# THIAMIN STABILITY IN A DEHYDRATED MODEL FOOD SYSTEM DURING STORAGE

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ABSTRACT

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By

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Stability of dehydrated foods is closely related to water activity, moisture content, and storage temperature. This study was designed to determine the effect of these parameters on the stability of thiamine in a dehydrated model food system stored in moisture vapor impermeable containers.

Sorption isotherm data obtained at 20, 25, 30, and 37°C for the model system was used to calculate the monomolecular moisture content at the respective storage temperatures. The model system was equilibrated at water activities below, at, and above the water activity corresponding to the calculated monomolecular moisture content for the adsorption and desorption isotherms. Samples were sealed in metal cans and stored under isothermal conditions to prevent any change in water activity during storage.

Thiamine levels were measured at monthly intervals using the potassium ferricyanide oxidation procedure. The rate of thiamine

degradation was determined at each water activity. In cases where thiamine losses were significant, losses followed first order kinetics. Loss rates were dependent on water activity, moisture content, and storage temperature.

Thiamine exhibited the greatest stability near or below the water activity corresponding to the monomolecular moisture content on the adsorption and desorption isotherms. Significant thiamine losses were measured in samples with water activities above the monomolecular values. Thiamine degradation was also shown to be a function of storage temperature above 30°C.

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Janice Ann Bach

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# INTRODUCTION

At present, limited information is available concerning the kinetics of nutrient losses in dehydrated food systems during storage. By determining the rate of thiamine degradation in a low moisture model food system as a function of water activity, moisture content and storage temperatures, optimum storage conditions can be established for maximum retention of thiamine.

In order to understand the chemical and biological deteriorative reaction in a dehydrated food system during storage, the physical state of water at low water activities must be understood. For several years water activity rather than total moisture content was thought to be the controlling factor in determining the chemical, microbiological, and enzymatic deterioration affecting food stability. However, more recent work has indicated that optimum stability conditions in a dehydrated food are determined by a combination of water activity and total moisture coordinates of a specified sorption isotherm.

The objective of this thesis was to determine the optimum storage stability of thiamine in a low moisture food system. The freeze-dried model food system was stored at temperatures typifying

actual yearly warehouse temperatures throughout the country. Water activities of the model system were adjusted to values below, at, and above the monomolecular moisture content on the adsorption and desorption isotherm. The hypothesis that monomolecular moisture content provides the greatest stability for the storage of low moisture foods was then evaluated. The effect of water activity and total moisture content on the food system was also evaluated by studying the sorption phenomena of the dehydrated model food system.

# REVIEW OF LITERATURE

## Sorption Phenomena in Dehydrated Foods

The sorption phenomena in foods describes the moisture-vapor equilibrium humidity (water activity) of dehydrated food. The amount of water held by the dehydrated food after equilibrium has been reached is a function of temperature and the water binding characteristics of the food components. Water activity is defined by the expression:

$$a_w = P/P_o$$

$a_w$ = water activity

$P$ = water vapor pressure exerted by the food

$P_o$ = vapor pressure of pure water at temperature $T_o$

$T_o$ = equilibrium temperature of system

Water activity is considered to be a measure of the amount of water which is available for chemical and enzymatic reactions and microbiological proliferation. Thus, water activity is regarded as an index of the water binding ability and is related to the stability of dehydrated food.

A moisture sorption isotherm is a plot of the water activity of a product versus its moisture content at a constant temperature. As the moisture content of dehydrated foods reaches 50% (dry weight basis), water activity decreases rapidly. This is the region described by the sorption isotherm curve. The sigmoid shape of the isotherm is due to qualitative differences in the affinity of food solids for water.

Labuza (1968) reviewed the sorption phenomena in dehydrated foods and divided the water sorption isotherm into three regions. These regions are dependent upon the type of water binding in a food. The first area of the sorption isotherm corresponds to the adsorption of a monomolecular film of water. This is followed by the second and third areas of the sorption isotherm, which describe the adsorption of multilayers of water and condensation of water in the pores of food material, respectively. Since the three regions overlap, the usefulness of any one mathematical model for describing isotherms is limited.

Chemical, physical, and thermodynamic studies have provided supporting evidence for these three types of water in foods. Rockland's (1969) work with nuclear magnetic resonance, electron spin resonance and phosphorescence decay on moisture-adjusted gelatin samples further helped to characterize these three regions of the moisture sorption isotherm. Rockland concluded that the bound water was associated with molecular groups:

Type 1 (monolayer) water binding was regarded as water molecules bound to ionic groups, such as carboxyl and amino groups.

Type 2 (multilayer) water binding was assumed to consist of water molecules hydrogen bonded to hydroxyl and amide groups.

Type 3 (capillary) was considered as unbound or free water, found in interstital pores in which capillary forces and soluble constituents caused vapor pressure lowering consistent with Raoult's law.

Three approaches have been presented in the literature to theoretically describe isotherms: the potential, the capillary condensation, and the kinetic approach.

The first approach (potential) was based on the development of a force field by the surface of a solid material. In describing sorption isotherms, Polanyi (1928) assumed the total work necessary to adsorb a molecule was equal to the work to overcome the field strength in bringing a molecule from a distance x to just above the solid's surface. While Polanyi's explanation for the sorption isotherm allowed for easy prediction of isotherms at any temperature once the initial isotherm was determined, this method failed to predict the monomolecular moisture content of solids. By further development of Polanyi's theory, Harkins and Jura (1944) described the sorption

isotherm based on the behavior of an adsorbed film as a liquid in a two-dimensional state.

The second approach used to theoretically describe sorption isotherms was based on the capillary condensation theory. Zsigmondy (1911) described the sorption isotherm with the capillary condensation theory using the Kelvin Equation to predict the amount of water absorbed. While this method was applicable in predicting the isotherm in the capillary region, it was of limited value in predicting water activities in the monolayer and multilayer regions. Henderson (1952) developed an empirical equation which has been used successfully to describe the complete isotherm for food products.

Langmuir's (1918) kinetic approach to theoretically describe isotherms was based on the adsorption of a monolayer of vapor on a solid surface. Since the molecules of a vapor striking a solid surface would not all rebound elastically, a higher concentration of vapor molecules near the surface could then result in adsorption to the surface of the solid. Langmuir's equation assumed that the ratio of the actual number of adsorbed molecules to the difference between the maximum number and the actual number was proportional to the vapor pressure. However, the Langmuir model did not hold for most food products.

Freundlich (1926) attempted to improve the Langmuir model; however, his model provided little applicability to food materials.

In both the Langmuir and Freundlich models, the isotherms were only useful in describing the monomolecular layer region of the sorption isotherm.

Brunauer, Emmett and Teller [BET] (1938) developed an equation, which described the adsorption of water molecules to product surfaces by Vander Waals forces. Three basic assumptions were made in deriving the BET equation: 1) more than one layer of water molecules is on the surface of a solid, 2) the energy of adsorption for molecules is equal to the heat of vaporization of water in all layers except the monolayer, and 3) energy of adsorption for the monolayer is the same for all molecules existing in that layer. The BET isotherm model has accurately described isotherms for food products up to 0.5 water activity. In addition, the monomolecular moisture content and the water surface area have been calculated using the BET equation. Labuza (1968) concluded that the BET equation has been most useful in predicting the monolayer value and heat of adsorption, which have been of concern in the processing and storage of dehydrated foods.

The adsorption isotherm curve is determined by adsorption of water by a completely dry material which is placed into atmospheres of increasing relative humidity. Desorption isotherms are obtained by placing a dry product having a high equilibrium relative humidity into atmospheres of decreasing relative humidity. At low vapor pressures, adsorbed molecules of water vapor can accumulate on a surface

where the energy of interaction is the greatest. Initial adsorption occurs in the form of single molecules of water followed by groups of water molecules. These small groups of water molecules increase in size until the surface is covered by one molecular thickness (BET monomolecular layer). As the density of the water molecules on the surface increases, the forces of interaction between the adsorbed molecules increases. Harkins (1952) concluded that the mean molecular area of nonpolar adsorbates is independent of the nature of the solid upon which the adsorption occurs at BET monolayer. Based on Harkins' findings, interactions in the water film become very large and the inhomogeneities on the surface of the solid do not influence the adsorption process. Duckworth and Smith (1963) showed that at and above the BET monolayer value, water soluble compounds have mobility in dry foods even though important differences exist in their interaction with the food components.

Adsorption and desorption isotherms illustrate the phenomenon of hysteresis. Hysteresis behavior consists of a higher moisture at a given water activity for isothermal dehydration of food products as compared to isothermal rehydration. Usually the desorption hysteresis loop ends at the monolayer value but it can extend down to a water activity of zero. Stitt (1958) claimed that the magnitude of hysteresis was dependent upon food composition, temperature, and pretreatment of a food material. Quin (1967) attributed the moisture-humidity

hysteresis to actual changes in the chemical bonding of the water and hydrogen bonding of the water molecules. Quin further theorized that the resistance to interposition by water could be greater for a material which has been dried, allowing for closer attraction among molecules other than water.

Labuza (1968) reviewed various theories of hysteresis and found the ink bottle theory of Rao (1941) to be the most plausible. This theory assumes that the capillaries caused by dehydration of the food solids possess narrow necks, followed by large cavities. Adsorption is controlled by the larger radius of the cavity. The capillary will not completely fill until the water activity corresponding to the larger radius is reached. The smaller radius of the neck of the cavity controls the unfilling or desorption of the capillary.

Rockland (1969) stated that moisture sorption isotherms of heterogeneous biological products represent the hygroscopic properties of their constituents. Salwin (1963) showed that starchy and high protein foods had greater water-holding capacity in comparison to foods which were high in sugar content. Fats in meats exerted the smallest water-holding capacity of the three primary food components (fats, carbohydrates, proteins). However, Taylor (1961) showed that fat did slow equilibration by coating the sample particles, thus preventing moisture transfer. According to Labuza (1968), pretreatment of foods such as heating had little effect on proteins but increased

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the amount of crystalline water impenetrable starch. Thus, smaller surface area was available for adsorption.

Taylor (1961), Stit (1958), Karel et al. (1964), Hofer and Mohler (1962), and Palmitker and Heldman (1971) provided detailed descriptions of procedures for obtaining moisture sorption isotherms for food products.

## Water Activity and Food Stability

Food stability refers to the relative resistance of foods to chemical, physical, enzymatic, and biological deterioration during processing and storage. Water activity is an index of the amount of water available in food for chemical, enzymatic, and microbiological deteriorative reactions and is therefore closely related to food stability. Recently total moisture content in addition to water activity is believed to play a role in the stability of food products. Rockland (1969) stated that water activity alone may not reflect the most precise physical-chemical state or susceptibility of the food to moisture dependent deterioration. Labuza et al. (1970) and Labuza (1971) discussed the basis for chemical stability of foods in relation to total moisture content and water activity.

Microbiological Effect

Scott (1957) reviewed the effect of water activity on microbial growth and concluded that reducing the water activity below an optimum growth level resulted in the following: a) an increase in lag period, b) a decrease in the growth rate, and c) a decrease in the amount of synthesized cell structure. The biological response to a particular water activity was found by Scott to be independent of the solute type and total moisture content of the substrate.

Locin et al. (1968) discussed the variable bactericidal effect of water activity by drying or addition of water soluble substances. Locin indicated that the duration of the operation, the final water activity attained, and the species of microorganism involved did not result in any significant population-reduction.

Bone (1969) tabulated the approximate lower limits of water activity for microbiological growth. Most species are limited to small but characteristic water activity ranges. Research on microbiological growth in freeze-dried foods after humidification indicated that as water activity decreased down to 0.70-0.85, death of the microorganism accelerated (Silverman and Goldblith 1965; Saleh and Goldblith 1966; Sinsky et al. 1967; and Chipley and May 1968).

Labuza (1972) concluded that water activity was not the sole controlling factor in microbiological growth in intermediate moisture

foods and that total moisture content must also be considered. Foods prepared by the desorption process have a higher moisture content than foods prepared by the adsorption process, given the same water activity. Therefore, Labuza based his conclusions on results which illustrated that growth minima are much higher if the food is prepared by an adsorption rather than a desorption technique.

## Enzymatic Effect

Comprehensive studies were published by Drapon (1961) and Acker (1962) on the effect of water activity and enzymatic reactions. Acker (1969) observed that enzymatic reactions are inhibited or occur at a very low rate at equilibrium moisture contents below the monolayer value. By illustrating how water serves as a medium, reactant or vehicle for substrate movement to the enzyme, Acker emphasized the importance of water in determining the enzymatic reaction rate and the degree to which the reaction proceeds.

## Chemical Effect

Rockland (1957) observed an optimum moisture level for shelled walnuts, above and below which darkening and rancid flavors and odors developed. The differential coefficient of moisture with respect to

relative humidity ($\Delta M/\Delta RH$) was calculated from the sorption isotherm and plotted against percent moisture by Rockland, to illustrate the stability characteristics of shelled walnuts during storage.

Salwin (1959, 1963) reported that the BET monomolecular value represented the moisture content where the greatest food stability was demonstrated. According to Salwin, the monomolecular water layer acted as a protective film against autoxidation of lipids. Uri (1956) theorized that a specific type of solvation of the coordination shells of metal ions by the polar water molecules caused the decrease in catalytic oxidation. Maloney et al. (1966) supported the theories of Salwin (1959, 1963) and Uri (1956) by establishing that water exerted an antioxidant effect on methyl linoleate using a freeze-dried model system consisting of methyl linoleate, microcrystalline cellulose and water. The protective effect of water occurred at a water activities from monomolecular moisture content to 0.5. Above or below these values the rate of lipid oxidation was greatly accelerated. Martinez and Labuza (1968) also illustrated the antioxidant effect of water by demonstrating optimum storage stability of freeze-dried salmon above the monolayer value. Labuza et al. (1969) attributed the protective effect of water on lipid oxidation to the hydration of metal ions which decrease their effectiveness and thus inhibit the free radical chain reaction. This protective effect was evident up to a water activity of 0.5. Heidelbaugh and Karel (1970) demonstrated increased oxidation

of model food systems in the capillary region of the isotherm was due to increased catalyst mobility and exposure of new catalytic sites.

Salwin (1959, 1963) reported that the rate of nonenzymatic browning was a function of moisture content. Lea (1958) reported that water promoted amino-carbonyl browning, which produced intermediate reducing compounds resulting in competition between lipid autoxidation and Maillard Browning. Karel and Labuza (1968) demonstrated that nonenzymatic browning increased with an increase in water activity up to the intermediate moisture range and then decreased. Browning was shown to result from the condensation of reducing sugars and free amino groups via the Maillard reaction. Labuza (1970) explained that the increase in browning above monolayer was a result of increased availability and mobility of reactants, while decreases at high water activities were a result of the dilution of reactants. Eichner and Karel (1972) suggested that the inhibitory effect of high moisture contents in model food systems against browning resulted from the formation of water as a product of condensation steps in the browning reaction.

Labuza (1972) presented a stability map which described chemical and biological deteriorative reaction rates as a function of water activity. This stability map could be used as a general guide in predicting the stability of food products.

## Thiamine Stability in Dehydrated Foods

Loss of thiamine in food products can primarily be attributed to process and storage conditions. Farrer (1955) extensively reviewed factors relating to thiamine degradation and determined that in most cases, thermal destruction of thiamine followed first order reaction kinetics and the Arrhenius equation. Thus, reaction velocity could be related to processing and storage temperature. Subsequent studies by Agrawal et al. (1963) and Sabry and Tannous (1961) also showed that the Arrhenius equation could be used successfully in predicting thiamine retention in foods. Recently Dwivedi and Arnold (1971) reported new mechanisms of thiamine degradation. Results showed high thiamine destruction rates and an activation energy of 30 Kcal/mole in aqueous solution. This activation energy was high in comparison to 18.5 Kcal/mole and 22 Kcal/mole reported for thiamine destruction in fresh and dehydrated pork, respectively (Farrer 1955, Rice et al. 1944).

Tappel (1955) and Yao (1956) concluded that freeze drying caused no appreciable losses of vitamins or amino acids, with the exception of thiamine. Thiamine loss in freeze dried chicken meat was reported by the Quartermaster Food and Container Institute (1961). However, Rowe et al. (1963) concluded that the thiamine content of chicken muscle was not altered by freeze-drying. Lempka and Prominski (1967) indicated that lyophilization of most meat products results in

chemically unchanged products which retain their flavor, odor, and appearance. Cain (1967) reported that a 3-15% thiamine loss occurred in dehydration of foods with approximately 2% loss with freeze drying.

With the exception of ascorbic acid, thiamine is affected by storage to a larger extent than other water soluble vitamins. Klose et al. (1943) found 0%, 48%, and 50% thiamine loss in spray dried eggs at -9.5, 21, and 37°C respectively over a 9-month storage period. Tressler et al. (1943) reported no thiamine loss in dehydrated vegetables with 4 months storage at temperatures ranging up to 24°C. Further study of similar samples by Continental Can Company (1944, 1945) demonstrated losses up to 76% in 3 months at 54.4°C. Morgan et al. (1945) examined thiamine stability in dehydrated broccoli, carrots, spinach, peas, and beans (2-6% moisture) and reported no nutritional losses in the dehydrated foods with the exception of 40% loss of thiamine in broccoli.

Rice et al. (1944) made an extensive study of thiamine destruction in dehydrated pork and indicated that no detectable loss of thiamine occurred after 3 weeks storage at -29 and 3°C. However, losses were shown at 27, 37, 49, and 63°C. Thiamine destruction rates of 0.072, 0.143, 1.5, and 8.9 weeks,$^{-1}$ respectively, were determined.

No thiamine loss was demonstrated by Thomas and Calloway (1961) in freeze dried meat products stored for one year at 20°C

and 2% moisture. These results were supported by Svabebsky (1967) who reported 100% thiamine retention in freeze-dried meat with a low moisture content and a water activity of 0.15. Svabebsky's data demonstrated that small temperature increases had little effect on thiamine stability of the freeze-dried meat at the described storage conditions. Nodolna (1970) supported these results by reporting large losses of thiamine (40%) in samples with high moisture contents regardless of the storage temperature.

The moisture content of dried products has been shown to affect thiamine destruction. Rice et al. (1944) and Nymon and Gartner (1948) demonstrated its importance in dehydrated pork. Hallenbeck and Obermeyer (1952) illustrated the importance of moisture content in the storage stability of thiamine in enriched flour stored for 4 months at 38°C. Losses of thiamine in the flour were 40% and 5% for thiamine hydrochloride and thiamine mononitrate, respectively. Herrman and Tungir (1966) varied the moisture content of flour and discovered that the rate of thiamine destruction passed through a maximum at a temperature of 100°C and moisture content of 13%. The storage stability of a corn-soy-milk food supplement was evaluated by Brookwalter et al. (1968) at 25, 38, and 48°C. The food supplement demonstrated an accelerated thiamine loss as moisture contents increased from 5 to 13.5%. These findings were supported by Nodolna et al. (1970) who

reported increased thiamine loss in dehydrated pea soup as moisture contents increased from 3 to 12%.

Early work concerning the stability of thiamine pointed to the importance of pH (Farrer 1941, Booth 1943, Farrer 1945). Labuza (1972) showed that as pH increased, the rate of thiamine destruction increased by several orders of magnitude over a pH range of 3.0 to 8.0, especially at pH greater than 7.0.

Farrer (1955) postulated two reactions for thiamine degradation, one involving the cleavage at the CH "bridge" between the pyrimidine and thiazole moieties and the other involving production of hydrogen sulfide by the breakdown of the thiazole ring. Dwivedi and Arnold (1973) extensively reviewed the chemistry of thiamine degradation in food products and model systems. They concluded that degradation was a function of pH, temperature, oxidation-reduction potential of systems, bisulfite content, presence of aldehydes, amines, and radiation. Small amounts of pseudo base and/or thiol forms of thiamine were discovered to be responsible for the production of hydrogen sulfide as a thiamine degradation product at pH 7.0 and above.

Thiamine reacted readily in Maillard type reactions when heated with glucose as a dry mixture (Lhoest 1957, 1958, Von der Poel, 1956). However, fructose, mannitol, and inositol retarded thiamine destruction (Ache and Ribeiro 1945 and Wei et al. 1962).

According to Farrer (1955), while protein protects thiamine, theories explaining the actual mechanism differ. Wada and Suzuke (1965) concluded that hydrogen sulfide groups in egg albumin exerted a protective effect on thiamine while Toda and Nakayama (1958) found that the formation of a thiamine-casein complex provided the greatest thiamine stability. Leichter and Joslyn (1969) discovered that soluble starch protected thiamine and theorized that an interfacial surface effect was involved. Morfee and Linka (1971) reported reversible bonding between thiamine and protein in a simulated milk model system. Bonding between sulfur-containing breakdown products was also demonstrated. Morfee and Linka were unable to explain the nature of bonding but postulated disulfide bonding as a possible explanation.

Farrer (1955) reviewed the catalytic effect of free metal ions on thiamine degradation and reported that metals could form complex ions with food constituents which could influence thiamine destruction. Spaleny (1960) found that metal chelating agents retarded the rate of thiamine degradation. Tanke (1969) suggested that formation and decomposition of thiamine copper complexes may be the cause of the gradual catalytic decomposition of thiamine by copper.

# EXPERIMENTAL PROCEDURES

## Model System Preparation

Cereal products comprise a large portion of existing low moisture foods. As a result of this the composition of the low moisture model food system used in this study was formulated to approximate that of cereal products (Table 1).

TABLE 1.--Composition of Freeze-dried Model Food System.

| Component | Per Cent |
|---|---|
| Protein | 10 |
| Fat | 1 |
| Carbohydrate | 75 |
| Reducing sugar | 5 |
| Sucrose | 5 |
| Salt | 2 |

The model system was prepared by adding water to the pre-blended dry ingredients until a thick slurry (25% total solids) formed. Following addition of the coconut oil, the model system was homogenized at 2,000 psig (first stage) in a Manton-Gaulin homogenizer. The system was cooled to ambient temperature and thiamine was added at a level of

1/2 RDA per 200 g (6 μg/g) of product on a dry weight basis. Thorough mixing of the model system insured even vitamin distribution.

The model system was layered onto freeze-drying trays, placed in the freeze-dryer and frozen at a platen temperature of -40°C. Drying was accomplished in 24 hours at 5 μ absolute pressure.

Each of the four model systems were prepared and freeze-dried under the same conditions. However, the systems were prepared on different days and subjected to slight variations in setting time between homogenization and freeze-drying, freeze-drying and equilibration, and time required for water activity equilibration. These factors might account for some variations in the four model systems; however, this effect should be insignificant.

## Adjustment of Water Activities of the Model System

Equilibrium moisture content isotherms were determined for the freeze-dried model system at 20, 25, 30, and 37°C. Measurements were conducted using a Cahn electrobalance and free water surfaces at the appropriate temperatures to maintain the desired water vapor pressures (Palnitkar and Heldman, 1971).

The adsorption and desorption isotherms of the model food systems were determined by measuring the moisture content at various

water activities. Initially the freeze-dried samples were adjusted to 0.00 water activity and then to higher water vapor pressure in increments along the adsorption isotherm. After a water activity of 0.95 was reached, the desorption isotherm was measured by equilibrating the model system at lower vapor pressures along the desorption isotherm.

Data from the moisture equilibrium isotherm was analyzed to determine the monomolecular moisture content using the Brunauer, Emmett and Teller (BET) equation [see Appendix]. By ploting $a_w/w(1-a_w)$ versus $a_w$, the data provided a linear relationship at the lower water activities (0-0.40). Utilizing the y-intercept ($1/w_mC$) and the slope of the curve ($C-1/w_mC$), the monomolecular moisture content ($w_m$) was evaluated (see Appendix).

The BET analysis was conducted on both adsorption and desorption isotherms so that monomolecular moisture contents were available for both conditions. These values were used to establish the water activities to be used for product storage.

Calculated BET values for the four model systems ranged from 0.22-0.275 $a_w$ for adsorption samples and 0.065-0.100 $a_w$ for desorption samples (Table 2). No set trend was clearly apparent with respect to temperature. Results indicate that both adsorption and desorption BET values for the four model systems do not vary much within the 20-37°C temperature range (see Figures 1 and 2).

TABLE 2.--Calculated and Equilibrated BET Water Activities of Freeze-dried Model Systems.

| Temperature °C | Adsorption | | Desorption | |
|---|---|---|---|---|
| | Calculated | Actual | Calculated | Actual |
| 20 | .235 | .220 | .100 | .100 |
| 25 | .255 | .255 | .95 | .055 |
| 30 | .220 | .305 | .065 | .100 |
| 37 | .275 | .280 | .090 | .035 |

Comparison of the calculated BET water activity values to the water activity values exhibited by the samples following moisture equilibration is shown in Table 2. The actual water activity of all samples was determined by evaluating the moisture content and the moisture sorption isotherms of the samples. The actual water activity of all samples agreed within 0.055 of the calculated monomolecular moisture content with the exception of the adsorption sample stored at 30°C, which differs from its calculated value by 0.075. This discrepancy occurred because the isotherm and corresponding BET value which were originally determined and used as a guide to adjust the 30°C adsorption sample were later found to be too high. The 30°C adsorption isotherm was redetermined and the BET value recalculated giving the new BET value of 0.220.

FIGURE 1. ADSORPTION ISOTHERMS FOR FREEZE-DRIED MODEL SYSTEM AT 20°, 25°, 30° AND 37°C.

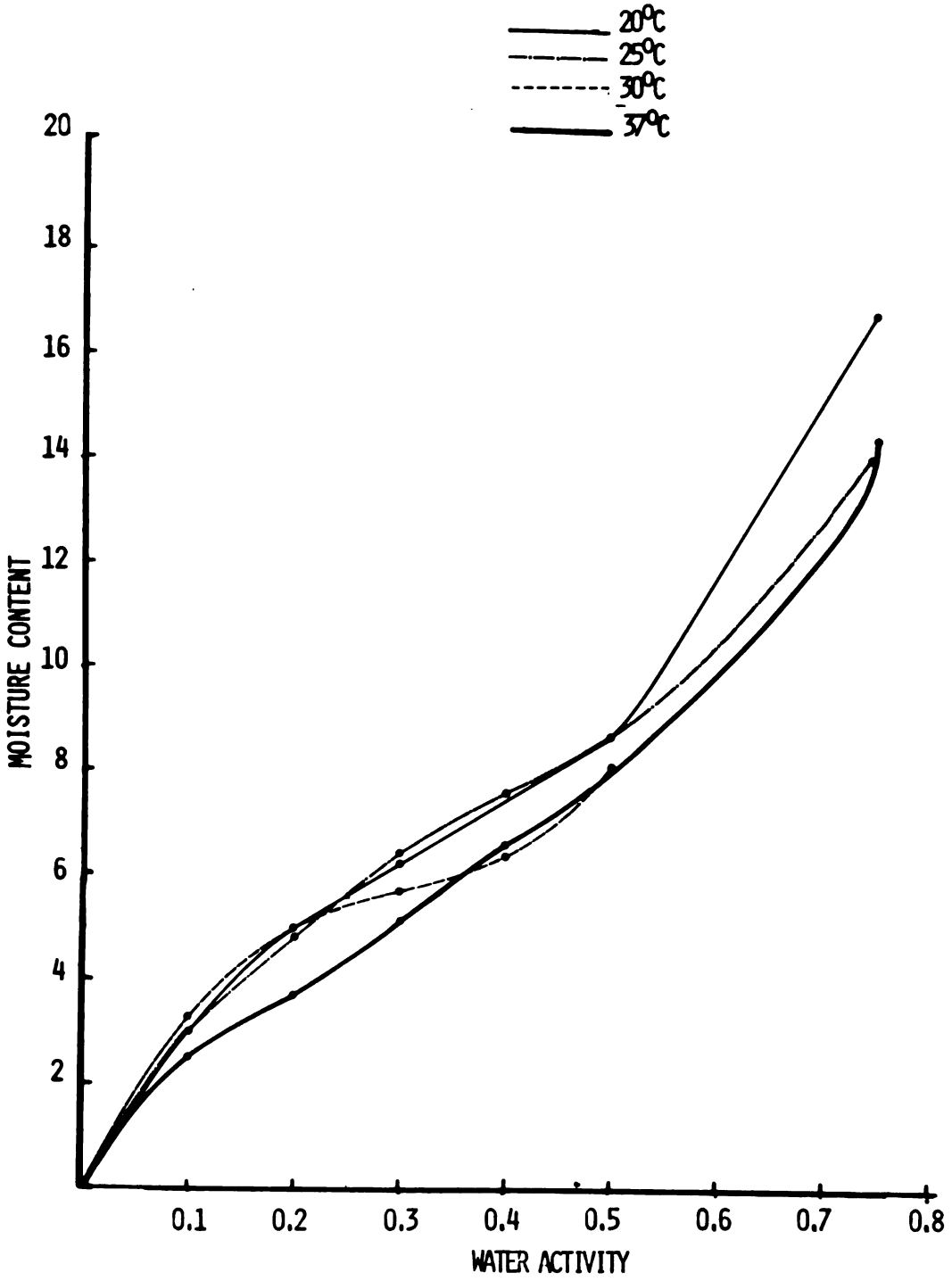
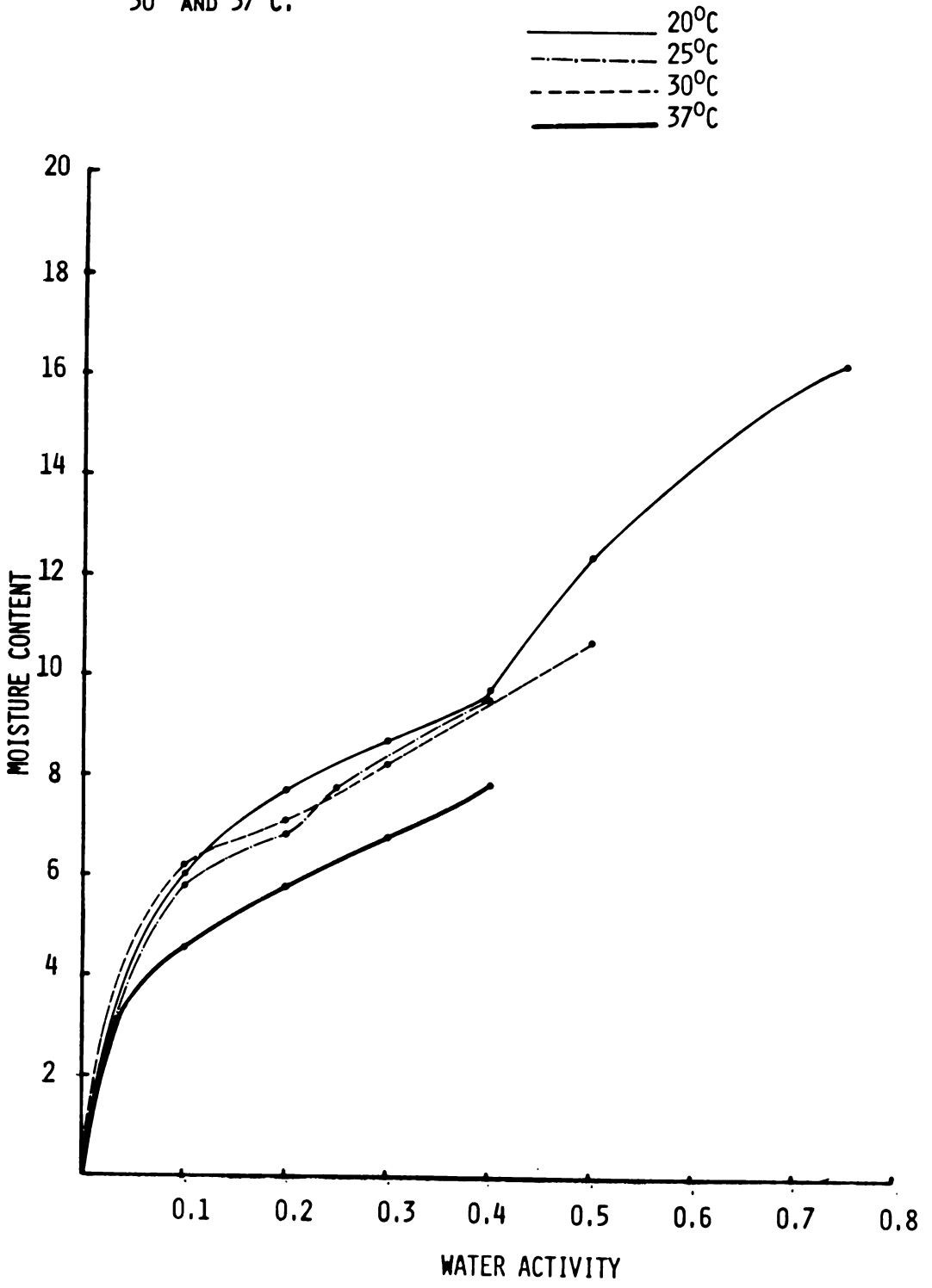


FIGURE 2. DESORPTION ISOTHERMS FOR FREEZE-DRIED MODEL SYSTEM AT 20°, 25°, 30° AND 37°C.



## Equilibrium of Product Samples

Samples with water activities below the BET values on the desorption isotherm were attained by packaging the dehydrated model food system in cryovac bags immediately after freeze-drying. The product was then pulverized and sealed in thermal-death-time cans. Packaging in this manner prevented any moisture transfer during storage. Samples with water activities below the BET value on the adsorption isotherm were prepared by exposing the freeze-dried model system to atmospheric conditions for 1-2 hours prior to canning.

Figure 3 illustrates the system which was used to adjust the moisture content of samples at and above the BET monomolecular moisture content. Freeze-dried model system samples were placed in the equilibration chamber in the form of thin slabs. Conditioned air was forced through the closed system in a clockwise direction as indicated by Figure 3. Air was drawn by the Aminco-Aire unit (inner and outer chamber) from the equilibration chamber through a spray of fine water droplets which are regulated to a constant temperature in the inner chamber. The air is then heated in the outer chamber of the Aminco-Aire unit where the dry bulb heaters are adjusted to temperatures equivalent to the storage temperature of the samples. This eliminates exposure of the model system to elevated temperatures which may induce undesirable deteriorative reactions in the freeze-dried food. When

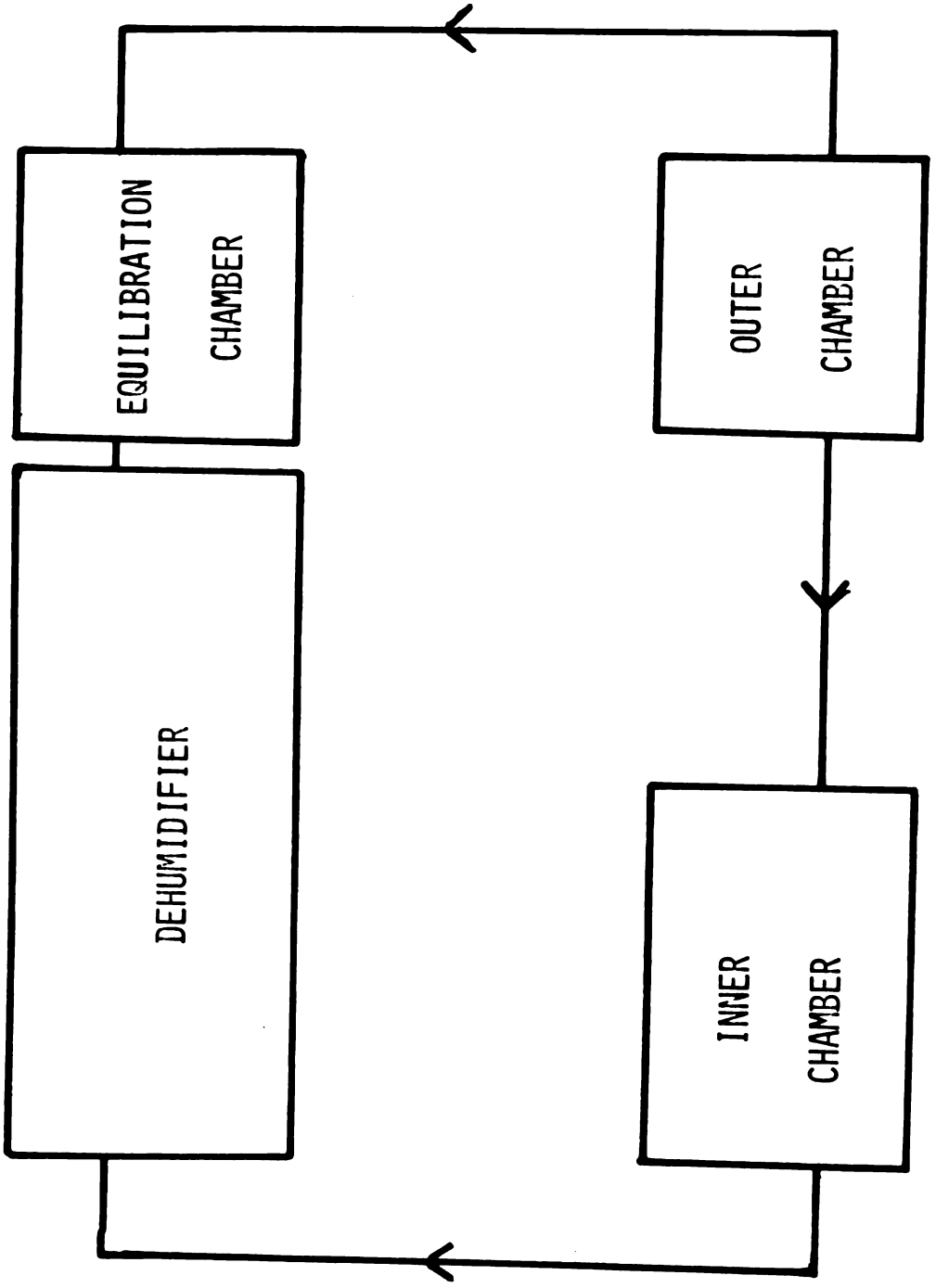



FIGURE 3. FLOW DIAGRAM FOR EQUILIBRATION OF MODEL SYSTEM TO WATER ACTIVITIES AT AND ABOVE THE BET MONOLAYER VALUE.

relative humidities lower than those provided by the Aminco-Aire conditioning unit were desired, a dehumidifier followed by cooling coils was attached to the system which further reduced the relative humidity of the air (Figure 3). Following equilibration of the model system, the samples at and above the BET value were packaged in the same manner as previously described.

## Storage of Product Samples

Storage of the canned model system was carried out at 20, 25, 30, and 37 ± 2°C.

## Total Moisture Content Determination

Total moisture content (dry weight basis) of the equilibrated and canned model system was determined by drying the samples in a vacuum oven at a vacuum pressure greater than 28 psig and at the same temperature the product was stored. Approximately 2 g of sample was weighed into a tared moisture dish and dried to a constant weight in approximately 48 hours. This method prevented exposure of the samples to high temperatures which could alter the chemical composition due to

rapid browning and degradation. A temperature gradient was used to aid in the transfer of moisture from the product during moisture determinations. This was accomplished by inserting a vacuum flask surrounded by dry ice in the line between the vacuum oven and pump. Air dried using concentrated sulfuric acid was admitted into the vacuum oven at a rate of 15-20 ml/minute to aid in the displacement of water vapor from the oven.

Determination of the moisture content in the model system samples required rapid weighing into closed containers due to the hygroscopic nature of the powdered model system.

Samples were run in duplicate.

## Water Activity Determination

The water activities of the four freeze-dried model systems at and above the BET monomolecular moisture content were adjusted using the system previously described (Figure 3).

Following the adjustment of moisture contents, the actual water activity of each canned model system below, at and above the monomolecular moisture content were determined using data from the isotherm curves at 20, 25, 30, and 37°C. Data in Tables 3 and 4 illustrate the

moisture content and corresponding water activities of samples stored at 20, 25, 30, and 37°C.

TABLE 3.--Moisture Content and Water Activity of Freeze-dried Model System Equilibrated on the Adsorption Curve.

| Temperature °C | Below BET | | At BET | | Above BET | |
|---|---|---|---|---|---|---|
| | Aw[a] | M.C.[b] | Aw[a] | M.C.[b] | Aw[a] | M.C.[b] |
| 20 | .100 | 2.32 | .220 | 5.64 | .520 | 8.99 |
| 25 | .035 | 1.37 | .255 | 5.27 | .410 | 7.60 |
| 30 | .025 | 0.73 | .305 | 5.54 | .520 | 8.36 |
| 37 | .005 | 0.16 | .280 | 4.76 | .635 | 10.70 |

[a]Water Activity

[b]Moisture Content

TABLE 4.--Moisture Content and Water Activity of Freeze-dried Model System Equilibrated on the Desorption Curve.

| Temperature °C | Below BET | | At BET | | Above BET | |
|---|---|---|---|---|---|---|
| | Aw[a] | M.C.[b] | Aw[a] | M.C.[b] | Aw[a] | M.C.[b] |
| 20 | .040 | 2.92 | .100 | 6.14 | .400 | 9.88 |
| 25 | .000 | 0.06 | .055 | 4.91 | .235 | 7.68 |
| 30 | .040 | 3.41 | .100 | 6.24 | .260 | 7.85 |
| 37 | .000 | .055 | .035 | 3.27 | .590 | 11.10 |

[a]Water Activity

[b]Moisture Content

## Thiamine Determination

Model system samples were taken from storage at equal time intervals and analyzed for their thiamine content using the following procedure. Five grams of sample were weighed into 100 ml volumetric flasks and 20 ml of 0.1 N hydrochloric acid were added. Samples were placed into an autoclave (250°F, 15 psig) for 20 minutes and then quickly cooled to ambient temperature. While stirring, 17 ml of 0.1 N sodium hydroxide was added to each flask and the samples diluted to the mark with distilled water. Samples were filtered through #41 Whatman filter paper. The thiamine content of the filtrate was determined using the automated procedure of Kirk (1974).

Samples were run in triplicate.

## RESULTS AND DISCUSSION

### Thiamine Stability and the Rate of Thiamine Destruction in Freeze-Dried Model System

Thiamine stability was shown to be inversely proportional to the rate of thiamine destruction in this storage study. Higher rates of thiamine destruction resulted in greater decreases in thiamine concentrations during storage when model system samples were compared (Tables 5 and 6).

TABLE 5.--Comparison of Thiamine Concentrations Determined by Analysis and Computed by the Kinfit Program for Model System Adjusted at the Monolayer Value on the Adsorption Isotherm and Stored at 37°C.

| Actual Thiamine Concentration (μg/g) | Computed Thiamine Concentration (μg/g) |
|---|---|
| 3.08 | 3.07 |
| 2.40 | 2.42 |
| 1.98 | 2.14 |
| 1.84 | 1.90 |
| 1.59 | 1.69 |
| 1.69 | 1.50 |
| Rate of Thiamine Degradation | $5.7 \times 10^{-3} \pm .67 \times 10^{-3}$ days$^{-1}$ |

TABLE 6.--Comparison of Thiamine Concentrations Determined by Analysis and Computed by the Kinfit Program for Model System Adjusted Below the Monolayer Value on the Adsorption Isotherm and Stored at 20°C.

| Actual Thiamine Concentration (μg/g) | Computed Thiamine Concentration (μg/g) |
|---|---|
| 5.04 | 4.983 |
| 5.06 | 4.978 |
| 4.90 | 4.971 |
| 4.88 | 4.965 |
| 4.82 | 4.958 |
| 5.04 | 4.951 |
| 5.02 | 4.945 |
| 5.02 | 4.939 |
| 4.92 | 4.933 |
| 4.80 | 4.926 |
| 4.85 | 4.920 |
| 5.00 | 4.913 |
| 4.94 | 4.907 |
| 4.90 | 4.900 |
| Rate of Thiamine Degradation | $.046 \times 10^{-3} \pm .042 \times 10^{-3}$ |

Concentrations of thiamine fluctuated slightly when water activities and storage temperatures of the freeze-dried model system provided conditions favorable for thiamine storage stability. Rate constants determined by analysis of these samples were low with high standard deviations. Water activities or storage temperatures which induced thiamine degradation in the freeze-dried model system resulted in decreased concentrations of thiamine. Resultant rate constants were higher and standard deviations were relatively lower, indicating better

agreement between thiamine concentrations determined by experimental analysis and concentrations computed by the Kinfit program (see Appendix).

Farrer (1955) extensively reviewed thiamine stability in foods and concluded that the loss of thiamine due to storage temperature could be predicted by first order reaction kinetics. In this study, the rate of thiamine degradation in a freeze-dried model system was assumed to follow first order kinetics based on Farrer's (1955) findings.

If a degrative reaction follows first order kinetics, a plot of the log concentration versus time results in a straight line. The rate constant or rate of degradation is determined from the slope of this plot.

The Kinfit computer program was used in this study to calculate the rate of thiamine degradation for each model system sample. Thiamine concentrations determined experimentally were used as input data. Final results were calculated as thiamine concentrations which resulted in a straight line on the log concentration versus time plot. A rate of thiamine degradation was determined by the Kinfit program based on this plot. The standard deviation computed by the Kinfit program indicated how well the thiamine concentrations determined by analysis agreed with concentrations calculated by the computer program.

Samples demonstrating low rates of reaction for thiamine loss with standard deviations greater than or equal to the rate constant were assumed to be stable. One reason for samples demonstrating low rate constants with high standard deviations was that thiamine degradation did not follow first order kinetics. The Kinfit computer program was used in the analysis of experimental data, and determined the rate constant for the first order reaction. If the data failed to follow first order kinetics, the computer model was invalid and low rate constants with high standard deviations resulted. Zero order kinetics was assumed to describe the rate of thiamine destruction in these samples. Based on this assumption, thiamine deteriorated at a rate independent of the concentration of reactants when rate constants were small and standard deviations were large. Another reason for the low rates of thiamine degradation with high standard deviations was data scattering.

All rates of thiamine degradation reported in this study can be converted to percent loss using the expression

$$\% \text{ loss} = 1 - e^{-kt}$$

k = rate of thiamine degradation

t = time of sample storage

(see Appendix).

## Effect of Water Activity on the Rate of Thiamine Destruction

### Freeze-Dried Model System Equilibrated on the Adsorption Isotherm

Data in Table 5 illustrates the influence of water activity on thiamine stability in samples equilibrated on the adsorption isotherm and stored at 20, 25, 30, and 37°C. The water activities of the samples increased below, at, and above the BET monomolecular moisture content (BET-MMC), respectively. Model system samples stored at 20°C showed a small but significant increase in the rate of thiamine destruction as the water activity of the samples increased. This was illustrated by the samples stored at 20°C having rate constants with small standard deviations and rate constants which differed by at least one standard deviation from each other.

At storage temperatures of 25, 30, and 37°C, the rate constants for the model systems equilibrated on the adsorption isotherm below and at the BET-MMC showed no significant difference. These samples had small rate constants which differed by less than one standard deviation (Table 7). This indicated thiamine stability. Samples stored at 25 and 30°C and adjusted to water activities on the adsorption curve below and at the BET-MMC had rate constants which were less than their

standard deviations. This illustrated thiamine stability, indicating a zero rather than a first order reaction rate.

TABLE 7.--Rate Constants and Standard Deviations for Thiamine Loss in Freeze-dried Model System Equilibrated on the Adsorption Isotherm at Water Activities Below, At, and Above the BET Monomolecular Moisture Content.

| Temperature °C | Below BET | | At BET | | Above BET | |
|---|---|---|---|---|---|---|
| | k[a] | σ[b] | k[a] | σ[b] | k[a] | σ[b] |
| 20 | .0307 | .0476 | .135 | .0404 | .229 | .0432 |
| 25 | .1750 | .2270 | .122 | .1820 | .622 | .1850 |
| 30 | .0700 | .1670 | .112 | .1550 | .435 | .1480 |
| 37 | .2420 | .2020 | .559 | .3460 | 5.700 | .6430 |

[a]rate constant, $10^{-3}$ $days^{-1}$

[b]standard deviation

At higher water activities (above BET-MMC), the rates of thiamine deterioration for the 25, 30, and 37°C samples were significantly greater than the rates at or below the monomolecular moisture content. Therefore, the higher the water activity was above BET-MMC, the faster the rate of thiamine destruction for samples equilibrated on the adsorption isotherm and stored at 25, 30, and 37°C.

Freeze-Dried Model System Equilibrated on the Desorption Isotherm

Model system adjusted to water activities below and at the BET monolayer value on the desorption isotherm and stored at 20, 30, and 37°C showed no significant differences in the rate of thiamine degradation (Table 8). These samples exhibited thiamine destruction rate constants differing by less than one standard deviation which was interpreted to demonstrate thiamine stability.

TABLE 8.--Rate Constants and Standard Deviations for Thiamine Loss in Freeze-dried Model System Equilibrated on the Desorption Isotherm at Water Activities Below, At, and Above the BET Monomolecular Moisture Content.

| Temperature °C | Below BET | | At BET | | Above BET | |
|---|---|---|---|---|---|---|
| | $k^a$ | $\sigma^b$ | $k^a$ | $\sigma^b$ | $k^a$ | $\sigma^b$ |
| 20 | .0961 | .0470 | .0221 | .0377 | .368 | .0673 |
| 25 | .1650 | .2670 | .4630 | .1940 | .485 | .2440 |
| 30 | .0877 | .1300 | .0269 | .0800 | .365 | .0861 |
| 37 | .4610 | .1150 | .7170 | .3070 | 3.670 | .5380 |

[a]rate constant, $10^{-3}$ $days^{-1}$

[b]standard deviation

Thiamine degradation rate constants for model systems with water activities above the BET-MMC on the desorption isotherm and stored at 20, 25, 30, and 37°C were significantly greater than the

rate constants of samples with lower water activities. These data support the observed trend for samples stored at 20, 25, 30, and 37°C with water activities equilibrated on the adsorption curve, i.e. the higher the water activity above the BET-MMC (up to 0.70), the faster the rate of thiamine degradation.

Samples equilibrated on the desorption isotherm and stored at 25°C showed an apparent increase in the rate constant as the water activity increased. However, the standard deviations were high enough for all samples stored at this temperature to indicate no significant difference between rates of thiamine degradation at the three different water activities. These results were supported by thiamine stability data at low temperatures reported by Rice and Beuk (1945) and Farrer (1955). Lower storage temperatures for the freeze-dried model system (20, 25, and 30°C) in addition to differences in product type, may have been responsible for the lower rates of thiamine destruction than previously reported by Rice (1944), Rice and Beuk (1945), and Bendex et al. (1951) in food products.

The high standard deviations of samples equilibrated on the desorption curve and stored at 25°C can also be explained by the shorter storage period of these samples (126 days) when compared to samples stored at 20 and 30°C (364 and 224 days). The slow rate of thiamine destruction at the lower storage temperatures necessitates a longer storage period of the model system to accurately determine

valid rate constants. Therefore it is more likely that the curve obtained by plotting log concentration versus time is a good representation of the whole thiamine destruction curve when a long storage period is used (Figure 4). The rate of thiamine destruction is determined by evaluating the slope of the curve. With a slow rate of thiamine deterioration and long storage period, a greater possibility exists that the rate constant obtained is representative of the actual thiamine destruction rate. Therefore it is questionable whether the calculated rate constant for the samples stored at 25°C above the BET-MMC correlates with its actual rate of thiamine destruction. It is also possible that when water activities increase above the BET-MMC, for the sample stored at 25°C, the rate of thiamine deterioration increases. However, the high standard deviations for the samples stored at 25°C prevents conformation of this trend. Thiamine was shown to be stable at water activities below and at the BET-MMC. Therefore, results of this study supported the hypothesis of Salwin (1959) that the BET-MMC afforded the optimum moisture content for storage stability when the total storage stability of a particular food was examined (i.e. fat soluble vitamins, fat, proteins, etc.).

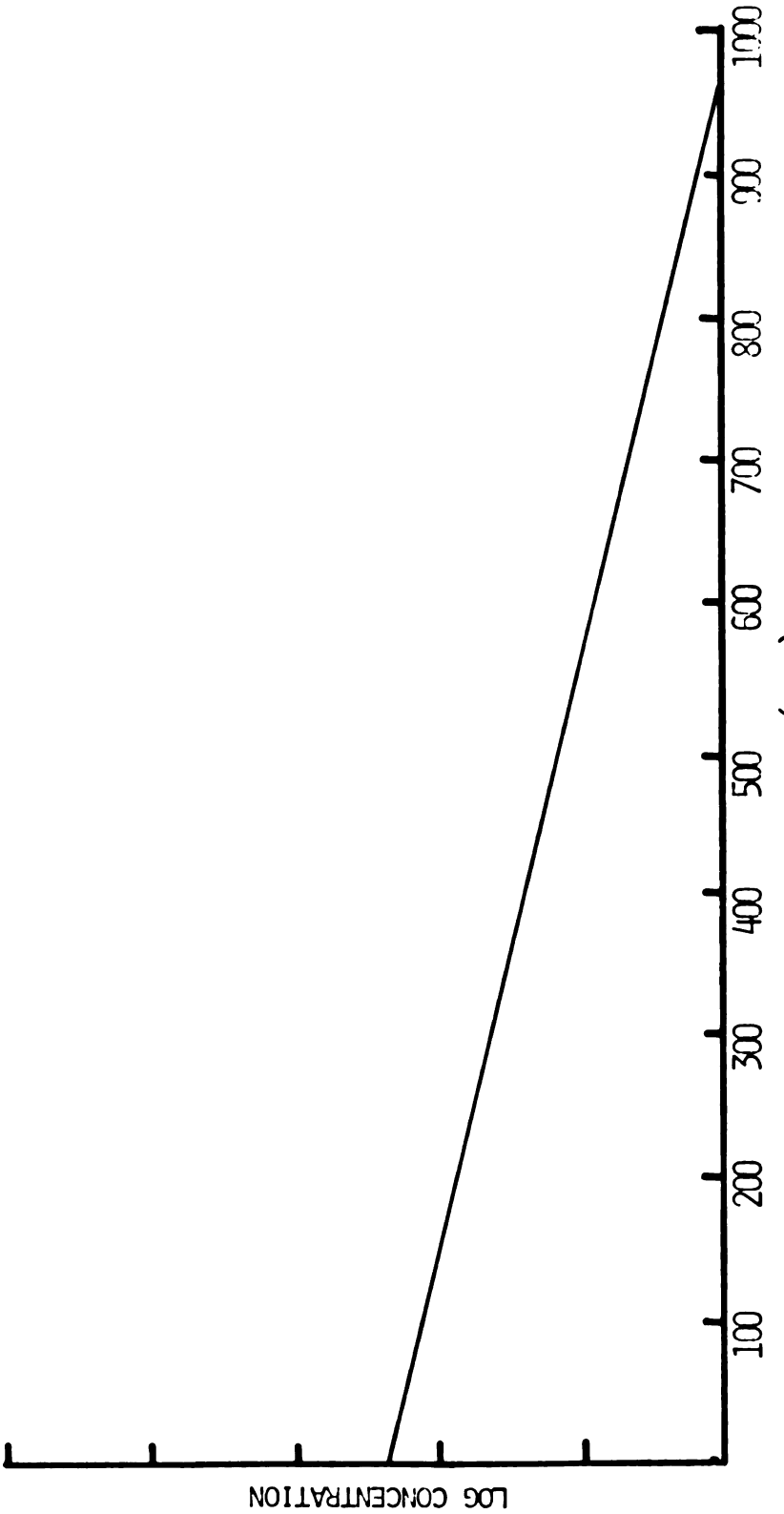



FIGURE 4. GRAPH OF LOG CONCENTRATION AS A FUNCTION OF TIME FOR A FIRST ORDER REACTION.

## Non-Enzymatic Browning and Thiamine Degradation in Freeze-Dried Model System

Results from Tables 7 and 8 show significantly higher rates of thiamine degradation above the BET-MMC in the multilayer and condensation region of the moisture sorption isotherm where the rate of non-enzymatic browning is accelerated (Lea, 1958; Karel and Labuza, 1968; Labuza et al., 1970). Visual observation of the freeze-dried model system showed significant browning of samples with higher water activities (0.5-0.7) and stored at 30 and 37°C.

Non-enzymatic browning in dehydrated foods is considered the most important type of chemical change that occurs at high water activities when microbial and enzymatic reactions have been inhibited (Lea, 1958). Water activities of the freeze-dried model system were sufficiently low to prevent microbial and enzymatic deterioration. However, the presence of reducing sugars and amino compounds provided necessary conditions for non-enzymatic browning to occur in the freeze-dried model system. High water activities, a pH of approximately 7.0 and a high storage temperature (Ellis, 1959) were conducive to Maillard browning in the freeze-dried model system.

In addition to proteins and amino acids, nitrogenous food constituents such as thiamine react readily in a Maillard-type reaction (Lea, 1958). Lhoest (1957) illustrated this in heating a dry

mixture of thiamine with glucose at 85°C. Vander Poel (1956) demonstrated loss of thiamine with the production of a brown discoloration and fluorescent compounds when heating thiamine in a glucose solution.

Interaction between lipid oxidation and non-enzymatic browning mechanisms could also effect the rate of thiamine degradation in the freeze-dried model system. While lipid oxidation products of aldehydes and ketones may initiate browning (Labuza, 1972), certain end products of browning demonstrate antioxidant properties. The melanoidins (products of non-enzymatic browning) formed by heating glucose and glycine together demonstrated a stabilizing effect on fat in biscuits (Franzke and Iwainski, 1954, 1955; Iwainski, 1956). Also, powerful antioxidant effects of crystalline amino-hexose-reductones in hydrogenated vegetable oils and in lard were shown by Evans et al. (1956).

## Effect of Temperature on the Rate of Thiamine Destruction

### Freeze-Dried Model System Equilibrated on the Adsorption Isotherm

Table 7 compares the rate of thiamine destruction of samples stored at four temperatures with water activities on the adsorption curve. By observing the rate constants for water activities below

the BET-MMC, the rates of thiamine destruction illustrated no significant difference for samples stored at 20, 25, 30, and 37°C. The low rate constants and high standard deviations indicated that the samples were stable to thiamine destruction and probably did not follow first order reaction kinetics (c.f. p. 34). The rate constants for samples with water activities at the BET-MMC showed no significant differences between the rate of thiamine destruction at 20, 25, 30, or 37°C. As with samples below the BET-MMC, these samples did not appear to follow first order reaction kinetics. Therefore, in samples equilibrated on the adsorption curve with water activities at the BET-MMC, thiamine was stable at temperatures of 20, 25, 30, and 37°C.

Samples with water activities above the BET-MMC on the adsorption curve and stored at 20, 25, and 30°C demonstrated rate constants which were similar. However, when the thiamine rate constants for samples at 20 and 30°C were compared, the rates differed by more than one standard deviation (Table 7). As a result of this, the rate of thiamine destruction was interpreted as being significantly higher for samples stored at 30°C than samples stored at 20°C. Thus, at water activities above the BET-MMC the effect of temperature on the rate of thiamine degradation became apparent, i.e. the higher the storage temperature of freeze-dried samples containing thiamine, the higher the rate of thiamine degradation. The rate constant for the model

system stored at 25°C was slightly higher than expected. This was explained by a shorter storage period for samples stored at 25°C than at 20 and 30°C. As a result of this, the calculated rate constant of the 25°C sample above the BET-MMC may not have correlated well with its actual rate. The rate constant for the model system stored at 37°C was significantly higher than the rates of thiamine destruction at lower temperatures with water activities above the BET-MMC (Table 7). Thus, samples with water activities above the BET-MMC on the adsorption curve showed significant thiamine degradation when stored at 37°C.

Freeze-Dried Model System Equilibrated on the Desorption Isotherm

The data in Table 8 illustrate no significant differences in the rate of thiamine degradation for samples with water activities below and at the BET-MMC on the desorption curve and stored at 20 and 30°C. These samples had low rate constants with large standard deviations which has been interpreted as indicating thiamine stability. The rate constant and standard deviation for model system stored at 25°C was higher than expected. As previously discussed, the shorter storage period for samples at 25°C, when compared to those stored at 20 or 30°C resulted in poor correlation between the calculated and

actual rate constant. Model system with a water activity below and at the BET-MMC equilibrated on the desorption curve and stored at 37°C exhibited a rate of thiamine degradation significantly larger than samples stored at 20 and 30°C. As shown with samples equilibrated on the adsorption isotherm at the BET-MMC, thiamine destruction in the model system at water activities below and at the BET-MMC on the desorption curve and stored at 37°C followed first order kinetics and demonstrated a temperature dependence.

Model system samples with water activities equilibrated above the BET-MMC on the desorption curve showed no significant difference in rate constants at storage temperatures of 20, 25, and 30°C. This was interpreted as indicating thiamine stability. Model system equilibrated on the desorption isotherm above the BET-MMC and stored at 37°C, demonstrated a thiamine destruction rate significantly higher than those of samples stored at the lower temperatures. This was explained by rate constants which differed by more than one standard deviation from each other, thus following first order kinetics.

Temperatures of 20, 25, 30, and 37°C were used in this study to approximate typical warehouse storage conditions of food products. While water activities of samples above the BET-MMC illustrated greater thiamine destruction than samples at lower water activities, it was not until samples were stored at 37°C that the higher water

activities significantly effected the nutritional shelf life of the dehydrated model food system.

## Arrhenius Plot

An Arrhenius plot could not be determined from the calculated thiamine rate constants of the freeze-dried model system. The reason for this was that the water activities of samples held at the four storage temperatures were not equal for samples below, at, or above the BET-MMC. However, the large differences in rate constants that existed between some of the samples stored at 20, 25, and 30°C, and samples stored at 37°C within a particular water activity range (i.e. below, at, and above the BET-MMC) were not explainable by the small differences in water activity. Degradation of thiamine in the freeze-dried model system proceeded at temperatures of 30°C and below in a manner which could not have been determined by extrapolation of an Arrhenius curve calculated by plotting rate constants of the system at storage at and above 37°C. This was explained by the degradation of thiamine in the freeze-dried model system following zero order rather than first order reaction kinetics under these conditions. Thus, it is important to exercise caution when using Arrhenius curves and extrapolating back to predict thiamine reaction rates at normal storage temperatures.

## Adsorption Versus Desorption

### Model System with Water Activities Below the BET Monolayer Value

Data in Table 9 compare the rate of thiamine degradation on the adsorption and desorption curve below the BET-MMC. For all temperatures studied (20, 25, 30, and 37°C) the rates of thiamine degradation were not significantly different from the adsorption to the desorption curve for any particular temperature. Thiamine in the freeze-dried model system was stable at these low water activities. The rate of degradation appeared to follow zero rather than first order kinetics because of the high standard deviation.

### Model System with Water Activities at the BET Monolayer Value

Data in Table 10 compares the rate of thiamine degradation in the adsorption and desorption curve at the BET-MMC. Samples stored at 20°C demonstrated a significant difference between the rate of thiamine degradation. Samples equilibrated on the adsorption isotherm had a water activity of 0.12 higher than the desorption sample and moisture contents which were quite close (0.5% difference). The sample with the higher water activity exhibited the higher thiamine

degradation rate constant. Model system stored at 25, 30, and 37°C showed no significant difference in the rates of thiamine degradation. While some differences in the rate of thiamine degradation occurred, the influence of water activity could not be adequately evaluated due to high standard deviations.

## Model System with Water Activities Above the BET Monolayer Value

Comparison of the rates of thiamine degradation between the adsorption and desorption curve above the BET-MMC are shown in Table 11. The sample stored at 20°C demonstrated the importance of moisture content on thiamine stability of low moisture foods. While the desorption sample had a lower water activity (0.40 vs 0.52), its moisture content was 9.88% compared to 8.99% for the adsorption sample. This was understandable since for a particular water activity, a higher corresponding moisture content exists on the desorption isotherm when compared to the adsorption isotherm. The model system stored at 20°C and equilibrated on the desorption isotherm exhibited a significantly higher rate of thiamine degradation. Data in Table 11 illustrate moisture contents of model system stored at 25°C differing only by 0.08% between the adsorption and desorption curve while the water activity of the sample equilibrated on the adsorption isotherm

TABLE 9.--Effect of Water Activity and Moisture Content on the Rate of Thiamine Deterioration for Model System Equilibrated on the Adsorption and Desorption Isotherms at Water Activities Below the BET Monomolecular Moisture Content.

| Temperature °C | Adsorption | | | |
|---|---|---|---|---|
| | Aw[a] | M.C.[b] | k[c] | σ[d] |
| 20 | .100 | 2.32 | .0307 | .0476 |
| 25 | .035 | 1.37 | .1750 | .2270 |
| 30 | .005 | 0.67 | .0700 | .1670 |
| 37 | .025 | 0.16 | .2420 | .2020 |
| | Desorption | | | |
| 20 | .040 | 2.92 | .0961 | .0470 |
| 25 | .000 | 0.06 | .1650 | .2670 |
| 30 | .020 | 3.41 | .0877 | .1300 |
| 37 | .000 | 0.06 | .4610 | .1150 |

[a]Water Activity

[b]Moisture Content

[c]Rate Constant, $10^{-3}$ $days^{-1}$

[d]Standard Deviation

surpassed that of the sample equilibrated on the desorption isotherm by 0.175. In this case water activity appeared to exert the greater influence in the stability of thiamine than the small moisture content difference. However, the standard deviations for the rates of thiamine destruction of samples stored at 25°C were too high to adequately evaluate the influence of water activity.

TABLE 10.--Effect of Water Activity and Moisture Content on the Rate of Thiamine Deterioration for Model System Equilibrated on the Adsorption and Desorption Isotherms at Water Activities At the BET Monomolecular Moisture Content.

| Temperature °C | Adsorption | | | |
|---|---|---|---|---|
| | Aw[a] | M.C.[b] | k[c] | σ[d] |
| 20 | .220 | 5.64 | .135 | .0404 |
| 25 | .255 | 5.27 | .122 | .1820 |
| 30 | .305 | 5.54 | .112 | .1550 |
| 37 | .280 | 4.76 | .559 | .3460 |
| | Desorption | | | |
| 20 | .100 | 6.14 | .0221 | .0377 |
| 25 | .055 | 4.91 | .4630 | .1940 |
| 30 | .100 | 6.24 | .0269 | .0800 |
| 37 | .035 | 3.27 | .7170 | .3070 |

[a]Water Activity

[b]Moisture Content

[c]Rate Constant, $10^{-3}$ days

[d]Standard Deviation

The rates of thiamine loss for model system stored at 30°C with a water activity above the BET-MMC suggested the same trend that was indicated in the samples stored at 25°C, i.e. water activity influenced thiamine stability to a greater extent than moisture content. Moisture contents in these samples differed by only 0.61% while water activities differed significantly (0.16 difference). The adsorption sample had the higher water activity and higher rate of thiamine

TABLE 11.--Effect of Water Activity and Moisture Content on the Rate of Thiamine Deterioration for Model System Equilibrated on the Adsorption and Desorption Isotherms at Water Activities Above the BET Monomolecular Moisture Content.

| Temperature °C | Adsorption | | | |
|---|---|---|---|---|
| | Aw[a] | M.C.[b] | k[c] | σ[d] |
| 20 | .520 | 8.99 | .229 | .0432 |
| 25 | .410 | 7.60 | .622 | .1850 |
| 30 | .520 | 8.36 | .435 | .1480 |
| 37 | .635 | 10.70 | 5.700 | .6430 |
| | Desorption | | | |
| 20 | .400 | 9.88 | .368 | .0673 |
| 25 | .235 | 7.68 | .485 | .2240 |
| 30 | .260 | 7.85 | .365 | .0861 |
| 37 | .590 | 11.10 | 3.670 | .5380 |

[a]Water Activity

[b]Moisture Content

[c]Rate Constant, $10^{-3}$ days

[d]Standard Deviation

degradation. However, high standard deviations of rate constants prevented adequate evaluation of the influence of water activity in these samples.

The moisture contents of model system stored at 37°C differed by only 0.38% while water activities of the adsorption and desorption samples differed by 0.07. Since water activities were significantly

different while moisture differed slightly, water activity exerted the greater influence on the rate of thiamine loss in these samples.

Results comparing adsorption to desorption samples agreed with Labuza (1971) and Rockland (1969) that both moisture content and water activity must be evaluated when studying the stability of dehydrated food systems. For a particular water activity, the corresponding moisture content will be higher on the desorption isotherm than the adsorption isotherm. When the difference in water activities was large and the moisture contents differed slightly for the adsorption and desorption model system at a particular temperature, water activity exerted the greater influence in thiamine stability. However, when the difference in moisture contents was great and the water activities were close for the adsorption and desorption samples at a particular temperature, moisture content exerted the greater influence in the stability of thiamine in the freeze-dried model system examined in this study.

# SUMMARY AND CONCLUSIONS

Freeze-dried model systems were stored at various temperatures (20, 25, 30, 37°C) and adjusted to water activities below, at, and above the monomolecular moisture content on their adsorption and desorption isotherms. Data indicated that as water activity increased from below to the BET monomolecular moisture content (BET-MMC) slight or insignificant differences in rate constants resulted. However, as water activity increased to above the BET-MMC, the rate constants significantly increased.

Rate constants for thiamine destruction in model system samples equilibrated to water activities below the BET-MMC on the adsorption and desorption isotherm did not illustrate a temperature dependence (20-30°C) for thiamine destruction at these low water activities.

Rate constants for thiamine destruction in model system samples with water activities at the BET-MMC, equilibrated on the adsorption isotherm did not demonstrate a temperature dependence at these water activities and storage temperatures of 20, 25, 30, and 37°C.

Rate constants for thiamine destruction in model system with water activities at the BET-MMC and equilibrated on the desorption isotherm demonstrated a temperature dependence at these water activities and storage temperatures of 20, 30, and 37°C.

Rate constants for thiamine destruction in model system equilibrated to water activities above the BET-MMC on the adsorption and desorption isotherm did not illustrate a temperature dependence for thiamine destruction at storage 20, 25, and 30°C. However, storage of the model system at 37°C showed an effect of temperature on the stability of thiamine. The rate constant for thiamine loss at this storage temperature was significantly higher than the rate constants at the lower storage temperatures. In addition, visual observations showed significant browning of samples adjusted to water activities above the BET-MMC and stored at 30 and 37°C.

In general, water activities of model system above the BET-MMC illustrated greater thiamine destruction than samples at lower water activities. However, not until samples were stored at 37°C did the higher water activities significantly effect the nutritional shelf life of the dehydrated model food system.

Comparison of thiamine rate losses between the adsorption and desorption isotherms were made for the model system stored at a particular temperature to determine the effect of moisture content. Rates of thiamine degradation in model system equilibrated to water

activities below and at the BET-MMC were low with little or no significant differences between the adsorption and desorption isotherms. When rate constants were compared between the adsorption and desorption curve, model system with water activities above the BET-MMC and stored at 20°C demonstrated thiamine stability dependence on moisture content while model system with water activities above the BET-MMC and stored at 37°C demonstrated thiamine stability dependence on water activity. These results indicated that both water activity and moisture content were important in determining thiamine stability in a dehydrated food system. Large differences in moisture contents and water activities which were close for the adsorption and desorption model system at a particular temperature resulted in moisture content exerting the greater influence in thiamine stability. However, when the difference in water activities was great and the moisture contents were close for the adsorption and desorption samples at a particular temperature, water activity was shown to influence in the stability of thiamine in the freeze-dried model system examined in this study.

With a slow rate of thiamine deterioration, a long storage period was expected to result in better correlation between calculated rate constants and the actual rate of thiamine destruction.

The hypothesis of Salwin (1959) that the BET-MMC optimized the storage stability of dehydrated foods was supported by results of this study.

# RESEARCH SUGGESTIONS

1. Browning rate determination may prove a relation between browning and thiamine degradation in the freeze-dried model system at water activities above the BET monolayer value.

2. Equilibration of the model system to the same water activities for a particular water activity range (i.e. below, at, above the BET-MMC) and storage at higher than 37°C would allow for calculation of an Arrhenius plot.

3. Addition of other vitamins to the model system would allow for evaluation of fat or water soluble vitamin interactions on thiamine degradation. This would also allow the evaluation of Salwin's (1959) hypothesis in a dehydrated food product containing vitamins both sensitive and stable to oxidative degradation.

4. Use of an unsaturated fat in the freeze-dried model system will demonstrate any effect lipid oxidation may have on the rate of non-enzymatic browning.

# APPENDIX

# APPENDIX

## Sorption Isotherm Data

a) 20°C Adsorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 2.96 | 0.0376 |
| 0.20 | 4.55 | 0.0550 |
| 0.30 | 6.18 | 0.0694 |
| 0.40 | 7.53 | 0.0886 |
| 0.50 | 8.64 | |
| 0.75 | 16.7 | |
| 0.95 | 28.4 | |

b) 20°C Desorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 6.11 | 0.0181 |
| 0.20 | 7.80 | 0.0320 |
| 0.30 | 8.73 | 0.0490 |
| 0.40 | 9.73 | 0.0685 |
| 0.50 | 12.40 | |
| 0.75 | 16.20 | |
| 0.95 | 26.90 | |

c) 25°C Adsorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 3.02 | 0.0368 |
| 0.20 | 4.82 | 0.0520 |
| 0.30 | 6.45 | 0.0664 |
| 0.40 | 7.57 | 0.0881 |

d) 25°C Desorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 5.77 | 0.0193 |
| 0.20 | 7.22 | 0.0346 |
| 0.25 | 7.77 | 0.0427 |
| 0.40 | 9.26 | 0.0720 |

e) 30°C Adsorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 3.29 | 0.0337 |
| 0.20 | 4.00 | 0.0625 |
| 0.30 | 5.63 | 0.0761 |
| 0.40 | 6.35 | 0.1049 |
| 0.50 | 8.04 | 0.1243 |

f) 30°C Desorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 6.32 | 0.0175 |
| 0.20 | 7.07 | 0.0354 |
| 0.30 | 8.20 | 0.0523 |
| 0.40 | 9.55 | 0.0698 |
| 0.50 | 10.70 | |
| 0.75 | 15.90 | |
| 0.95 | 30.00 | |

g) 37°C Adsorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 2.58 | 0.0431 |
| 0.20 | 3.65 | 0.0684 |
| 0.30 | 5.17 | 0.0829 |
| 0.40 | 6.59 | 0.1020 |
| 0.75 | 14.50 | |
| 0.95 | 57.80 | |

h) 37°C Desorption

| Water Activity | % Moisture Dry Basis | $a_w/w(1-a_w)$ |
|---|---|---|
| 0.10 | 4.46 | 0.0249 |
| 0.20 | 5.82 | 0.0430 |
| 0.30 | 6.67 | 0.0643 |
| 0.40 | 7.82 | 0.0854 |

## BET Calculation of Monomolecular Moisture Content

a) Formula

$$a_w/w(1-a_w) = 1/w_mC + C-1/w_mC \times a_w$$

$a_w$ = Water Activity

$w$ = Moisture Content

$C$ = Energy Constant

$w_m$ = Monomolecular Moisture Content

b) Plot

$a_w$ versus $a_w/w(1-a_w)$ is plotted. A linear relationship between 0.10 and 0.40 $a_w$ exists. The y intercept and the slope of the line is determined from this plot. The BET equation can then be used to calculate C and $w_m$.

c) Sample Calculation--25°C Adsorption Sample

| $a_w$ | $a_w/w(1-a_w)$ |
|---|---|
| 0.1 | 0.0368 |
| 0.2 | 0.0519 |
| 0.3 | 0.0664 |
| 0.4 | 0.0881 |

y intercept = $1/w_mC$ = .0198

slope = $C-1/w_mC$ = .165

C = 9.44

$w_m$ = 5.34

$a_w$ = 0.255 (using sorption isotherm)

## Kinfit Analysis of Data

A general non-linear curvefitting and equation solving program developed by Vincent A. Nicely and James L. Dye was used to determine the rate of thiamine degradation, the standard deviation of the rate constant, and the multiple correlation coefficient. The statistical information obtained allowed for a test for the best of data to an equation without imposing special constraints upon the data to be evaluated. Numerical integration procedures were used to fit the data to differential equations (Dye and Nicely, 1971). The program minimized the functional

$$\phi = \sum_{i=1}^{n} W_i F_i^2$$

$n$ = number of points

$F_i$ = residual which approaches zero for all i as the parameters approach their best values if the data were free of errors

$W_i$ = a weight which gives maximum likelihood estimates of the independent normal distribution.

The variance of the data was estimated based on samples run in triplicate for a particular water activity and temperature. Initial concentrations and standard error were estimated by calculations using the Least Squares Fit-Exponential, program No. 1003-2 T3, on a Wang calculator-computer.

The program used to analyze the data has been deposited in the Program Library at the Michigan State University Computer Library, East Lansing, Michigan 48823.

Percent Loss of Thiamine in a Dehydrated Model Food System on the Desorption Curve Stored at 20, 25, 30, and 37°C for Six Months

| Temperature | Percent Loss of Samples with Water Activities | | |
|---|---|---|---|
| | Below BET | At BET | Above BET |
| Adsorption | | | |
| 20°C | 0.514 | 2.24 | 3.77 |
| 25°C | 2.90 | ---- | 9.92 |
| 30°C | 1.17 | 1.86 | 7.05 |
| 37°C | 3.98 | 12.30 | 61.60 |
| Desorption | | | |
| 20°C | 1.60 | 0.371 | 6.00 |
| 25°C | 2.73 | 7.480 | 7.79 |
| 30°C | 1.46 | 0.451 | 5.95 |
| 37°C | 7.45 | 11.400 | 46.00 |

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