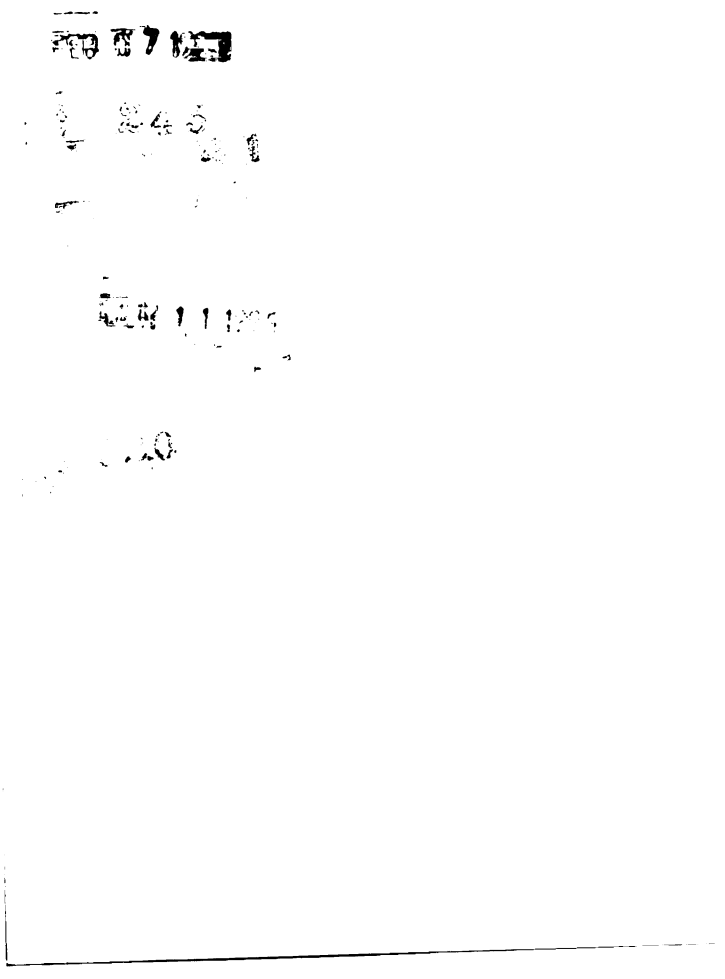


DEGRADATION OF ASCORBIC ACID IN A DEHYDRATED MODEL FOOD SYSTEM DURING STORAGE

By

Daniel B. Dennison

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1978

ABSTRACT

DEGRADATION OF ASCORBIC ACID IN A DEHYDRATED MODEL FOOD SYSTEM DURING STORAGE

By

Daniel B. Dennison

The storage stability of reduced and total ascorbic acid in a dehydrated model food system designed to simulate a ready-to-eat breakfast cereal was studied as a function of water activity, moisture content, oxygen and temperature. Sorption isotherm data obtained at 10, 20, 30 and 37°C for the model system were used to calculate the Brunauer, Emmet, Teller (BET) monomolecular moisture content at these respective temperatures. The model system, which contained 11.25 mg of ascorbic acid per 100 g dry weight basis (25% Recommended Dietary Allowance, RDA) was equilibrated at water activities below, at and above the water activity corresponding to the calculated BET monomolecular moisture content for the adsorption isotherm. The samples were sealed in thermal death time (TDT) and 303 cans and stored under isothermal conditions to prevent any shift in water activity. The TDT cans were filled with the model system

so that no headspace remained and limited gaseous oxygen was available due to the inter and intrastitial spaces. The 303 cans were filled with an equal mass of model system as the TDT, thus providing a large excess of gaseous oxygen in the headspace. The effect of air and moisture vapor transmission on the storage stability of ascorbic acid in the model system was studied by packaging the model system in 1-oz. paperboard boxes with waxed liners. The samples were stored at 30°C and 10, 40 and 85% relative humidities.

Reduced, dehydro and total ascorbic acid concentrations were determined as a function of storage time by an automated o-phenylenediamine fluorometric assay procedure. Under all storage conditions, reduced and total ascorbic acid losses could be satisfactorily described by first-order kinetics.

Ascorbic acid destruction in the model system stored in cans or boxes was linearly dependent on the water activity, exhibiting maximum stability below the BET monomolecular moisture content. These results contradict the hypothesis that the BET monomolecular moisture content should represent the water activity of greatest stability.

Comparison of the rate constants for total and reduced ascorbic acid degradation in TDT and 303 cans at identical conditions of water activity and storage

temperature showed a dependence on the availability of gaseous oxygen. The results were interpreted in terms of the consumption of dissolved oxygen in the degradation of ascorbic acid and the transfer of gaseous oxygen into the product moisture which would be governed by the equilibrium constant $K = (O_2)_d/(O_2)_g$, where $(O_2)_d$ and $(O_2)_g$ represent the concentration of dissolved and gaseous oxygen present in the system, respectively.

The influence of riboflavin and vitamin A on the degradation of ascorbic acid in the dehydrated model system was studied as a function of water activity and storage temperature. Ascorbic acid degradation was found to be unaffected by the presence of either vitamin.

The catalytic influence of trace minerals (Fe, Cu, Zn, Ca) on the rate of ascorbic acid degradation was studied as a function of water activity. No catalysis by the added metals was observed as 0.10 and 0.40 a_w, except for copper at 0.40 a_w. This is interpreted as a lack of metal ion mobility and/or insolubility at low water activities. At 0.65 a_w, which was in the capillary region of the adsorption isotherm, a 2-3 fold increase in degradation rate over the nonfortified system was observed for each form of added trace mineral with the exception of zinc oxide, which did not exhibit catalysis. These results are explained of the basis of Fe and Cu

ion mobility in the capillary region of the isotherm, whereas Ca and Zn catalysis are interpreted as altering the activity coefficient of oxygen.

Activation parameters were calculated for the degradation of ascorbic acid in the model system stored in metal cans. The free energy of activation remained constant at all a_w, thus it was concluded that the degradation of ascorbic acid followed only one mechanism.

ACKNOWLEDGMENTS

The author wishes to express special gratitude to Dr. James R. Kirk. His advice, personal direction and expert counsel provided invaluable guidance throughout this investigation and during the writing of this dissertation.

The author also expresses grateful appreciation to the members of his graduate committee, Drs. J. R. Brunner, D. R. Heldman, L. L. Bieber and R. W. Luecke for their review of the manuscript. A special note of appreciation is extended to the members of the Department of Food Science and Human Nutrition, Michigan State University, for their continual interest and encouragement.

The author expresses his most sincere appreciation to his wife, Judy, for constant inspiration and encouragement throughout his graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Fortification of food products is not a recent idea. As noted by Bauernfeind (1971), Boussingault, in 1833, first proposed the fortification of table salt with iodine in South America to prevent goiter. The initial impetus for nutrient fortification in the United States came principally from an executive World War II order in 1941 requiring the addition of thiamin, riboflavin, niacin and iron to white bread and flour to combat beriberi, ariboflavinosis, pellagra and iron deficiency anemia. From the initial addition of vitamins to flour, the practice of nutrient fortification of most foods has been commonplace and, in many instances, expected by the consumer.

The principle consideration that should govern any fortification program is public health. The resolution of nutrient deficiencies in humans must involve a multi-phase approach. Aylward and Morton (1971) outlined six areas for the improvement of the nutritional status of the general population: (1) improvement in the initial quality of plant or animal product, (2) changes in dietary

habits and household practices, (3) improved methods of processing, preservation and storage, (4) biological enoblements, (5) physical treatment and (6) addition of supplements. The changing food patterns and the increased use of manufactured foods have led to the acceptance of food enrichment policies in more than thirty countries. Bauernfeind (1971) has presented an extensive compilation of fortified foods utilized in various countries.

Extensive fortification of cereals, flours and other low moisture products has been employed for a number of years in the United States. Addition of nutrients above the label claim is generally required in order to compensate for incomplete distribution, nutrient degradation during processing and storage and analytical error (Borenstein, 1971).

Labuza (1968) and Borenstein (1971) have noted that little kinetic data are available describing the stability of vitamins during processing and storage of foods. The influence of factors such as pH, moisture content, oxygen, product composition, trace metals on vitamin stability are unknown or difficult to extrapolate from one product to another.

Ascorbic acid is frequently not found in fortified products such as flour and ready-to-eat breakfast cereals because of its instability. Ascorbic acid destruction is known to be affected by pH, moisture content, oxygen and

trace metals, but a detailed knowledge of the physio-chemical properties governing the loss and mechanism of ascorbic acid deterioration during storage of dehydrated foods is negligible.

The purpose of this research was to investigate the decomposition of ascorbic acid in dehydrated foods as a function of selected product and storage variables. A model food system was chosen to simulate the composition of a ready-to-eat breakfast cereal. The goals were to obtain kinetic data describing the degradation of ascorbic acid as influenced by water activity, temperature, moisture content, oxygen availability and interaction with other nutrients, including trace metals. It was anticipated that thermodynamic activation parameters could be calculated and thus provide information regarding the degradative process of ascorbic acid.

LITERATURE REVIEW

Sorption Phenomena

The common goal of food preservation is the extension of the product shelf-life to permit convenient storage and distribution. The most important consideration in food preservation is controlling the growth of microorganisms in the product. One method of preservation is reduction of the availability of water to the microorganisms by dehydration. However, other physical and chemical reactions may still occur in dehydrated foods which adversely affect food quality. An understanding of the relationship between the state of water and these processes will contribute to an improved quality of the product following storage.

Water Activity and the Sorption Isotherm

Water is prevalent throughout nature, as would be anticipated, and is very important to the structure, texture and chemical interactions of foods. The thermodynamic state of water in a food system is given by the chemical potential (Labuza, 1976)

$$\mu = \mu_o + RT \ln a_w \qquad (1)$$

where μ_o = standard state chemical potential for water, R = gas constant, T = absolute temperature and a_w = thermodynamic activity of water. The activity of water may be defined as

$$a_w = \frac{P}{P_o} = \frac{\%\ ERH}{100} \qquad (2)$$

where: P_o = vapor pressure of pure water, P = vapor pressure of water in the food product and % ERH = per cent relative humidity at which the system neither gains nor loses water.

The removal of water from a food system and its relationship to the water activity are depicted in Figure 1. During the initial stages of water removal, the water activity of the product remains close to unity (Figure 1A). On continued removal of water, the product undergoes a relatively rapid decrease in water activity per unit loss of moisture content (g H_2O/g solid). This phenomenon is typical of food systems and the curve is referred to as the moisture sorption isotherm.

Equation 2 would predict the ratio of the moisture content to water activity to be independent of the determination of the sorption isotherm. That is, whether the equilibrium moisture is obtained via the removal of water (desorption) or the addition of water (adsorption). Close examination of the sorption isotherm

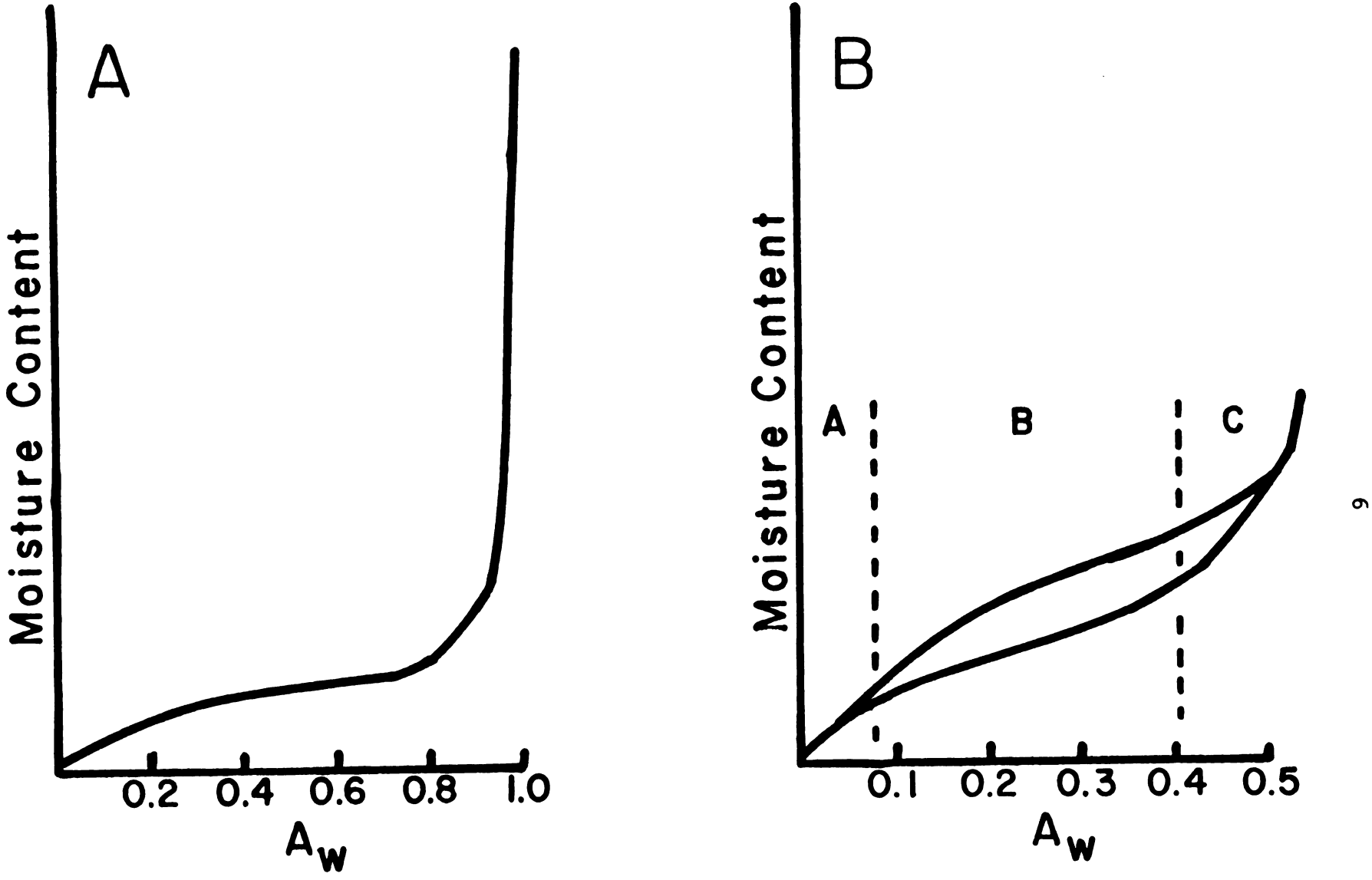


Figure 1. General moisture sorption isotherm for foods (A) and effect of hystersis on moisture sorption isotherm (B).

determined by these two methods reveals a phenomenon referred to as hystersis (Figure 1B). This effect shows that dehydrated systems which reach their equilibrium moisture content by desorption of moisture contain a greater moisture content at the same water activity than systems which reach moisture equilibrium via the adsorption of moisture. Brunauer (1945) identified five general types of adsorption isotherms, designated by Roman numerals. The sorption isotherm shown in Figure 1 is referred to as type II. The type II isotherm is common in the case of physical adsorption and is believed to correspond to multilayer formation. The type II isotherm is typical of most dehydrated food products (Labuza, 1968).

In an effort to make the moisture sorption isotherm more manageable, Labuza (1968) divided it into three regions to define the state of water in a dehydrated food system (Figure 1B). Region A corresponds to the adsorption of a monomolecular layer of water which is tightly bound and unavailable for reaction (Labuza, 1976). The water molecules in this region are considered as bound to carboxyl and amino groups (Rockland, 1969) and reactions which depend on solvation by water are not measured (Fennema, 1976). Region B is designated the multilayer region and corresponds to the adsorption of additional layers of water molecules in which the water is more loosely bound, probably via hydrogen bonding to hydroxyl

and amide groups. Region C is referred to as the capillary condensation area and describes water which exists in the capillaries of the dehydrated food. This water is relatively free to act as a solvent. The reason it has a value of less than 1.0 is that capillary forces and soluble constituents cause a lowering of the vapor pressure of this water in accord with Raoult's law. These regions of the moisture sorption isotherm are general and are not defined at precise water activities. Considerable overlap between regions exists for various products.

Theoretical Treatments of the Sorption Isotherm

Various theories for the adsorption of nonpolar gases on homogeneous and heterogeneous surfaces have recently been reviewed by Adamson (1976). There are four theoretical approaches which describe the sorption isotherm to varying degrees of success: (1) kinetic, (2) potential, (3) equation of state and (4) capillary condensation. In addition, several empirical equations have been proposed.

The kinetic approach proposed by Langmuir (1918) for monolayer adsorption of gases was extended by Brunauer, Emmett and Teller (1938) to apply to multilayer adsorption. Their derivation led to the BET equation:

$$\frac{a_w}{(1-a_w)m} = \frac{1}{m_o c} + \frac{(c-1)a_w}{m_o c} \qquad (3)$$

where a_w = water activity, m = moisture content at a_w, m_o = monomolecular moisture content and c = constant related to the bonding energy. Three basic assumptions were required for the derivation of the BET equation:

(1) Sorption occurs only on specific sites.

(2) Heat of sorption is constant for the first layer, and equals the total heat of vaporization plus a constant.

(3) Heat of sorption equals the heat of vaporization for layers above the monolayer.

The major limitation of the BET equation is the restricted water activity range (0.0 - 0.5) over which it is applicable. The popularity of the BET equation is derived from its ability to predict the monomolecular moisture content and a hypothesis by Salwin (1959, 1962, 1963) that the monomolecular moisture content should correspond to the water activity for maximum storage stability of a product.

The BET monomolecular moisture content may also be used to calculate the water surface area, S_o:

$$S_o = m_o \cdot \frac{N}{H_2O} \cdot A \qquad (4)$$

where N = Avagadro's number, H_2O = molecular weight of water and A = area of a water molecule, 10.6 X $10^{-20}m^2$ (Labuza, 1968). The water surface area value is generally several orders of magnitude greater than that determined by nitrogen surface areas (Berlin et al., 1966) or gas permeability measurements (Fox et al., 1963). This difference in areas is rationalized by the ability of water to plasticize long chain polymers exposing interior sites for adsorption and that the water molecule is smaller than nitrogen and thus is capable of entering smaller pores.

The second approach to describing the sorption isotherm is due to Polanyi (1928) and based on the force field potential caused by the surface of the solid material. The underlying assumption was that the total work necessary to adsorb a gas molecule must equal the work necessary to overcome the field strength as the molecule approaches the surface of the solid and the work of condensation. Frenkel (1946) extended the theory by making the reasonable assumption that the interaction between the solid and the adsorbed gas molecule is principally of the dispersion type and the potential should decrease with the inverse cube of the distance. This led to an equation for the moisture content, m:

$$m = -\alpha + \beta \ (\ln a_w) \qquad (5)$$

where α and β are constants. Although this method successfully predicts an isotherm once an initial curve has been determined, the primary disadvantage is its inability to predict the monomolecular moisture content.

An alternative method to the formulation of the force field potential for determining the sorption isotherm is that proposed by de Boer and Zwikker (1929). The adsorption of gaseous molecules was explained by assuming the polar adsorbent induces dipoles in the first layer of adsorbed gas molecules which in turn induces dipoles in the second layer, etc. This treatment suggests the presence of strong orientational effects in the multilayers; however, it suffers from the same shortcomings as the dispersion theory.

The third approach is that of Harkins and Jura (1944) which utilizes a two-dimensional equation of state for the film pressure. The Harkins-Jura equation takes the form:

$$\ln a_w = B - A/v^2 \qquad (6)$$

where A and B are constants and v = amount of adsorbed gas.

The capillary condensation of the vapor, utilizing the Kelvin equation (Zsigmondy, 1911), is the only approach that predicts the effect of hystersis. The

adsorption curve is dependent on the radius of curvature of the capillary r, thus the capillary filling would be described by

$$a_w = \exp\left(-\frac{2\gamma}{r}\cos\theta V_o/RT\right) \quad (7)$$

whereas on desorption, the retreat of the meniscus of curvature was 2r and

$$a_w = \exp\left(-\frac{\gamma}{r}\cos\theta V_o/RT\right) \quad (8)$$

In equation (7) and (8), γ = liquid surface tension, θ = the contact angle of liquid in pores and V = molar volume. Although an appealing hypothesis, Lubuza (1968) has reviewed the problems associated with applying the Kelvin equation to food systems:

(1) The equation is not applicable to situations where the pores may be of molecular dimensions.

(2) The assumption of zero contact angle may lead to errors.

(3) Surface tension of liquid in pores may vary depending on the food matrix.

(4) Difficult to measure effects of nonideality of vapor pressure lowering due to solutes.

Adamson (1976) and Labuza (1968) have noted that the agreement of a theoretical equation with the

experimental data is necessary but insufficient proof of its premises. The theoretical equation may well satisfy the objectives of the user, or an empirical approach may be equally suitable. Three empirical equations should be mentioned.

Henderson (1952) described the sorption isotherm empirically by:

$$1 - a_w = e^{-cv^n} \tag{9}$$

where c and n are constants and v = the amount of adsorbed gas. Rockland (1957) has reported some success with food systems for equation 9, but found other cases where a composite isotherm of three straight lines described the experimental data better. This led Rockland to the proposal of "local isotherms," which correspond very closely with the three regions of the sorption isotherm identified by Labuza (1968).

More recently, Caurie (1970) proposed that the "natural" water sorption isotherm could be represented mathematically by:

$$\ln C = \ln C_o - a_w \ln r \tag{10}$$

where $C = \frac{100 - \%H_2O}{\%H_2O}$ at any a_w, C_o = C at zero a_w and r = magnitude of the gradient. This expression was further developed to yield

$$\ln C = \frac{1}{0.045M_o} - a_w \ln r \qquad (11)$$

where M_o is the optimum safe moisture content for food stability. Subsequently, Caurie suggested M to be numerically equal to the water binding energy, Q, in kcal and that M_c is equivalent to the sorptive energy, Q_c (Caurie, 1971a). The value obtained for M_o is somewhat greater than the BET monomolecular moisture content. This has led Caurie (1971b) to postulate that the sorption of water vapor in dehydrated foods is a penetration of the sorbate throughout the sorbent rather than the surface phenomenon assumed in the BET theory.

Physical State of Water

The description of the physical state of water in dehydrated food systems is still incomplete. Much of the problem arises from an inadequate understanding of pure water and solute-water interactions. The physiochemical factors of water important to the consideration of dehydrated foods have been summarized by Labuza (1976):

(1) Solvent properties--the ability of water to dissolve molecules, to participate as a reactant, act as a diffusion medium for other molecules, and to concentrate molecules in the aqueous phase.

(2) Solute effects--the depression of water activity by increasing solute concentration in accordance with Raoult's law.

(3) Structural effects--the three-dimensional structural effects (size and shape of the capillaries) in dehydrated food systems on lowering the water activity.

(4) Solid surface interactions--the strong binding of water molecules to specific sites on the protein or other macromolecules of the food systems.

The state of water in dehydrated systems has been studied by a variety of methods, such as differential thermal analysis (DTA), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), electron spin resonance (ESR) and phosphorescence. Results of each technique suggest that water exists in at least two states in dehydrated food systems. This has led to the concept of bound water, which shows physical properties different from free water (Kuprianoff, 1958).

One criterion for bound water has been to measure the nonfreezable water which remains on cooling a sample to sub-zero temperatures. The proton resonance signal from flour samples adjusted to different moisture contents was monitored as the temperature decreased by Toledo et al. (1968). A dramatic decrease in signal

intensity occurred between 30 and 20°F which they attributed to the freezing of water. Below this temperature, the signal for different samples was similar and the intensity decreased slowly with continued cooling. This was interpreted as reflecting the presence of unfrozen or bound water. However, Toledo et al. (1968) were unable to determine the temperature at which the freezing process was complete.

Differential thermal analysis revealed the temperature (within narrow limits) at which a phase change from ice to liquid occurs (Duckworth, 1972). Using samples of carefully adjusted moisture contents, they determined the nonfreezable water to correspond to the maximum moisture content at which there is no evidence of a phase transition signal. Furthermore, the question of the completeness of the freezing process could be easily determined and in some cases erroneously high values for nonfreezable water determined by other methods have been reported.

Of more practical importance is the binding of water at ambient temperature since water binding may be a temperature dependent phenomenon. Shanbhag et al. (1970) have reported an NMR method for determination of "bound water capacity" (BWC). Careful adjustment and determination of moisture content were critical, and as later reported by Mousseri et al. (1974), up to five days for samples at the BWC moisture content were required to achieve equilibrium.

Rockland (1969) has presented qualitative NMR, ESR and phosphorescence results for water behavior in gelatin. The NMR evidence showed a decreased band width for water protons with increasing moisture content. ESR signals from irradiation induced free radicals were found to decay more quickly with increasing moisture content. Phosphorescent decay times in the irradiated moisture-adjusted gelatin samples were also found to decrease with increasing moisture content, reflecting the stability of the highly reactive triplet state. Rockland (1969) further found that the physical behavior of the gelatin sample as measured by these techniques correlated very well with his proposed "local isotherm" concept (Rockland, 1957).

The ability of water in dehydrated foods to solubilize chemical species and to allow diffusion of these species was reported by Duckworth and Smith (1963). They showed that migration of glucose, calcium and sulfate occurred at moisture contents corresponding to monolayer coverage. Thus, the solvent properties of water cannot be neglected in dehydrated systems.

Thermodynamic Activation Parameters

One main task of chemistry is to interpret and predict reactivity. No general agreement prevails as to the physiochemical quantity which should serve as a measure of reactivity. Frequently, reactivity is

expressed by either the equilibrium constant, K, or the rate constant, k, both of which are strongly temperature dependent. An alternative approach is to separate the reactivity into temperature dependent (enthalpic) and temperature independent (entropic) parts. In kinetics, classical Arrhenius theory or the absolute rate theory yield an activation enthalpy ($\Delta H^{\ddagger}$) and an activation entropy ($\Delta S^{\ddagger}$). The activation parameters are far less sensitive to temperature, and within a narrow temperature range may be taken as invariant (Schaleger and Long, 1963).

These parameters, entropy of activation, enthalpy of activation and free energy of activation are calculated from an equation based on the theory of absolute reaction rates (Eyring, 1935):

$$k = \frac{k_b T}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta H^{\ddagger}/RT} \qquad (12)$$

where k is the "best" value for the reaction rate constant at a given temperature (T) and:

$k_b = 1.38 \times 10^{-16}$ erg $^{o}K^{-1}$ (Boltzman's constant)

$h = 6.63 \times 10^{-27}$ erg sec (Plank's constant)

$R = 1.987$ cal/^{o}K-mole (gas constant)

$\Delta H^{\ddagger} = E_a - RT$ (for solutions).

The free energy of activation may then be calculated by

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \qquad (13)$$

Enthalpy-entropy relationships are of two types, (1) structural relationships for similar compounds undergoing the same reaction and (2) effects of moderate solvent changes on a specific reaction. Frequently, for both cases, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ tend to change in a compensating manner and

$$\Delta H^{\ddagger} = \beta \Delta S^{\ddagger} + \Delta H^{\ddagger}_{o} \qquad (14)$$

where β is in ^{o}K and $\Delta H^{\ddagger}_{o}$ is the intercept at $\Delta S^{\ddagger} = 0$. This function is termed the compensation law or isokinetic relationship (Leffler, 1955) and if obeyed results in only small changes in $\Delta G^{\ddagger}$, the free energy of activation.

The significance of the isokinetic temperature may be viewed as a constant, characteristic of the reaction series and dependent on the experimental temperature interval. Further, the value for the isokinetic temperature may be either positive or negative and occur either above or below the experimental temperature range. Thus the significance is not the value of the isokinetic temperature, but whether or not the isokinetic relationship holds (Exner, 1973).

Blackadder and Hinchelwood (1958a,b) distinguished three types of reaction series based on the activation parameters: (1) series with constant entropy, reactivity enthalpy controlled, interpretation based on electronic effects; (2) series with constant enthalpy, reactivity

entropy controlled, interpretation based on steric and/or solvent effects; (3) changes in $\Delta H^{\ddagger}$ are paralleled by changes in $\Delta S^{\ddagger}$ such that the resulting effect on reactivity is less than if controlled by one parameter, interpretation based on steric and solvent effects. More recently, an anti-compensation type series has been detected (Exner, 1949, 1972).

The existence of an enthalpy-entropy relationship possesses important mechanistic implications. The isokinetic relationship is expected to be followed only by a reaction series in which structural or solvent changes do not result in a change in mechanism. Large changes in enthalpy or entropy of activation have often been considered indicative of changes in mechanism, but a more reliable criterion of a change in mechanism is the constancy of the free energy of activation (Leffler, 1955).

Reactions in Dehydrated Foods

Labuza (1968) summarized the general behavior of the major classes of reactions in dehydrated foods as a function of water activity as shown by the stability map (Figure 2). This provides a guide to the types of reactions that are dominant at a particular water activity and some discussion as to the role of water is warranted.

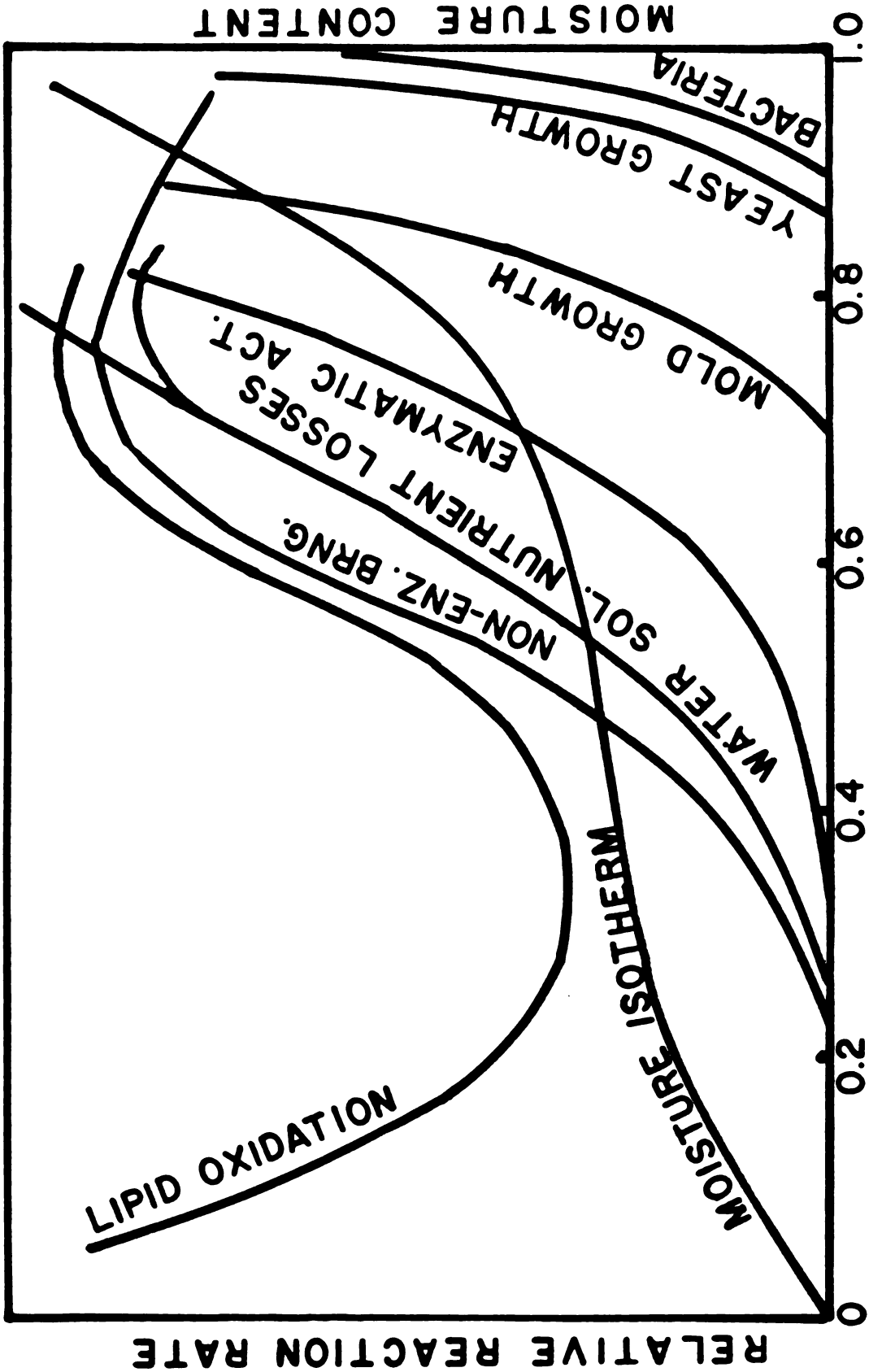


Figure 2. Stability profile of dehydrated foods (from Labuza, 1976).

Microbiological

The initial concern in dehydration is the elimination of microbial growth. Scott (1957), in a comprehensive review, suggested that water activity, rather than moisture content, determined the availability of water for microbial growth. He reported water activity limits for bacteria, molds and xerophilic fungi to be 0.91, 0.80 and 0.65, respectively. Furthermore, environmental factors such as pH, oxygen, pressure, temperature and nutritional adequacy may necessitate a higher minimum water activity for microbial growth.

Recent research has identified additional effects of water activity on microorganisms (Karel, 1973; Labuza, 1972):

(1) Microorganisms are most sensitive to heat, light, and chemicals at high water activities. However, an insensitivity develops upon moisture removal.

(2) Toxin production often occurs at water activities lower than the minimum required for microbial growth.

(3) Microbial growth is sensitive to the hystersis effect. The minimum water activity for microbial growth may be a higher water activity for adsorption than desorption.

In a review of xerophilic fungi, Pitt (1976) has noted the importance of the predominant solute present in the medium at low a_w, and the different responses of the organism with different solutes.

Enzymatic

Enzymatic reactions that can occur in low moisture foods which have not been heat treated are well known. Acker (1962, 1969a,b) has concluded that enzymatic reactions usually occur when fluid water is available, that is, above the monomolecular moisture content. The liquid water is required for solubilization and diffusion or transport of the substrate to the enzyme. Acker and Weise (1972a,b) have noted lipid splitting enzymatic activity at water activities below the BET monomolecular moisture content (as low as 10% RH) if the triglycerides are in a fluid state and can be mobilized. Duden (1971) reported hydrolytic activity in freeze-dried vegetables at relative humidities of 1% concluding that the transport medium for the substrate was the vapor phase.

In a recent study of the thermal inactivation of wheat ribonuclease at varying low moisture levels, Multon and Guilbot (1976) reported a two-stage process. The first stage, which was more rapid than the second, was interpreted as a water catalyzed step in which a reorganization of bond energies between water and polar sites occurred. The second stage reflected the thermal effect.

Chemical

Nonenzymatic browning and lipid oxidation are two classes of chemical reactions in dehydrated foods which illustrate two distinctly different roles for water.

Comprehensive reviews of nonenzymatic browning and a general discussion of carbonyl-amine reactions can be found in the literature (Reynolds, 1962, 1965, 1969 and Feeney et al., 1976). The rate of browning has been found to reach a maximum at a water activity between 0.3 to 0.7 depending on the food matrix, the most common range being 0.5 - 0.7. Two explanations have been proposed for this maximum in browning rate. Loncin et al. (1968) have suggested that the water produced in the reaction increases the moisture content resulting in product inhibition. However, Labuza et al. (1970) believe the maximum browning rate occurs at the point of greatest reactant concentration and that the decreased browning rate on increasing water activity is simply due to a dilution of the reactants.

The kinetics and mechanisms for lipid oxidation have been reviewed by Labuza (1971) and Schultz et al. (1962). Early studies showed improved stability toward oxidation with increasing moisture content (Stevens et al., 1948; Matz et al., 1955; Martin, 1958), however, the interaction of water in lipid oxidation was unclear. Investigations by Maloney et al. (1966), Labuza et al.

(1966) and Karel et al. (1967) have suggested several functions of water in retarding lipid oxidation:

(1) The water molecules may hydrogen bond to hydroperoxides at the lipid-water interface, thus, slowing initiation. This antioxidant effect increases until the interface becomes saturated with water.

(2) Water may hydrate with trace metals reducing their catalytic activity.

(3) Water may react to form insoluble metal hydroxides, thus removing the metal catalysts from the reaction phase.

(4) The lifetime of free radicals decreases as the water content is increased.

The reaction profile for lipid oxidation is found to increase as the water activity approaches that of the intermediate moisture range. Labuza et al. (1970) have attributed this to the greater solubility and mobility of the trace metals, thus overcoming the antioxidant properties of water.

Ascorbic Acid

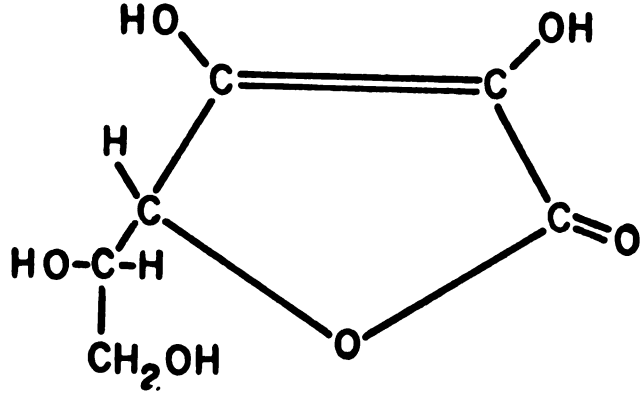
Zilva and co-workers (1923a,b; 1924a,b; 1925; 1927) isolated and reported the general properties of a compound with antiscorbutic activity in the mid-1920s.

The substance was found to be labile and exhibit strong oxidizing power. Freshly oxidized solutions of this antiscorbutic compound also possessed physiological activity. Tillmans et al. (1932a,b,c) resolved the anomaly when they concluded that both the oxidized and reduced forms possessed antiscorbutic activity, and that the forms were reversible. Szent-Gyorgi (1928) isolated an optically active, acidic reducing sugar with the empirical formula $C_6H_8O_6$. The subsequent demonstration that this isolated sugar with the antiscorbutic factor of Zilva was L-ascorbic acid was confirmed with its synthesis.

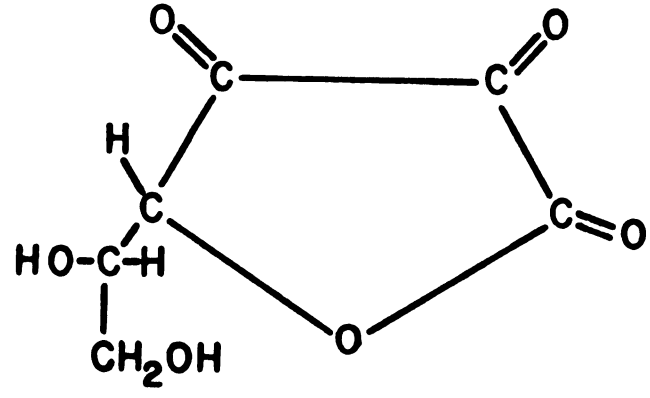
Structure and Properties

L-Ascorbic acid (Figure 3) is a white crystalline solid melting at 192°C. It is an optically active ($[\alpha]_o$ = +23° in water) lactone ring system and shows selective absorption of ultraviolet light at 245 nm to 265 nm as the pH is increased. The hydroxyl groups on carbons 2 and 3 are enolizable due to the conjugated system. L-Ascorbic acid possesses two acidic hydrogens with pKa's in water of 4.25 and 11.79 (Figure 4). The solubility of L-ascorbic acid is 32g/100 ml water (Bauernfeind and Pinkert, 1972).

Dehydroascorbic acid (Figure 3), formed by the removal of two equivalents of hydrogen from L-ascorbic acid, retains the basic ring structure although it no longer contains a conjugated system. The acidity associated with L-ascorbic acid is lost on oxidation to



L-ascorbic acid



L-dehydroascorbic acid

Figure 3. Molecular structure of biologically active forms of ascorbic acid.

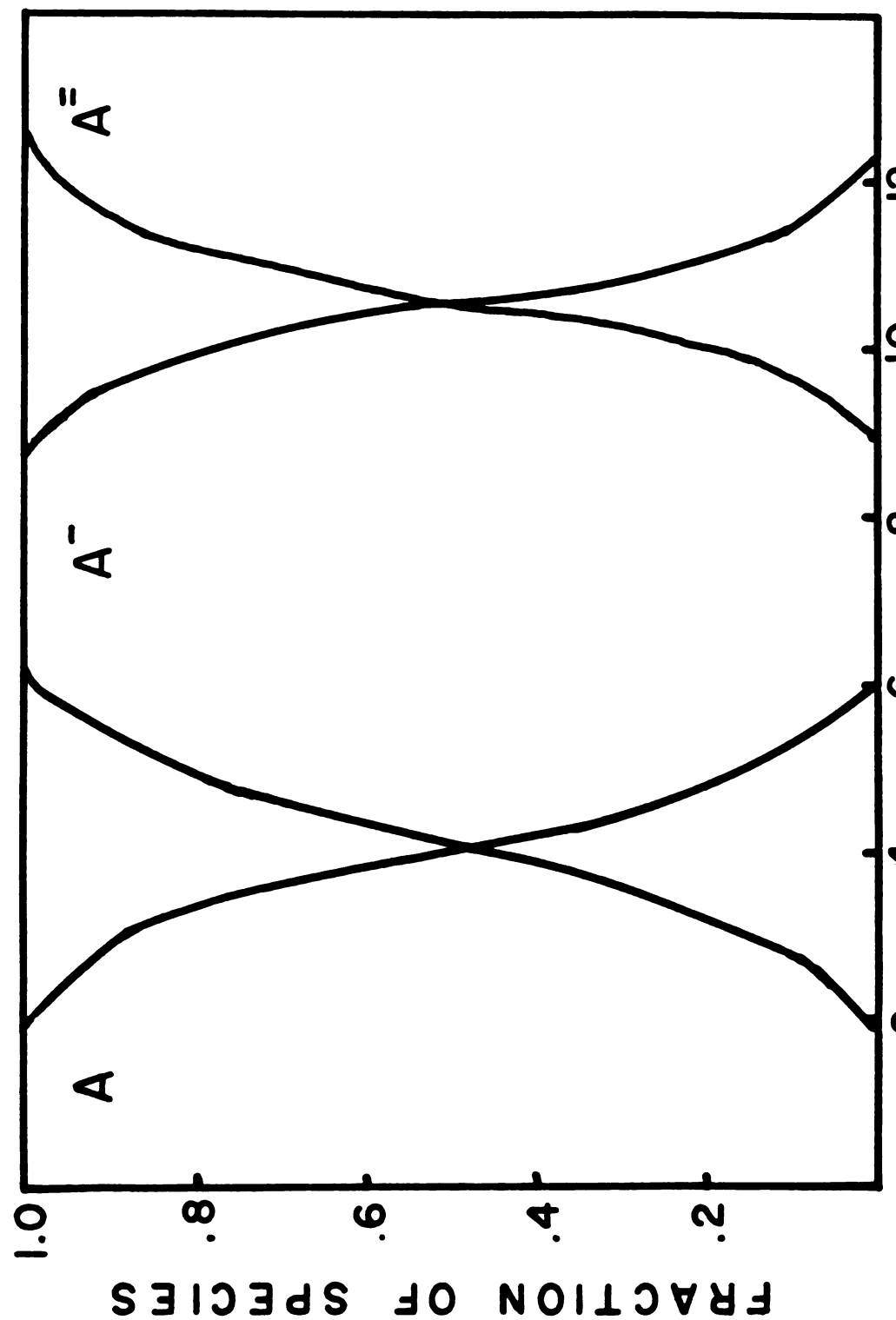


Figure 4. Fraction of ascorbic acid species in solution as a function of pH.

dehydroascorbic acid. The planar structure of L-ascorbic acid was confirmed by x-ray crystallographic analysis (Cox et al., 1932).

Reactivity in Solution

Ascorbic acid is stable in the dry crystalline state. In solution, it reacts readily giving a variety of products depending upon the reaction conditions. Oxygen, pH, metal ion catalysis and temperature are reported as the principle factors determining the decomposition of ascorbic acid. Although extensively studied by numerous investigators, uncertainty still exists as to the mechanism of degradation. A discussion of the importance of these factors is most easily presented in terms of the presence or absence of oxygen.

The anaerobic decomposition of ascorbic acid in aqueous solutions has been reported to be pH dependent. Finholt et al. (1963) found that the maximum rate of anaerobic degradation of ascorbic acid occurred at pH 4.0 at 96°C. Huelin et al. (1971) reported an increase in destruction rate as a function of pH at 50 and 100°C, reaching a maximum at pH 2.3, decreasing to a minimum at pH 4 and increasing again to pH 6, the highest pH of the study. The discrepancy between these two studies is difficult to rationalize; however, Finholt et al. (1963) did find a catalytic effect due to the buffer type and concentration used to maintain the pH. No primary salt

effect on the anaerobic degradation rate was found. Finholt et al. (1963) suggested that the rate maximum at pH 4 was due to the complexation of ascorbic acid and the monoionic species of ascorbic acid as a reactive intermediate.

Various reaction products from ascorbic acid degradation have been reported in the literature. Huelin et al. (1971) measured carbon dioxide and furfural formation during the anaerobic degradation of ascorbic acid. One mole of CO_2 was formed per mole of ascorbic acid over the pH range 2.2-6.0, but the yield of furfural decreased with increasing pH. Investigations of Coggiola (1963) identified, 2,5-dihydro-2-furoic acid as an end product of the anaerobic degradation of ascorbic acid and Kurata and Sakurai (1967) have reported 3-deoxy-L-pentosone as a probable intermediate breakdown product.

The anaerobic catalysis of ascorbic acid by cupric ion at pH 2.1 - 3.5 has been reported by Shtamm et al. (1974). The reaction was second-order with respect to copper (II) concentration, first-order in ascorbic acid and inversely proportional to hydrogen-ion concentration. Additionally, Huelin (1953) and Huelin et al. (1971) have reported the catalytic properties of fructose, sucrose, fructose-1-phosphate and fructose-1,6-diphosphate in the anaerobic decomposition of ascorbic acid. The fructofuranose form of fructose was found to be the

catalytic agent. Sucrose catalysis was attributed to its partial hydrolysis in the reaction medium.

The aerobic oxidation of ascorbic acid has received intensive investigation. The reaction rate is known to depend on oxygen, pH, metal catalyst, buffer and temperature. The rate of the spontaneous oxidation of ascorbic acid increases with pH, showing maxima at pH 5.0 and 11.5 (Csuros and Petro, 1955). This pH dependence has been confirmed by Miller and Joslyn (1949) and Khan and Martell (1967) for the pH range 2 - 6. Weissberger et al. (1943) found no rate maxima, although the same pH dependence of the reaction from pH 4.7 - 9.2 was observed. The monoionic and diionic species of ascorbic acid have been interpreted as being more reactive in the presence of oxygen than the neutral species. The reported order of ascorbic acid oxidation with respect to hydrogen-ion varies, -0.5, -1.0 and -2.0, Miller and Joslyn (1949), Khan and Martell (1967) and Dekker and Dickinson (1940), respectively.

The oxygen dependence of ascorbic acid oxidation has been reported to be first-order above 0.2 atm oxygen (Khan and Martell, 1967). Recently, Shtamm and Skurlator (1974a) have reported a half-order dependence on oxygen, which has been supported by the results of Jameson and Blackburn (1975; 1976a,b). Jameson and Blackburn also

suggest that the oxygen dependence data of Khan and Martell (1967) more adequately fits half-order kinetics.

Reports of the catalytic properties of transition metals, notably copper and iron, on the oxidation of ascorbic acid abound in the literature. In many cases the rates are found to increase linearly with electrolyte concentration. Existing theories do not account for the rate enhancement exhibited by ion-molecule reactions upon the addition of neutral electrolytes.

It is generally assumed that all rate changes resulting from the presence of neutral electrolytes may be described by the Bronsted equation (Bronsted, 1922):

$$k = k_o \frac{\gamma_A \gamma_B}{\gamma_{\ddagger}} \qquad (15)$$

where γ_A, γ_B and $\gamma_{\ddagger}$ are the activity coefficients for the reactants and the transition state, respectively. Once the applicability of this equation was accepted, the major problem in developing a theory of salt effects was determining the relationship of the activity coefficients of the reactants to the added electrolyte concentration. The Debye-Huckel theory provided the first relationship of this type in the form of the Debye-Huckel limiting law,

$$\text{Log } \gamma = -QZ^2 \sqrt{I}\,, \qquad (16)$$

where Q is a constant for a given solvent, Z is the valency of the ion and I is the ionic strength. The

limiting law is valid only below concentrations of 0.01M. Following the procedure of Scatchard (1922), equation (16) may be substituted into the rate equation (15) giving

$$\text{Log } k = \text{Log } k_o + 2QZ_AZ_B \sqrt{I} \ . \qquad (17)$$

For aqueous solutions at 25°C, the value of Q is approximately 0.51 (Laidler, 1965). This equation has been shown to describe salt effects for a large number of ion-ion reactions (Figure 5), even though the applicability of this equation is exceeded. According to equation (17) a plot of log k vs. $\sqrt{I}$ will give a straight line of slope $1.02Z_AA_B$. For the case where one reactant is a neutral molecule, the product Z_AZ_B in equation (17) is zero and no salt effect would be predicted. The fact that salt effects do exist for ion-molecule reactions has often been explained by assuming that the solutions were not dilute enough to allow application of the Debye-Huckel limiting law. To surmount this shortcoming several relationships using higher powers of I and purely emperical parameters have been developed.

Weissberger and LuValle (1944) reported that only the monoionic ascorbic acid moiety was susceptible to Cu(II) catalysis. More recently, studies by Khan and Martell (1967) have shown the oxidation of ascorbic acid to be linearly dependent on the concentration of Cu(II) and Fe(III) ions. Ogata et al. (1968) found that the

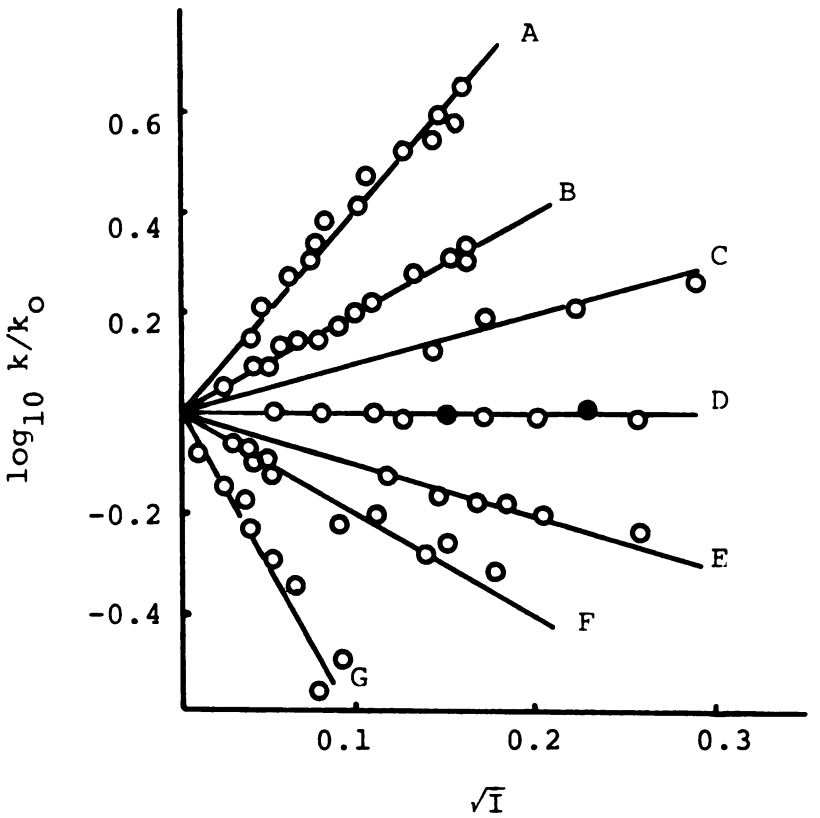


Figure 5. Plots $\log_{10} k/k_o$ vs. $\sqrt{I}$ for ionic reactions of various types. The lines are drawn with slopes equal to $Z_A Z_B$ (Laidler, 1965).

A	$Co(NH_3)_5Br^{2+} + Hg^{2+}$	$Z_A Z_B = 4$
B	$S_2O_8^{2-} + I^-$	$Z_A Z_B = 2$
C	$Co(OC_2H_5)N{:}NO_2^- + OH^-$	$Z_A Z_B = 1$
D	$(Cr(UREA)_6)^{3+} + H_2O$	$Z_A Z_B = 0$
	$CH_3COOC_2H_5 + OH^-$	$Z_A Z_B = 0$
E	$H^+ + Br^- + H_2O_2$	$Z_A Z_B = -1$
F	$Co(NH_3)_5Br^{2+} + OH^-$	$Z_A Z_B = -2$
G	$Fe^{2+} + Co(C_2O_4)_3^{3-}$	$Z_A Z_B = -6$

catalytic ability of Cu(II) ions was dependent on the anion of the salt in the order $CuCl_2 > Cu(NO_3) \sim CuSO_4$. Shtamm and co-workers (1974a,c) and Jameson and Blackburn (1976a,b) have also reported the catalytic properties of Cu(II) ions in ascorbic acid degradation. Pekkarinan (1974) has reported evidence supporting Fe(III) catalysis of ascorbic acid. Other catalytic agents which have been reported include metal chelates, vanadyl and uranyl ions, and iron (III) chelates of aminopolycarboxlyic acids (Khan and Martell, 1968a,b, 1969). The mechanistic interpretation of metal catalyzed ascorbic acid degradation is varied, ranging from an ascorbate-metal-oxygen complex involving a one electron transfer to oxygen (Khan and Martell, 1967) to the formation of a metal-metal dinuclear-ascorbate-oxygen complex with a two electron transfer to oxygen (Jameson and Blackburn, 1975).

Stability in Food Products

The availability of kinetic data pertaining to the destruction of ascorbic acid in food products is much more limited than for destruction in model solutions. Singh et al. (1976) studied the disappearance of ascorbic acid in a liquid infant formula as a function of initial oxygen concentration and light intensity at 7.2°C. The rate of ascorbic acid loss was found to be dependent on the presence of oxygen and obeyed overall second-order kinetics. If the oxygen level was maintained, pseudo

first-order loss of ascorbic acid was observed. The rate of ascorbic acid loss also increased linearly with light intensity up to 1600 lux. A companion study by Mack et al. (1976) showed a first-order relationship for the uptake of oxygen in the same infant formula.

Stability of ascorbic acid in canned tomato juice was studied by Lee et al. (1977) as a function of pH, Cu(II) and temperature. First-order reaction kinetics were observed in the anaerobic system. The reported activation energy was 3.3 kcal/mole, which was less than that found by Pope (1972) for a similar tomato juice system. Lee et al. (1977) reported that the degradation rate of ascorbic acid reached a maximum at pH 4.06, and was linearly dependent on the concentration of copper at each pH.

Karel and Nickerson (1964) reported the destruction rates of ascorbic acid in dehydrated orange juice crystals increased with increasing a_w, but no dependence on the storage atmosphere (air or vacuum) was observed. The authors concluded that there was no value of a_w below which destruction of ascorbic acid ceased.

Vojnovich and Pfeifer (1970) studied the stability of reduced ascorbic acid in a corn-soy-milk (CSM) blend at three moisture contents. Lee and Labuza (1975) also studied the rate of ascorbic acid destruction in an intermediate moisture (0.32-0.84 a_w) glycerol, corn oil

and microcrystalline cellulose model system. Both of these studies showed increased ascorbic acid degradation rates with increasing a_w. Lee and Labuza (1975) also examined the effects of moisture sorption hystersis on ascorbic acid stability and found greater degradation of ascorbic acid on the desorption leg of the isotherm. However, activation energies calculated for ascorbic acid degradation were the same (~20 kcal/mole) for both legs of the isotherm.

Recently, Waletzko and Labuza (1976) studied ascorbic acid stability in an intermediate moisture food system (a_w=0.85) in an accelerated shelf-life test. They reported that ascorbic acid degraded rapidly by first-order kinetics whether packed in an air or N_2/H_2 atmosphere. Ascorbic acid was more stable, however, in the product packaged in the N_2/H_2 atmosphere.

The retention of ascorbic acid in tomato juice crystals as a function of a_w was reported by Riemer (1977). First-order kinetics were obeyed for the loss of reduced ascorbic acid and dehydroascorbic acid. Activation energies ranging from 16.2 - 24.6 kcal/mole were calculated. As with the study of Karel and Nickerson (1964) no effect of oxygen was observed. Ascorbic acid stability in products with high acidity may reflect a pH stabilizing effect.

The stability of ascorbic acid in foods is generally improved at storage temperatures below 0°C. However, Grant and Alburn (1965) reported the rate of ascorbic acid oxidation to be greater at -11°C than at 1°C in 0.02M acetate buffer of pH 5.0 and 5.5. Freeze concentration or the increase in concentration of the solutes in the frozen state over the unfrozen state has been presented as the most probable rationale for this phenomenon (Pincock and Kiovsy, 1966). The detailed study by Thompson and Fennema (1971) on the effect of freezing on ascorbic acid stability has shown the discrepancy to be a function of the solute concentration which governs the solubility of oxygen. Thus, the higher the solute concentration, which would be the normal pattern on freezing of foods, the less dissolved oxygen and the slower the oxidation rate of ascorbic acid at subfreezing temperatures.

Predictions of Product Storage Stability

The prediction of the storage stability of food products has received increased attention recently in view of nutritional labelling, cost and time requirements for conventional stability studies. Numerous mathematical techniques have been proposed for the prediction of product quality and deterioration rates of specific reaction types (e.g., nonenzymatic browning, lipid oxidation).

The most commonly used model is that which depends on a detailed understanding of the kinetics of the reaction being modeled. This requirement necessitates the experimental determination of those parameters affecting the reaction kinetics.

Kwolek and Bookwalter (1971) developed a mathematical model for storage stability based on time-temperature data. The Arrhenius equation was satisfactory in predicting flavor and peroxide values.

Mathematical models proposed for predicting the stability of space foods in semi-permeable packages, where lipid oxidation was the only deteriorative mechanism of concern, were sufficiently accurate to aid in package design (Karel and Labuza, 1969; Simon et al., 1971).

Mizrahi et al. (1970a,b) and Karel et al. (1971) developed a computer model for the prediction of the storage life of dehydrated cabbage as a function of non-enzymatic browning. The feasibility of using accelerated storage tests for determining the necessary kinetic parameters have been evaluated. Conditions of high storage temperature and high moisture content provided experimental data in a short time that could be used to predict the degree of browning at ambient storage conditions. Correlation analysis between accelerated shelf life test data and long-term storage studies indicated that accelerated storage tests could be utilized in the development of prediction equations.

The storage stability of potato chips that undergo loss of quality resulting from moisture adsorption and oxidative rancidity was studied by Quast et al. (1972) and Quast and Karel (1973). A mixed model based on reaction kinetics and empirical data fitting was used to describe the deterioration process. The agreement between the actual storage tests and the computer prediction was good, although the procedure was less accurate for accelerated test studies.

Wanniger (1972) proposed a mathematical model for prediction of ascorbic acid storage stability using the equation $\ln k = -E_a/RT + a \ln H_2O + b$, where a and b are constants and the other symbols possess their normal meaning. Utilizing the data of Vojnovich and Pfeifer (1970), he reported excellent correlation between the experimental and predicted results.

A basic computer model for simulation of nutrient stability in semi-permeable packages was postulated by Heldman (1974). The loss of the nutrient was considered to be a function of oxygen and water activity; storage temperature was not incorporated into the model.

Lee et al. (1976) developed a computer simulation for determining ascorbic acid stability in canned tomato juice. Copper ion concentration, pH and storage temperature were the parameters incorporated into the model. The experimental and predicted results were in excellent

agreement (±3%). The simulation also contained the additional capacity to calculate the effect of seasonal variations as a result of storage temperature fluctuations using the Fourier transform series.

Recently, Mizrahi and Karel (1977) proposed a "no model" accelerated test method for the determination of the deterioration rate of dehydrated products due to moisture sensitive reactions. This technique is based on theoretical considerations that the deterioration rate is inversely proportional to the rate of moisture gain at any given moisture content. This procedure was successfully applied to the prediction of ascorbic acid loss in tomato power and browning in dehydrated cabbage.

From this overview of methods for predicting product stability, it is obvious that presently used models generally rely on one index of quality, e.g. browning, lipid oxidation, nutrient loss, organoleptic properties. To date, modeling proposals have lacked general acceptance and utility. The reason for this appears to be their inability to consider simultaneous deteriorative reactions.

EXPERIMENTAL PROCEDURES

Model Food System Composition

The composition of the dehydrated model food system is given in Table 1. The model system was designed to simulate a ready-to-eat breakfast cereal.

Model Food System Preparation

The ingredients, except coconut oil, were mixed dry in a ribbon blender, water was then added to give a slurry of approximately 40% total solids. The slurry was heated to 60°C, the coconut oil was then added and the system was homogenized in a Manton-Gaulin homogenizer at 2000 psig (1st stage, 1500 psig; 2nd stage, 500 psig). The pH of the homogenized slurry was 6.8.

The model system for all studies was fortified with USP reduced ascorbic acid at a level of 25% NAS/NRC RDA (11.25 mg ascorbic acid) per 100g model system (dry weight basis). The ascorbic acid was thoroughly mixed into the homogenized model system slurry after it had cooled to ambient temperature.

Following fortification, the model system slurry was layered onto stainless steel freeze-drying trays,

placed in a Virtis Model FFD 42 WS Freeze-Dryer and frozen at a platen temperature of -40°C. The model system was then dried to 5μ absolute pressure at a platen temperature of 110°C.

Table 1. Composition of model food system

Component	%[d]
Protein[a]	10.2
Fat	1.0
Carbohydrate[b]	76.6
Reducing Sugar[c]	5.1
Sucrose	5.1
Salt	2.0

[a]Soya protein--Promine D. Central Soya.

[b]Food Grade Powdered Starch--A.E. Staley, Inc., and Corn Syrup Solids 15 D.E., American Maize.

[c]Supplied by the corn syrup solids, % dry weight basis.

[d]Calculated on dry weight basis.

Addition of Other Nutrients to the Model System

Fortification of the dehydrated model food system with other nutrients for the interaction studies was accomplished in a manner similar to the addition of ascorbic acid.

In the case of the multivitamin system, vitamin A, as retinyl acetate, was added to the model system slurry

prior to homogenization using the coconut oil as the carrier. Riboflavin and ascorbic acid were then added as aqueous solutions to the homogenized model system slurry after it had cooled to ambient temperature. The model system was then freeze-dried as described above. Both vitamin A (retinyl acetate, Sigma) and riboflavin (Sigma) were added at a level of 25% NAS/NRC RDA (0.287mg and 0.450mg, respectively) per 100g model system (dry weight basis).

For the mineral fortification study, the individual mineral was added at the levels indicated in Table 2 to the homogenized model system slurry. All salts were of reagent grade quality. The model system was then freeze-dried as described above.

Model Food System Equilibration

Water activities for the model system were adjusted using equilibrium moisture content isotherm data (Figure 6) determined by Bach (1974) for the freeze-dried model system at 10, 20, 30 and 37°C using the method of Palnitkar and Heldman (1971). All experiments in this study were performed on the adsorption leg of the sorption hystersis loop. Equilibration was accomplished by placing thin slabs of the freeze-dried model system into an equilibration chamber and forcing conditioned air of the desired a_w and temperature from an Aminco-Aire unit through the closed system (Figure 7).

Table 2. Mineral supplement, source and %RDA employed in mineral fortification study.

Mineral Supplement	Source	%RDA	mg per 100g d.b.
Control	--	--	--
$FeSO_4 \cdot 7H_2O$	Mallinckrodt	10	9.0
		25	22.4
$FeCl_2 \cdot 4H_2O$	Mallinckrodt	10	6.4
		25	16.0
Fe	Fisher	10	1.8
		25	4.5
$ZnCl_2$	Mallinckrodt	10	3.1
		25	7.8
$ZnSO_4 \cdot 7H_2O$	Mallinckrodt	10	6.6
		25	16.5
ZnO	Fisher	10	1.9
		25	4.7
$CaCO_3$	Mallinckrodt	10	300.3
		25	750.5
$CuCl_2 \cdot 2H_2O$	Baker		2.7
			6.7
$CuSO_4 \cdot 5H_2O$	Mallinckrodt		3.9
			9.8

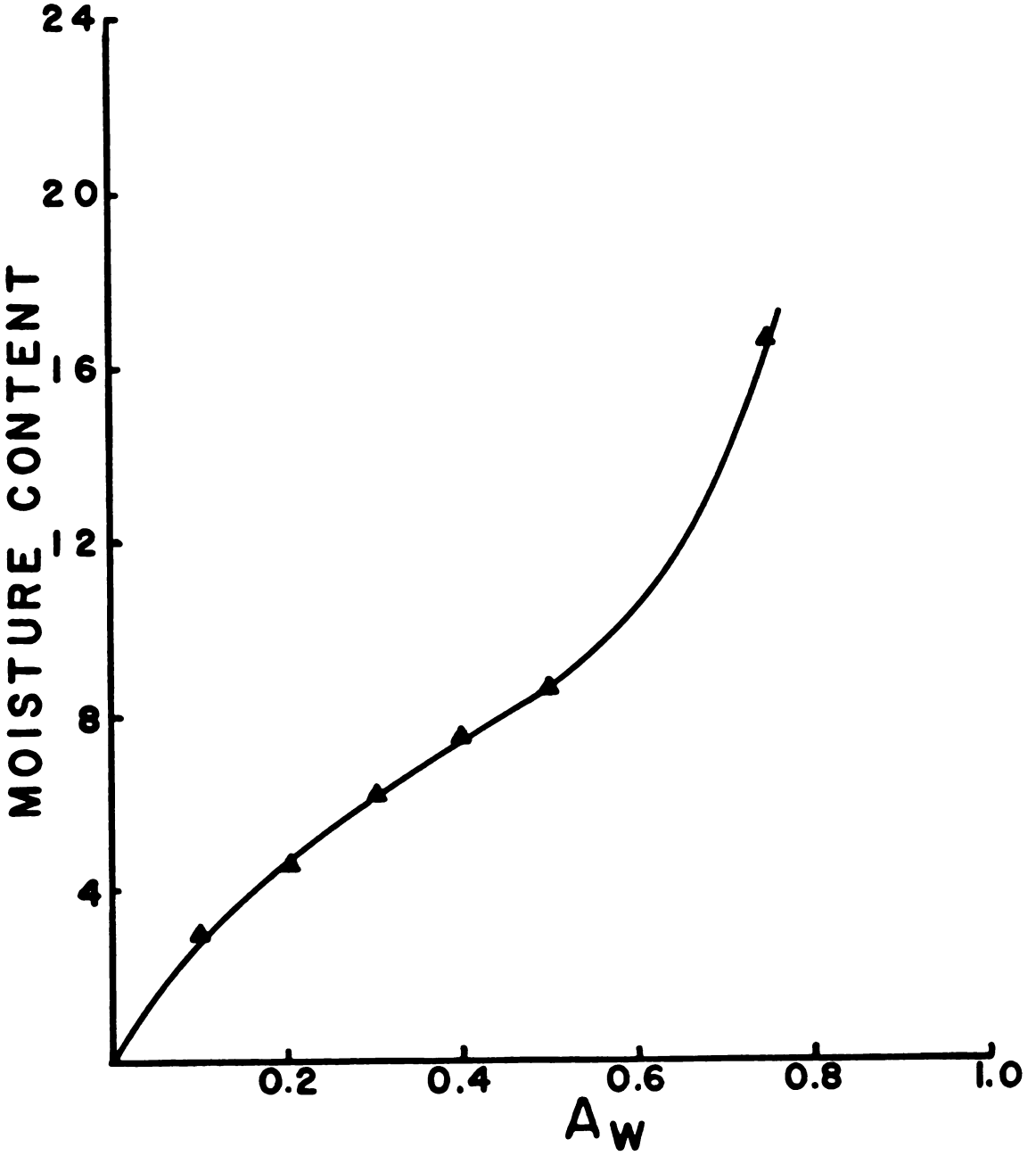


Figure 6. Adsorption isotherm for the dehydrated model food system at 20°C (from Bach, 1974).

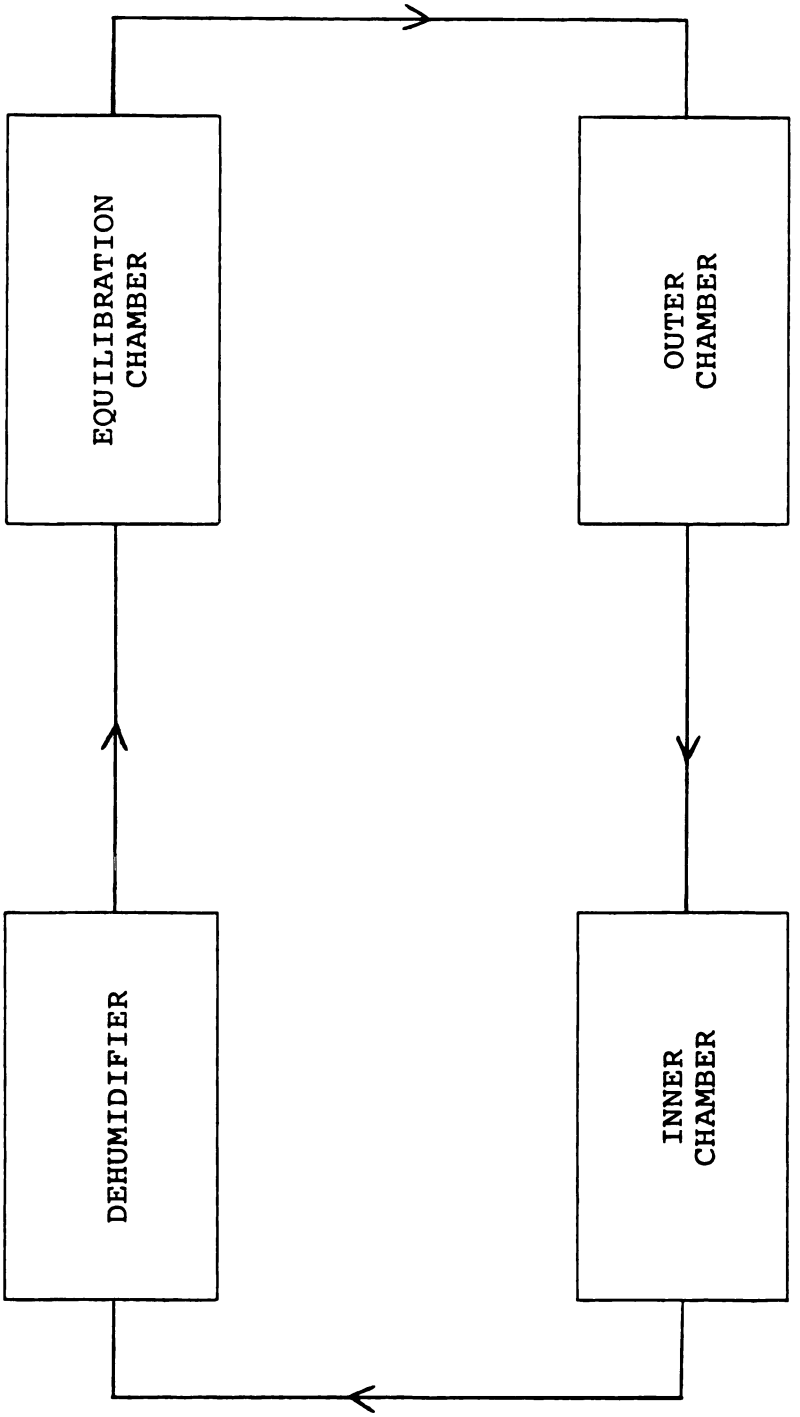


Figure 7. Block diagram for equilibration of dehydrated model food system (inner and outer chamber represent Aminco-Aire unit).

A dehumidifier and cooling coil were placed in the closed system when relative humidities were required which were lower than could be provided by the Aminco-Aire unit alone. Samples were equilibrated in approximately twenty-four hours.

Model System Packaging and Storage

Following equilibration of the model system to the desired equilibrium moisture content, the product was immediately packaged in either enameled metal containers (TDT-208x006 or 303- 303x406) or 1-oz. paperboard boxes. The metal containers prevented the transfer of air and moisture vapor into or out of the package. All containers were filled with the same mass of model system, approximately 15g. This amount of the model system filled the TDT cans leaving no headspace in the container, whereas the 303 cans permitted a large headspace in the container.

Paperboard 1-oz. cereal boxes (3cm x 7cm x 10.3 cm) containing waxed liners (thickness 0.009cm) were packaged with unequilibrated freeze-dried model system. Moisture transfer coefficient for the liner and paperboard box plus liner were equal (7.25×10^{-5} gH_2O-cm/m^2-h-mmHg) as reported by Purwadaria (1976).

The packaged model system was then stored in constant temperature cubicles of the appropriate temperatures. The cubicle temperatures were estimated to be ±1°C of the set temperatures of 10, 20, 30 and 37°C.

Relative humidities in the cubicles were estimated to vary no more than ±2% of the 10, 40 and 85% RH storage conditions.

Moisture Content Measurement

Moisture content (dry weight basis) of equilibrated model system was determined by drying the samples in a vacuum oven at a vacuum of 28 inches Hg at the same temperature at which the product was equilibrated and stored. An ethanol-dry ice cold trap was inserted in the line between the vacuum oven and the vacuum pump to aid in the transfer of moisture from the product. Dry air was admitted into the vacuum oven at a rate of 15-20 ml/min to aid in the displacement of water vapor from the drying chamber. All samples were dried until they reached a constant weight. Using this method, the water activity of each sample could be determined to ensure that the a_w had not changed during storage. The validity of this method for water activity determination of a large number of samples was confirmed by the vapor pressure manometric method of Sood and Heldman (1974).

Ascorbic Acid Determination

Ascorbic acid was measured by the continuous flow o-phenylenediamine micro-fluorometric procedure described by Kirk and Ting (1975). Ascorbic acid was extracted from the sample matrix with a 3% m-phosphoric acid--3%

glacial acetic acid solution. Samples were filtered and the extract introduced to a Technicon autoanalyzer. Dehydroascorbic acid was permitted to condense with o-phenylenediamine forming a fluorophor which was detected fluorometrically with an excitation wavelength of 360nm and emmission wavelength of 436nm. Total ascorbic acid was measured as dehydroascorbic acid following oxidation of reduced ascorbic acid with 2,6-dichloroindophenol. Boric acid was utilized as the blanking reagent. Reduced ascorbic acid was determined from the difference of total minus dehyroascorbic acid.

Data Analysis

The loss of ascorbic acid was analyzed by the first-order kinetic equation for all storage conditions studied:

$$- \frac{d\ (C)}{dt} = kC \qquad (18)$$

where (C) = molar concentration of ascorbic acid; t = time (days); and k = first-order rate constant ($days^{-1}$). Ascorbic acid levels for zero time storage were determined after each aliquot of the dehydrated model system was equilibrated to the desired water activity. Ascorbic acid determinations were performed at preset intervals for a minimum of two half-lives, except for samples stored at 10°C in TDT cans which exhibited an extremely long half-life.

The temperature dependence for ascorbic acid degradation was analyzed according to the Arrhenius equation:

$$k = A \exp (-E_a/RT) \qquad (19)$$

where k = first-order rate constant; A = Arrhenius pre-exponential; E_a = activation energy (cal/mole); R = gas constant (1.987 cal/^{o}K-mole) and T = absolute temperature (^{o}K). The reaction rate constants and the activation energies were calculated by linear regression analysis and by a computer program, KINFIT.

The KINFIT program differs from the usual least squares techniques in that numerical integration procedures are used to provide a fit to the desired differential equation (Dye and Nicely, 1971; Singh et al., 1975). This method of calculation assists in accounting for errors in vitamin assays and small variations in storage times. This program is specifically written for chemical reactions.

Thermodynamic activation parameters for the destruction of ascorbic acid were calculated based on the theory of absolute reaction rates. The appropriate equations are outlined in the literature review.

Experimental Design

Various factors such as temperature, pH, oxygen, metal ion concentration and water activity have been

reported as influencing the degradation rate of ascorbic acid in solution or dehydrated systems. As noted by Labuza (1968) and Borenstein (1971), a dearth of kinetic data exists on the storage stability of ascorbic acid and other nutrients in food systems. It was, therefore, the purpose of this study to examine some of these factors and obtain satisfactory kinetic data for ascorbic acid degradation in a dehydrated model food system.

Ascorbic Acid Stability in TDT Cans

The model food system was prepared, fortified with ascorbic acid only and equilibrated as previously described. Equilibration conditions were 0.10, 0.24, 0.40, 0.50 and 0.65 a_w at 10, 20, 30 and 37°C. Following equilibration, the model system was then sealed in TDT cans and stored at the respective equilibration temperatures. This provided a total of twenty conditions with which to examine the effects of water activity and temperature on the storage stability of ascorbic acid. The 0.10 a_w represents a condition below the BET monomolecular moisture content, 0.24 a_w is the experimentally determined BET monomolecular moisture content, 0.40 and 0.50 a_w are conditions in the monolayer region and 0.65 a_w approaches the capillary region of the adsorption isotherm.

Ascorbic Acid--Vitamin Interactions in TDT Cans

The model system was prepared, fortified with ascorbic acid and vitamins A and B_2 and equilibrated as previously described. Equilibration conditions were 0.10, 0.24, 0.40, 0.50 and 0.65 a_w at 10, 20, 30 and 37°C. Following equilibration, the model system was then sealed in TDT cans and stored at the respective equilibration temperatures. This provided twenty conditions with which to examine the effects of vitamin interactions, water activity and temperature on the storage stability of ascorbic acid.

Ascorbic Acid Stability in Paperboard Boxes

The model food system was prepared, fortified with ascorbic acid only and with ascorbic acid, vitamins A and B_2 and packaged unequilibrated in 1-oz. paperboard boxes with waxed liners. The packages were stored in cubicles at 10, 40 and 85% RH at 30°C. This permitted an examination of the effects of moisture vapor and air transmission on the storage characteristics of ascorbic acid.

Ascorbic Acid Stability in 303 Cans

The model food system was prepared, fortified with ascorbic acid only and equilibrated as previously described. Equilibration conditions were 0.10, 0.40 and

0.65 a_w at 10, 20, 30 and 37°C. After equilibration, approximately 15g of the model system was then sealed in 303 cans and stored at the respective equilibration temperatures. This provided twelve conditions for the examination of the influence of a large gaseous oxygen reservoir on the storage stability of ascorbic acid.

Ascorbic Acid--Mineral Interaction in 303 Cans

The model system was prepared, fortified with the minerals in Table 2 and equilibrated as previously described. Equilibration conditions were 0.10, 0.40 and 0.65 a_w at 30°C. After equilibration, approximately 15g of the model system was then sealed in 303 cans and stored at 30°C. Zero, 10 and 25% RDA fortification levels were employed to evaluate the effects of mineral concentration at selected a_w on the storage characteristics of ascorbic acid. In addition, the effect of the anion of the mineral employed could also be evaluated.

RESULTS

Ascorbic Acid Stability in TDT Cans

The effects of water activity, moisture content and storage temperature on the stability of ascorbic acid were determined by studying the destruction of reduced (RAA), dehydro (DAA) and total (TAA) ascorbic acid in a dehydrated model food system. The model system was equilibrated to 0.10, 0.24, 0.40, 0.50 and 0.65 a_w at 10, 20, 30 and 37°C, packaged in TDT cans, sealed and then stored isothermally at their respective equilibration temperatures. The dependence of TAA and RAA destruction on a_w at 30°C are shown in the first-order kinetic plots of Figures 8 and 9. At all a_w and storage temperatures, the loss of TAA and RAA could be satisfactorily described by first-order kinetics. Correlation coefficients associated with these plots were > 0.95.

The first-order rate constants and half-lives for TAA and RAA degradation are presented in Table 3. The experimentally determined TAA and RAA concentrations at zero time storage were in good agreement with calculated ascorbic acid levels at t = 0 computed by the

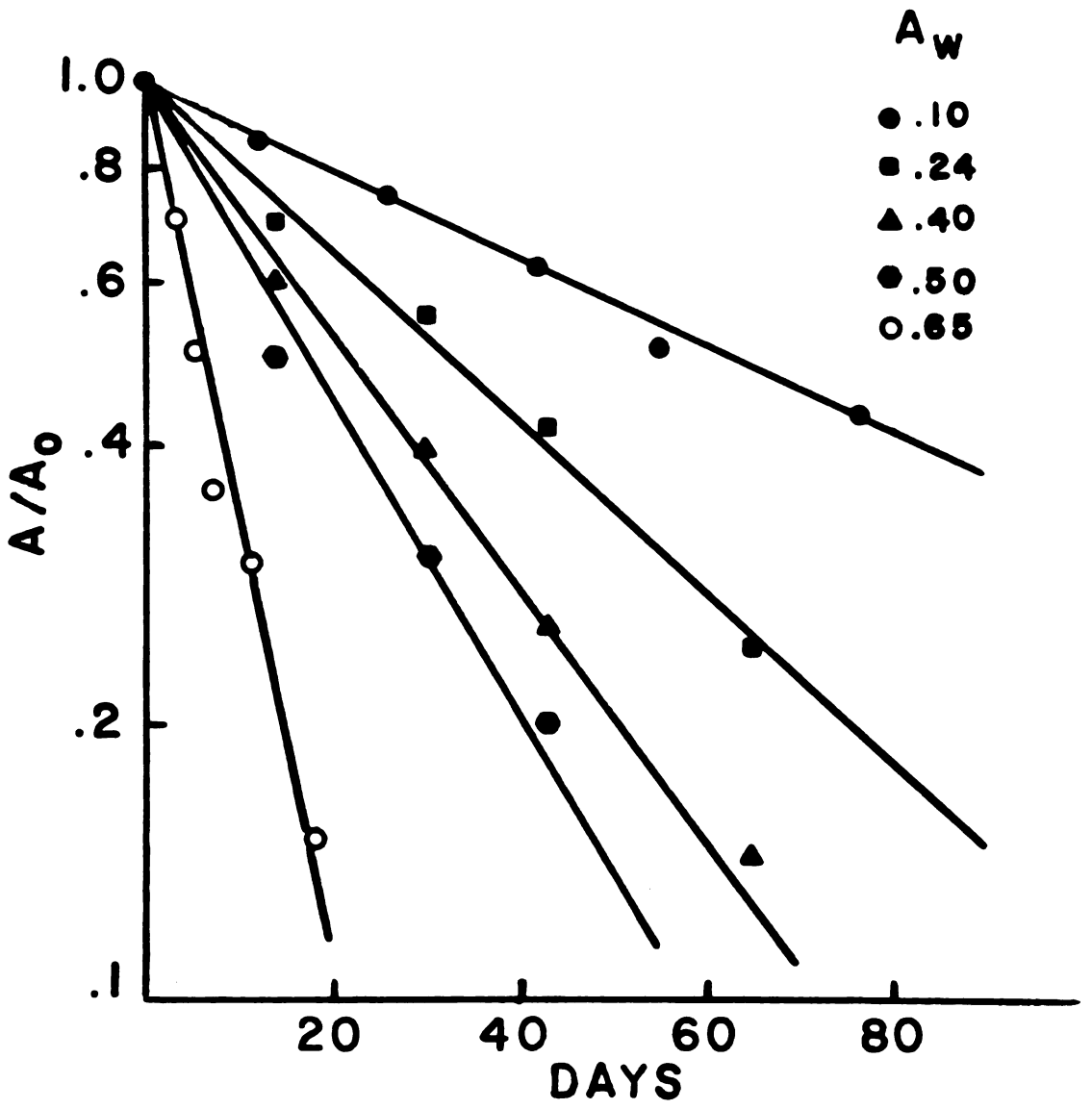


Figure 8. Fraction of TAA remaining vs. time for selected a_w at 30°C in the ascorbic acid only fortified model system stored in TDT cans.

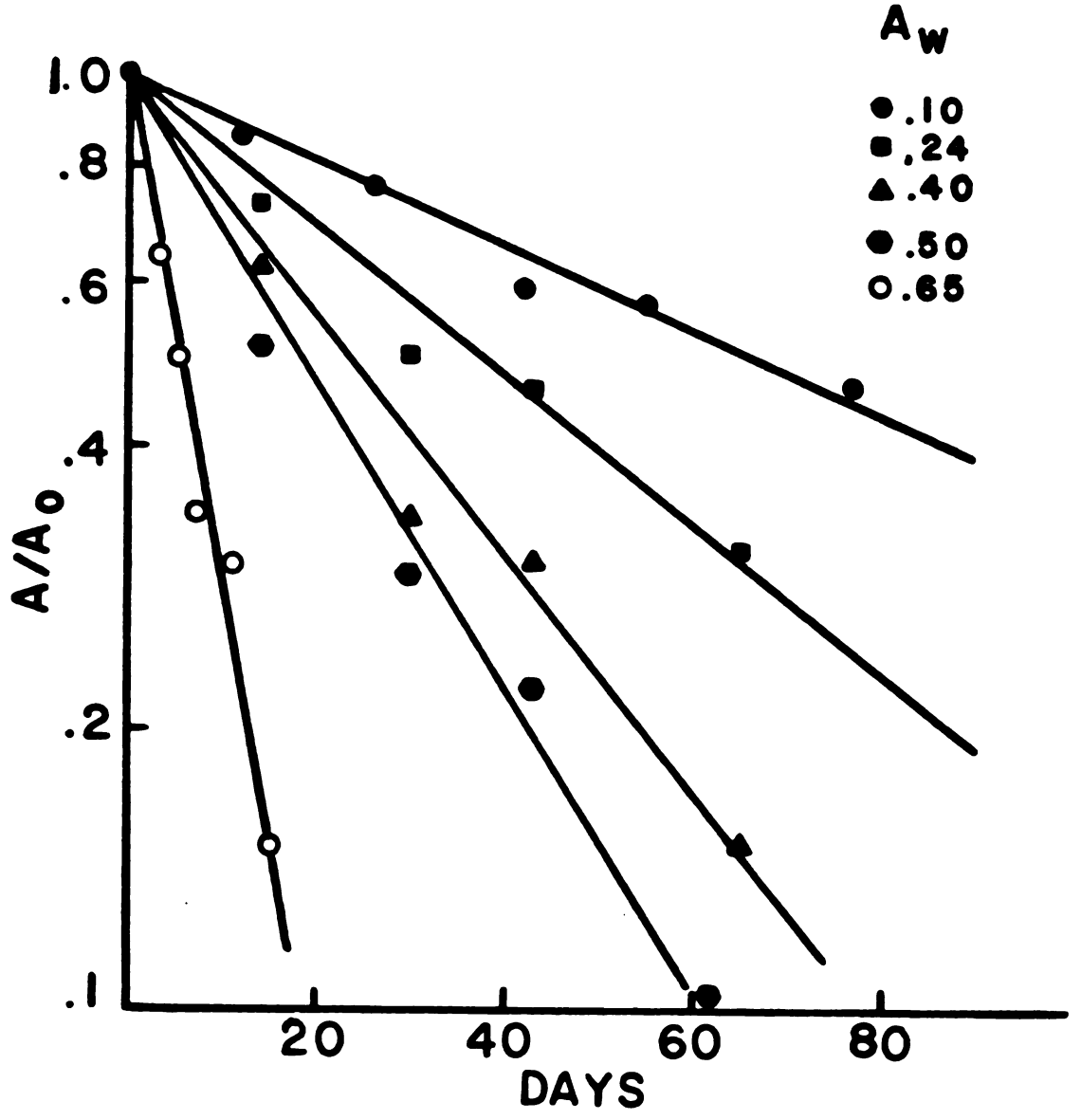


Figure 9. Fraction of RAA remaining vs. time for selected a_w at 30°C in the ascorbic acid only fortified model system stored in TDT cans.

Table 3. Rate constants and half-lives for TAA and RAA loss as a function of water activity and storage temperature in ascorbic acid fortified dehydrated model food system packaged in thermal death time cans.

Temp °C	a_w	TAA			RAA		
		k^a	σ^b	$t_{½}^c$	k^a	σ^b	$t_{½}^c$
10	0.10	0.31	0.03	224	0.43	0.07	161
	0.24	0.37	0.04	187	0.45	0.05	154
	0.40	0.42	0.05	165	0.47	0.09	147
	0.50	0.49	0.03	141	0.58	0.05	119
	0.65	0.50	0.02	139	0.55	0.05	126
20	0.10	0.45	0.06	154	0.65	0.01	107
	0.24	0.95	0.12	73	1.34	0.21	52
	0.40	1.28	0.11	54	1.69	0.22	41
	0.50	1.12	0.08	62	1.30	0.13	53
	0.65	1.44	0.28	48	1.93	0.54	36
30	0.10	0.91	0.08	76	1.11	0.15	63
	0.24	1.78	0.23	39	2.30	0.31	30
	0.40	3.13	0.26	22	3.84	0.42	18
	0.50	3.99	0.34	17	4.63	0.50	15
	0.65	4.77	0.42	15	5.29	0.51	13
37	0.10	0.98	0.11	71	1.23	0.20	56
	0.24	5.01	0.36	14	4.44	0.16	16
	0.40	7.03	0.24	10	7.87	0.28	9
	0.50	9.24	0.59	8	8.90	0.57	8
	0.65	15.74	0.66	4	16.85	1.11	4

[a]First Order Rate Constant, x 10^{-2} $days^{-1}$ (KINFIT analysis).

[b]Standard Deviation, x 10^{-2}.

[c]Half-Life, days.

KINFIT program. The standard deviation of the rate constants calculated by the KINFIT program was, in general, less than 10% of the respective rate constants.

Data in Table 3 show that the stability of RAA and TAA in the model system stored in TDT cans decreased with increasing a_w and storage temperature. Rate constants for RAA destruction are slightly greater than those describing TAA losses at the same conditions. Little significance is placed on this rate difference because of the standard deviations associated with the rate constants describing RAA losses are in part a function of the RAA determination (TAA-DAA=RAA).

The stability of DAA showed a strikingly different pattern than that observed for RAA and TAA. During the initial portion of the storage study, the concentration of DAA increased at 10°C, 0.10 and 0.24 a_w and 20°C, 0.10 a_w followed by a decrease after approximately twenty-five days of storage (Figure 10). No increase in DAA levels were observed at the other storage conditions and DAA loss at the other storage conditions appeared to fit a first-order equation. The rate constants for the loss of DAA, however, were not calculated for reasons which will be presented in the discussion.

The effect of temperature on the rate constants describing the loss of TAA as a function of a_w at 10, 20, 30 and 37°C is shown in Figure 11. A similar temperature

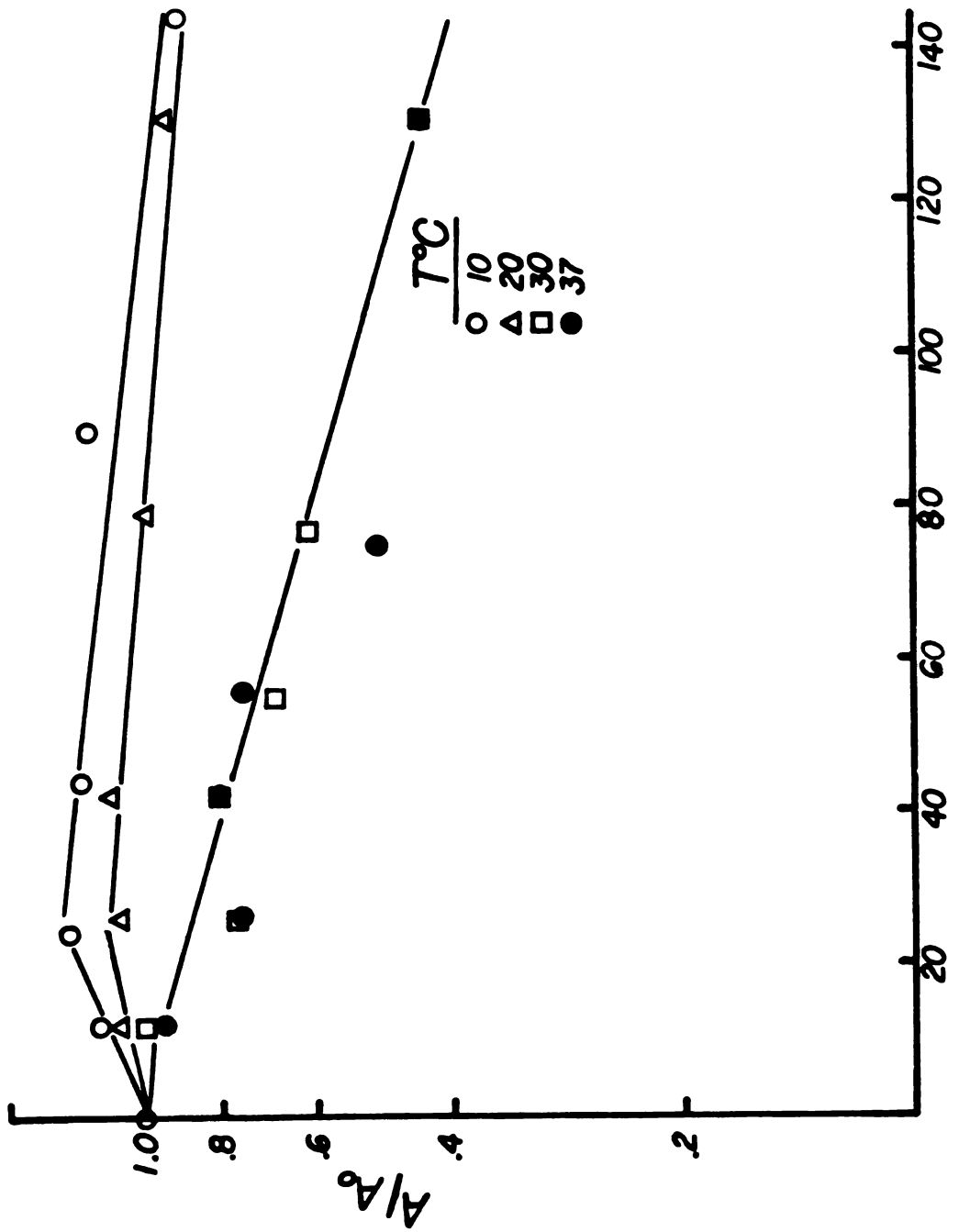


Figure 10. Fraction of DAA remaining vs. time for 0.10 a_w at selected storage temperatures in the ascorbic acid only fortified model system stored in TDT cans.

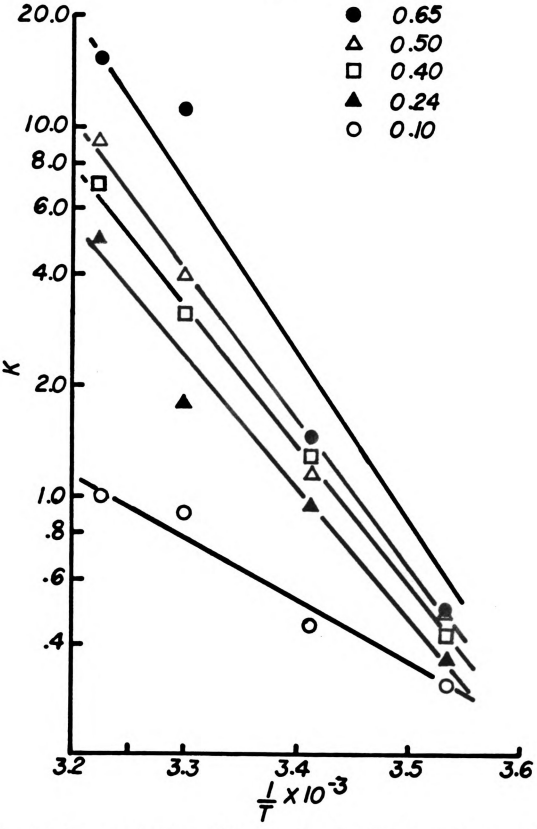


Figure 11. Arrhenius plot for the activation energy of TAA loss in the ascorbic acid only fortified model system stored in TDT cans as a function of a_w.

dependence of the rate constant for RAA loss was also found. Calculated activation energies (E_a) for TAA and RAA destruction as a function of a_w are reported in Table 4. The activation energies for a_w equal to or above the monomolecular moisture content (a_w = 0.24) averaged 18.0±1.6 kcal/mole for TAA loss and 17.4±2.2 kcal/mole for RAA loss. The activation energy for both TAA and RAA destruction at a_w of 0.10 were confirmed by the t-test to be significantly different from the activation energies for the other storage conditions at the 95% confidence level.

Table 4. Activation energies for TAA and RAA loss in ascorbic acid fortified dehydrated model food systems packaged in TDT cans.

a_w	Activation Energy (kcal/mole)	
	TAA	RAA
0.10	8.1	7.1
0.24	15.9	14.2
0.40	17.6	17.8
0.50	19.2	18.1
0.65	19.2	19.3

Ascorbic Acid--Vitamin Interactions in TDT Cans

The model food system for this study was fortified with ascorbic acid, vitamins A and B_2, equilibrated at 0.10, 0.24, 0.40, 0.50 and 0.65 a_w at 10, 20, 30 and

37°C, sealed in TDT cans and stored isothermally at their respective equilibration temperatures. The possible effects of these added nutrients on the stability of ascorbic acid were evaluated as a function of a_w and storage temperature.

The loss of RAA and TAA in the multivitamin model system could be adequately described by first-order kinetics. The degradation constants and half-lives are presented in Table 5. Correlation coefficients were > 0.95 and the standard deviations of the rate constants as calculated by the KINFIT computer program were less than 10%. The degradation rate of TAA and RAA in the multivitamin fortified model system increased with increasing a_w, similar to that found for the model system containing only ascorbic acid. There was no significant difference in rate constants for TAA and RAA loss. Model system containing vitamins A, B_2 and C, which was equilibrated at a_w of 0.10 to 0.65 and stored at 10°C in TDT cans showed apparent increases in RAA and TAA stability under these conditions (Table 5) when compared to the model system containing only ascorbic acid and stored under similar conditions (Table 3).

The pattern of small initial accumulation of DAA at low a_w and storage temperatures found in the ascorbic acid model system (Figure 10) was also observed for the

Table 5. Rate constants and half-lives for TAA and RAA loss as a function of water activity and storage temperature in multivitamin dehydrated model food system packaged in thermal death time cans.

Temp °C	a_w	TAA			RAA		
		k^a	σ^b	$t_{½}^c$	k^a	σ^b	$t_{½}^c$
10	0.10	0.18	0.05	385	0.14	0.04	495
	0.24	0.31	0.03	224	0.33	0.12	210
	0.40	0.43	0.03	161	0.41	0.11	169
	0.50	0.46	0.06	151	0.52	0.22	133
	0.65	0.45	0.04	154	0.54	0.07	128
20	0.10	0.49	0.10	141	0.84	0.14	83
	0.24	1.03	0.13	67	1.75	0.34	40
	0.40	1.46	0.21	47	2.46	0.78	28
	0.50	1.18	0.11	59	2.43	0.37	28
	0.65	1.31	0.24	53	2.88	0.22	24
30	0.10	1.03	0.69	67	1.70	0.38	40
	0.24	3.63	0.28	19	3.43	0.24	20
	0.40	3.46	0.30	20	3.81	0.52	18
	0.50	3.28	0.30	21	4.40	0.54	16
	0.65	5.96	0.77	12	7.38	0.85	9
37	0.10	0.98	0.19	71	1.21	0.09	62
	0.24	3.14	0.19	22	--	--	--
	0.40	5.20	0.55	13	6.01	0.73	12
	0.50	5.08	0.49	14	6.64	1.52	10
	0.65	8.46	0.78	8	10.64	0.95	7

[a]First Order Rate Constant, x 10^{-2} $days^{-1}$ (KINFIT analysis).

[b]Standard Deviation, x 10^{-2}.

[c]Half-Life, days.

multivitamin fortified model system. At higher temperatures, the concentration of DAA decreased steadily with time as noted previously.

Activation energies calculated from the kinetics data describing the destruction of TAA and RAA in the multivitamin model stored in TDT cans are presented in Table 6. The values are similar to those calculated for the ascorbic acid fortified system with the exception of the 0.10 a_w condition (Table 4). Activation energies for a_w equal to or above the monomolecular moisture content averaged 16.5±2.8 kcal/mole for TAA loss and 16.0±3.1 kcal/mole for RAA loss.

Table 6. Activation energies for TAA and RAA loss in multivitamin fortified dehydrated model system packaged in TDT cans.

a_w	Activation Energy (kcal/mole)	
	TAA	RAA
0.10	11.5	13.1
0.24	13.5	11.8
0.40	16.8	16.6
0.50	15.9	16.1
0.65	20.0	19.3

Ascorbic Acid Stability in Paperboard Boxes

Two model systems were used in this study, one fortified with ascorbic acid only and the other with

vitamins A, B_2 and ascorbic acid. Both model systems were packaged, unequilibrated, in paperboard boxes containing waxed liners and stored in cubicles at 10, 40 and 85% relative humidity at 30°C. This study was designed to evaluate the influence of air and moisture vapor transmission on the storage stability of ascorbic acid.

Destruction of TAA in the ascorbic acid only model system stored in boxes at 10, 40 and 85% RH are described by first-order kinetics (Figure 12). Similar conformity to first-order kinetics were found for the loss of RAA in the ascorbic acid only fortified model system and the loss of both TAA and RAA in the multivitamin fortified model systems. It is recognized that the moisture content of the boxed model systems changed during storage and the effect of this change on the destruction rate of ascorbic acid is discussed later. The calculated first-order rate constants and half-lives for the degradation of TAA and RAA in the ascorbic acid and multivitamin fortified dehydrated model systems packaged in paperboard boxes are presented in Table 7. Higher relative humidities were associated with greater destruction rates of TAA and RAA. Little difference is noted in the destruction rates for TAA versus RAA in the ascorbic acid only system. There is a slightly greater rate of loss of RAA in the multivitamin fortified system.

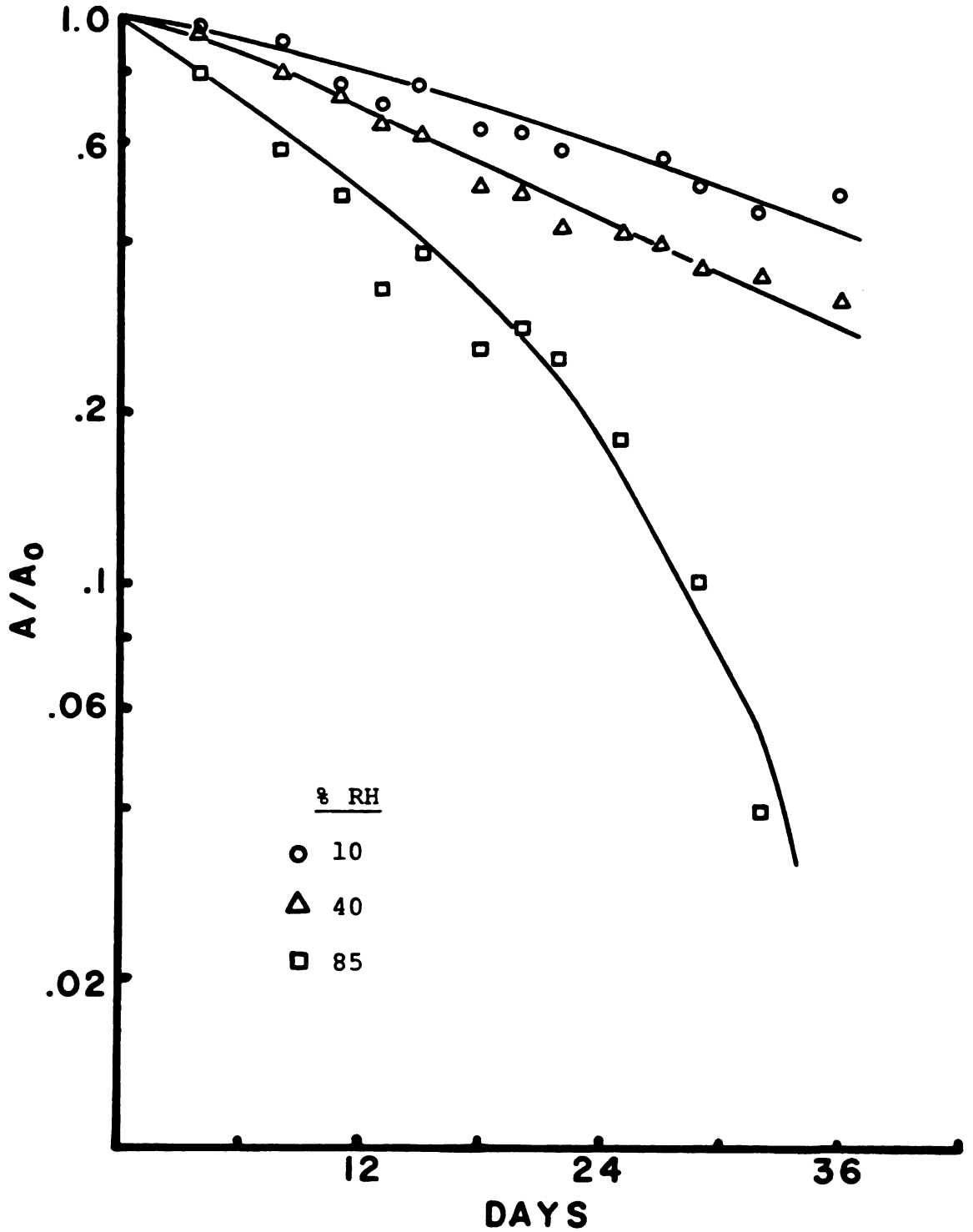


Figure 12. Fraction of TAA remaining vs. time at selected relative humidities at 30°C in the ascorbic acid only fortified model system packaged in paperboard boxes.

Table 7. Rate constants and half-lives for TAA and RAA loss at 30°C as a function of water activity in ascorbic acid and multivitamin fortified dehydrated model food systems packaged in paperboard boxes.

Model System	% RH	TAA			RAA		
		k^a	σ^b	$t_{½}^c$	k^a	σ^b	$t_{½}^c$
Ascorbic Acid Only	10	2.45	0.19	28	2.66	0.21	26
	40	3.63	0.16	19	3.77	0.25	18
	85	7.01	0.45	10	7.06	0.46	10
Multi-vitamin	10	2.01	0.34	34	2.93	0.24	24
	40	3.00	0.21	23	3.48	0.33	20
	85	10.88	0.19	6	13.44	1.91	5

[a]First Order Rate Constant, x 10^{-2} $days^{-1}$ (KINFIT analysis).

[b]Standard Deviation, x 10^{-2}.

[c]Half-Life, days.

Ascorbic Acid Stability in 303 Cans

The degradation of total, reduced and dehydro ascorbic acid in dehydrated model food system packaged in 303 cans was studied as a function of water activity and storage temperature. The model system was equilibrated to 0.10, 0.40 and 0.65 a_w at 10, 20, 30 and 37°C, sealed in 303 cans and stored isothermally at their respective equilibration temperatures. The experimental results for the loss of TAA stored in 303 cans at 0.10, 0.40 and 0.65 a_w at 20°C and at 10, 20, 30 and 37°C at 0.40 a_w are presented in Figure 13. These data conformed to the first-order kinetic function as did the destruction data at all a_w and storage temperatures. Figure 13 shows the dependence of TAA stability on a_w as well as temperature. A similar first-order dependence was found for the experimental data for RAA destruction in the model system stored in 303 cans.

The first-order rate constants and half-lives for TAA and RAA degradation in the model system stored in 303 cans are presented in Table 8. These data show an increase in the rate of TAA and RAA loss with increasing water activity at a constant temperature. No significant difference is apparent in the rate constants for TAA and RAA destruction at the same storage conditions of temperature and water activity.

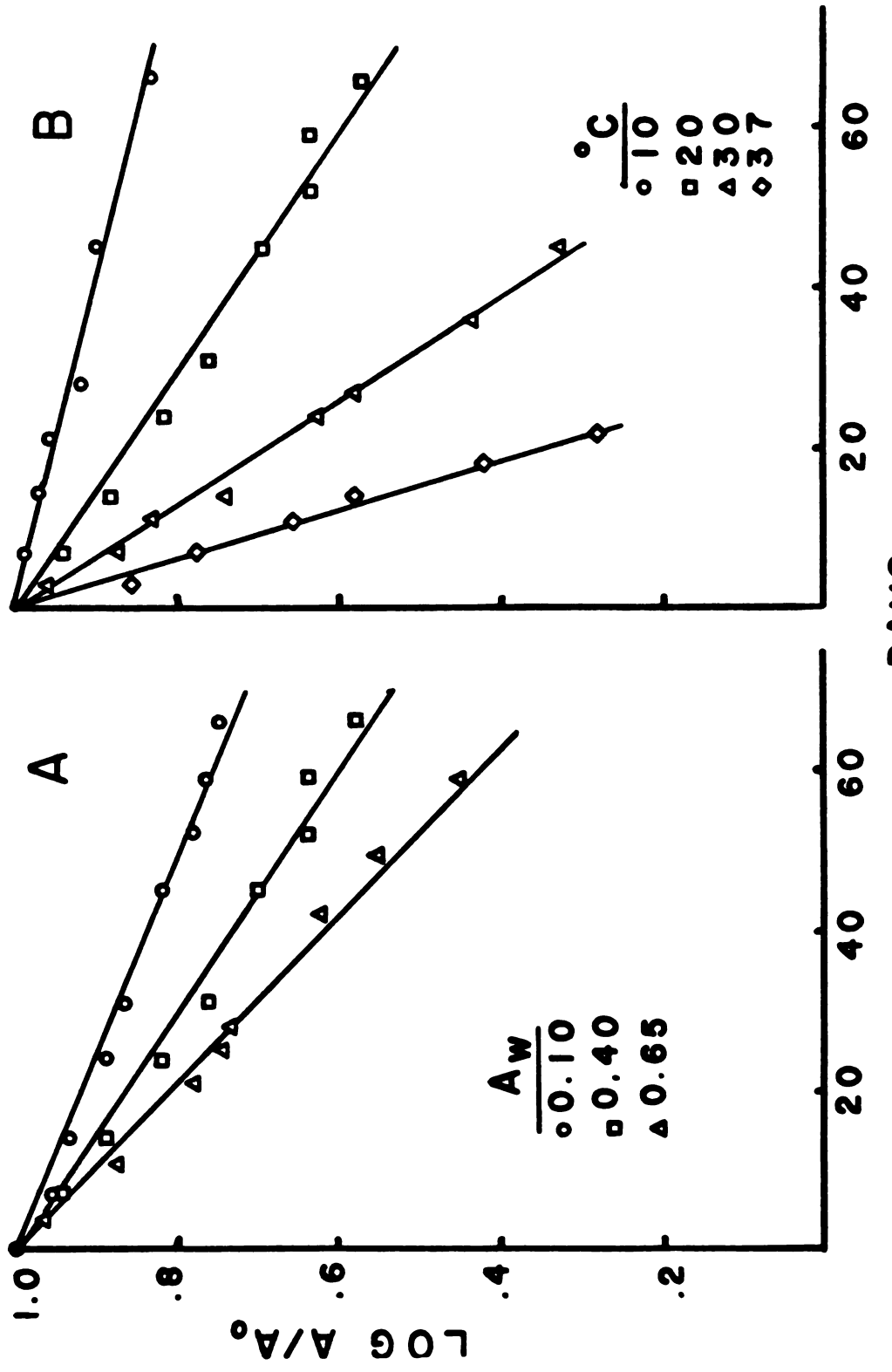


Figure 13. Fraction of TAA remaining vs. time as a function of (a) water activity at 20°C and (b) temperature at 0.40 a_w for the ascorbic acid only model system stored in 303 cans.

Table 8. Rate constants and half-lives for TAA and RAA loss as a function of water activity and storage temperatures in a dehydrated model food system packaged in 303 cans.

Temp °C	a_w	TAA			RAA		
		k^a	σ^b	$t_{½}^c$	k^a	σ^b	$t_{½}^c$
10	0.10	0.33	0.03	210	0.34	0.51	204
	0.40	0.60	0.04	116	0.63	0.61	110
	0.65	0.69	0.09	100	0.81	1.21	86
20	0.10	0.86	0.10	81	0.84	1.27	83
	0.24	1.10	0.07	63	1.03	0.90	67
	0.40	1.46	0.08	47	1.47	0.97	47
	0.65	2.03	0.15	34	2.04	2.00	34
30	0.10	1.36	0.76	51	1.29	1.09	54
	0.40	3.66	0.25	19	3.12	2.04	22
	0.65	5.55	0.29	12	5.11	4.98	14
37	0.10	1.77	2.33	39	1.86	2.18	37
	0.40	7.25	0.74	10	7.59	9.10	9
	0.65	12.07	4.31	6	11.67	26.20	6

[a]First-order rate constant, x 10^{-2} $days^{-1}$.

[b]Standard deviation, x 10^{-3}.

[c]Half-life, days.

The temperature dependence of TAA and RAA loss could be described by the Arrhenius equation. The activation energies for TAA and RAA degradation in the ascorbic acid fortified model system stored in 303 cans is presented in Table 9. The activation energies for 0.40 and 0.65 a_w are within the range of experimental error of those found for TAA and RAA degradation in the TDT can studies. However, at 0.10 a_w the activation energies were 10.7 kcal/mole for TAA and RAA degradation in the model system stored in 303 metal cans versus 7-8 kcal/mole for the TDT containers.

Table 9. Activation energies for TAA and RAA loss in ascorbic acid fortified dehydrated model food system packaged in 303 cans.

a_w	Activation Energy (kcal/mole)	
	TAA	RAA
0.10	10.7	10.7
0.40	16.0	15.6
0.65	18.3	17.0

Ascorbic Acid--Mineral Interaction in 303 Cans

Selected trace minerals were added individually to the model system prior to freeze-drying to evaluate their effect on the storage stability of ascorbic acid.

The minerals were added at two different concentration levels (Table 2). Three forms of iron ($FeSO_4$, $FeCl_2$, Fe) and zinc ($ZnSO_4$, $ZnCl_2$, ZnO) as well as two forms of copper ($CuSO_4$, $CuCl_2$) and $CaCO_3$ were selected for the study. The freeze-dried model systems were then equilibrated to 0.10, 0.40 and 0.65 a_w at 30°C and sealed in 303 cans and stored isothermally at 30°C.

TAA and RAA losses could be described by first-order kinetics at all storage conditions. Correlation coefficients were > 0.93 and the standard deviation of the rate constants were approximately 10% of the rate constants. The experimentally determined rate constants and half-lives are presented in Tables 10 and 11 for TAA and RAA degradation, respectively. No difference in TAA and RAA destruction rates was observed, and a similar dependence on water activity was found as for the other studies.

No rate enhancement in ascorbic acid destruction over the nonfortified system due to any of the minerals studied was observed for the 0.10 and 0.40 a_w storage conditions. The only exception to this is the slight catalysis in the $CuCl_2$ and $CuSO_4$ fortified systems at 0.40 a_w.

At 0.65 a_w, which is in the capillary region of the adsorption isotherm, a 2-3 fold increase in the degradation rate of TAA and RAA over the nonfortified

Table 10. Rate constants and half-lives for TAA loss as a function of mineral fortification and water activity in a dehydrated model food system stored at 30°C in 303 cans.

Mineral Supplement	%RDA	0.10 a_w		0.40 a_w		0.65 a_w	
		k^a	$t_{½}^b$	k^a	$t_{½}^b$	k^a	$t_{½}^b$
None	--	1.92	36	3.50	20	5.09	14
$FeSO_4$	10	1.31	53	2.20	31	11.22	6
	25	1.81	38	2.11	33	11.62	6
$FeCl_2$	10	1.33	52	2.57	27	10.48	7
	25	1.59	44	2.94	24	9.41	7
Fe	10	1.31	53	2.69	26	8.65	8
	25	1.53	45	2.25	31	17.28	4
$ZnCl_2$	10	1.40	50	2.38	29	17.25	4
	25	1.62	43	2.47	28	25.65	3
$ZnSO_4$	10	1.44	48	2.91	24	18.60	4
	25	1.75	40	2.30	30	17.53	4
ZnO	10	1.34	52	2.25	31	3.19	22
	25	1.63	43	2.50	28	3.91	18
$CaCO_3$	10	1.45	48	2.97	23	10.57	7
	25	1.46	47	2.78	24	17.39	4
$CuCl_2$		1.36	51	3.86	18	5.51	13
		1.38	50	4.50	15	11.67	6
$CuSO_4$		1.17	59	5.25	13	5.00	14
		1.52	46	6.37	11	9.00	8

[a]First-order rate constant, x 10^{-2} $days^{-1}$.

[b]Half-life, days.

Table 11. Rate constants and half-lives for RAA loss as a function of mineral fortification and water activity in a dehydrated model food system stored at 30°C in 303 cans.

Mineral Supplement	%RDA	0.10 a_w		0.40 a_w		0.65 a_w	
		k^a	$t_{½}^b$	k^a	$t_{½}^b$	k^a	$t_{½}^b$
None	--	2.04	33	3.45	20	5.01	14
$FeSO_4$	10	1.74	40	2.47	28	11.48	6
	25	2.16	32	2.44	28	11.73	6
$FeCl_2$	10	1.70	41	2.97	23	10.07	7
	25	1.72	40	2.69	26	9.87	7
Fe	10	1.32	52	2.64	26	9.38	7
	25	1.65	42	2.20	31	17.51	4
$ZnCl_2$	10	1.96	35	2.55	27	17.06	4
	25	2.13	33	2.47	28	23.19	3
$ZnSO_4$	10	1.87	37	2.93	24	18.13	4
	25	1.77	39	3.02	23	18.65	4
ZnO	10	1.74	40	2.52	28	3.37	21
	25	1.63	43	2.46	28	3.90	17
$CaCO_3$	10	1.61	43	3.30	21	10.41	7
	25	1.81	38	3.11	22	17.72	4
$CuCl_2$		1.28	54	3.01	23	5.94	12
		1.75	40	5.93	12	12.01	5
$CuSO_4$		1.40	50	4.00	17	4.72	15
		1.80	39	5.92	12	9.18	8

[a]First-order rate constant, x 10^{-2} $days^{-1}$.

[b]Half-life, days.

system is noted for each trace mineral added, regardless of form. The only exception is zinc oxide which did not exhibit catalysis.

Thermodynamic Activation Parameters

The thermodynamic activation parameters associated with the degradation reaction for ascorbic acid were calculated from the first-order rate constants for TAA and RAA destruction. The calculated entropy of activation ($\Delta S^{\ddagger}$), enthalpy of activation ($\Delta H^{\ddagger}$) and free energy of activation ($\Delta G^{\ddagger}$) for TAA and RAA degradation for the three temperature dependent studies are presented in Tables 12, 13 and 14. The free energy of activation for TAA and RAA loss in each study remains constant at the various water activities, whereas an increase in both the entropy and enthalpy of activation is noted with increasing water activity.

Table 12. Activation parameters for TAA and RAA degradation in ascorbic acid fortified model system stored in TDT cans.

a_w	E_a (kcal/mole)	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta G^{\ddagger}$ (kcal/mole)
		TAA		
0.10	8.1	7.5	-43	20.2
0.24	15.9	15.3	-15	19.8
0.40	17.6	17.0	- 8	19.5
0.50	19.2	18.6	- 3	19.4
0.65	19.2	18.6	- 2	19.2
		RAA		
0.10	7.1	6.5	-46	20.1
0.24	14.2	13.6	-20	19.7
0.40	17.8	17.2	- 7	19.4
0.50	18.1	17.5	- 6	19.3
0.65	19.3	18.7	- 2	19.2

Table 13. Activation parameters for TAA and RAA degradation in multivitamin fortified model system stored in TDT cans.

a_w	E_a (kcal/mole)	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta G^{\ddagger}$ (kcal/mole)
		TAA		
0.10	11.5	10.9	-31	20.2
0.24	13.5	12.9	-22	19.4
0.40	16.8	17.2	- 7	19.4
0.50	15.9	15.3	-14	19.5
0.65	20.0	19.4	1	19.1
		RAA		
0.10	13.1	12.5	-25	19.9
0.24	11.8	11.2	-28	19.4
0.40	16.6	16.0	-11	19.4
0.50	16.1	15.5	-13	19.3
0.65	19.3	18.7	- 1	19.0

Table 14. Activation parameters for TAA and RAA degradation in ascorbic acid fortified model system stored in 303 cans.

a_w	E_a (kcal/mole)	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta G^{\ddagger}$ (kcal/mole)
		TAA		
0.10	10.7	10.1	-33	20.0
0.40	16.0	15.4	-13	19.4
0.65	18.3	17.7	- 5	19.2
		RAA		
0.10	10.7	10.1	-33	20.0
0.40	15.6	15.0	-15	19.5
0.65	17.0	16.4	- 9	19.2

DISCUSSION

The storage stability of ascorbic acid in low moisture dehydrated food products was studied utilizing a model food system designed to simulate a ready-to-eat breakfast cereal. The model system provided a product of known composition with which to conduct the study. Model system preparation, as previously described, was relatively simple and ensured a homogeneous distribution of vitamins and minerals.

Ascorbic Acid Stability in TDT Cans

The degradation of total (TAA) and reduced (RAA) ascorbic acid in the low moisture dehydrated model food system are adequately described by first-order kinetics with correlation coefficients ≥ 0.95 (Table 3). This first-order dependence was observed at all storage conditions of water activity and temperature. Data in Table 3 show that the storage stability of TAA and RAA in the model system stored in TDT cans decreased as the water activity increased from 0.10 to 0.65 at each storage temperature. These data contradict the hypothesis

of Salwin (1959, 1962) that the BET monomolecular moisture content should represent the equilibrium moisture content for maximum storage stability of the product. The BET monomolecular moisture content has been determined to be equal to an a_w of 0.24 in the dehydrated model system (Bach, 1974), yet ascorbic acid degradation was significantly reduced at 0.10 a_w. Ascorbic acid degradation at a measurable rate at 0.10 a_w also conflicts with the view of Fennema (1976), that reactions which depend on solvation would not be measurable at water activities below the monomolecular moisture content. The dependence of RAA loss on water activity has been observed by other investigators (Karel and Nickerson, 1964; Vojnovich and Pfeifer, 1970; Lee and Labuza, 1975) but their studies did not include a_w below the BET monomolecular moisture content. The dependence of TAA loss on water activity and its conformity to first-order kinetics has not been reported.

The rate constants for RAA loss are, in general, slightly greater than the rate constants for TAA loss at corresponding storage conditions. It is important to note that little significance can be attached to the difference in rate constants for TAA and RAA losses.

Bauernfeind and Pinkert (1970) have summarized the possible degradation pathways for reduced ascorbic acid. A major route for RAA degradation at neutral pH

is through dehydro (DAA) ascorbic acid. Following the scheme shown in equation (20) it is evident that if DAA is more stable than

$$\text{RAA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{DAA} \xrightarrow{k_2} \text{products} \qquad (20)$$

RAA ($k_2 < k_1$), one would expect the rate constant describing RAA loss to be significantly greater than that for TAA loss. In fact it would be fortuitous if TAA loss could be described by first-order kinetics. In this study, the rate of loss of TAA and RAA were essentially equal at corresponding conditions, suggesting that either RAA does not degrad via DAA, or that the degradation rate for DAA is greater than for RAA ($k_2 > k_1$). This latter view of ascorbic acid degradation is supported by the investigations of Khan and Martell (1967) in which they utilized the 2, 4-dinitrophenylhydrazone method of Roe (1943) to measure the formation of dehydroascorbic acid with time.

Examination of the data in Figure 10 shows that the concentration of DAA during the initial stages of the storage study increased followed by a subsequent decrease in DAA concentration at 10°C, 0.10 and 0.24 a_w and 20°C, 0.10 a_w. At all other conditions a constant decrease in DAA levels with time was noted. This is interpreted as further evidence that RAA degrades

principally via DAA (equation 20). Kinetic treatment of the data describing the destruction of DAA with time was not attempted because it had not been independently confirmed that RAA degraded solely via DAA in the dehydrated model system. The data for DAA loss does suggest, however, that the rate of DAA degradation was faster than the rate of RAA degradation.

The temperature dependence of TAA and RAA degradation in dehydrated model system stored in TDT cans was described by the Arrhenius equation (Table 4). The activation energies calculated for a_w equal to and above the monomolecular moisture content (a_w = 0.24) were in the range of 15-19 kcal/mole, and showed a slight dependence on the water activity. These values were not significantly different from the activation energies reported by Lee and Labuza (1975) for the destruction of RAA in an intermediate moisture model food system at a_w 0.32-0.84 on both the adsorption and desorption legs of the sorption isotherm. The activation energy for TAA and RAA destruction in the model system stored at 0.10 a_w in TDT cans was 7-8 kcal/mole. This value was determined by the t-test to be significantly different from the activation energies at the other water activities. No stability studies of ascorbic acid in systems at water activities below the BET monomolecular moisture content have been reported. Lee et al. (1977), however, reported the

activation energy for the anaerobic destruction of ascorbic acid in canned tomato juice (pH = 4.06) to be 3.3 kcal/mole. The observed change in activation energy for ascorbic acid destruction in the model system at the 0.10 a_w storage condition could be interpreted as a change in degradative mechanism. Based on these limited data such a conclusion may be tenuous.

Ascorbic Acid--Vitamin Interaction in TDT Cans

Kinetics data were obtained for TAA and RAA loss in the same model system which was fortified with vitamins A and B_2 and then stored in TDT cans. The calculated first-order rate constants and half-lives for TAA and RAA loss are presented in Table 5. Correlation coefficients for the determination of the rate constants were ≥ 0.95. In general, the stability of TAA and RAA in this multivitamin study showed a similar dependence on water activity as was found for the system containing only ascorbic acid. The degradation rate for TAA and RAA loss in the multivitamin fortified system increased with a_w. As was found in the preceding study, the BET monomolecular moisture content did not prove to be the a_w offering the greatest stability to ascorbic acid.

There was no difference in degradation rates between TAA and RAA loss in the multivitamin study. DAA

levels were measurable and followed a pattern similar to that observed in the model system containing only ascorbic acid. The measurement of DAA concentration with time during the multivitamin study presents further supporting evidence that the principal degradative pathway for RAA in the model system was via DAA. As in the preceding study, the loss of DAA in the multivitamin fortified system could be inferred to be faster than the loss of RAA.

Comparison of TAA and RAA degradation constants between the multivitamin model system and the model system containing only ascorbic acid showed no difference, except at 10°C and low a_w where an apparent increase in ascorbic acid stability was observed in the multivitamin system. This difference may be due to the relatively long storage period which did not permit assays beyond one-half life at these conditions. From these data, it is concluded that neither vitamin A nor B_2 significantly interacted with ascorbic acid to either catalyze or inhibit the degradation of ascorbic acid. The exceptional stability of riboflavin in dehydrated products reported by Borenstein (1971) and Dennison et al. (1977) support this conclusion.

The temperature dependence of TAA and RAA degradation in the multivitamin fortified model system stored in TDT cans was described by the Arrhenius equation

(Table 6). The activation energies calculated for water activities equal to and above the BET monomolecular moisture content were 15-19 kcal/mole. These E_a values were not significantly different from the activation energies found for TAA and RAA degradation in the model system containing only ascorbic acid and stored in TDT cans. The higher E_a value at 0.10 a_w for the multivitamin system over the model system with ascorbic acid only probably reflects the experimental imprecision associated with the long storage period.

Ascorbic Acid Stability in Paperboard Boxes

The experimental data for the degradation of ascorbic acid in model systems, which were fortified with ascorbic acid only and ascorbic acid, riboflavin and vitamin A, and stored in paperboard boxes, was treated by first-order kinetics (Table 7). The standard deviations associated with the rate constants were relatively large and the correlation coefficients were approximately 0.90. The model system was packaged unequilibrated and therefore the moisture content and water activity changed during storage. Experimental data in Figure 14 reported by Purwadaria (1976) using the same model system and boxes demonstrated the time dependence required for the unequilibrated model system to reach moisture content equilibrium with the storage atmosphere

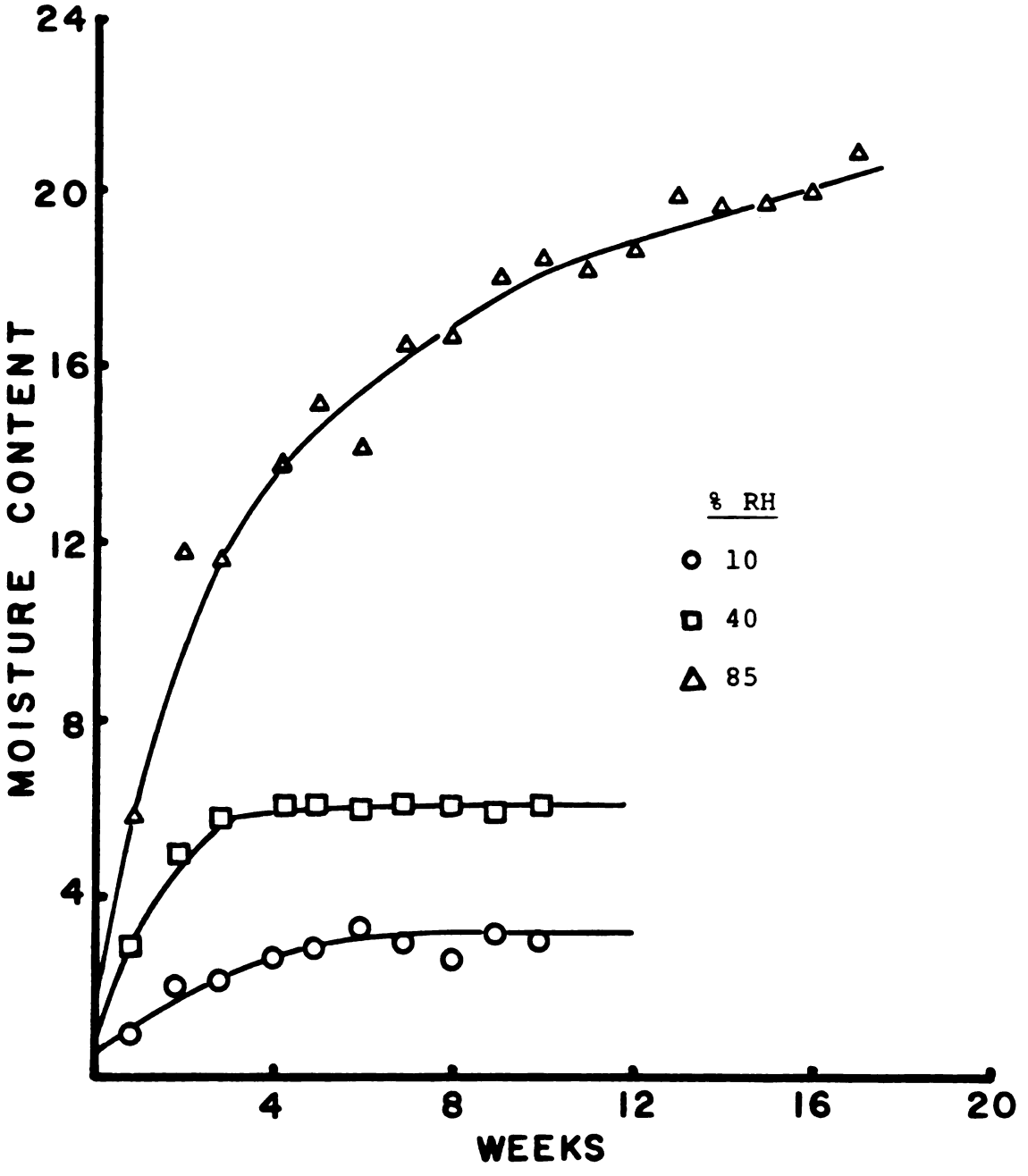


Figure 14. Moisture content equilibration during storage at selected relative humidities in dehydrated model systems packaged unequilibrated in paperboard boxes (from Purwadaria, 1977).

at 30°C. The rate of ascorbic acid degradation in the previous studies was found to be dependent on a_w, therefore, the rate of TAA and RAA loss in the model system packaged in paperboard boxes would be expected to increase with time until the moisture in the model system achieves equilibrium with the storage atmosphere.

Treatment of the experimental data for TAA and RAA loss in the model system stored in paperboard boxes at 10% RH by first-order kinetics is believed to result in little error in the calculated destruction rates because of the short storage period required for moisture equilibration at this condition (Figure 14). Determination of the rate constants for ascorbic acid destruction in the boxed model system stored at 40 and 85% RH present a more complex situation. For simplicity, the experimental data for TAA and RAA destruction in the paperboard boxes at 40 and 85% RH were analyzed by the first-order rate function in order to compare these data with similar rate data obtained for the model system stored in TDT cans at constant water activity.

The experimental first-order rate constants and half-lives for TAA and RAA loss in both model systems stored at 10, 40 and 85% RH at 30°C in paperboard boxes are presented in Table 7. The stability of ascorbic acid was dependent on the relative humidity of the storage atmosphere and there was little difference in

degradation constants for TAA and RAA loss either in the ascorbic acid only fortified model system or the multivitamin fortified model system. These results are similar to those found in the previous studies for ascorbic acid stability in TDT cans.

There was a marked difference in the half-lives for TAA and RAA loss in the model system packaged in paperboard boxes at 10% RH and 30°C versus the TDT cans at 0.10 a_w and 30°C (28 vs. 76 days, respectively). This observed difference in half-lives decreases as the relative humidity of the storage atmosphere increases. The use of the paperboard boxes permitted relatively free transmission of atmospheric gases and moisture vapor across the waxed liner as shown by the moisture vapor transfer coefficient (7.25×10^{-5} g H_2O - cm/m^2 - h mmHg) reported by Purwadaria (1976). Since the product was packaged unequilibrated at a very low moisture content, the driving force for moisture equilibration would be into the product resulting in a gradual increase in the moisture content. Under these conditions, the observed rate of TAA and RAA loss at 40 and 85% RH should be less if the system had been equilibrated to these a_w conditions prior to storage. The increasing moisture content and a_w (Figure 14) with storage time are interpreted to account for the differences in the observed destruction rates of TAA and RAA in the model system

stored at 40 and 85% RH and 30°C in the boxes and the model system packaged in TDT cans at 0.40 and 0.65 a_w and stored at 30°C.

The dramatic differences observed for TAA and RAA stability in the model food system equilibrated to 0.10 a_w and stored in TDT cans at 30°C versus the model system packaged in boxes and stored at 10% RH at 30°C cannot be accounted for on the basis of moisture content or a_w differentials. Equilibration of the boxed model system at 10% RH is achieved in about two weeks (Figure 14), yet the rate of loss of TAA and RAA in the model system is 2-3 times faster than in the TDT containers. Since the model systems were identical in composition, essentially of equal a_w, and the transfer of atmospheric gases could occur only in the boxed model system, these data suggest the involvement of oxygen in the stability of TAA and RAA in the dehydrated food system at neutral pH. The influence of oxygen on the stability of ascorbic acid has been reported by Waletzko and Labuza (1976) in an intermediate moisture model system at 0.85 a_w. Karel and Nickerson (1964), however, reported no significant difference in the rate of ascorbic acid destruction in dehydrated orange juice crystals stored in air or vacuum. Data from this latter study probably reflects the stabilizing influence of low pH and/or the chelation of metal ions

by the acids present in the soluble solids of the dehydrated orange juice crystals.

Ascorbic Acid Stability in 303 Cans

The comparison of the destruction rate at 30°C for TAA and RAA at 10% RH in the boxed model system versus the 0.10 a_w condition in the TDT cans suggested the involvement of oxygen in the degradative process. This led to a storage study in which a large reservoir of gaseous oxygen could be provided with a constant water activity. The 303 can was selected as the storage container and was filled with the model food system fortified with ascorbic acid equal to the mass (~15g) utilized in the TDT can study. The model system stored in 303 and TDT cans at all storage conditions was identical in composition and source of ingredients. The water activities and moisture contents of the two were identical within experimental error; therefore, there should be no effect of viscosity, reactant mobility or new catalytic sites on the stability of ascorbic acid. The only variable in the two systems is the relative amount of gaseous oxygen at any condition of temperature and water activity.

The first-order degradation constants and half-lives for TAA and RAA loss in the dehydrated model system packaged in the 303 metal cans are presented in Table 8. The dependence of the rate constants for TAA and RAA

degradation in the 303 cans on water activity and storage temperature was similar to that found for ascorbic acid destruction in the TDT cans. The experimental rate constants for TAA and RAA loss in the 303 cans were found to be greater than the corresponding rate constants in the TDT cans.

The relationship between the rate constant and a_w was shown to be linear between 0.10-0.65 a_w and is valid for water activities below the capillary region of the adsorption isotherm for the model system at all temperatures studied (Figure 15). Mathematically, this function takes the form

$$k_{obs} = k_o + (\text{slope})\ a_w \qquad (21)$$

where k_o is the intercept at zero water activity. This relationship was also applied to the rate constants for ascorbic acid degradation in the TDT can study. Although the rate constants for TAA and RAA loss in the TDT can are less than the corresponding rate constants for the same product in 303 cans, they exhibit the same linear relationship of k_{obs} vs. a_w (Figure 15).

Calculation of the slopes obtained for the observed rate constant vs. a_w function (equation 21) at constant temperature are presented in Table 15 for TAA and RAA destruction in the model system stored in 303

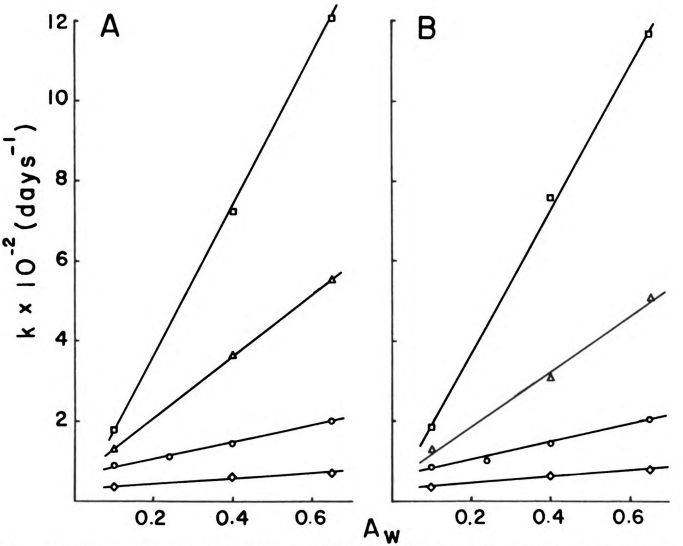


Figure 15. Relationship of the rate of TAA degradation vs. water activity at constant storage temperature in 303 (A) and TDT (B) cans (◇ 10, ○ 20, △ 30, □ 37°C).

Table 15. Linear regression of rate constant vs. water activity for TAA and RAA degradation in model system stored in 303 and TDT containers.

container/°C	TAA			RAA		
	$k_o \times 10^{-3}$	slope	ratio	$k_o \times 10^{-3}$	slope	ratio
303/10	2.86	.0066	1.78	2.64	.0086	3.31
TDT/10	2.80	.0037		3.96	.0026	
303/20	6.15	.0215	1.33	5.64	.0225	1.18
TDT/20	4.37	.0162		6.57	.0191	
303/30	6.02	.0762	1.05	5.22	.0692	0.88
TDT/30	1.58	.0729		4.57	.0788	
303/37	-1.43	.1871	0.95	1.87	.1788	0.91
TDT/37	-5.28	.1965		-4.77	.1964	

and TDT cans. The ratio of the slopes for the 303 to TDT containers decreased with increasing temperature, and at 37°C the difference in slopes are within experimental error. This relationship parallels the decrease of oxygen solubility in water with increasing temperature. The meaning of k_o, which is obtained from equation 21 would be the degradation constant at 0.0 a_w at the respective temperatures. The relatively large standard deviations associated with the k_o values are a function of the limited number of data points and the experimental precision and are believed to be the reason for the disparity in these values at the various temperatures.

The 303-TDT storage containers represent a closed system, mass may neither enter nor leave the container; however, mass may transfer across phase boundaries within the container. The transfer of gaseous oxygen into the product moisture at a given water activity and temperature would be governed by the equilibrium constant

$$K = \frac{(O_2)_d}{(O_2)_g} \qquad (22)$$

where $(O_2)_d$ and $(O_2)_g$ represent the concentration of dissolved and gaseous oxygen, respectively. As the dissolved oxygen in the moisture of the dehydrated model system is consumed via the destruction of ascorbic acid,

the concentration of dissolved oxygen would be maintained in accordance with this equilibrium constant.

The model system used to study the stability of TAA and RAA was prepared as described in the methods section, equilibrated to the appropriate a_w and packaged in 303 cans and TDT cans, each with an equal mass of product (15g). This left no headspace in the TDT container, although a limited amount of gaseous oxygen was present in the intra and intersitial spaces of the product. The maximum concentration of dissolved oxygen that could be present in any of the equilibrated model system used (303 or TDT can studies) was estimated assuming a moisture content of 10%, which corresponds to an a_w of 0.65 at 37°C. Using the solubility of oxygen in water at 0°C (4.89 $cm^3/100cm^3$), even though this temperature will yield an artificially high value, the maximum oxygen content from air which could be present in the moisture of the model system would be 4.36×10^{-7} moles of oxygen/cm^3 of water. Assuming complete solubility, calculation of the moles of ascorbic acid in the fortified model system yielded 6.10×10^{-6} moles of ascorbic acid/cm^3 of water in the model system at 0.65 a_w and 37°C (based on 11.25 mg ascorbic acid/100 g model system, dry weight basis). These data show that the maximum dissolved oxygen content was approximately a factor of 10 less than the

theoretical stoichiometric ratio of one mole of oxygen/ mole of ascorbic acid reported by Hand and Greisan (1942) for the oxidation of ascorbic acid in a system with pH = 7.0. Thus, all equilibrated samples used in this study, whether packaged in cans or boxes contained dissolved oxygen at a concentration less than that required for a 1 to 1 mole ratio of oxygen to ascorbic acid.

No headspace was left in the TDT can after filling it with model system. Thus, the moles of gaseous oxygen which could be present in the TDT cans would vary depending upon the inter and intrastitial spaces in the packaged model system. Using estimates that 20 to 40% of the total volume of the TDT can could be occupied by air, the total moles of gaseous oxygen were calculated and ranged from 2.8×10^{-5} to 5.4×10^{-5}. This would represent approximately a 10-fold excess of moles of gaseous oxygen/mole of ascorbic acid in the 15g of model system in the TDT can. The moles of gaseous oxygen present in the 303 can were estimated to be 5.1×10^{-3}, providing an approximately 1000 fold excess of gaseous oxygen/ mole of ascorbic acid in the 303 can. Thus, for an equal mass of product, the dissolved oxygen concentration would be more nearly maintained at its initial level in the container with the larger headspace.

Oxygen solubility in pure water is known to decrease with an increase in temperature. Joslyn and

Supplee (1949) have reported that the solubility of oxygen decreases as a function of soluble solids in pure sugar solutions at constant temperature. Therefore, as a function of temperature and soluble solids the dissolved oxygen content in the moisture of the model food system would be expected to be significantly lower than that calculated for pure water. Assuming that the ascorbic acid content (moles/ml) in the moisture of the food product can only decrease in concentration with increasing water activity and that the concentration of dissolved oxygen (moles/ml) would be constant regardless of the water activity, the initial ratio of dissolved oxygen to ascorbic acid would increase as a function of water activity. This relationship was approximated by assuming oxygen solubility in the model system to be comparable to that in pure water, and plotting the $(O_2)/(RAA)$ ratio against a_w (Figure 16). A sharp increase in the slope of the curve is noted in the region corresponding to capillarity of the adsorption isotherm and suggests an increase in the rate of ascorbic acid degradation not in accord with equation 21. This hypothesis is supported by the ascorbic acid stability data reported by Lee and Labuza (1975) for an intermediate moisture food system equilibrated on either the adsorption or desorption leg of the equilibrium moisture sorption isotherm. Lee and Labuza (1975) found an increase in

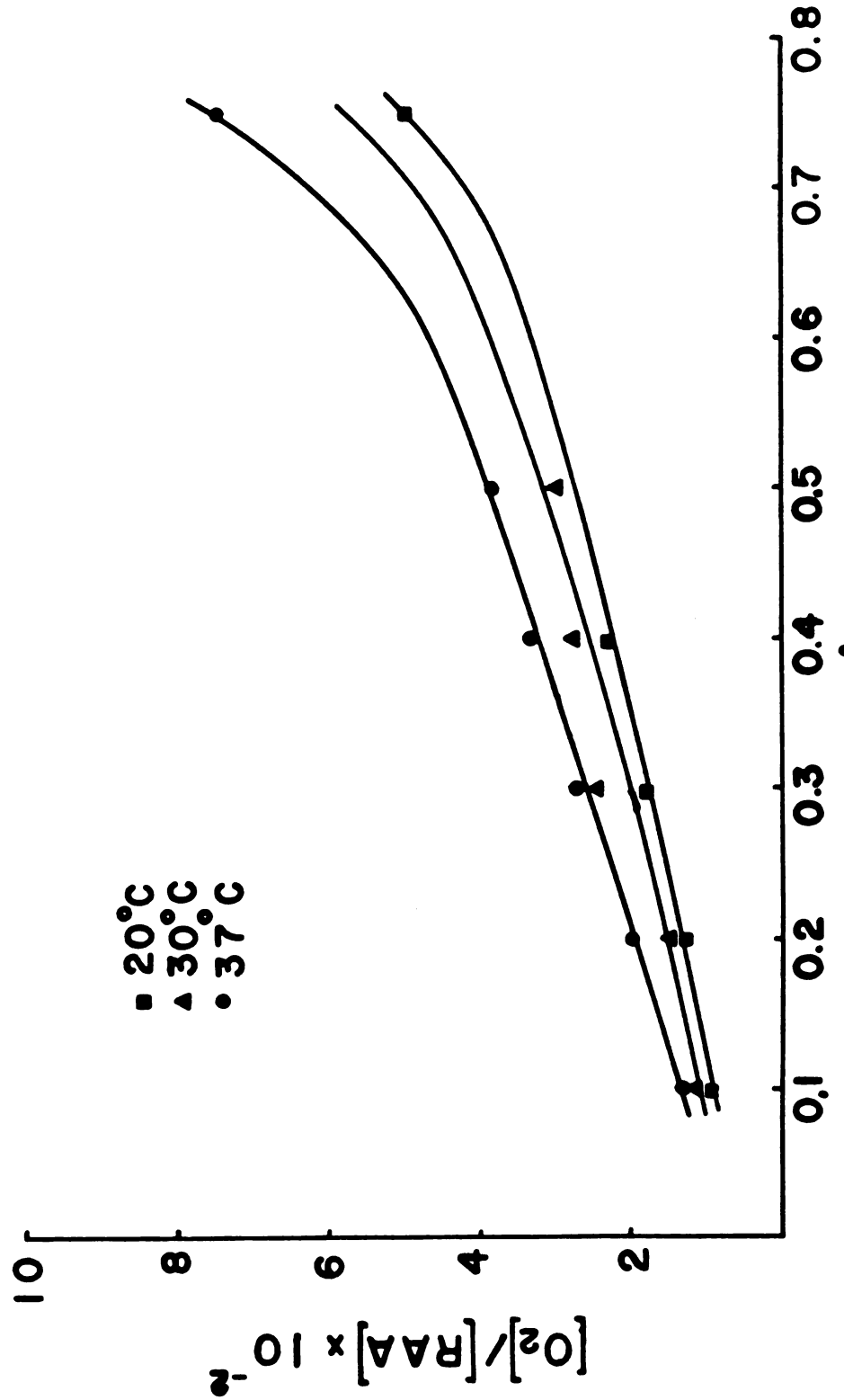


Figure 16. Ratio of (O_2)/ (AA) as a function of water activity.

the rate of ascorbic acid destruction as a function of the water activity (0.32-0.84). A sharp increase in the slope at 0.65-0.70 a_w is noted when their data are treated according to equation 21 (Figure 17). This break point corresponds to the capillary region of the sorption isotherm.

The activation energies calculated for TAA and RAA loss in the 303 cans at 0.40 and 0.65 a_w (15-19 kcal/mole) in this study are within the range of those found in the TDT can study. Similar activation energies (16-20 kcal/mole) have been reported by Lee and Labuza (1975) for the degradation of RAA in an intermediate moisture model food system. Activation energies for TAA and RAA degradation in the model system equilibrated to 0.10 a_w and packaged in 303 cans were found to be 10.7 kcal/mole versus 7-8 kcal/mole for the same model system stored in the TDT containers. The discontinuity in activation energies and the preceding discussion on oxygen might suggest an anaerobic mechanism for ascorbic acid destruction at 0.10 a_w. Lee et al. (1977) suggested that above pH 4.1, the E_a for the degradation of ascorbic acid under anaerobic conditions was a function of pH according to

$$E_a = 1.840\ (pH) - 4.178. \qquad (23)$$

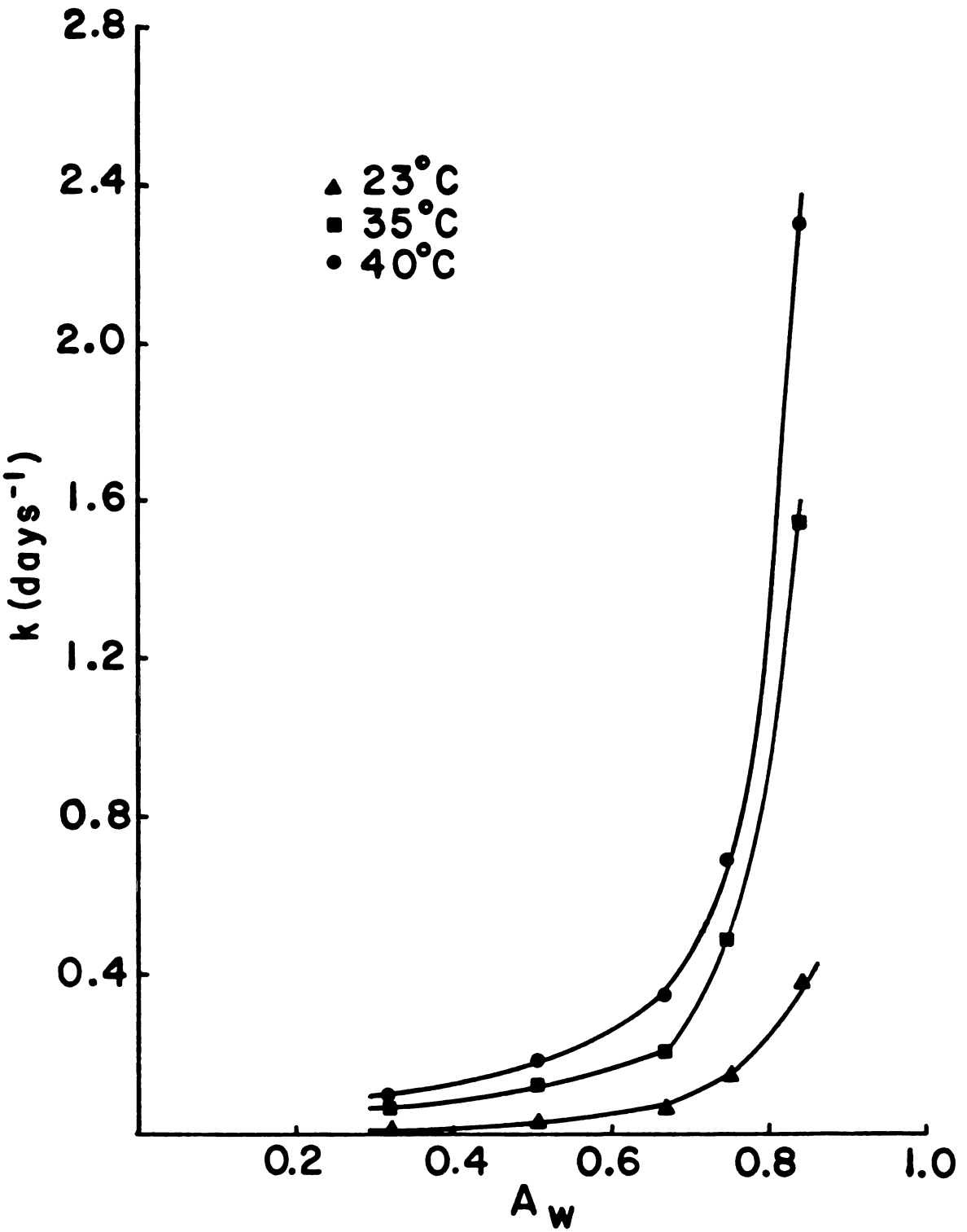


Figure 17. Relationship of the rate of ascorbic acid degradation vs. water activity at constant temperature for an intermediate moisture model food system (data from Lee and Labuza, 1975).

Applying this equation to the model food system at pH 6.8, a predicted E_a of 8.5 kcal/mole is obtained, which correlates favorably with experimental results for the model food system stored in the TDT can. These data might suggest the possible involvement of an anaerobic mechanism for the degradation of ascorbic acid in the 0.10 a_w model system stored in the TDT can, but as emphasized earlier, this is a tenuous conclusion.

Lee and Labuza (1975) have postulated that the observed rate increase with water activity for ascorbic acid degradation could not be rationalized by assuming a dilution of the reaction species because the measured half-lives did not remain constant. They attributed the increased rate of destruction with increasing water activity to the viscosity of their system. Using NMR relaxation studies which showed that the viscosity was inversely proportional to the moisture content of the aqueous phase, Lee and Labuza (1975) suggested that as the moisture content increased, the mobility of the reaction species and/or catalysts would increase, resulting in higher degradation rates of ascorbic acid. Since viscosity was a linear function and the destruction rates a hyperbolic function of moisture content, they concluded that several mechanisms must be expected by which water in controlling the reaction, most likely through a dilution effect and a control of the diffusion rate.

As previously discussed, the increasing moisture content of the system results in a dilution of the ascorbic acid concentration, but the oxygen concentration would remain constant. Assuming ascorbic acid degradation to follow overall second-order kinetics and to be first-order in both ascorbic acid and oxygen as reported by Khan and Martell (1967), the ratio of oxygen to ascorbic acid would increase as the water activity increases, hence the reaction rate should increase. Thus, the availability of oxygen in a low moisture food system is interpreted as the major factor governing the degradation of ascorbic acid. This rationale would also apply to the effect of the desorption system on the stability of ascorbic acid.

Ascorbic Acid--Mineral Interaction in 303 Cans

The effects of trace minerals on the stability of ascorbic acid in dehydrated foods have not been previously studied. Extensive investigations concerning metal ion catalyzed oxidation of ascorbic acid in solution have been reported in the literature. Khan and Martell (1967) have postulated an ascorbate-metal-oxygen complex as the intermediate for copper and iron catalysis of ascorbic acid. More recently, Blackburn and Jameson (1975) have suggested a metal-metal dinuclear ascorbate-oxygen complex to be the intermediate.

The influence of mineral fortification on the storage stability of TAA and RAA in the model food system packaged in 303 cans was studied at 30°C and 0.10, 0.40 and 0.65 a_w. The experimental data were treated by first-order kinetics and the rate constants and half-lives for TAA and RAA loss are presented in Tables 10 and 11. It is apparent from the rate constants for TAA and RAA degradation that there was no catalytic activity associated with any of the metals studied at 0.10 a_w. Water activity of 0.10 is less than the BET monomolecular moisture content and, therefore, extremely limited mobility of the metal ions would be anticipated.

No rate enhancement, due to metal ion catalysis for TAA and RAA degradation, was observed at 0.40 a_w, except for the slight catalysis exhibited by $CuCl_2$ and $CuSO_4$. Apparently, the copper(II) ion possesses slightly greater mobility than the other minerals studied at 0.40 a_w. Khan and Martell (1967) have suggested that the rate determining step of metal catalyzed ascorbic acid oxidation involves a one electron-transfer from the ascorbate anion to the metal ion, thus requiring a stable lower valence form of the metal ion for electron acceptance. This rationale may explain the lack of observed catalysis by calcium and zinc ions. At or below 0.40 a_w, the kinetic data from this study for copper and iron catalysis are interpreted as

reflecting the reduced ability of the aqueous phase to solubilize and/or mobilize the metal ions in the model system.

At 0.65 a_w, which is in the capillary region of the adsorption isotherm, a 2-3 fold increase in the degradation rate of TAA and RAA is noted for each of the added trace minerals as compared to the model system not fortified with minerals. The exception was zinc oxide, which did not exhibit a catalytic effect. Catalysis by iron and copper are anticipated in view of published results in the literature. Iron is probably oxidized to Fe(III) before catalyzing the ascorbic acid reaction. It is difficult to discern the precise effect of mineral concentration of the reaction, but the degradation rate appears to increase with increasing copper or iron levels. This dependence on concentration is in accord with the results of Khan and Martell (1967) and Ogata et al. (1968). There appears to be a negligible, if any, effect of the anion on the catalytic properties of copper and iron. Mapson (1945) and Ogata et al. (1968) have suggested that the chloride ion possesses some catalytic properties, but such an effect was not observed in this study.

Reaction data indicate that free mobility of the Cu(II) or Fe(II) may require the complete hydration of the metal ion in its octahedral configuration, which may

only be possible in the free water of the capillary region. Below this region, the metal ions may have one or more charged species from the product matrix as substituted liquids, effectively immobilizing or limiting the migration of the ions.

The effect of various zinc salts on the catalysis of ascorbic acid degradation was unexpected. The absence of a catalytic effect associated with ZnO can be explained in view of its characteristic insolubility in aqueous solution. The catalysis of ascorbic acid degradation by zinc chloride and zinc sulfate was unexpected because Zn(II) is reportedly inactive as a catalyst of ascorbic acid oxidation due to the lack of a stable lower valence state (Khan and Martell, 1967). The same expectation and rationale apply to calcium carbonate.

The effect of added electrolytes on the activity of oxygen in aqueous solution has been reviewed by Long and McDevit (1951). The activity coefficients of oxygen increased linearly with the electrolyte concentration, and was dependent on both the cation and anion present. This phenomenon could explain the rate enhancement observed for ascorbic acid degradation in the model system with added $CaCO_3$, $ZnCl_2$, and $ZnSO_4$. Assuming the Bronsted equation discussed in the literature review to be valid for ascorbic acid degradation,

$$k = k_o \frac{\gamma_{AA} \ \gamma_{O^2}}{\gamma_{\ddagger}} \qquad (24)$$

any increase in the activity coefficient of oxygen should result in an increased degradation rate. The importance of this electrolyte effect may not be detectable in aqueous solution where the ascorbic acid degradation is studied in the presence of a large excess of oxygen. In the model system, calculations have shown the ascorbic acid concentration to be approximately 10-fold greater than the dissolved oxygen content. Thus, an increase in the activity of oxygen in the model system could have a marked effect.

The rate data for ascorbic acid degradation in the dehydrated food system at 0.65 a_w is interpreted as reflecting two types of behavior: (1) catalysis by copper and iron ions as suggested by Khan and Martell (1967) and (2) rate enhancement due to the increased activity coefficient of oxygen on the addition of calcium and zinc electrolytes. From a practical standpoint, trace mineral fortification of a low moisture food would have negligible effect on ascorbic acid stability at moderately low water activities.

Thermodynamic Activation Parameters

The activation parameters for TAA and RAA degradation in the dehydrated model food system are presented

in Tables 12, 13 and 14. As depicted graphically, $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ increased in value as a function of water activity (Figures 18 and 19), while the free energy of activation, $\Delta G^{\ddagger}$, remained constant. The effect of water activity on the destruction of ascorbic acid in the dehydrated model system could be interpreted in a manner analogous to chemical reactions in various water-alcohol mixtures, i.e., as a solvent effect. Solvent effects, such as degree of solvation, dielectric constant and hydrogen bonding, should be important to the reactivity of the dehydrated system.

Activation parameters calculated for the degradation of TAA and RAA in the dehydrated model system indicate that the degradation of ascorbic acid obeys the isokinetic relationship (equation 14), as illustrated by Figures 20 and 21. The results from the TDT and 303 can studies appear to fall on the same isokinetic line, yielding β values of 273°K and 276°K for TAA and RAA destruction, respectively. These β values are less than the temperature range of the study, which implies that the degradation reaction of ascorbic acid is controlled by the entropy of activation (Blackadder and Hinchelwood, 1958a,b). The validity of this isokinetic relationship suggests that, although there is a large change in $\Delta H^{\ddagger}$ (hence E_a) and $\Delta S^{\ddagger}$ with a_w, the constant

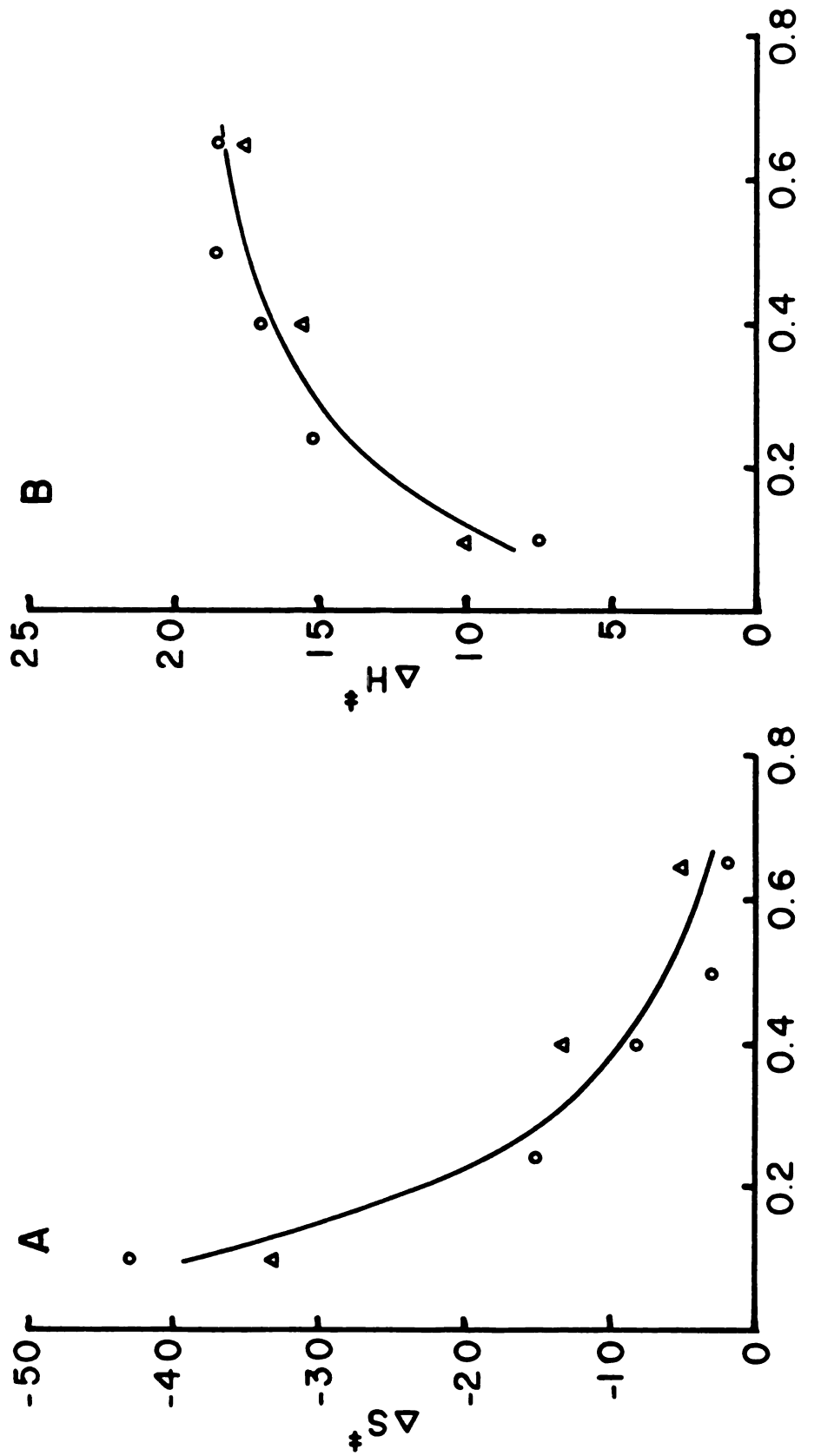


Figure 18. Dependence of entropy of activation (A) and enthalpy of activation (B) on water activity for TAA loss in the dehydrated model system stored in TDT cans (o) and 303 cans (Δ).

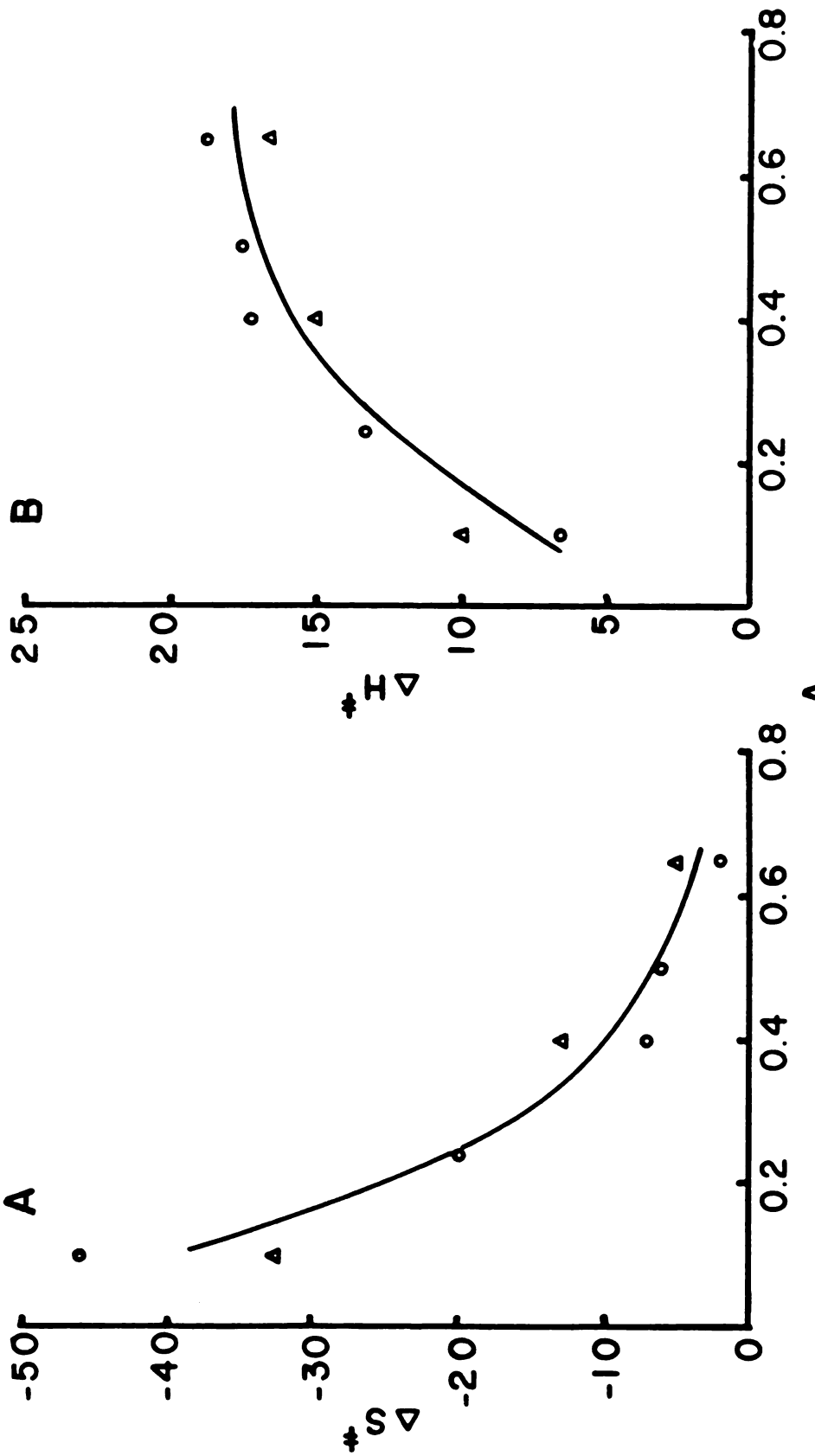


Figure 19. Dependence of entropy of activation (A) and enthalpy of activation (B) on water activity for RAA loss in the dehydrated model system stored in TDT cans (o) and 303 cans (Δ).

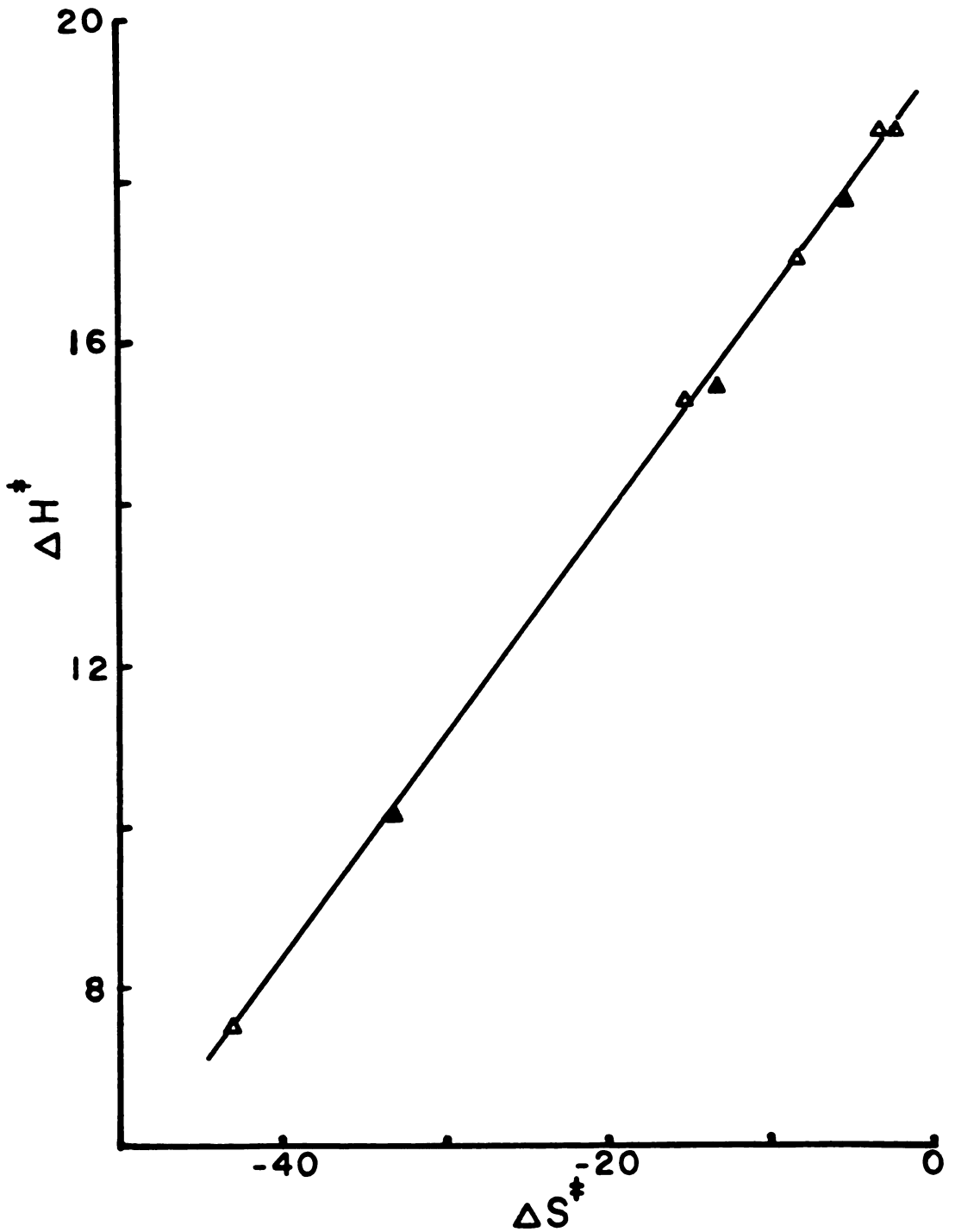


Figure 20. Isokinetic relationship of TAA degradation in dehydrated model system stored in TDT cans (Δ) and 303 cans (▲).

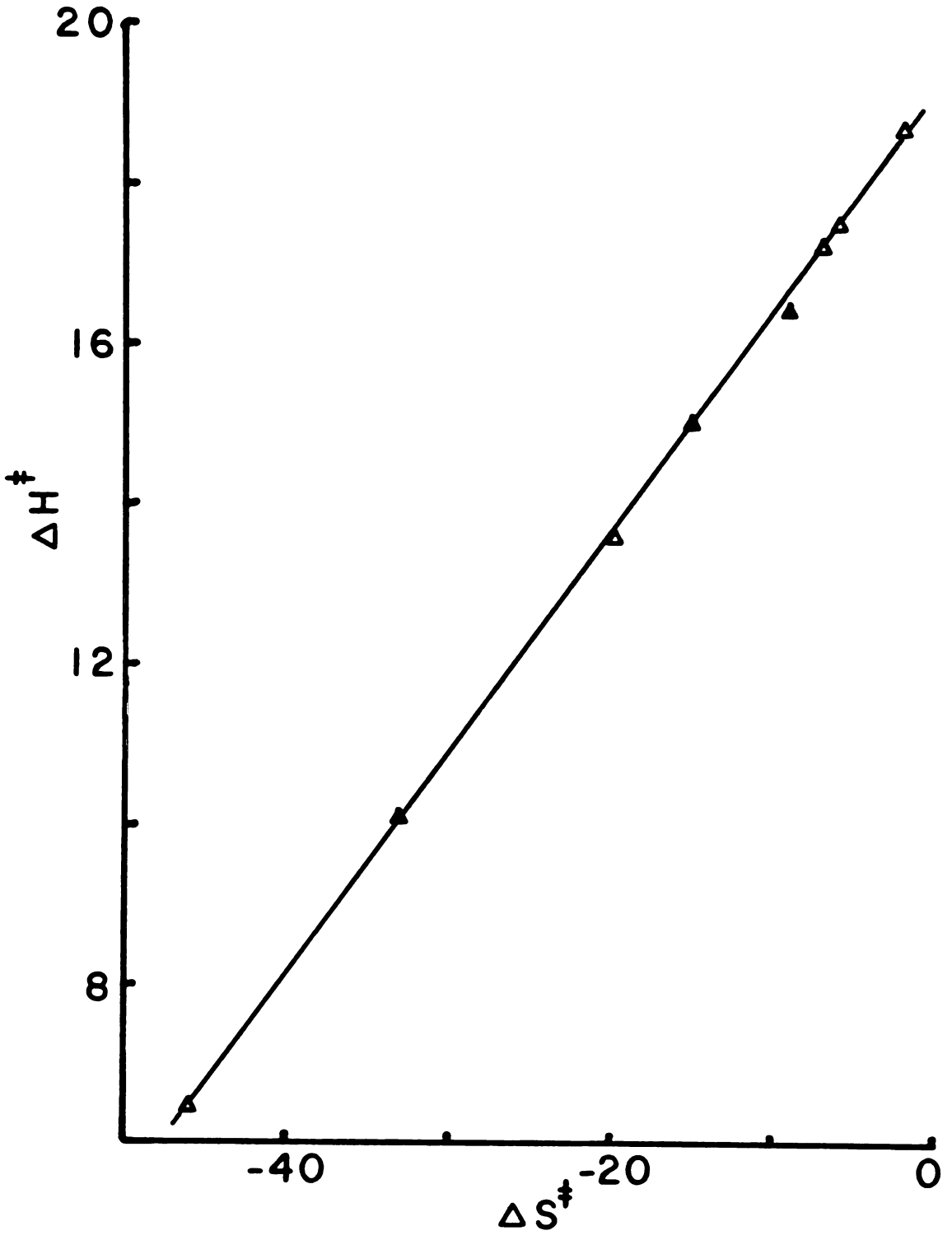


Figure 21. Isokinetic relationship of RAA degradation in dehydrated model system stored in TDT cans (△) and 303 cans (▲).

free energy of activation indicates the degradation reaction of ascorbic acid in the model system, in all likelihood, follows one mechanism.

The data presented earlier for the involvement of oxygen in the degradation of ascorbic acid suggest a possible rationale for the observed dependence of $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ on a_w. The entropy of activation is a measure of the randomness of the activated complex relative to the reactants. A negative $\Delta S^{\ddagger}$ represents a loss in entropy or loss of freedom as the reactants proceed through the transition state, whereas a positive $\Delta S^{\ddagger}$ suggests a loosely bound activated complex and a gain in freedom. A decrease in $\Delta S^{\ddagger}$ with increasing water activity may be due to increased solvation of the reactants and the activated complex or a decreased effective charge on the transition state. At 0.10 a_w in the model system, the $\Delta S^{\ddagger}$ of -43 e.u. obtained for TAA degradation in the TDT can (Table 12) indicates a loss in entropy and the formation of a tightly bound activated complex. As the system approaches 0.65 a_w, the entropy of activation approaches zero, suggesting little or no difference in the internal degrees of freedom of the transition state versus the reactants. The experimental data for ascorbic acid degradation in the model system show a dependence on the availability of oxygen. Thus,

a possible reaction intermediate would be the ascorbic acid-oxygen complex postulated by Khan and Martell (1967). Since the free energy of activation is constant, the change in $\Delta S^{\ddagger}$ could be interpreted as an increase in degree of solvation of both the reactants and transition state, as the water activity increases to 0.65. In a like manner, the change in $\Delta H^{\ddagger}$ as a function of a_w reflects increased solvation of the reactants and transition state with increased water activity, but the degradation of ascorbic acid in the model system is entropy controlled.

Little experimental data are available from the literature which may be treated in a similar manner. The activation parameters of Lee and Labuza (1975) for the degradation of ascorbic acid in an intermediate moisture system (a_w = 0.32 - 0.84) have been calculated and are presented in Table 16 and Figure 22. The free energies of activation were about 18 kcal/mole compared to the 19 - 20 kcal/mole found in the present study. The enthalpy and entropy of activation were also constant at approximately 18 kcal/mole and zero e.u., respectively. The activation parameters calculated for the adsorption and desorption studies did not indicate a dependence on the sorption hystersis phenomenon. These calculations suggest that the degradation of ascorbic acid in the

Table 16. Activation parameters for reduced ascorbic acid degradation in an intermediate moisture model system.[a]

a_w	$\Delta H^{\ddagger}$ (kcal/mole)		$\Delta S^{\ddagger}$ (e.u.)		$\Delta G^{\ddagger}$ (kcal/mole)	
	run 1	run 2	run 1	run 2	run 1	run 2
			adsorption			
0.32	20.9	21.3	+6	+7	19.1	19.2
0.51	18.4	22.0	-1	+11	18.7	18.8
0.67	16.1	18.4	-8	0	18.4	18.4
0.75	17.2	15.8	-2	-7	17.9	17.8
0.84	17.6	17.8	+1	+2	17.4	17.3
			desorption			
0.32	19.4	19.9	+1	+3	19.0	19.1
0.51	18.6	22.0	0	+11	18.7	18.7
0.67	18.4	16.7	+1	-5	18.1	18.3
0.75	18.5	21.3	+3	+13	17.5	17.5
0.84	15.8	19.8	-4	+9	17.0	17.0

[a]Activation parameters calculated from data of Lee and Labuza (1975).

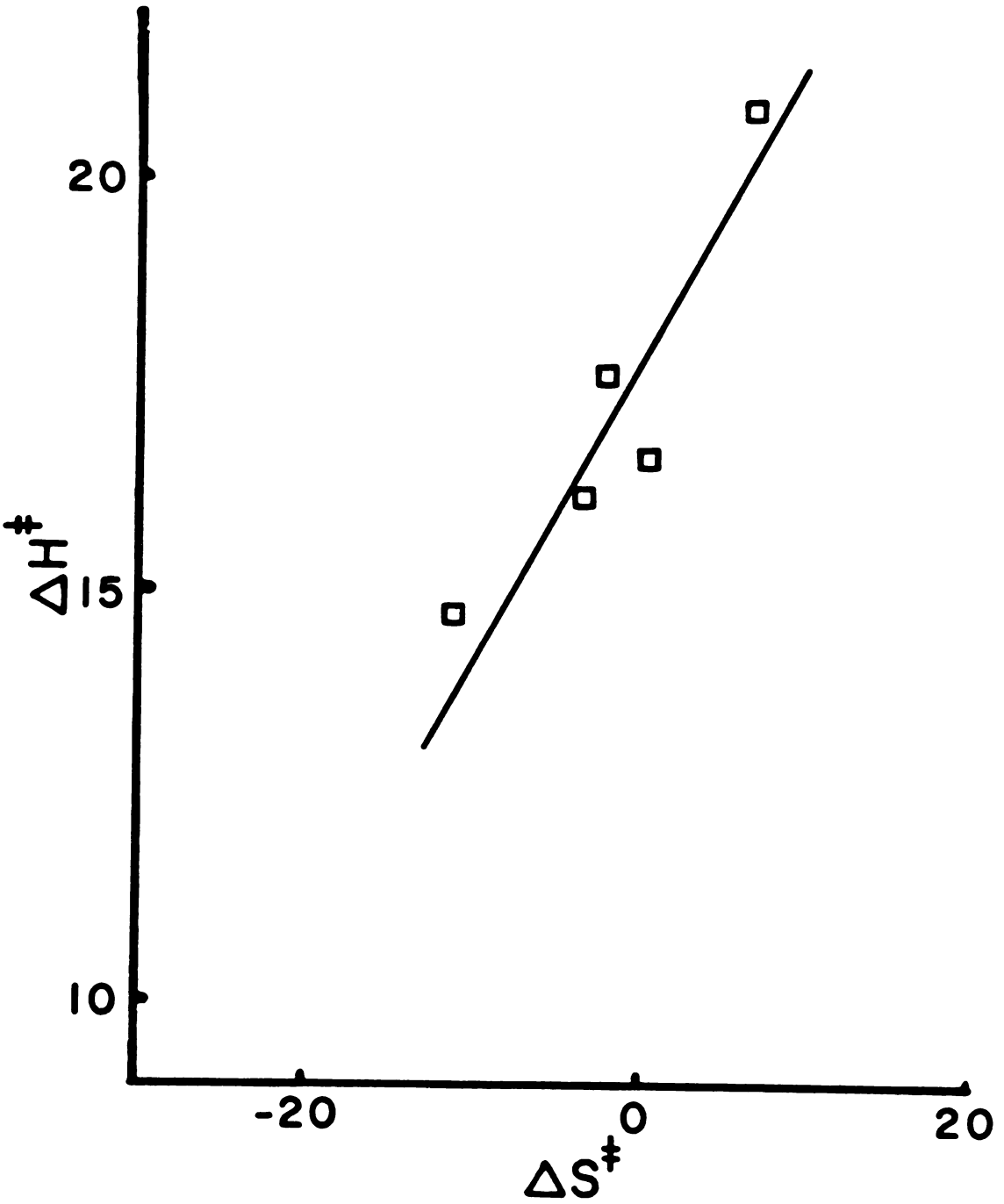


Figure 22. Isokinetic relationship for ascorbic acid degradation in an intermediate moisture model system (data from Lee and Labuza, 1975).

intermediate moisture model system of Lee and Labuza (1975) followed a similar pathway as found in the present study.

Limited data are available for the calculation of activation parameters for the degradation of ascorbic acid and other nutrients in low moisture food systems. However, information of this type may lead to a better understanding of the role of water in nutrient stability, nonenzymatic browning and lipid oxidation.

SUMMARY AND CONCLUSIONS

The storage stability of total, reduced and dehydroascorbic acid in a low moisture dehydrated model food system was investigated as a function of storage temperature, water activity, oxygen availability, vitamin interactions and trace mineral catalysis.

The destruction of TAA and RAA was dependent on the water activity and storage temperature, and its loss could be adequately described by first-order kinetics. Maximum stability of TAA and RAA was observed at low storage temperatures and 0.10 a_w, which was below the BET monomolecular moisture content. A linear relationship between the degradation constants for TAA and RAA loss with water activity was found, which contradicts the hypothesis of Salwin (1959) that the BET monomolecular moisture content should correspond to the water activity of greatest stability. The data presented tends to support the suggestion by Karel and Nickerson (1964) that there is no water activity below which the degradation of ascorbic acid ceases.

Dehydroascorbic acid was measurable during the storage study. DAA concentration decreased at storage temperatures and water activities above 20°C and 0.24 a_w, respectively. An exception was noted at 10°C and low a_w in which there was a small initial increase in DAA concentration followed by a gradual decrease after 25 days. It was concluded that the RAA degraded to DAA, which underwent further destruction.

Oxidation reactions in dehydrated food systems have been shown to be a function of reduced viscosity promoting mobility of reactants, dissolution of precipitated catalysts and swelling of solid matrices exposing new catalytic sites (Labuza et al., 1970). These factors certainly explain to some extent the increase in reaction rates for TAA and RAA in dehydrated food systems. However, the identical model system was prepared and equilibrated at the same a_w, and stored in TDT and 303 cans so that the only variable was the amount of gaseous oxygen available. Factors other than oxygen would have been internally compensated for in this study. The increased destruction rates for ascorbic acid in the 303 cans was attributed to the presence of the large gaseous oxygen reservoir in the 303 can and the maintenance of the initial dissolved oxygen concentration according to the equilibrium constant.

The effects of added riboflavin and vitamin A on the storage stability of ascorbic acid was studied using the same model system. Comparison of degradation rates for TAA and RAA loss in the multi-vitamin fortified model system versus the ascorbic acid fortified model system showed no significant effect.

The catalytic properties of added trace minerals (Fe, Cu, Zn, Ca) on the degradation of ascorbic acid was investigated at 30°C and three water activities. At and below 0.40 a_w, no rate enhancement of ascorbic acid degradation was observed over the nonmineral fortified model system due to the presence of these minerals, except for copper at 0.40 a_w. At 0.65 a_w, which is in the capillary region of the adsorption isotherm, a 2-3 fold increase in degradation rate over the nonmineral fortified system was observed for each form of the added trace mineral. The lone exception was zinc oxide, which did not exhibit catalysis due to its inherent insolubility. The catalytic effect of Fe and Cu ions in the capillary region of the adsorption isotherm were explained on the basis of the mobility of these ions. Catalysis by soluble zinc electrolytes and calcium ions was attributed to the electrolyte effect on increasing the activity coefficient of oxygen, thus increasing the rate of ascorbic acid degradation.

Activation parameters were calculated for the degradation of ascorbic acid in the model system stored in TDT and 303 cans. Although a significant change in the energy of activation was noted as a function of water activity, the free energy of activation remained constant. It is concluded that the degradation of ascorbic acid in the dehydrated model system followed the same mechanism at all water activities studied.

REFERENCES

REFERENCES

Acker, L. 1962. Enzymatic reactions in foods of low moisture content. Adv. Food Res. 11:263.

Acker, L. 1969a. Enzyme activity at low water contents. Recent Adv. Food Sci. 3:239.

Acker, L. 1969b. Water activity and enzyme activity. Food Tech. 23:1257.

Acker, L. and R. Weise. 1972a. Lipase behavior in systems of low water content. I. Influence of the physical state of the substrate on enzymic lipolysis. Lebensmittel - Wiss. u. Technol. 5:181.

Acker, L. and R. Weise. 1972b. Behavior of lipase in systems of low water content. II. Enzymic lipolysis in the range of extreme low-water activity. Z. Lebensmittelunters. u. - Forsch. 150:205.

Adamson, A. W. 1976. Physical Chemistry of Surfaces, Wiley and Sons, New York.

Aylward, F. and I. D. Morton. 1971. Vitamin fortification of foods. Proc. 3rd. Int. Congr. Food Sci. Technol. 192.

Bauernfeind, J. C. and D. M. Pinkert. 1970. Food processing with ascorbic acid. Adv. Food Res. 18:219.

Berlin, E., P. G. Kliman and M. J. Pallansch. 1966. Surface areas and densities of freeze-dried foods. Ag. Food Chem. 14:15.

Blackadder, D. A. and C. Hinchelwood. 1958a. Kinetics of the rearrangement and oxidation of hydrazobenzene in solution. (1). Rearrangement and spontaneous oxidation. 3. Chem. Soc. 2720.

Blackadder, D. A. and C. Hinchelwood. 1958b. Kinetics of the rearrangement and oxidation of hydrazobenzene in solution. (11). Catalyzed oxidation. 3. Chem. Soc. 2728.

Bronsted, J. N. 1922. Theory of the rate of chemical reactions. Z. Physik. Chem. 102:169.

Brunauer, S. 1945. The Adsorption of Gases and Vapors. Princeton University Press. Princeton, New Jersey.

Brunauer, S., P. H. Emmet and E. Teller. 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60:309.

Caurie, M. 1970. A new model equation for predicting safe storage moisture levels for optimum stability of dehydrated foods. J. Fd. Technol. 5:301.

Caurie, M. 1971a. A practical approach to water sorption isotherms and the basis for the determination of optimum moisture levels of dehydrated foods. J. Fd. Technol. 6:85.

Caurie, M. 1971b. A single layer moisture absorption theory as a basis for the stability and availability of moisture in dehydrated foods. J. Fd. Technol. 6:193.

Coggiola, I. M. 1963. 2,5-dihydro-2-furoic acid: A product of the anaerobic decomposition of ascorbic acid. Nature, London. 200:954.

Cox, E. G. and T. H. Goodwin. 1936. Crystalline structure of the sugars (III) ascorbic acid and related compounds. J. Chem. Soc. 769.

Csuros, Z. and J. Petro. 1955. Autoxidation of ascorbic acid as a function of temperature. Acta Chim. Acad. Sci. Hung. 7:199.

de Boer, J. H. and C. Zwikker. 1929. Adsorption as a result of polarization-adsorption isotherm. Z. Phys. Chem. 133:407.

Dekker, A. D. and R. G. Dickinson. 1940. Oxidation of ascorbic acid by oxygen with cupric ion as catalyst. J. Am. Chem. Soc. 62:2165.

Dennison, D., J. Kirk, J. Bach, P. Kokoczka and D. Heldman. 1977. J. Food Process. Preserv. 1:43.

Duden, R. 1971. Enzymic reactions in foods with very low water activity. Lebensmittel.-Wiss. u. Technol. 4:205.

Dye, J. L. and V. A. Nicely. 1971. A general purpose curve-fitting program for class and research use. J. Chem. Edu. 48:443.

Duckworth, R. B. 1971. Differential thermal analysis of frozen food systems. I. The determination of unfreezable water. J. Fd. Technol. 6:317.

Duckworth, R. B. and G. M. Smith. 1963. Diffusion of solutes at low moisture levels. Recent Adv. Food Sci. 3:230.

Eichner, K. 1976. The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates, in Water Relations of Foods: ed. R. B. Duckworth. Academic Press, New York.

Exner, O. 1964. Enthalpy-entropy relation. Coll. Czech. Chem. Commun. 29:1094.

Exner, O. 1973. The enthalpy-entropy relationship. Prog. Phys. Org. Chem. 11:411.

Eyring, H. 1935. Activated complex and the absolute rate of reactions. J. Chem. Phys. 3:107.

Feeney, R. E., G. Blankenhorn and H. B. F. Dixon. 1976. Carbonyl-amine reactions in protein chemistry. Adv. Prot. Chem. 29:135.

Fennema, O. R. 1976. "Water and ice" in Principles of Food Science, pt. 1, Food Chemistry. ed. O. R. Fennema, Marcel Dekker, Inc., New York.

Finholt, P., R. B. Paulssen and T. Higucki. 1963. Rate of anaerobic degradation of ascorbic acid in aqueous solution. J. Pharm. Sci. 52:948.

Fox, K. K., V. H. Holsinger, M. K. Harper, N. Howard, L. S. Pryor and M. J. Pallansch. 1963. Measurement of the surface areas of milk powders by a permeability procedure. Food Tech. 17:127.

Frenkel, Y. I. 1946. Kinetic Theory of Liquids. The Clarendon Press, Oxford.

Grant, N. H. and H. E. Alburn. 1965. Fast reactions of ascorbic acid and H_2O_2 in ice, a presumptive early environment. Biochemistry 4:1913.

Hand, D. B. and E. C. Greisen. 1942. Oxidation and reduction of vitamin C. J. Am. Chem. Soc. 64:358.

Harkins, W. D. and G. Jura. 1944. A vapor adsorption method for determination of the area of a solid without assumption of molecular area. J. Am. Chem. Soc. 66:1366.

Heldman, D. R. 1974. Computer simulation of vitamin stability in foods. Paper presented at seminar on Stability of Vitamins in Foods for the Association of Vitamin Chemists, Chicago, IL.

Henderson, S. M. 1952. A basic conception of equilibrium moisture. Agr. Eng. 33:24.

Huelin, F. E. 1953. Studies on the anaerobic decomposition of ascorbic acid. Food Res. 18:633.

Huelin, F. E., I. M. Coggiola, G. S. Sidhu and B. H. Kennet. 1971. The anaerobic decomposition of ascorbic acid in the pH range of foods and in more acid solutions. J. Sci. Fd. Agric. 22:540.

Jameson, R. F. and N. J. Blackburn. 1975. The role of Cu-Cu dinuclear complexes in the oxidation of ascorbic acid by O_2. J. Inorg. Nucl. Chem. 37:809.

Jameson, R. F. and N. J. Blackburn. 1976a. Role of copper dimers and the participation of copper (III) in the copper catalyzed autoxidation of ascorbic acid. Part II. Kinetics and mechanism in 0.100 mol dm^{-3} potassium nitrate. J. C. S. Dalton 534.

Jameson, R. F. and N. J. Blackburn. 1976b. Role of copper dimers and the participation of copper (III) in the copper catalyzed autoxidation of ascorbic acid. Part III. Kinetics and mechanism in 0.100 mol dm^{-3} potassium chloride. J. C. S. Dalton 1596.

Joslyn, M. A. and H. Supplee. 1949. Effect of sugars on conversion of dehydroascorbic acid. Food Res. 14:216.

Karel, M. 1973. Recent research and development in the field of low-moisture and intermediate foods. CRC Crit. Rev. Food Tech. 4:329.

Karel, M. and J. T. R. Nickerson. 1964. Effect of relative humidity, air and vacuum on browning of dehydrated orange juice. Food Technol. 18:104.

Karel, M., T. P. Labuza and J. F. Maloney. 1967. Chemical changes in freeze-dried foods and model systems. Cryobiology, 3:288.

Karel, M., S. Mizrahi and T. P. Labuza. 1971. Computer prediction of food storage. Modern Pack. 44:54.

Khan, M. M. Taqui and A. E. Martell. 1967a. Metal ion and chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation. J. Am. Chem. Soc. 89:4176.

Khan, M. M. Taqui and A. E. Martell. 1967b. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. II. Cupric and ferric chelate catalyzed oxidation. J. Am. Chem. Soc. 89:7104.

Khan, M. M. Taqui and A. E. Martell. 1968a. The kinetics of the reaction of iron(III) chelates of amino-polycarboxylic acids with ascorbic acid. J. Am. Chem. Soc. 90:3386.

Khan, M. M. Taqui and A. E. Martell. 1968b. Kinetics of metal ion and metal chelate catalyzed oxidation of ascorbic acid. III. Vanadyl ion catalyzed oxidation. J. Am. Chem. Soc. 90:6011.

Khan, M. M. Taqui and A. E. Martell. 1969. Kinetics of metal ion and metal chelate catalyzed oxidation of ascorbic acid. IV. Uranyl ion catalyzed oxidation. J. Am. Chem. Soc. 91:4668.

Kirk, J. R. and N. Ting. 1975. Fluorometric assay for total vitamin C using continuous flow analysis. J. Food Sci. 40:463.

Kurata, T. and Y. Sakurai. 1967. Degradation of L-ascorbic acid and mechanism of non-enzymatic browning reaction. II. Nonoxidative degradation of L-ascorbic acid including formation of 3-deoxy-L-pentosene. Agric. & Biol. Chem. 31:170.

Kurpianoff, J. 1959. "Bound water in foods," Fundamental Aspects of the Dehydration of Foodstuffs, the Macmillian Company, New York, NY.

Kwolek, W. F. and G. M. Bookwalter. 1971. Predicting storage stability from time-temperature data. Food Tech. 25:1025.

Labuza, T. P. 1968. Sorption phenomena in foods. Food Tech. 22:263.

Labuza, T. P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Tech. 2:355.

Labuza, T. P. 1976. "Interpretation of sorption data in relation to the state of constituent water" in Water Relations of Foods. ed. R. B. Duckworth. Academic Press, New York.

Labuza, T. P., J. F. Maloney and M. Karel. 1966. Autoxidation of methyl linoleate in freeze-dried model systems. II. Effect of water on cobalt catalyzed oxidation. J. Food Sci. 31:885.

Labuza, T. P., S. R. Tannenbaum and M. Karel. 1970. Water content and stability of low-moisture and intermediate moisture foods. Food Tech. 24:543.

Labuza, T. P., S. Cassil and A. J. Sinsky. 1972. Stability of intermediate moisture foods. 2: Microbiology. J. Food Sci. 37:160.

Laidler, K. J. 1965. Chemical Kinetics, second edition, McGraw-Hill, New York.

Langmuir, I. 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. J. Am. Chem. Soc. 40:1361.

Lee, S. H. and T. P. Labuza. 1975. Destruction of ascorbic acid as a function of water activity. J. Food Sci. 40:370.

Lee, Y. C., J. R. Kirk, C. L. Bedford and D. R. Heldman. 1977. Kinetics and computer simulation of ascorbic acid stability of tomato juice as functions of temperature, pH and metal catalyst. J. Food Sci. 42:640.

Leffler, J. E. 1955. The interpretation of enthalpy and entropy data. J. Org. Chem. 31:533.

Loncin, M., J. J. Bimbenet and J. Lenges. 1969. Influence of the activity of water on the spoilage of foodstuffs. J. Food Technol. 3:131.

Mack, T. E., D. R. Heldman and R. P. Singh. 1976. Kinetics of oxygen uptake in liquid food. J. Food Sci. 41:309.

Maloney, J. F., M. Karel, and T. P. Labuza. 1966. Autoxidation of methyl linoleate in freeze-dried model systems. I. Effect of water on the autocatlyzed oxidation. J. Food Sci. 31:878.

Mapson, L. W. 1945. Influence of halides on the oxidation of ascorbic acid. 2. Action of Cl^- on the cupric-cuprous system. Biochem. J. 39:228.

Martin, M. F. 1958. Factors in the development of oxidative rancidity in ready to eat oat flakes. J. Sci. Food Agric. 9:817.

Matz, S., C. S. McWilliams, R. A. Larsen, J. H. Mitchell, Jr., M. McMullen and B. Layman. 1955. The effect of variations in moisture content on the storage deterioration rate of cake mixes. Food Tech. 9:276.

Miller, J. and M. A. Joslyn. 1949. Effect of sugars on the oxidation of ascorbic acid. III. Influence of pH and type of buffer. Food Res. 14:354.

Mizrahi, S., T. P. Labuza and M. Karel. 1970a. Computer aided predictions of extent of browning in dehydrated cabbage. J. Food Sci. 35:799.

Mizrahi, J., T. P. Labuza, and M. Karel. 1970b. Feasibility of accelerated tests for browning in dehydrated cabbage. J. Food Sci. 35:804.

Mizrahi, S. and M. Karel. 1977. Accelerated stability tests of moisture sensitive products in permeable packages by programming rate of moisture contents. J. Food Sci. 42:1575.

Mousseri, J., M. P. Steinberg, A. I. Nelson and L. S. Wei. 1974. Bound water capacity of corn starch and its derivatives by NMR. J. Food Sci. 39:114.

Multon, J. L. and A. Guillot. 1976. "Water activity in relation to the thermal in activation of enzymic proteins," in Water Relations of Foods. ed. R. B. Duckworth, Academic Press. New York.

Ogata, Y., Y. Kosugi and T. Morimato. 1968. Kinetics of cupric salt-catalyzed autoxidation of L-ascorbic acid in aqueous solutions. Tetrahedron 24:4057.

Palnitkar, M. P. and D. R. Heldman. 1971. Equilibrium moisture characteristics of freeze-dried beef components. J. Food Sci. 35:799.

Pekkarinan, L. 1974. The mechanism of the autoxidation of ascorbic acid catalyzed by iron salts in citric acid solution. Finn. Chem. Lett. 233.

Pincock, R. E. and T. E. Kiovsy. 1966. Kinetics of reactions in frozen solutions. J. Chem. Edu. 43:358.

Pitt, J. I. 1976. "Xerophilic fungi and the spoilage of foods of plant origin," in Water Relations of Foods. ed. R. B. Duckworth. Academic Press, New York.

Polanyi, M. 1928. Anwendurg der langmuirschen theorie auf die adsorption von gasen an holzkohle. Z. Physik Chem. A138:459.

Pope, G. G. 1972. Effect of time, temperature and fortification level on the retention of ascorbic acid in fortified tomato juice. Ph.D. thesis, Ohio State University, Columbus, Ohio.

Purwadaria, H. K. 1977. Simulation of nutrient stability in dry foods during storage. M. S. Thesis, Michigan State University, East Lansing, MI.

Quast, D. G., M. Karel and W. M. Rand. 1972. Development of a mathematical model for oxidation of potato chips as a function of oxygen pressure, extent of oxidation and equilibrium relative humidity. J. Food Sci. 37:673.

Quast, D. G. and M. Karel. 1973. Simulating shelf-life. Modern Pack. 46:50.

Reimer, J. 1977. Prediction of vitamin C. retention in a stored dehydrated food. Ph.D. thesis, Massachusetts Institute of Technology, Boston, MA.

Reynolds, T. M. 1963. Chemistry of nonenzymic browning. I. The reaction between aldoses and amines. Adv. Food Res. 12:1.

Reynolds, T. M. 1965. Chemistry of nonenzymic browning. II. Adv. Food Res. 14:167.

Reynolds, T. M. 1969. Nonenzymic browning: sugar-amino interactions, in Symposium on Foods: Carbohydrates and Their Roles, ed. H. W. Schultz, R. F. Cain and R. W. Wrolstad. Avi. Publishing Company, Inc. Westport, Conn.

Rockland, L. B. 1957. A new treatment of hygroscopic equilibria. Food Res. 22:604.

Rockland, L. B. 1969. Water activity and storage stability. Food Tech. 23:1241.

Salwin, H. 1959. Defining minimum moisture contents for dehydrated foods. Food Tech. 13:594.

Salwin, H. 1962. The role of moisture in deteriorative reactions of dehydrated foods. Proceedings of Conference on Freeze-Drying of Foods. National Academy of Sciences National Research Council.

Salwin, H. 1963. Moisture levels required for stability in dehydrated foods. Food Tech. 17:1114.

Scatchard, G. 1922. Statistical mechanics and reaction rates in liquid solutions. Chem. Rev. 10:229.

Schaleger, L. L. and F. A. Long. 1963. Entropies of activation and mechanism of reactions in solutions. Prog. Phys. Org. Chem. 1:1.

Schultz, H. W., E. Day and R. Sinnhuber. 1962. Lipids and their oxidation. Avi. Publishing Company, Inc. Westport, Conn.

Scott, W. J. 1957. Water relations of food spoilage organisms. Adv. Food Res. 7:83.

Shanbhag, S., M. P. Steinberg and A. I. Nelson. 1970. Bound water defined and determined at constant temperature by wide-line NMR. J. Food Sci. 35:612.

Shtamm, E. V. and Yu. I. Shurlator. 1974a. Catalysis of the oxidation of ascorbic acid by copper(II) ions. I. Kinetics of the oxidation of ascorbic acid in the copper(II) - ascorbic acid - molecular oxygen system. Zhur. Fiz. Khin. 48:1454.

Shtamm, E. V. and Yu. I. Skurlator. 1974b. Catalysis of the oxidation of ascorbic acid by copper(II) ions. IV. Kinetics of the oxidation of ascorbic acid in the copper(II) - ascorbic acid - hydrogen peroxide system. Zhur. Fiz. Khin. 48:1857.

Shtamm, E. V., A. P. Purmal and Yu. I. Skurlator. 1974a. Catalysis of the oxidation of ascorbic acid by copper(II) ions. II. Anaerobic oxidation of ascorbic acid by copper(II) ions. Zhur Fiz. Khin. 48:2229.

Shtamm, E. V., A. P. Purmal and Yu. I. Skurlator. 1974b. Catalysis of the oxidation of ascorbic acid by copper(II) ions. III. Mechanism of oxidation by molecular oxygen. The role of copper(I) ions. Zhur. Fiz. Khin. 48:2233.

Simon, I. B., T. P. Labuza, and M. Karel. 1971. Computer aided prediction of food storage stability: oxidative deterioration of a shrimp product. J. Food Sci. 36:280.

Singh, R. P., D. R. Heldman and J. R. Kirk. 1976. Kinetics of quality degradation: ascorbic acid oxidation in infant formula during storage. J. Food Sci. 41:304.

Sood, V. C. and D. R. Heldman. 1974. Analysis of a vapor pressure manometer for measurement of water activity in nonfat dry milk. J. Food Sci. 39:1011.

Stevens, H. H. and J. B. Thompson. 1948. The effect of shortening stability on commercially produced army ration biscuits. II. Development of oxidation during storage. J. Amer. Oil Chemists Soc. 25:389.

Szent-Gyorgi, A. 1928. Observations on the function of peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative. Biochem. J. 22:1387.

Thompson, L. U. and O. Fennema. 1971. Effect of freezing on oxidation of L-ascorbic acid. J. Agr. Food Chem. 19:121.

Tillmans, J., P. Hirsch and W. Hirsch. 1932a. Reduction capacity of plant foodstuffs and its relation to vitamin C(I) reducing substances of lemon juice. Z. Untersuch. Lebensm. 63:1.

Tillmans, J., P. Hirsch and F. Siebert. 1932b. Reduction capacity of plant foodstuffs and its relation to vitamin C(II) reducing substance of lemon juice as a stabilizer for the true vitamin. Z. Untersuch. Lebensm. 63:21.

Tillmans, J., P. Hirsch and J. Jackisch. 1932c. Reduction capacity of plant foodstuffs and its relation to vitamin C(III) quantities of reducing substance in various fruits and vegetables. Z. Untersuch. Lebensm. 63:241.

Toledo, R., M. P. Steinberg and A. I. Nelson. 1968. Quantitative determination of bound water by NMR. J. Food Sci. 33:315.

Vojnovich, C. and V. F. Pfeifer. 1970. Stability of ascorbic acid in blends with wheat flour, CSM and infant cereals. Cereal Sci. Today 19:317.

Waletzko, P. and T. P. Labuza. 1976. Accelerated shelf-life testing of an intermediate moisture food in air and in oxygen-free atmosphere. J. Food Sci. 41:1338.

Wanninger, L. A., Jr. 1972. Mathematical model predicts stability of ascorbic acid in food products. Food Tech. 26:42.

Weissberger, A., J. E. LuValle and D. S. Thomas, Jr. 1943. Oxidation processes. XVI. The autoxidation of ascorbic acid. J. Am. Chem. Soc. 65:1934.

Weissberger, A. and J. E. LuValle. 1944. Oxidation processes. XVII. The autoxidation of ascorbic acid in the presence of copper. J. Am. Chem. Soc. 66:700.

Zilva, S. S. 1923a. Influence of reaction on the oxidation of the antiscorbutic factor in lemon juice. Biochem. J. 17:410.

Zilva, S. S. 1923b. Note on the conservation of the potency of concentrated antiscorbutic extracts. Biochem. J. 17:416.

Zilva, S. S. 1924a. Note on the conservation of the potency of concentrated antiscorbutic preparations. II. Biochem. J. 18:186.

Zilva, S. S. 1924b. Antiscorbutic fraction of lemon juice. Biochem. J. 18:632.

Zilva, S. S. 1925. The antiscorbutic fraction of lemon juice. III. Biochem. J. 19:589.

Zilva, S. S. 1927. Note on the precipitation of the antiscorbutic factor from lemon juice. Biochem. J. 21:354.

Zsigmondy, R. 1911. Uber die struktur des gels der kieselsaure theorie der entwasserung. Z. Anorg. Chem. 71:356.