food products is notably more resistant to heat than pure thiamine in aqueous or buffer solutions. This would indicate that many factors other than the ones mentioned previously could affect the thermal degradation of

thiamine. These are still not very well established, however they cannot be ignored, and they will be discussed briefly.

Farrer (1955) and several others since then reported that acids, gelatin, albumin, proteins, α and β amino acids, calcium hydrogen phosphate, soluble starch, gums, dextrins, fructose, invertase and inositol have a protective or stabilizing effect upon thiamine. Borate, thiosulfate, acetate, carbonate, monohydrogen phosphate, potassium dihydrogen phosphate, oxidizing agents (like potassium bromate, a dough improver), γ , δ , ε -aliphatic amino acids, p-aminobenzoic acid, copper, gamma radiation, ultraviolet light and ultrasonic waves would accelerate thiamine destruction. Fe, Zn, Al, Sn and Ni could modify the destruction reaction or be without effect, depending on the conditions obtained in the solutions. No effect on thiamine stability has been observed with lithium, sodium, potassium, chloride, potassium nitrite, sulfate, iodide, magnesium ions, glucose, ethyl alcohol, glycine, xanthine and riboflavin. Thiaminases, enzymes found mainly in fresh water fish, shellfish and molluscs, are capable of destroying thiamine. The percent of moisture in the food, together with other factors, also influences thiamine destruction. The rate of destruction is more likely to increase as the product becomes more concentrated. Finally, Farrer(1955) commented that under normal conditions found in foods, most of these factors do not play a very significant role in thiamine degradation.

2.3 THIAMINE LOSSES IN BREAD

Even though no work has been done on thiamine kinetics in bread, many studies on thiamine losses in bread and baked products were reported. The data of these studies are presented in Appendix A.

A review of the data in the literature (as presented in Appendix A) allows one to conclude that the thiamine losses of baked bread are of the order of 20%, whatever the conditions. The only exceptions are the 43 and 47% losses found by Tabekhia and D'Appolonia (1979). Among all these authors, two different conclusions were reached. Some authors, Schultz et al. (1942) and Coppock et al. (1956). agreed that bread made from flours of different extractions show similar retention rates, whereas Farrer (1949) and Dawson and Martin (1942) found higher losses from high-extraction flours. Goldberg and Thorpe (1946) cited in Coppock et al. (1956) also agreed with the latter, but attributed this result to a longer baking time for the wholemeal (100% extraction) bread.

There are several theories explaining the differences in thiamine retention between low and high extractions flours. Dawson and Martin (1942) suggested two hypotheses. The first was that these differences were due to changes in pH, although Farrer (1949) cited in Coppock et al. (1956) commented that in Dawson and Martin's study little difference in the pH values were detected in the bread. The second hy-

pothesis was that the free thiamine was absorbed by the yeast during fermentation and then was protected during subsequent baking. They suggested that this would explain higher losses at high extraction rates, especially if the vitamin in the yeast was less susceptible to heat and if thiamine was bound to bran, and therefore less available to the yeast. Here again Farrer (1949) criticized them by saying that thiamine in yeast was in the cocarboxylase form, which was more thermolabile than the free form.

An interesting explanation for higher losses at higher extraction rates is related to the ash or mineral content of the flours. Dawson and Martin (1942) and Farrer (1955) showed a linear relationship when ash content, which is higher as extraction rates increased, was plotted against thiamine destruction. Farrer (1955) also observed that the pH increased with the percent of extraction (from pH 5.68 with 75% extraction to pH 5.98 with 100%). He concluded that because he had shown in previous work (Farrer, 1945) only a slight increase in the k values between pH 5.0 and 6.0 in phosphate buffer solutions (which is the predominant anion in cereals) then the increase in the destruction rates may be due not only to the pH but also to the increase in inorganic constituents. Coppock et al. (1956) commented that they disagreed with the ash theory since they showed similar thiamine losses for all the different extractions.

Only one study by Van der Mijl Dekker (1951) cited in Coppock et al. (1956) showed smaller losses at higher extraction rates. This was attributed by other authors (Farrer, 1955; Coppock et al., 1956) to the use of the diazotation procedure, a very poor method for thiamine analysis.

The source of thiamine would be a factor that affects its stability. As previously mentioned, the cocarboxylase form (which is the form of thiamine in yeast) is more vulnerable to heat. Farrer (1955) suggested that more cocarboxylase could be present in the higher extraction flours, which would explain the higher thiamine losses. However, some authors referenced by Farrer (1955), agreed that wheat contains little cocarboxylase and little or no protein bound thiamine. The cocarboxylase content of 74.9, 85, 98 and 100% extraction flours were reported to be 8.2, 12.9, 11.3 and 10% respectively.

A study described by Coppock et al. (1956) using internal temperatures of various 450 g (1 lb) bread loaves, cakes and biscuits found a linear relationship between the product of minutes heated above 80°C and final temperature minus 80°C versus average percent loss. This experiment took into consideration the bulk of the product.

And finally, Coppock et al. (1956) concluded from this latter study, as well as other studies and their own work, that 'thiamine destruction in baked products is mainly thermal and only to a relatively small extent affected by other factors'.

Although a number of studies have been carried out on thiamine losses in bread, these have been conducted over a wide range of conditions, making it difficult to compare them. In addition, there have been only a few studies attempting to investigate the mechanism of thiamine degradation in food using the kinetic approach, and none have been done on bread. As stated earlier, the kinetic approach allows one to obtain standardized data to make comparative studies.

The objectives of this thesis were to study the destruction of thiamine (using the kinetic approach) in:

1. a phosphate buffer solution at pH 6.0 over the temperature range 80 to 120°C.
2. a phosphate buffer solution at 120°C over the pH range 4.0 to 7.0.
3. in a semi-model enriched white and whole wheat bread system at natural pH over the nominal oven temperature range 177 to 288°C (350 to 550°F).

Furthermore, thermal destruction in bread was to be studied to determine whether any difference in the destruction of the natural and synthetic sources of thiamine occurred by comparing their rate of destruction.

III

MATERIALS AND METHODS

All thiamine analyses were done by the thiochrome method described by the Association of Vitamin Chemists (1966). The fluorescence was measured with an Aminco-Bowman Spectrofluorometer (American Instruments Co. Inc, Silver Spring, MD) and was recorded on a photomultiplier- microphotometer. The wave lengths used were 365 nm for excitation, and 435 nm for the output. Blanks were used in each run.

An Accumet Model 230 pH/ion meter (Fisher Co., Pittsburg, PA) was used to check pH values.

All chemicals used were reagent grade and/or prepared as specified in the procedure.

3.1 MODEL SYSTEM

3.1.1 Vitamin analysis

Because the analyses were made on pure solutions, the extraction and purification steps were omitted from the thiochrome method. For each run, two standards and two reference controls (unheated samples representing 100% thiamine) were measured.

3.1.2 Preparation of buffered thiamine solutions

Intermediate buffered thiamine solutions were made by adding 5.0 ml of stock thiamine solution (see Thiochrome method, p.130 in Assoc. of Vit. Chem., 1966) to a buffer solution and diluting to 100 ml. Two different working buffer solutions were used. One, for the test tubes, was made by adding 5.0 ml of intermediate thiamine solution to a buffer solution and diluting to 100 ml. The concentration of this working buffer was 0.25 µg thiamine/ml. The other working buffered thiamine solution, for the TDT (thermal death time) tubes, was made with 30.0 ml of intermediate buffered thiamine diluted to 100 ml with a buffer solution, giving a concentration of 1.5 µg thiamine/ml.

3.1.3 Treatment of samples

The samples heated at temperatures lower than 100°C were heated in a water bath using test tubes closed with a metal cap. Those heated at temperatures higher than 100°C were heated in a oil bath using TDT tubes sealed in an oxy/acetylene flame. Both baths were equipped with temperature controls. After heating, the tubes were cooled immediately in an ice bath. HCl (0.01N) solution was added to the samples to adjust the pH to between 3.5 and 4.5. The final volume of all samples was 6.8 ml. The final concentration of all unheated samples was 0.2193 µg thiamine/ml. The samples were then stored at 4°C in an ice bath until needed for analysis.

Preliminary work showed that the ice holding time and storage at 4°C up to 30 hr did not have any significant effect on thiamine concentration.

For each heating time, a total of 2 to 6 replicates were done over several days.

3.1.4 Come-up times

Come-up times for 80, 90 and 100°C were estimated from heat penetration data obtained from two test tubes monitored with thermocouples. The come-up times were found to be 3.6 min for 80°C, 4.0 min for 90°C, and 2.3 min for 100°C. In the assays using the TDT tubes, the come-up times were estimated to be 0.6 min according to the procedure described by Sognefest and Benjamin (1944). In the present study the come-up time corrections were not applied to the data, since the estimated come-up times were very small and the cooling lag factor will compensate for the heating lag factor.

3.1.5 Temperature effect

The buffer system used was a 0.2 M phosphate buffer (Sorensen) (Gomori, 1955) at pH 6.0. Destruction rates were determined at temperatures of 80, 90, 100, 110, 115 and 120°C.

The kinetic rate data were obtained graphically by plotting the logarithm of percent thiamine retention against time for different temperatures. The lines were all forced through 100% thiamine retention. Slopes and coefficient of determination (r^2) were calculated by linear regression

equation using appropriate formula for slopes forced through the origin (Zar, 1974, p.214). The destruction rates, k, expressed in reciprocal minutes, were calculated by the formula:

$$k = -2.303 \text{ slope}$$

Half-life values (τ 1/2) were calculated by the formula:

$$\tau\ 1/2 = \frac{0.693}{k}$$

Log k values were plotted against the reciprocal of the absolute temperature (^{o}K) x 1000. Slopes were determined by the method of least squares. The energy of activation, Ea, expressed in kilocalories per mole, was calculated by the formula:

$$k = A\, e^{-Ea/RT}$$

$$Ea = -2.303\ R\ (\text{slope})$$

where R (gas constant) = 1.987 kcal/mole K^{o}

3.1.6 pH effect

The destruction rates were determined at 120°C and pH of 4.0, 4.5, 5.0, 6.0 and 7.0. This temperature was taken as being representative of average bread temperature while baking. The pH 6.0 data were previously determined in section 2.1.5. Phosphate buffer (0.2M) (Sorensen) (Gomori, 1955) was used for pH 6.0 and 7.0 and 0.2M phosphate-0.1M citric acid buffer (McIlvaine, 1921) for the pH below 6.0. The kinetic rate data and τ 1/2 values for the pH effect were ob-

tained in the same way as for the determination of the temperature effect.

To evaluate the effect of the pH on the stability of thiamine, log k values were ploted against pH.

3.2 BREAD SYSTEM

3.2.1 Thiamine analysis

The enzyme used for the extraction step was acid phosphatase (0.4 units/mg) at a concentration of 0.25 g/100 ml. This step was not omitted since preliminary work showed higher thiamine recovery for commercial enriched white and whole wheat breads when using this enzyme. The purification procedure was carried out as well, because preliminary determination showed that without this step, the solutions were not clear and blank values were very high (see thiochrome procedure 2 (b)(6), in Assoc. of Vitamin Chemists, 1966). Ionac C-102 (modified alumino silicate resin 30-80 mesh-85%, by MCB, Los Angeles, CA) and activated Decalso (Permutit-T by the Fisher Co., Pittsburg, PA) were used as column material.

Two standards were used for each run. Two samples of dough after proofing (before baking) were analysed at each run as the reference representing 100% thiamine retention. Each sample was finely ground in a Cuisinart Food Processor in preparation for the extraction procedure.

3.2.2 pH determination

In the 12 g bread assays, the pH of the bread was measured in a constantly stirred slurry made of bread crumbs, or dough, and distilled water.

3.2.3 Moisture determination

The moisture in the samples (dough and baked product) were determined by the standard method (AOAC, 1980) using the ground up material left over from the extraction step. Results could therefore be calculated on dry weight basis.

3.2.4 Apparatus for heating

A Despatch laboratory electric oven (Minneapolis, MN) with Robertshaw thermostat control was used to heat all the samples.

3.2.5 Flour samples

The commercial enriched all purpose white flour used in all assays was labelled as containing 0.45 mg of thiamine per 100 g of flour.

3.2.6 Enriched white flour slurry assay

Mixtures of 100 g of enriched flour and 150 g of tap water were heated at a nominal oven temperature of 246°C (475°F) for 60, 75 and 90 min. Duplicate determinations for each baking time were used. Moisture analysis was done on

each baking time mixture for each pair of duplicates. Analysis was performed right after cooling the samples in an ice bath. Percent thiamine retentions were calculated.

3.2.7 One pound loaves (enriched white bread)

The bread formula and procedure were those used by Tabekhia and D'Appolonia (1979). Bread loaves were also baked at an oven temperature of 221°C (430°F). Two slices from each loaf were stored at -10°C prior to analysis.

3.2.7.1 Experiment 1

Two loaves of bread were baked for 30 minutes. An average moisture content, using duplicates, was determined on slices from both loaves. Percent thiamine retentions were recorded for each bread.

3.2.7.2 Experiment 2

Three loaves of bread were baked for 15, 37 and 60 min. No moisture analyses were done. Percent thiamine retentions were recorded for each duplicate for the different baking times.

3.2.8 12 g loaves (enriched white and whole wheat bread)

The following formula, similar to straight-dough formula from AACC (1969), was used:

Flour.....120.0 g

Water..... 70.0 g

Sugar..... 6.5 g

Shortening..... 4.0 g

Yeast..... 2.7 g

Salt..... 1.5 g

A straight-dough method with 3 hr fermentation and 55 min proofing done in a fermentation cabinet at 36-38°C was used. After the fermentation period, the dough was allowed to rest for 10 minutes, then it was divided into approximately 20 g loaves (12 g flour each), and finally molded by hand and placed into bread pans (dimensions: top: 5.8 cm x 3.1 cm; bottom: 5.0 cm x 2.3 cm; height: 2.3 cm).

3.2.8.1 Treatment of samples

Bread loaves were baked for 4 different time periods at 4 different nominal oven temperatures. Enriched white and whole wheat bread loaves were baked in different batches. The bread was considered to be underbaked for the 2 first time intervals. For the 3rd time, it was normal baked, and the last one was overbaked. Normal bake refers to the stage during baking where the colour of the crust and degree of doneness are optimum for consumption in terms of its functional and organoleptic properties. Underbaked means when the bread has not been baked long enough to reach the normal bake stage, and overbaked when it has been baked beyond the normal bake stage. An illustration, on the following page (Figure 1), of the enriched white and whole wheat bread

shows the loaves baked at the 4 different baking time periods.

After each baking time, the loaves were removed from the oven, allowed to cool at room temperature, and then were frozen (-10°C). Just before the extraction procedure, the bread was thawed at room temperature, or at 37°C. The entire bread loaf was used for the extraction step in the thiochrome procedure. Each run included two replicates of each baking time at a given temperature for each type of bread.

3.2.8.2 Experiment 1

The nominal oven temperatures used were 163, 191, 218 and 246°C (325, 375, 425 and 475°F). Two replicates, baked in the same oven load, for each time and temperature treatment were used. No moisture analyses were done. Only the oven temperature was monitored, using thermocouples. pH of bread after baking was recorded. The percent thiamine retentions were calculated from these experimental results.

3.2.8.3 Experiment 2

The nominal oven temperatures used were 177, 218, 246 and 288°C (350, 425, 475 and 550°F). Four replicates, baked in the same oven load, were used for each time and temperature treatment. An average moisture content, using duplicates, was determined on each pair of replicates analysed in the

Figure 1: Enriched white and whole wheat loaves baked at 4 different baking time periods.

the same run. pH of dough before proofing, dough after proofing and bread after baking were recorded.

Heat penetration data.

The oven, crust and bread crumb temperatures were monitored with a 0.12 mm diam. (crust) and 0.60 mm diam. (middle and oven) copper-constantan thermocouple on a Digitec Datalogger (United Systems Corporation, Dayton, OH), which recorded the temperature at one minute intervals. In each oven load, two bread pans were equipped with thermocouples. One thermocouple was placed in the middle of the loaf, and another was inserted just beneath the top surface of the dough. One thermocouple monitored the oven temperature in the middle of the oven, just above the loaves. The mass average temperatures (MAT) at minute intervals for each bread treatment were calculated by using the Stumbo (1965) formula that applies to a cylinder shaped mass:

$$MAT\ (^{o}C) = {}^{o}Tcrust - 0.27({}^{o}Tcrust - {}^{o}Tmiddle)$$

This Stumbo formula is only an approximation of the real average bread temperature for a given time. Heat penetration curves (mass average temperature versus time) were plotted for each oven load, taking the average of the two bread MAT data. In addition, the heat penetration data for the crust and the middle were plotted for enriched white bread baked at 218°C (425°F) (nominal temperature). The average oven temperature for each load was also calculated.

Treatment of data.

Degradation reaction rates were obtained graphically by plotting the logarithm of percent thiamine retention against time for the various baking temperatures. All the lines were forced through 100% thiamine retention. All slopes and r^2 were calculated by the method of least squares (Zar, 1974, p. 214).

Half-life values (τ 1/2) were calculated by the formula:

$$\tau\ 1/2 = \frac{0.301}{\text{slope}}$$

Interpretation of data.

The slopes were used to compare the stability of thiamine in 1) the enriched white and whole wheat breads for each different temperature; 2) the same type of bread at different oven temperatures.

Statistical analysis

Slopes for the results of data pertaining to bread types (1 above) were compared using the Students' t-test. Slopes for the results of data for different oven temperatures (2 above) were compared using F-test and SNK (Student-Newman-Keul).

Deviation from linearity, viz. whether Y is a linear function of X, was tested for each slope. The test is analogous to a one-way ANOVA. Essentially, the total variance is divided into between groups SS and within groups SS, and the between groups SS was further divided so that the devia-

tion from linearity SS is equal to the between groups SS minus the regression SS. If the F value is high, viz. the deviation from linearity MS is greater than the within groups MS, then the null hypothesis that the regression is linear is rejected. Results were considered to be significant at the maximum limit of the 5% probability level. (Zar, 1974, chap.16 and 17, and Appendix B)

IV

RESULTS

4.1 MODEL SYSTEM

A sample calculation of percent thiamine retention is given in Appendix C.

4.1.1 Temperature effect

Table 2 shows the average percent retention ± the standard deviation for thiamine hydrochloride in phosphate buffer (pH 6.0) for different times at temperatures ranging from 80 to 120°C. Coefficients of variation for the percent thiamine retention data varied from 0.0 to 29.8%. Thermal destruction curves for these data are given in Figure 2 and 3. The extremely good fit of the experimental points to a straight line is strong evidence that the thermal destruction of thiamine in phosphate buffer is first order in nature.

Table 3 gives the k values and the coefficients of determination (r^2) for each of these destruction curves. The very high r^2 values also demonstrate the excellent fit of the curves. These results reveal that thiamine stability decreases as temperature increases.

Table 2

Retention of thiamine hydrochloride in phosphate buffer (pH 6.0) at different heating times between 80 and 120°C.

Time	Thiamine retention[a] (%)					
	80°C	90°C	100°C	110°C	115°C	120°C
10 min						72.0±0.0(2)
20 min					69.4±2.5(3)	51.8±4.5(4)
30 min				72.6±3.8(4)		37.0±1.8(4)
40 min					49.4±8.6(4)	27.2±8.1(4)
50 min						24.1±3.9(3)
60 min		87.0±1.9(6)	77.6±3.8(4)	49.9±2.2(3)	30.6±6.0(4)	15.6±2.2(3)
80 min					29.1±0.3(2)	
90 min				37.3±5.7(4)		
100 min					17.1±1.7(3)	
120 min		80.6±4.2(4)	66.9±2.6(4)	25.9±1.8(4)	9.6±0.7(2)	
3 hrs		76.0±1.5(4)	55.5±3.4(4)	13.6±1.2(4)		
4 hrs		64.9±1.8(3)	47.1±4.6(4)			
5 hrs			38.3±5.1(4)			
6 hrs		58.9±4.0(4)	32.4±2.1(4)			
7 hrs		49.2±3.5(4)				
8 hrs	80.0±4.4(4)					
12 hrs		32.8±3.8(2)				
24 hrs	45.5±1.8(4)					
46 hrs	22.4±3.5(4)					
72 hrs	9.8±1.8(4)					

[a]Values are presented as means ± standard deviations. Numbers in parentheses refer to number of replicates.

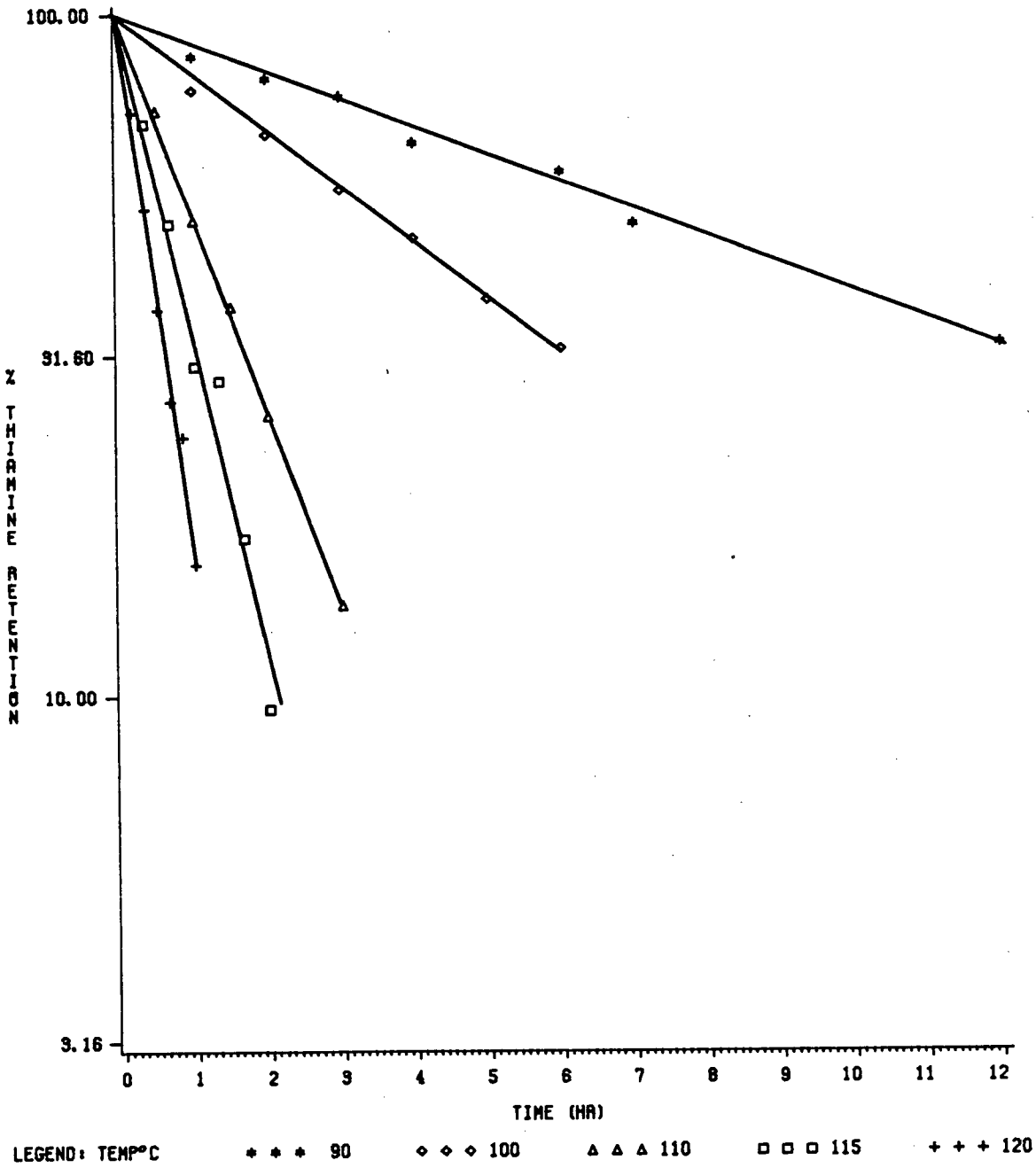

Figure 2. Retention curves for thiamine hydrochloride in phosphate buffer (pH 6.0).

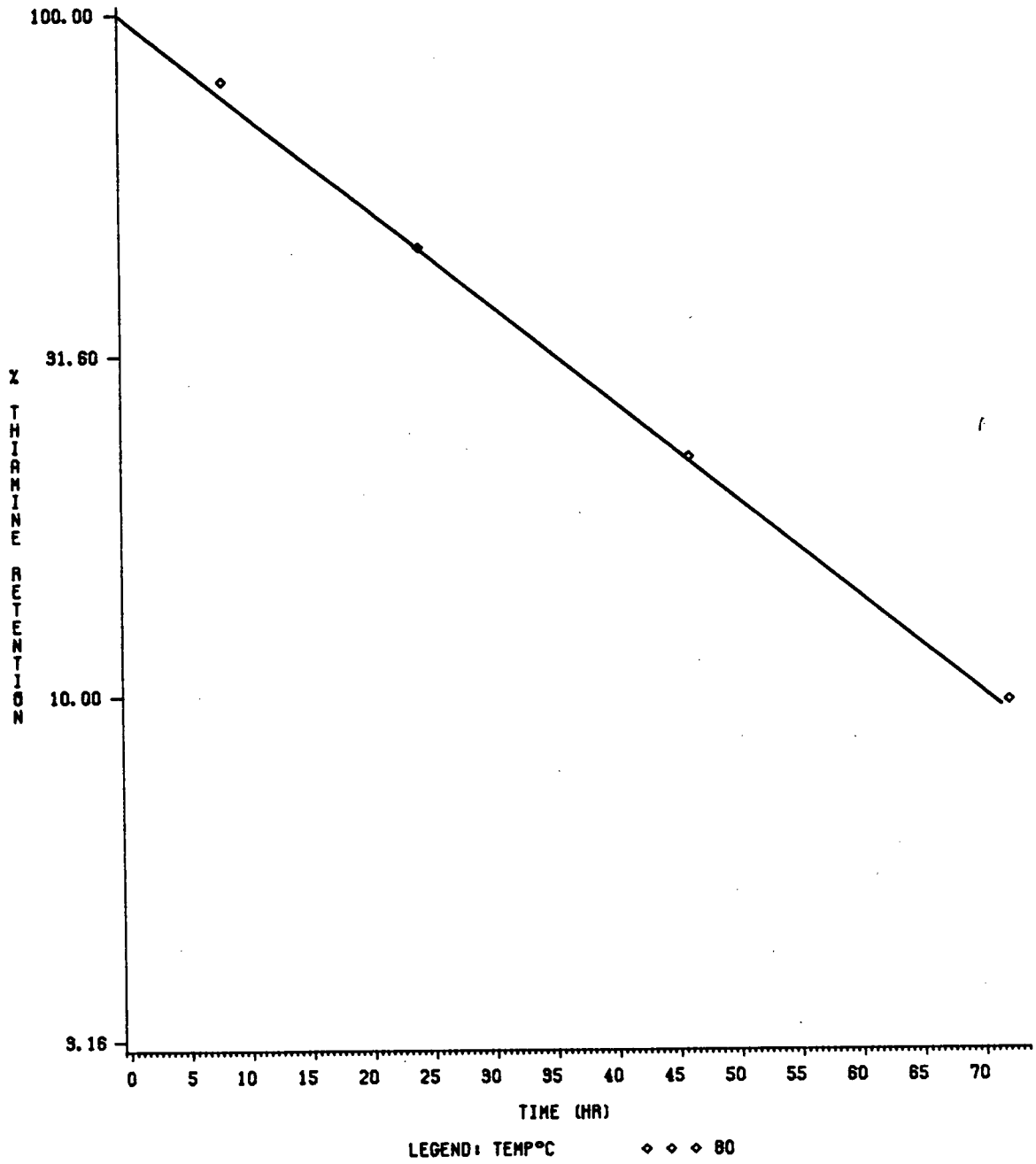

Figure 3. Retention curve for thiamine hydrochloride in phosphate buffer (pH 6.0).

TABLE 3

k, r^2 and $t_{1/2}$ values for thiamine HCl in phosphate buffer (pH 6.0) in the temperature range 80 to 120°C.

Temp (°C)	k (min^{-1})	r^2	$t_{1/2}$ (min)
80	0.0005477	0.9952	1265
90	0.001602	0.9862	433
100	0.003199	0.9894	217
110	0.01116	0.9952	62
115	0.01827	0.9871	38
120	0.03132	0.9845	22

Table 3 gives the half-life values for the different heating temperatures.

In Figure 4, the linearity of the Arrhenius plot indicates that the data conform to the Arrhenius equation. The energy of activation, Ea, obtained from this curve for the phosphate buffer system is 34.2 kcal/mole, with a r^2 value of 0.8375, which shows a good fit of the data to the curve.

4.1.2 pH effect

The average percent thiamine retention ± the standard deviation for thiamine hydrochloride in phosphate buffer at pH 4.0 to 7.0 heated at 120°C for different lengths of time are given in Table 4. Coefficients of variation for the percent thiamine retention values among the different replicates vary from 0.0 to 25.5%. Figure 5 shows the destruction curves plotted from these data. Like in the temperature assay, the experimental points fit very well to the line. The k values of these destruction curves with the r^2 values are given in Table 5. High r^2 values confirm the good fit of the lines.

Half-lives for the different pH are given in Table 5.

When the k values are plotted against pH (Figure 6), the graph shows that thiamine stability decreases with pH, with greater losses when the pH reaches 6.0. One can observe that the experimental points fit on a irregular curve shaped line.

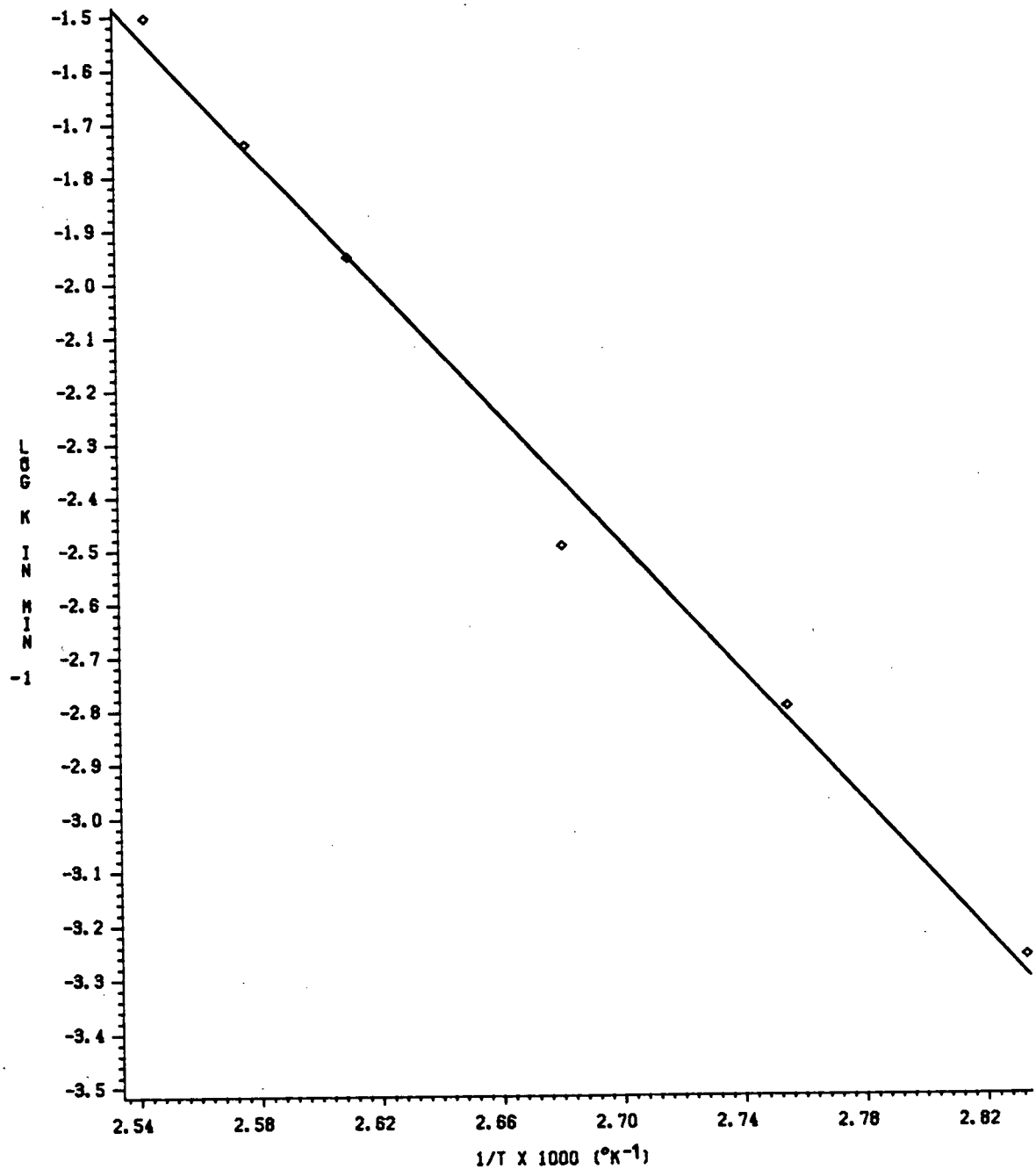

Figure 4. Arrhenius plot for the thermal degradation of thiamine hydrochloride in phosphate buffer (pH 6.0).

Table 4

Retention of thiamine hydrochloride in phosphate buffer (120°C) at different heating times between pH 4.0 and 7.0.

Time (Min)	Thiamine retention[a] (%)					
	4.0	4.5	5.0	5.5	6.0	7.0
3						48.4±3.9(3)
7						11.2±1.2(3)
10					72.0±0.0(2)	5.6±1.1(2)
12						3.7±0.2(2)
15				83.2±4.9(4)		
20					51.8±4.5(4)	
30			63.0±4.6(3)	63.1±2.8(4)	37.0±1.8(4)	
32			61.5 (1)			
38		58.3±0.5(2)				
40	62.8±2.5(4)				27.2±8.1(4)	
45				55.7±0.7(2)		
50					24.1±3.9(3)	
60			39.2±7.4(6)	42.3±4.1(6)	15.6±2.2(3)	
78		36.8±1.5(2)				
80	43.9±0.8(3)					
90			25.5±3.1(6)	36.1±3.9(3)		
110		27.1±6.9(2)				
120	32.3±2.3(2)	21.5±1.8(2)	19.6±3.4(5)			
160	21.8±2.5(3)					

[a]Values are presented as means ± standard deviations. Numbers in parenthesis refer to number of replicates.

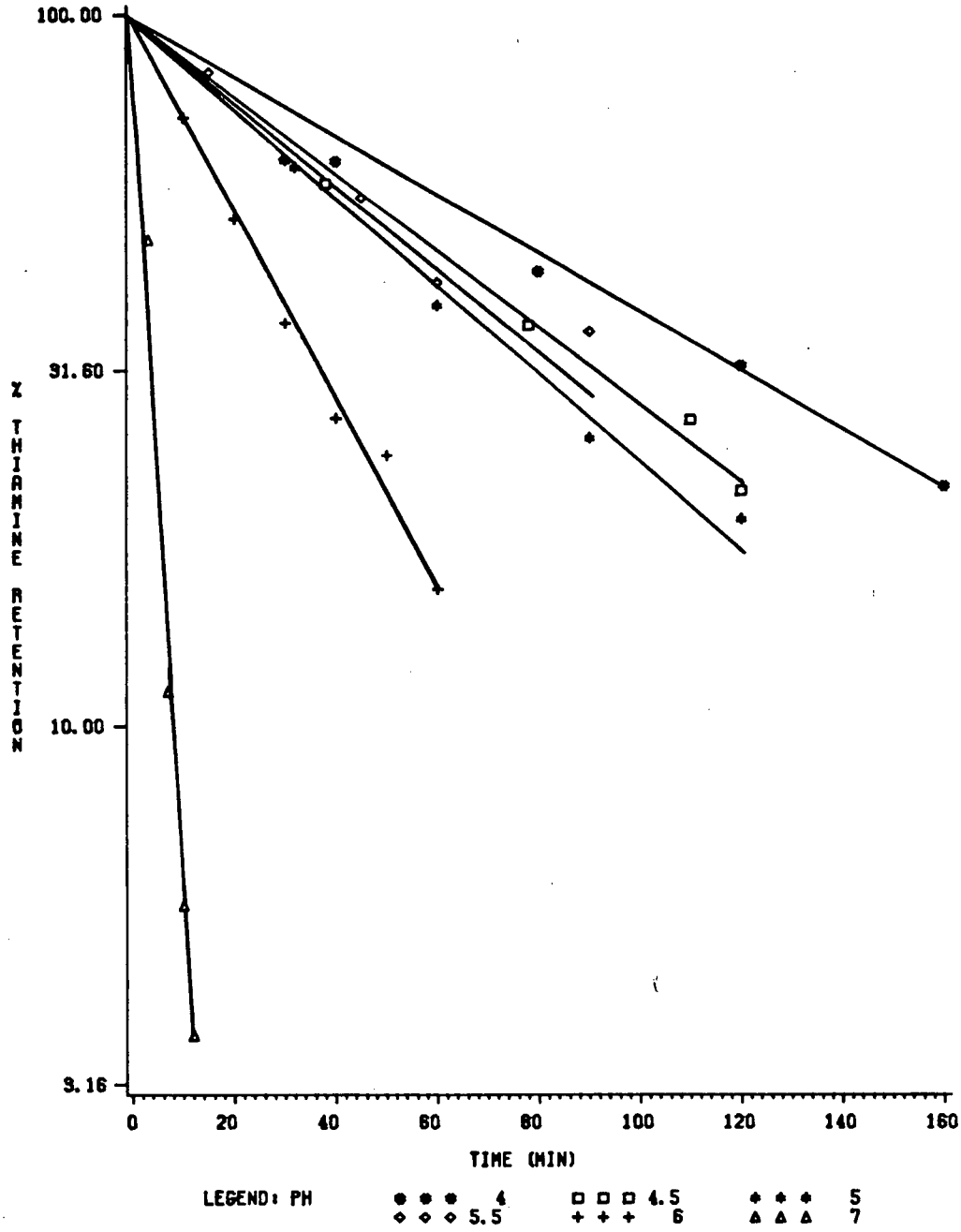

Figure 5. Retention curves for thiamine hydrochloride in phosphate buffer at 120°C at various pHs.

TABLE 5

k, r^2 and $t_{1/2}$ values for thiamine HCl in phosphate buffer (120°C) in the pH range 4.0 to 7.0.

pH	k (min^{-1})	r^2	$t_{1/2}$ (min)
4.0	0.009733	0.9942	71
4.5	0.01261	0.9915	55
5.0	0.01459	0.9838	47
5.5	0.01299	0.9769	53
6.0	0.03132	0.9845	22
7.0	0.2870	0.9951	3

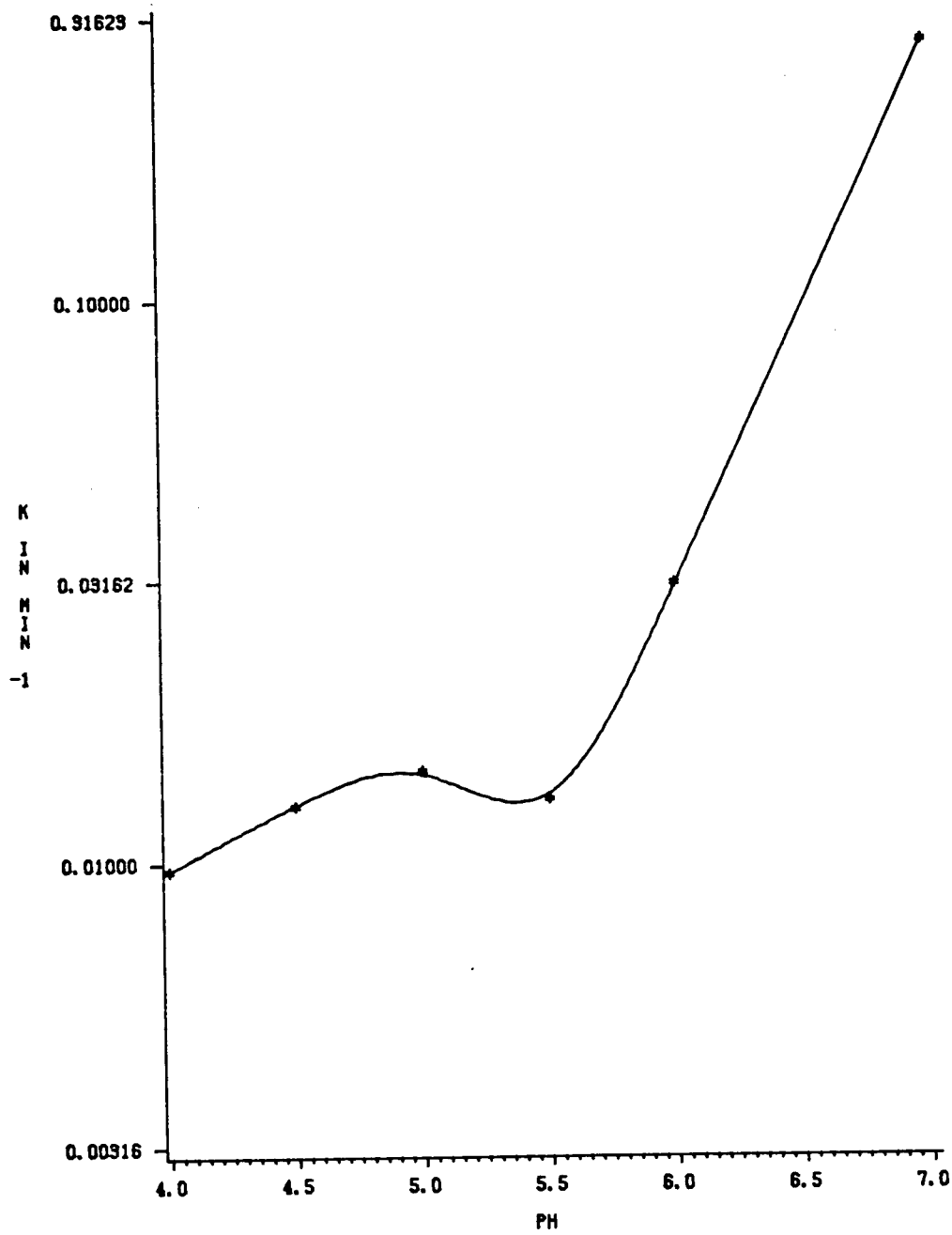

Figure 6. The effect of pH on the destruction rate constant for thiamine hydrochloride in phosphate buffer at 120°C.

4.2 BREAD SYSTEM

A sample calculation of percent thiamine retention is given in Appendix C.

4.2.1 Enriched white flour slurry assay

Results for the average thiamine retention are shown in Table 6. After 60 minutes of baking at 246°C (475°F), no appreciable thiamine destruction was found, whereas for both 75 and 90 minutes of baking, about 30% of thiamine was destroyed.

4.2.2 One pound loaves (enriched white bread)

4.2.2.1 Experiment 1

After baking 450 g (1 lb) loaves of bread for 30 minutes at 221°C (430°F) (oven temperature), no destruction of thiamine was found. The percent thiamine results were actually slightly higher than 100%: 103.3%, for loaf 1, and 105.2%, for loaf 2.

4.2.2.2 Experiment 2

Table 7 shows the mean percent thiamine retention of 450 g (1 lb) loaves made from enriched white bread and baked for different times. The results are inconclusive because they are on a wet weight basis. Because bread becomes dryer as baking time increases, and because percent thiamine results

TABLE 6

Thiamine retention for enriched flour slurry for different baking times at 246°C (475°F) (nominal).

Baking time (min)	Thiamine retention* (%)		
60	97.6	±	6.0
75	72.1	±	16.7
90	70.8	±	6.9

*Mean of duplicates ± standard deviations.

TABLE 7

Thiamine retention in 450 g (1 lb) loaves of enriched white bread baked at 221°C (430°F) for different times.

Baking time (min)	Thiamine retention* (%)		
15	126.3	±	4.8
37	112.3	±	21.3
60	97.9	±	1.3

*Means of duplicates ± standard deviations.

are based on the bread dough, which has a higher moisture content than the baked bread, each percent thiamine result is therefore calculated on a different weight basis and thus cannot be compared to each other. However, if the dry weight data of the dough and bread at 30 min baking from Experiment 1 are considered in the calculation of the % thiamine retention for the 37 min baking, the result is 97.6% retention. This result agrees very well with the one from Experiment 1.

4.2.3 12g loaves (enriched white and whole wheat bread)

4.2.3.1 Experiment 1

The mean pH value of two replicates (one baked at 191°C and the other one at 246°C - nominal temperatures) was 5.08 for enriched white bread and 5.63 for whole wheat bread. pH of whole wheat bread was slighty higher than enriched bread.

Table 8 shows the mean percent thiamine retention for enriched white and whole wheat bread at four different oven temperatures, 163, 191, 218 and 246°C (325, 375, 425 and 475°F), for different baking times. Because the results were calculated on a wet weight basis instead of on a dry weight basis, they are inconclusive (see explanation in previous section). This is shown by the very irregular patterns of thiamine destruction with time and different oven temperatures. The greatest thiamine destruction obtained was 26.1% for enriched white bread at 163°C (325°F) for 45 minutes.

4.2.3.2 Experiment 2

The pH values of dough and baked bread for enriched and whole wheat bread are given in Table 9. Whole wheat bread as well as the dough have a slightly higher pH than enriched white bread and dough. The dough after proofing and the baked bread, for each type of bread, have essentially the same pH values. The higher degree of acidity demonstrated by the dough after proofing, compared to the dough before proofing, is a result of yeast activity during the fermentation process.

Heat penetration data.

The recorded average oven temperatures during the baking of the enriched white and whole wheat bread are shown in Table 10. The oven temperatures for both types of bread at each nominal temperature are in good agreement. Considering that the oven doors were opened four times during each baking period and that the oven has its own temperature cycle, the standard deviations are quite small. The coefficients of variation for 177, 218 and 246°C (350, 425 and 475°F) are less than 5% and less than 6% for 288°C (550°F).

The heat penetration curves, where the mass average temperature (MAT) is plotted against time, are given for all the different oven temperatures for enriched white bread in Figure 7, and for whole wheat bread in Figure 8.

TABLE 8

Thiamine retention of white and whole wheat bread baked at 4 different temperatures for several baking times (Exp.1).

Nominal oven temp (°C)	Enriched white		Whole wheat	
	Baking time (min)	Thiamine retention* (%)	Baking time (min)	Thiamine retention* (%)
163 (325°F)	10	90.7	10	115.1
	20	81.7	20	122.5
	32	101.1	31	98.3
	45	73.7	43	102.3
191 (375°F)	7	97.2	7	131.8
	13	98.8	13	108.5
	20	95.4	20	120.8
	27	97.8	27	79.7
218 (425°F)	4	94.5	4	93.6
	8	80.5	8	98.5
	13	82.3	13	104.1
	19	94.2	19	94.9
246 (475°F)	3	119.9	3	71.9
	7	102.3	7	95.8
	9	88.4	10	109.3
	12	100.6	13	104.7

*Values are means of 2 replicates.

TABLE 9

pH values for white and whole wheat dough and bread baked at 252°C (485°F) (nominal) for 17 min (Experiment 2).

	pH*	
	Enriched white	Whole wheat
Dough before proofing	5.35	6.03
Dough after proofing	4.93	5.40
Baked bread	5.03	5.38

*Values are means of 2 replicates.

TABLE 10

Oven temperatures for enriched white and whole wheat bread baked in different loads at 4 nominal oven temperatures.

Nominal oven temp (°C)	Oven temperature (°C)	
	Enriched white*	Whole wheat*
177 (350°F)	152.2 ± 7.3	154.9 ± 6.8
218 (425°F)	172.6 ± 5.6	177.6 ± 6.9
246 (475°F)	189.1 ± 8.3	189.5 ± 6.3
288 (550°F)	215.8 + 12.1	217.7 + 11.9

*Values are means ± standard deviations.

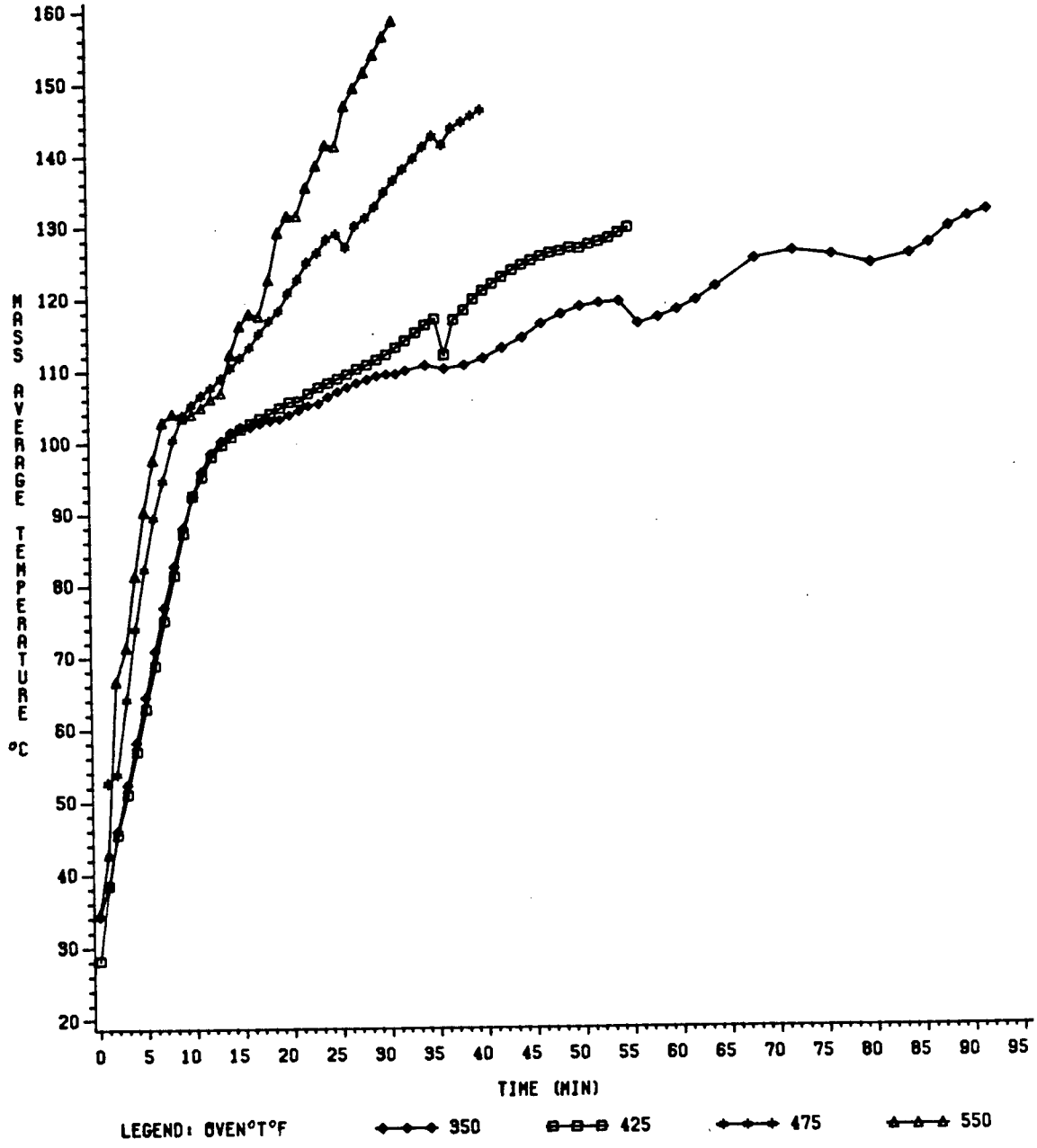

Figure 7. Heat penetration curves for enriched white bread baked at 4 nominal oven temperatures.

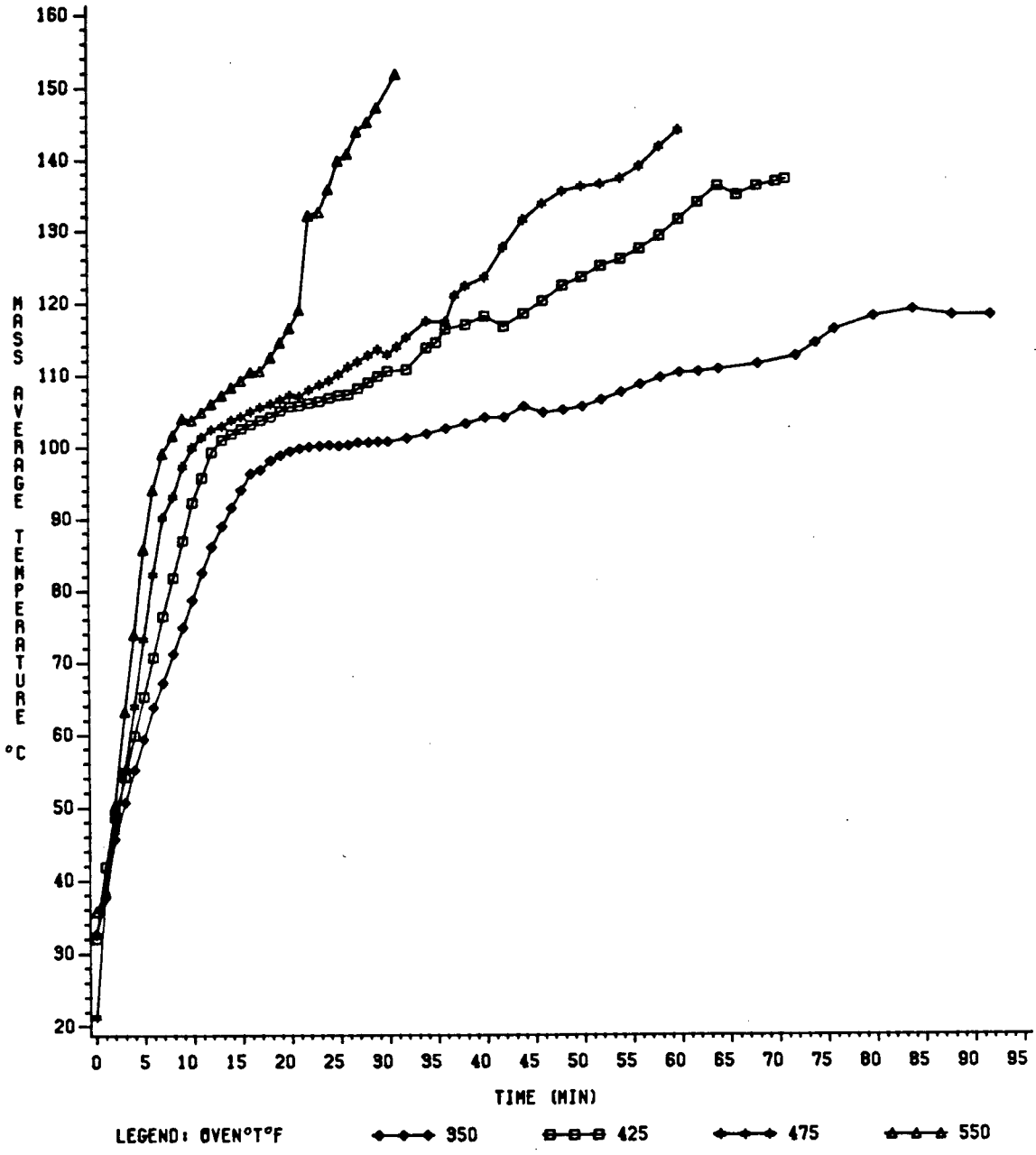

Figure 8. Heat penetration curves for whole wheat bread baked at 4 nominal oven temperatures.

Figure 9 shows the heat penetration curves for the middle and crust of enriched white bread at 218°C (425°F) (nominal temperature).

Thiamine retention results.

Table 11 summarizes the results of thiamine retention in enriched white and whole wheat bread at different baking times at 177, 218, 246 and 288°C (350, 425, 475 and 550°F (nominal oven temperatures). Variability in % thiamine retention values among the 4 replicates as expressed by the coefficients of variation were 2.8 to 35.7% for enriched white bread, and 3.7 to 23.4% for whole wheat bread. Variability of thiamine retention for whole wheat bread appears to be smaller than for enriched white bread.

Figures 10, 11, 12 and 13 show the thiamine destruction curves for each different oven temperature comparing enriched white and whole wheat bread on the same graph. From these experimental data, it is evident that thiamine retention for both types of bread decreases with baking time for all baking temperatures. Since most of the data points seemed to form a straight line, regression equations were calculated from the data and the lines were drawn through the points and the slopes were calculated. The slopes with r^2 values are given in Table 12. When the statistical hypothesis Ho: the population regression is linear is tested (Zar, 1974, p. 215), only the curve for the enriched white

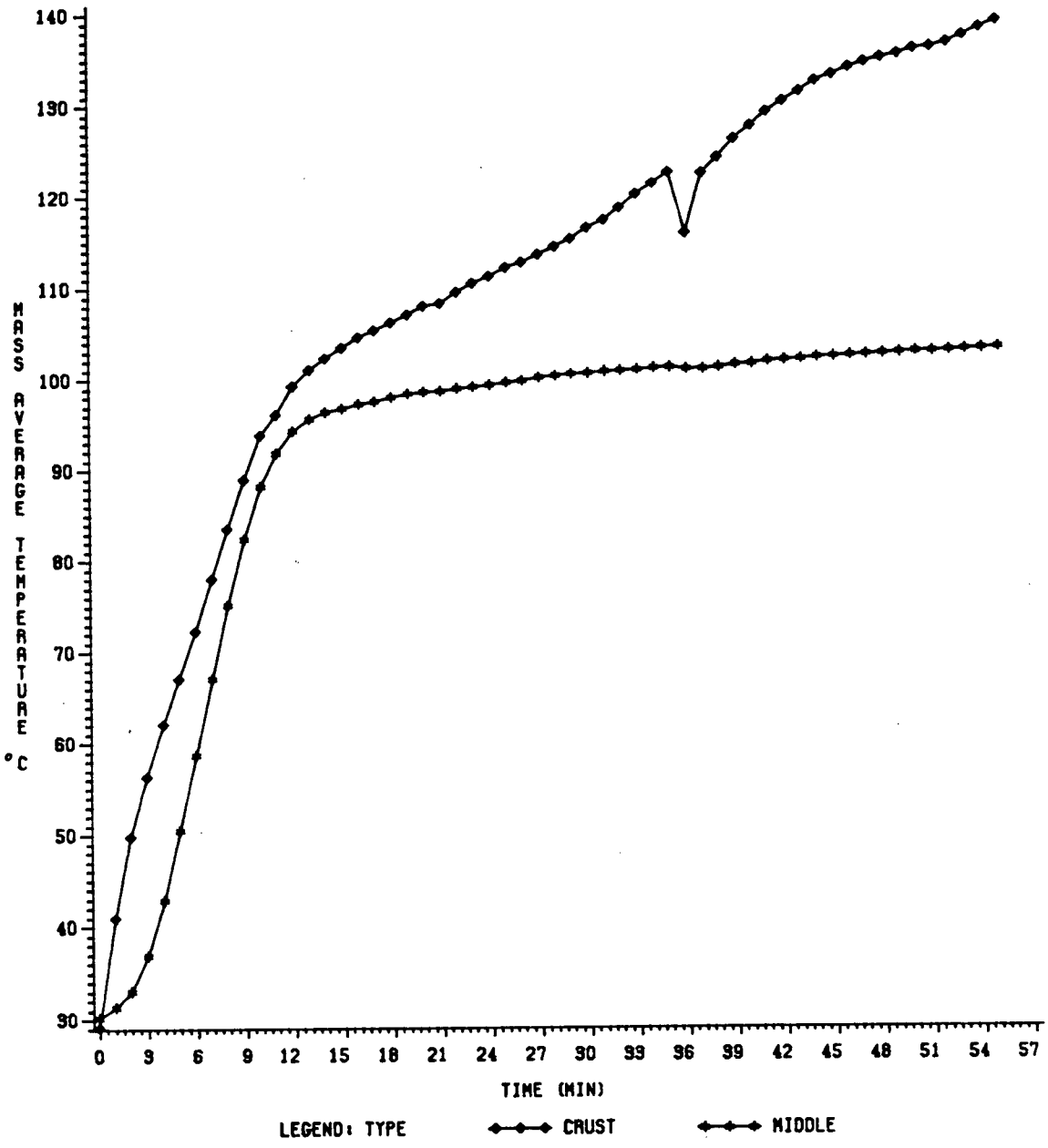

Figure 9. Heat penetration curves for the middle and crust of enriched white bread baked at 218°C (425°F) (nominal).

TABLE 11

Thiamine retention of white and whole wheat bread baked at 4 different temperatures for several baking times (Exp.2).

Nominal oven temp (°C)	Enriched white		Whole wheat	
	Baking time (min)	Thiamine retention** (%)	Baking time (min)	Thiamine retention** (%)
175 (350°F)	15	72.3 ± 1.7	16	78.0 ± 4.4
	35	63.6 ± 6.6	40	66.1 ± 2.5*
	55	53.3 ± 1.5	65	52.2 ± 1.9
	92	51.6 ± 7.1	90	43.5 ± 9.4
220 (425°F)	10	88.3 ± 31.5	10	92.9 ± 11.2
	20	89.0 ± 18.3	25	71.0 ± 4.2
	35	57.2 ± 7.9	40	59.0 ± 10.1
	55	42.3 ± 3.1	71	20.7 ± 4.3
245 (475°F)	6	76.2 ± 21.0	7	83.0 ± 7.3
	15	72.4 ± 16.2	20	70.2 ± 7.2
	25	71.7 ± 10.8	35	52.2 ± 8.2*
	40	56.1 ± 8.1	60	26.3 ± 8.2
280 (550°F)	5	91.5 ± 12.4	6	83.0 ± 9.4
	12	86.8 ± 10.3	15	67.0 ± 5.7
	22	65.3 ± 7.0	22	60.4 ± 14.1
	31	45.0 ± 7.8	31	35.3 ± 7.7

**Values are means ± standard deviations for 4 replicates.
*Only 3 replicates were used.

bread at 177°C (350°F) deviates significantly from linearity at the 5% probability level. Values of r^2 are quite significant, especially for the whole wheat bread. The lowest r^2 value obtained was for enriched white bread at 246°C (475°F) (r^2=0.7618).

For all oven temperatures, the slopes for whole wheat bread are higher than the ones for enriched white bread. Only the curves for 177°C (350°F) did not show any significant difference (see Appendix B). These results demonstrate that thiamine in whole wheat bread (a natural source) is less stable than thiamine in enriched white bread (a synthetic form).

Thiamine destruction lines for all different oven temperatures for each type of bread were also drawn (see Figures 14 and 15). When comparing the slopes from each graph, the slope values increase as oven temperature increases, which means that thiamine become less stable as oven temperature rises. When each line is compared to each other (SNK test) at 5% level, 218 and 246°C (425 and 475°F) lines for enriched white and whole wheat bread do not show a significant difference (see Appendix B).

Half-life values are given in Table 13.

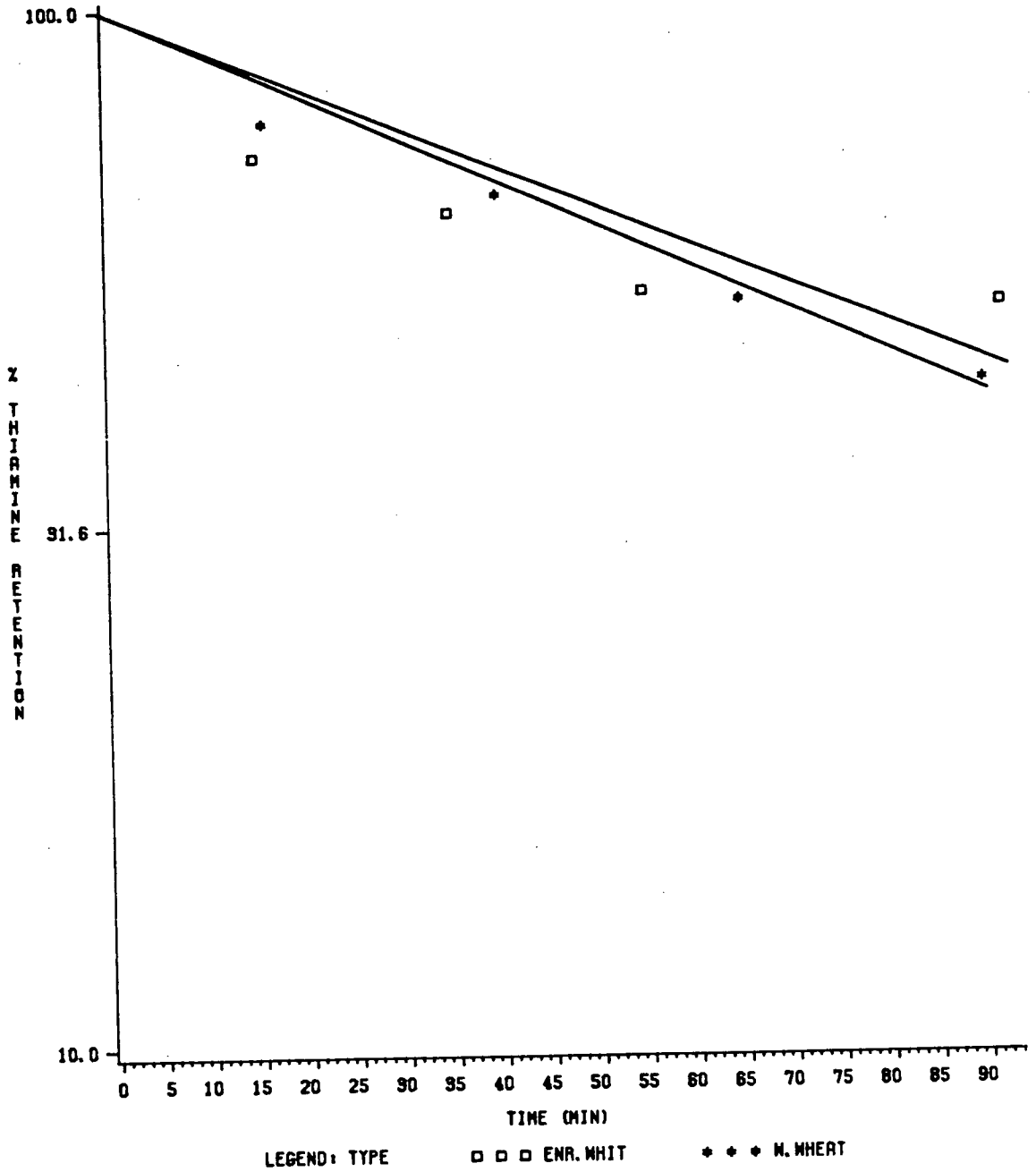

Figure 10. Thiamine retention curves for enriched white and whole wheat bread baked at 177°C (350°F) (nominal).

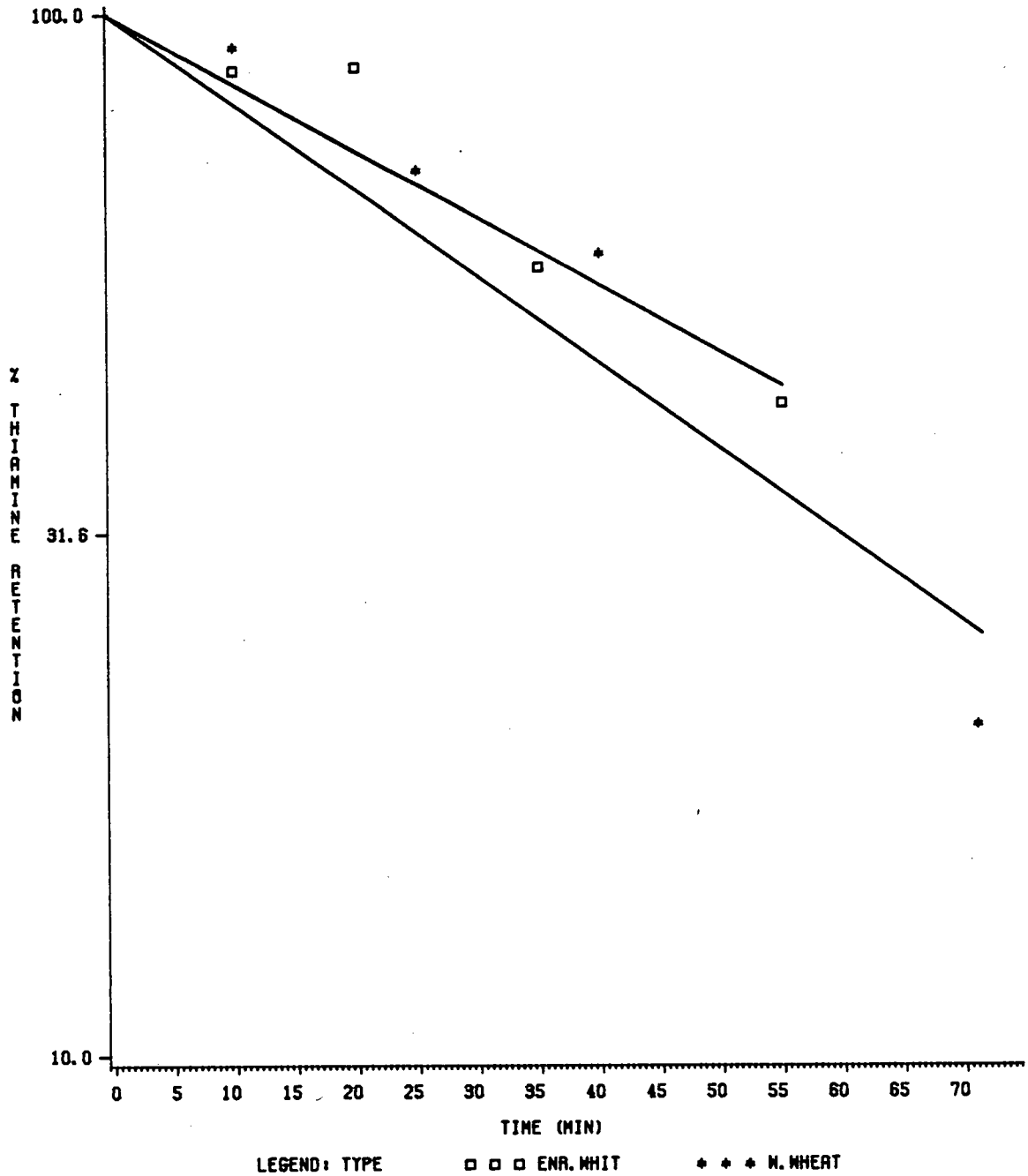

Figure 11. Thiamine retention curves for enriched white and whole wheat bread baked at 218°C (425°F) (nominal).

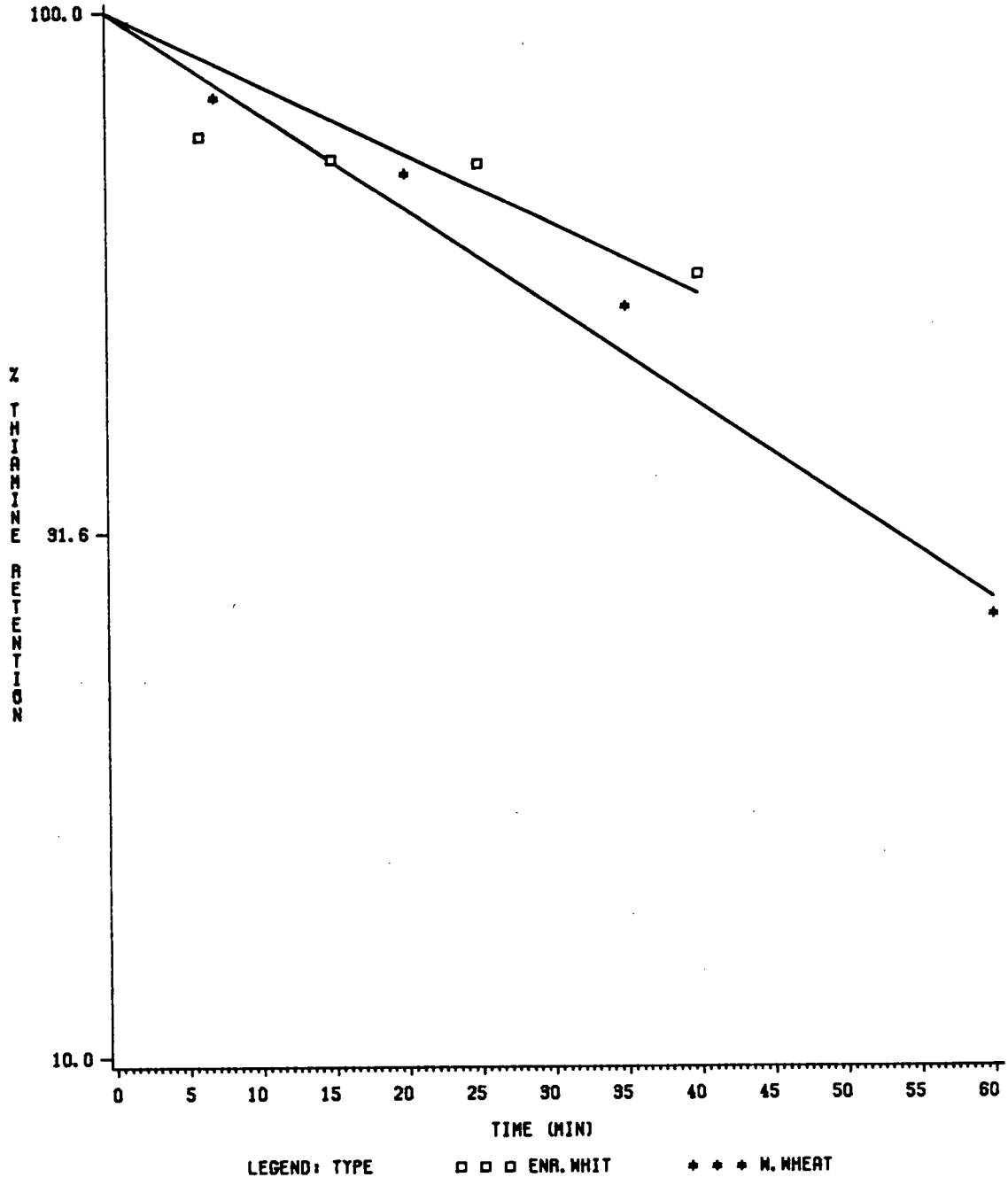

Figure 12. Thiamine retention curves for enriched white and whole wheat bread baked at 246°C (475°F) (nominal).

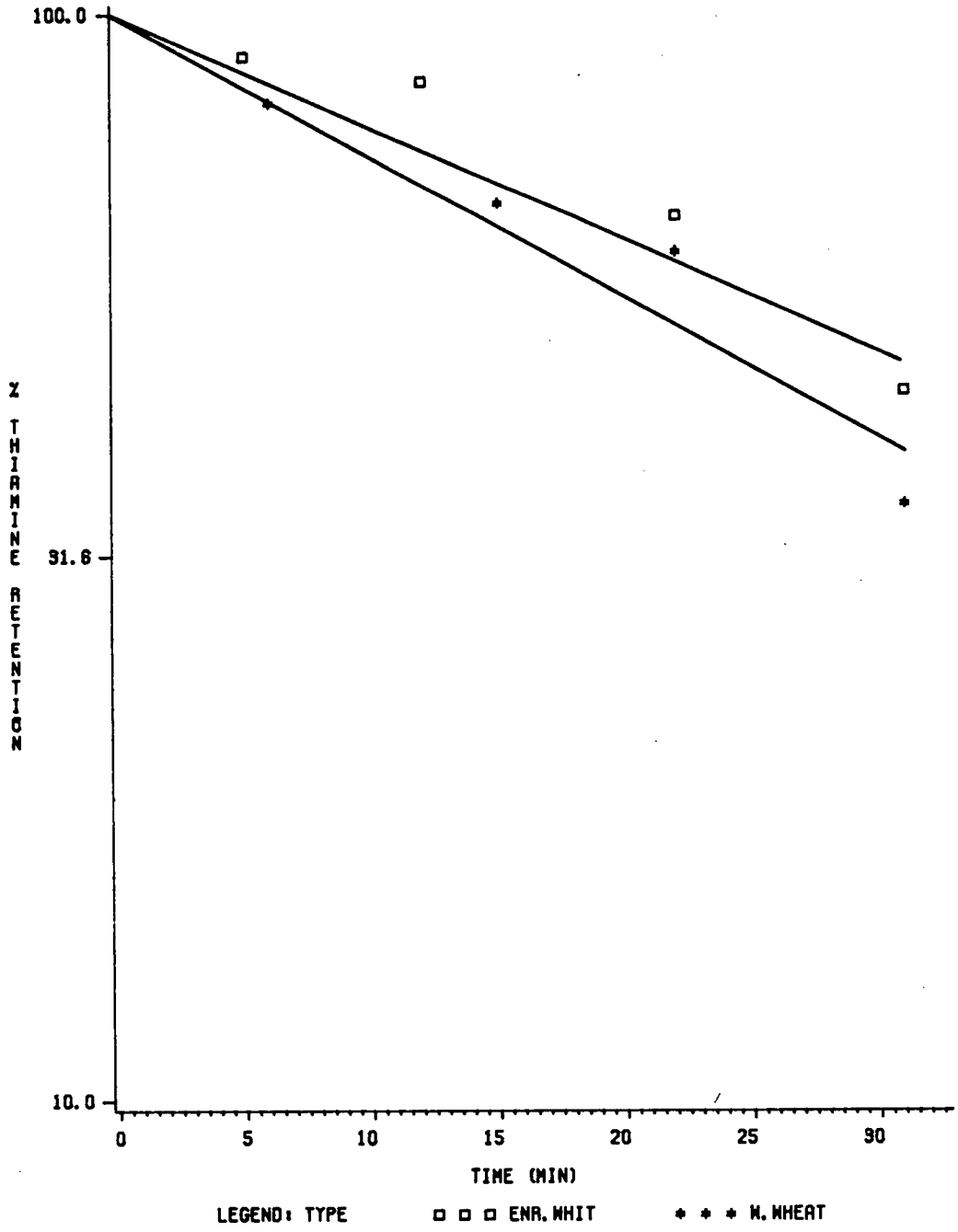

Figure 13. Thiamine retention curves for enriched white and whole wheat bread baked at 288°C (550°C) (nominal).

TABLE 12

Slope and r^2 values of thiamine destruction curves for white and whole wheat bread baked at 177, 218, 246 and 288°C.

Nominal oven temp (°C)	Enriched white		Whole wheat	
	Slope (min^{-1})	r^2	Slope (min^{-1})	r^2
177 (350°F)	0.003931	0.8956	0.004264	0.9619
218 (425°F)	0.006545*	0.8576	0.008491*	0.9333
246 (475°F)	0.006800**	0.7618	0.009475**	0.9487
288 (550°F)	0.01039*	0.9327	0.01317*	0.9187

*Difference between slopes in the same row is significant at $0.02<p<0.05$.
**Difference between slopes in the same row is significant at $0.01<p<0.02$.

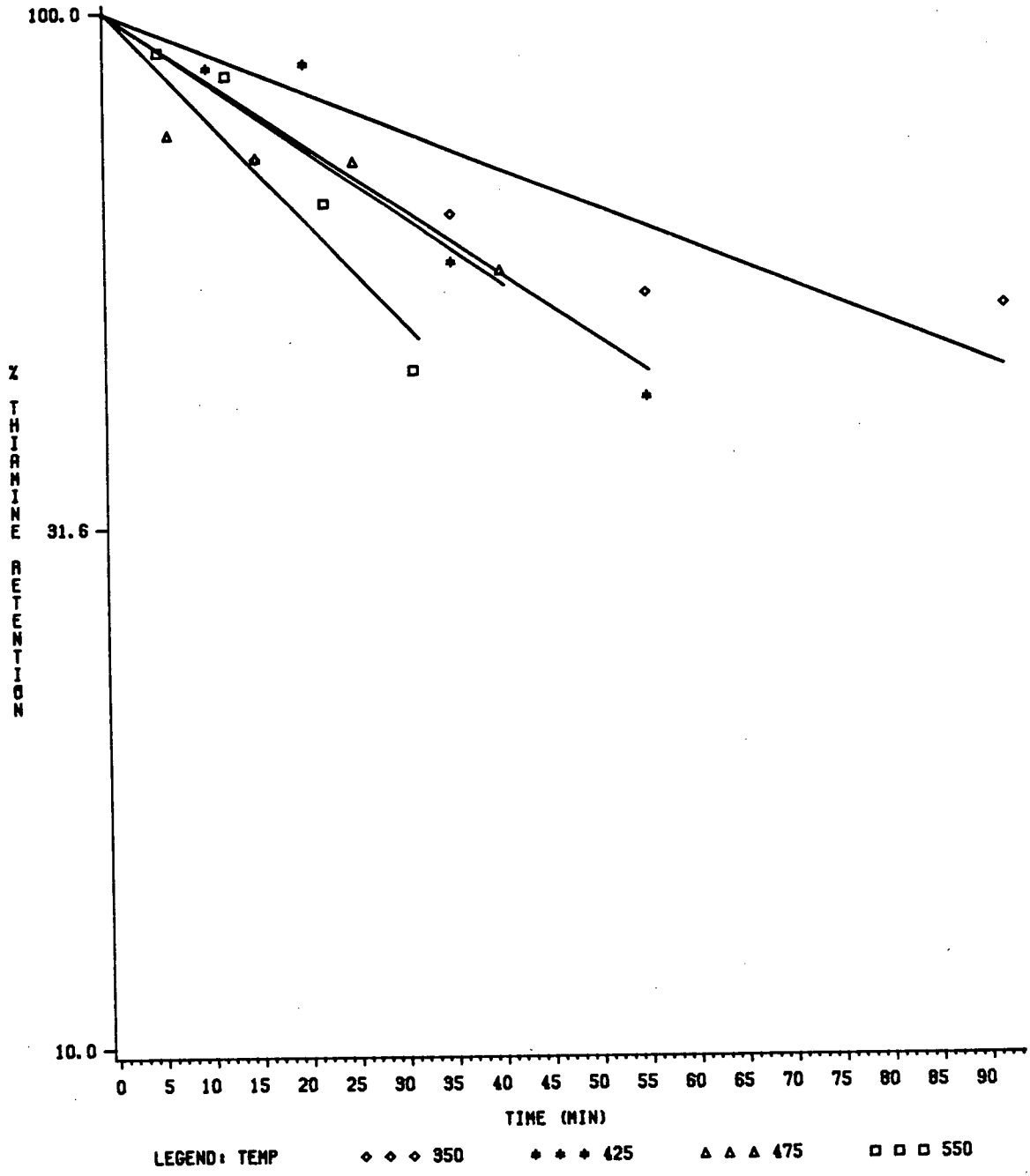

Figure 14. Thiamine retention curves for enriched white bread baked at 177, 218, 246 and 288°C (nominal).

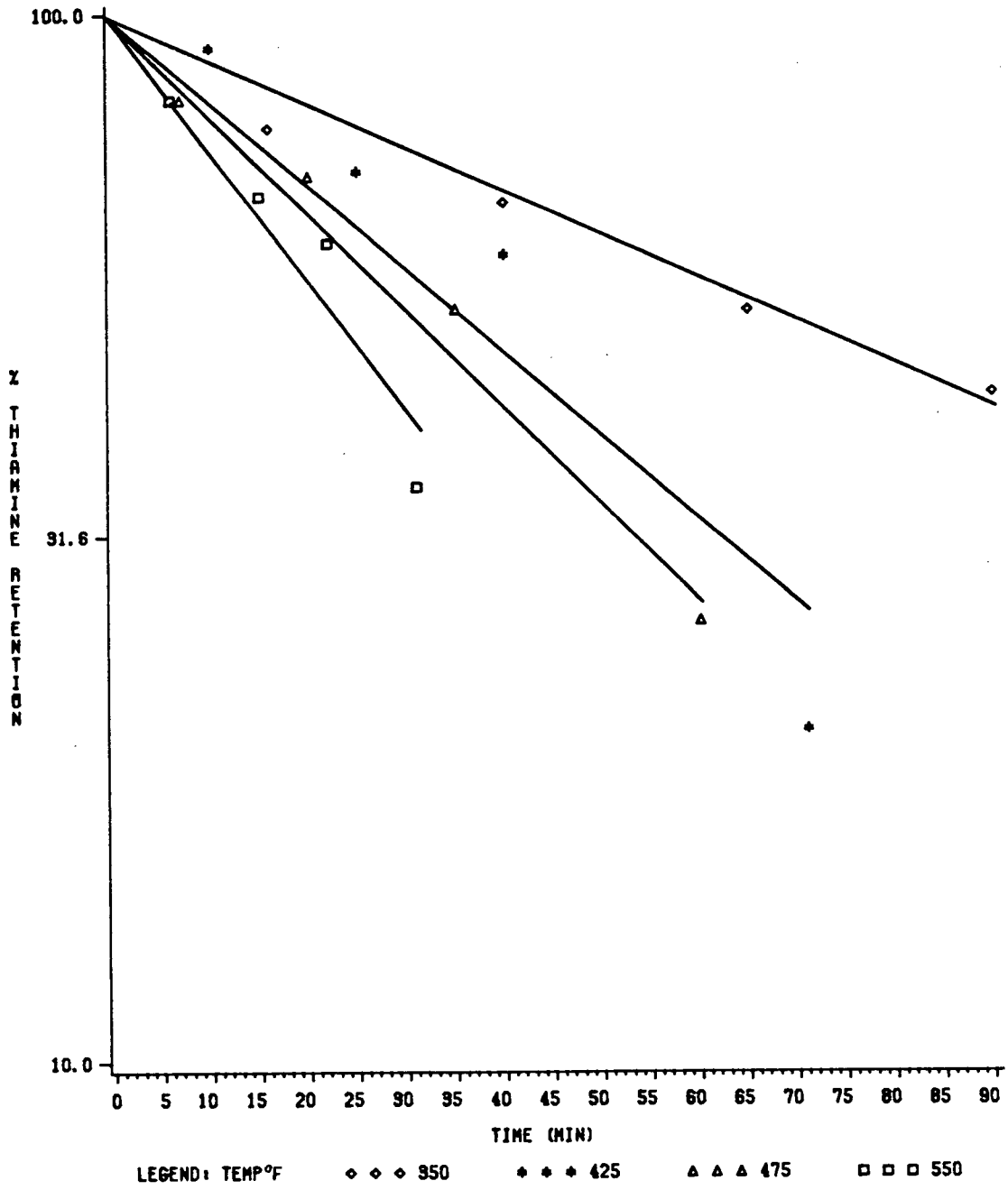

Figure 15. Thiamine retention curves for whole wheat bread baked at 177, 218, 246 and 288°C (nominal).

TABLE 13

Half-life values for thiamine in enriched white and whole wheat bread baked at 177, 218, 246 and 288°C (nominal).

Nominal oven temp (°C)	τ 1/2 (min)	
	Enriched White	Whole wheat
177 (350°F)	77	71
218 (425°F)	46	36
246 (475°F)	44	32
288 (550°F)	29	23

V

DISCUSSION

This study on kinetics of thiamine degradation in a phosphate buffer confirms again the work done by the many authors mentioned earlier where thermal degradation of thiamine follows a first order reaction and adheres to the Arrhenius equation. The Ea value of 34.2 kcal/mole found in this experiment is slightly higher than values recorded by Feliciotti and Esselen (1957) and Mulley *et al.* (1975) in the same buffer system (see Table 1). A likely explanation for the higher value is that all of the destruction curves were forced through 100% retention, whereas these other workers did not correct their curves accordingly. When the present data were not forced through 100% an Ea value of 27.8 kcal/mole was obtained, which is closer to the values found by these authors.

The pH experiment agrees with the findings of the researchers cited earlier, where thiamine destruction is greater at higher pH. Furthermore, the results show a higher loss between 6.0 and 7.0, which is also found by Lincoln *et al.* (1944), Pace and Whitacre (1953) and Feliciotti and Esselen (1957). The pattern of the curve of log k versus pH in Figure 16 agrees fairly well with the ones of Farrer

(1945), Feliciotti and Esselen (1957) and Mulley et al. (1975b). The only obvious difference is that in this study there appears to be a small drop in the k value at pH 5.5 when compared to the trend of the 3 preceeding points.

All the k and $\tau 1/2$ values found in the model system are in the same range as those found in other studies (Table 1). When the results of different authors, using similar systems, are compared, the values obtained are not identical. These differences among k and $\tau 1/2$ values of different authors and this work may be due to experimental variation in the buffer system used, in the thiamine analysis procedure and/or in the considerations for the treatment of data (e.g. forcing slopes through 100% thiamine retention).

The pH values of baked white bread in experiment 1 and 2 agree with the values given by Pomeranz and Shellenberger (1973) where pH of white bread crumb varies from pH 5.1 to 5.4.

It is surprising to see that there is no loss of thiamine during normal baking of 450 g (1 lb) loaves of enriched white bread. This is especially interesting because 30 to 50 percent thiamine destruction was found in the 12g bread formula at normal baking and 30% in the slurry assay at 75 and 90 minutes (although 0% was found at 60 minutes). Certainly this 0% destruction in 1 lb loaves does not correspond well with the 20% destruction results of other workers, and is in sharp contrast to the 50% destruction result

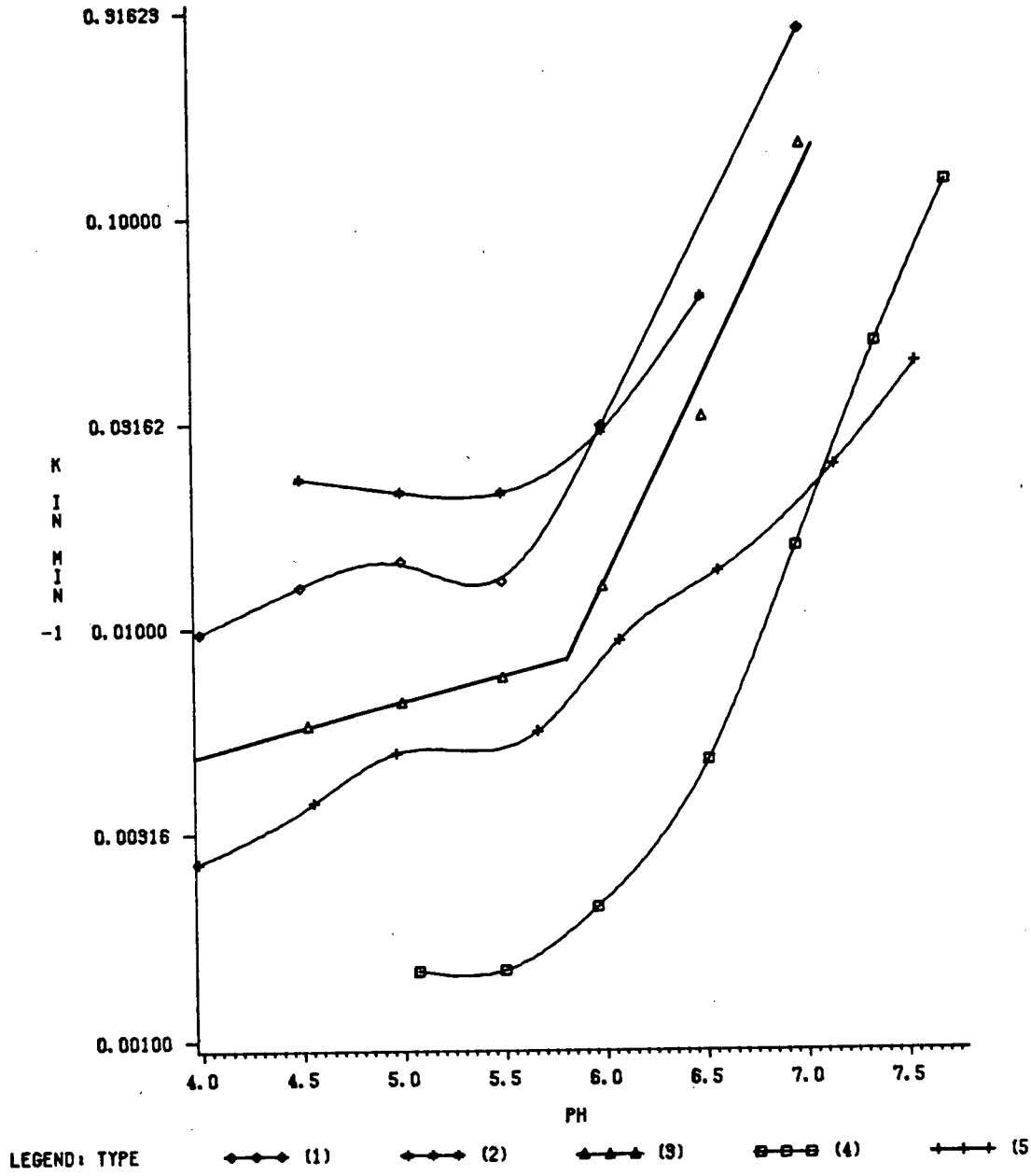

Figure 16. A review of pH/k curves for thiamine in buffered solutions. (1) from this work. (2) Mulley et al. (1979b) - phosphate buffer at 129°C. (3) Feliciotti and Esselen (1957) - phosphate buffer at 119°C. (4) Farrer (1945) - phosphate buffer at 100°C. (5) Farrer (1945) - phosphate-citric acid buffer at 100°C.

of Tabekhia and D'Appolonia (1979), where the same conditions were used.

One explanation for the 0% destruction is that yeast might have been active still during the initial period of baking. Because the mass of the bread is large, it takes a while for the middle of the bread to reach a temperature where the yeast is killed. Some thiamine would then be produced during this initial period, which could account for an error in the percent retention calculation. The reason why other workers obtained up to 20% destruction could be because the production of thiamine might vary with the variety of yeast, and the type they used produced less thiamine. More 450 g (1 lb) loaf experiments should be done at the normal bake and over bake stage to confirm these results.

The 12 g bread formula was used instead of the 450 g (1 lb) loaves mainly because it was easier to handle. However, these breads are very small and the proportion of surface crust to volume is much higher compared to 450 g (1 lb) loaves. Because most of the thiamine destruction occurs in the crust, one would expect more destruction in these smaller loaves than in the 450 g (1 lb) loaves. This is well demonstrated by the 30 to 50 percent thiamine destruction found in the 12 g loaves at the normal bake time compared to the 0% found in the 1 lb loaves here and the 20% average found by the other workers cited in Appendix A.

Because of the unsteady temperatures in the bread during baking, as shown by the heat penetration curves, it was not possible to calculate the kinetic rate constant (k) and the energy of activation (Ea) for thiamine destruction in bread by means of the steady state approach. Bread being a conduction heating food with changing temperatures, it is probably better analyzed by the a more complex non-steady state approach. This was not done for this work. However, experimental results showed that log percent thiamine retention decreased at a constant rate with time. Thus these slopes made it possible to compare mathematically the difference between whole wheat and enriched white bread.

When comparing the slopes for enriched white and whole wheat bread baked at different oven temperatures, the 218 and 246°C (425 and 475°F) slopes were found to be not significantly different. This can be explained for the whole wheat by the fact that their MAT curves are similar for the two temperatures, therefore the same percent destruction would be expected. However, this explanation cannot be true for the enriched white bread since their MAT curves for 218 and 246°C (425 and 475°F) are quite different. However, the difference in oven temperature between these two nominal oven temperatures is only 16.5°C for enriched white and 11.9°C for whole wheat, whereas the difference between the other temperatures are much higher (see Table 10). This later observation is a more likely explanation for the non-sig-

nificant difference between the slopes for 218 and 246°C (425 and 475°F).

Slopes show a higher thiamine instability as oven temperature increases. But because bread is baked in less time as oven temperature increases, during normal baking of bread there is not more destruction of thiamine at the higher temperature.

For all curves, it appears that the MAT increases according to a pattern approximated by two straight lines. First, the MAT increases very rapidly for 6 to 15 minutes, with slopes increasing as oven nominal temperature increases. Then, forming a second line, the MAT continues to increase at a constant rate, but slower than the first line. This rate also is greater with increasingly higher oven temperatures. The 288°C (550°F) curve is the only curve where the experimental points would not fit well on this hypothetical 2nd straight line pattern. Also, when curves for enriched white and whole wheat bread are compared for the same oven temperature, they do not agree very well with each other, especially the curves at 246°C (475°F). MAT curves for enriched white bread are all steeper than the whole wheat bread curves. This is likely due to the different composition and moisture retention of the two types of bread, having different heat penetration properties. Whole wheat bread would have a greater moisture retention, because of the high fiber content. Like for Figure 7 and 8, the two

straight lines pattern can be observed. Both curves show a rapid increase in temperature (being slighty higher for the crust) for the 12 first minutes. Afterwards, the curve for middle of the bread is almost horizontal (slighty positive slope), whereas for the crust, the temperature increases at a constant rate. This almost constant temperature for the middle of the bread is about 100°C on the graph. The temperature data for all the other bread treatments also reveal that the middle temperature data follow a very similar pattern with the temperature never exceeding 100 to 108°C. From the MAT formula and graphs, it is obvious that the crust temperature is the one that governs the MAT of the bread loaf.

Because MAT curves were all lower for whole wheat bread compared to enriched white, less thiamine destruction should be expected. But in fact the destruction curves demonstrated more thiamine destruction for whole wheat bread. This observation on MAT curves reinforces the thiamine destruction data which showed that thiamine in whole wheat bread is more vulnerable to temperature than synthetic thiamine in enriched white bread.

Two different theories could be proposed to explain the lower stability of thiamine to heat for whole wheat bread. The first is the pH effect. In this study, whole wheat bread had a slightly higher pH than enriched white bread, and it has been demonstrated that thiamine stability de-

creases with an increase in pH. As a second theory there is the ash or inorganic constituents explanation proposed by Dawson and Martin (1942) and Farrer (1955), where higher thiamine losses are found with higher extractions of flour. Since k values are very similar between pH 5.0 and 5.5, higher thiamine losses in whole wheat are most probably caused by both higher pH and an increase in the inorganic constituents. This theory was also proposed by Farrer (1955).

To take into consideration the pH effect only, other experiments should be done using the same type of bread but varying the pH, for example, by adding an acid like vinegar to the bread, or using a sour-dough type bread. To verify the ash theory, different percent extraction flours with controlled pH could be used.

VI

CONCLUSION

Thiamine destruction in phosphate buffer follows a first order reaction and the Arrhenius equation, where Ea was 34.2 kcal/mole. It increased as pH increased, with greater destruction at pH 6.0 and above.

Kinetic data could not be obtained with bread system because of unsteady temperature change of the bread during baking. However, a linear relationship was obtained when logarithm of percent thiamine retention was plotted against time, showing higher destruction with time. When slopes were compared, the stability of thiamine decreased with higher oven temperatures, and thiamine in whole wheat bread (a natural source) was less stable than in enriched white bread (a synthetic source). This difference might be explained by higher pH and higher ash content in the whole wheat bread.

Baking losses were found to be 30 to 50% for normal baking of 12g loaves. To interpret these results in terms of a normal loaf (450 g or 1 lb), one would expect to get less destruction in larger loaves of both types of bread, although not necessarily 0%.

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Appendix A

THIAMINE LOSSES IN BREAD

Type of bread	Baking time (min)	Baking temperature	% loss	Reference
White Whole wheat Rye			almost 0	Morgan and Frederick (1935)*[2]
Regular white High B_1 white Whole wheat			8 5 9	Hoffman et al. (1940)
Regular white High B_1 white Whole wheat	light toasting		0 4,12 0	
Regular white High B_1 white Whole wheat	medium toasting		0 10,17 3	
Regular white High B_1 white Whole wheat	heavy toasting		0 24,21 40	
Melba toast Reg. white High B_1			 9 26	
Whole wheat	45	190-218°C	15	Aughey and Daniel (1940)
White	20 30	425°F 475°F	6-8 6-8	Harrel, et al.[1] (1941)
White High B_1 white			11 3-14	Dawson and Martin (1941)
Enriched white 85% extraction 95% extraction 100% extraction (stone ground) Hovis(wheat germ)	45-48	510-520°F	22 27 33 35 19	Dawson and Martin (1942)

(continued)

Type of bread	Baking time (min)	Baking temperature	% loss	Reference
Enriched white (1 lb)	10	410°F	3	Schultz et al. (1942)
	20		13	
	30		21	
	40		33	
Whole wheat (1 lb)	10		2	
	20		17	
	30		23	
	40		33	
High B_1 white (1 lb)[1]	10		6	
	20		14	
	30		23	
	40		32	
Commercial scale	'commercial conditions' (corresponding to 30 min laboratory bakes)			
Enriched white			21	
High B_1 white			22	
Whole wheat			26	
White (1.25 lb)	35	440°F	17	Meckel and Anderson (1945)[1]
White (10 lb)	44		14	
	80	360-440°F	21	
White (4 lb)	75		19	
	80	360-475°F	24	
British 85% extr.	Good baking conditions		16	Goldberg and Thorpe (1946)[1]
British 80% extr.			15	
South Africa 95% extr.			29	
Canned bread			15	Brenner et al.[1] (1948a)
White	30	400°F	17	Zaehringer and Personius (1949)[1]
	40		20	
	50		26	
Bread rolls (various)	15		7-12	
	20		8-16	
	25		12-22	
75% extraction (0.35% ashes)			18.0	Farrer (1949)[2]
85% extraction (0.49% ashes)			21.7	

(continued)

Type of bread	Baking time (min)	Baking temperature	% loss	Reference
98% extraction (0.97% ashes)			30.0	
100% extraction (1.06% ashes)			31.0	
Wheat	30	225-240°F	11-20	Pulkki et al. (1950)[1]
Rye	45	240-260°F	15-25	
Melba toast			20	Menger (1952)[2]
Loaves			15	Pace and Whitacre (1953)[2]
Muffins			21	
Sticks			34	
Wheat			12-14	Bukin et al. (1954)[2]
Rye			30	
White patent flour	30	475°F	21.0	Coppock et al. (1956)
80% extr.(unbleached,untreated)			17.3	
80% extr.(with dough improver)			20.7	
80% extr.(with chlorine dioxide)			19.1	
100% extr. (no additives)			23.3	
American army type canned bread			19.6	
National bread				
5mm thickness	Toasting		31.0	
9mm			14.7	
12mm			13.4	
(all loaves were 1 lb loaves)				
Enriched white (1 lb)				Tabekhia and D'Appolonia (1979)**
Conventional	8	430°F	4	
method	13		10	
	18		10	
	23		21	
	30		47	

(continued)

Type of bread	Baking time (min)	Baking temperature	% loss	Reference
Continuous method	8	430°F	8	Tabekhia and D'Appolonia (1979)**
	13		12	
	18		15	
	23		27	
	30		43	

*Feeding tests.
**Approximate values from their Fig. 1.
[1]Referenced in Farrer (1955).
[2]Referenced in Coppock et al. (1956).

Appendix B

STATISTICAL ANALYSIS - 12G LOAVES (EXPERIMENT 2)

B.1 T-TEST

$$t = \frac{b_1 - b_2}{Sb_1 - b_2}$$

	350°F		475°F	
	Enr. white	W. wheat	Enr. white	W. wheat
ΣX^2	51756	55124	9944	19871
ΣY^2	0.8931	1.0419	0.6035	1.8804
ΣXY	203.4679	235.0443	67.6186	188.2795
n	16	15	16	15
b	0.003931	0.004264	0.006800	0.009475
Residual SS	0.09323	0.03973	0.1437	0.09640
Residual DF	15	14	15	14
$(S^2y.x)p$	0.004585		0.008281	
Sb_1-b_2	0.0004144		0.001118	
t exp	-0.8035		-2.3931	
v	29		29	
t.05(2),29	±2.045		±2.045	
Conclusion	Bew=Bww (p=0.2140)		Bew≠Bww (p=0.0204)	

	425°F		550°F	
	Enr. white	W. wheat	Enr. white	W. wheat
ΣX^2	19000	29464	6120	6824
ΣY^2	0.9491	2.2763	0.7081	1.2881
ΣXY	124.3641	250.1912	63.5770	89.8601
n	16	16	16	16
b	0.006545	0.008491	0.01039	0.01317
Residual SS	0.1351	0.1519	0.04765	0.1048
Residual DF	15	15	15	15
$(S^2y.x)p$	0.009566		0.0050807	

(continued)

	425°F		550°F	
	Enr. white	W. wheat	Enr. white	W. wheat
Sb_1-b_2	0.0009100		0.001255	
t_{exp}	2.1384		2.2154	
v	30		30	
$t_{.05(2),30}$	±2.042		±2.042	
Conclusion	Bew≠Bww (p=0.0117)		Bew≠Bww (p=0.01720)	

B.2 F-STATISTIC

$$F = \frac{\frac{SSc - SSp}{k - 1}}{\frac{SSp}{DFp}}$$

	Enr. white		W. wheat	
	Res. SS	Res. DF	Res. SS	Res. DF
Regression 350	0.09323	15	0.03973	14
Regression 425	0.1351	15	0.1519	15
Regression 475	0.1437	15	0.0964	14
Regression 550	0.04765	15	0.1048	15
Pooled regression	0.4197	60	0.3928	58
Common regression	0.7270	59	1.2502	57
F experimental	14.6396		42.2034	
$F_{.05(1),3,DFp}$	2.76		2.77	
Conclusion	b350≠b425≠b475≠b550 (p<<0.0001)		b350≠b425≠b475≠b550 (p<<0.0001)	

B.3 SNK TEST

$$q = \frac{b_1 - b_2}{S.E.}$$

B.3.1 Enriched white bread

See following page.

B.3.2 Whole wheat bread

See following page.

B.4 DEVIATION FROM LINEARITY

H_o: The population regression is linear.

H_1: The population regression is not linear.

$$F = \frac{\text{MS deviation from linearity}}{\text{MS within groups}}$$

Example of anova table for whole wheat at 550°F:

Source of variation	SS	DF	MS
Total	1.288089		
Between groups	1.211828		
Linear regression	1.183316		
Deviation from linearity	0.0285119	2	0.014256
Within groups	0.0762604	13	0.0058662

$F = 2.4302$

$F.05(1),2,13 = 3.89$

conclusion: accept H_o ($p=0.1269$)

Enriched white bread

Comparison (1 vs 2)	Difference ($b_1 - b_2$)	S.E.	q	p	q.05,60,p	Probability p	Conclusion
550 vs 350	0.006459	0.0007995	8.0792	4	3.737	p<0.001	b550≠b350
550 vs 425	0.003848	0.0008693	4.4266	3	3.399	0.01<p<0.005	b550≠b425
550 vs 475	0.003590	0.0009609	3.7361	2	2.829	0.025<p<0.01	b550≠b475
475 vs 350	0.002869	0.0006476	4.4304	3	3.399	0.01<p<0.005	b475≠b350
475 vs 425	0.0002550	0.0007320	0.3483	2	2.829	p>0.50	b475=b425
425 vs 350	0.002614	0.0005017	5.2105	2	2.829	p<0.001	b425≠b350

350 425 475 550

Whole wheat bread

Comparison (1 vs 2)	Difference ($b_1 - b_2$)	S.E.	q	p	q.05,58,p	Probability p	Conclusion
550 vs 350	0.008906	0.0007467	11.9267	4	3.737	p<0.001	b550≠b350
550 vs 425	0.004679	0.0007817	5.9855	3	3.399	p<0.001	b550≠b425
550 vs 475	0.003695	0.0008164	4.5257	2	2.829	0.001<p<.005	b550≠b475
475 vs 350	0.005211	0.0004815	10.8230	3	3.399	p<0.001	b475≠b350
475 vs 425	0.0009840	0.0005341	1.8428	2	2.829	0.10<p<0.20	b475=b425
425 vs 350	0.004227	0.0004199	10.0650	2	2.829	p<0.001	b425≠b350

350 425 475 550

Summary:

	F exp	F.05(1),2,DFwi	Conclusion	p
Enr.white 350	26.050	3.89	reject H_o	p<<0.0001
Enr.white 425	1.395	3.89	accept H_o	0.2826
Enr.white 475	2.908	3.89	accept H_o	0.0904
Enr.white 550	2.107	3.89	accept H_o	0.1612
W.wheat 350	1.432	3.81	accept H_o	0.2769
W.wheat 425	1.027	3.89	accept H_o	0.3853
W.wheat 475	1.252	3.81	accept H_o	0.3207

Appendix C

EXAMPLE OF CALCULATION FOR % THIAMINE RETENTION

C.1 MODEL SYSTEM

Example: Sample at 100°C after 4 hrs of heating.

	Photomultiplier reading
'0' (no heating)	50.5
'0' blank	0.3
Standard	50.0
Standard blank	0.3
Sample	25.8
Sample blank	0.3

$$\text{Thiamine } \mu g/ml = \frac{\text{Sample reading - Sample blank reading}}{\text{Standard reading - Stand.blank reading}} \times \frac{1}{5}$$

$$\mu g \text{ thiamine/ml '0'} = \frac{50.5 - 0.3}{50.0 - 0.3} \times \frac{1}{5} = 0.2020 \ \mu g/ml \qquad (1)$$

$$\mu g \text{ thiamine/ml sample} = \frac{25.8 - 0.3}{50.0 - 0.3} \times \frac{1}{5} = 0.1026 \ \mu g/ml \qquad (2)$$

$$\% \text{ thiamine retention} = \frac{(2)}{(1)} \times 100 = 50.8\%$$

C.2 BREAD SYSTEM

Example: Whole wheat bread baked at 350°F for 65 min (2nd run).

	Photomultiplier reading
Dough	93.0
Dough blank	1.8
Standard	85.85
Sample	63.5

Sample blank 5.6
Sample weight: 9.0 g dry weight: 0.8506 g/g
Dough weight: 10.0 g dry weight: 0.5906 g/g

$$\text{Thiam.}\mu\text{g/g sample} = \frac{\text{S.reading - S.blank read.}}{\text{Stan.read.-St.blank read.}} \times \frac{1}{5} \times \frac{100}{\text{dry sample wt (g)}}$$

$$\mu\text{g thiam./g sample} = \frac{(63.5 - 5.6)}{(85.85-0.65)} \times \frac{1}{5} \times \frac{100}{(9 \times 0.8506)}$$

$$= 1.7745\ \mu\text{g/g} \qquad (3)$$

$$\mu\text{g thiam./g dough} = \frac{(93.0 - 1.8)}{(85.58-0.65)} \times \frac{1}{5} \times \frac{100}{(10 \times 0.5906)}$$

$$= 3.5950\ \mu\text{g/g} \qquad (4)$$

$$\%\ \text{thiamine retention} = \frac{(3)}{(4)} \times 100 = 49.4\%$$

Dry weight calculation:

$$\frac{\text{(dry sample wt + container wt) - container wt}}{\text{wet sample wt}} = \text{dry wt g/g}$$

$$\text{Duplicate 1 : } \frac{4.7082 - 1.2991}{4.0000} = 0.8523\ \text{g/g} \qquad (1)$$

$$\text{Duplicate 2 : } \frac{4.7004 - 1.3047}{4.0000} = 0.8589\ \text{g/g} \qquad (2)$$

$$\text{Average dry wt g/g} = \frac{(1) + (2)}{2} = 0.8506\ \text{g/g}$$