STORAGE STABILITY AND IMPROVEMENT OF INTERMEDIATE MOISTURE FOODS

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ABSTRACT

This study was designed to determine methods for improvement of shelf-life stability of intermediate moisture foods. It was found that vitamin C is the most limiting vitamin from a nutritional standpoint with its rate of destruction increasing with a_w. Techniques for microbial challenge studies were developed. It was found that organisms have a higher growth a_w limit if the IMF is prepared by the adsorption process and long times (about 6 months) are needed for challenge studies. Several alternative antimycotic systems were found. It was also found that the vegetative cells of pathogens have a maximum heat resistance in the IMF a_w range. Non-enzymatic browning is a problem if reducing sugars are present. If glycols are in the formula, the IMF should have as high an a_w as possible. The reverse is true if lipid oxidation occurs. In addition, to prevent rancidity, anti-oxidants and a low O_2 atmosphere are necessary. The package also must be a good moisture barrier. The use of high temperature (45^oC) is useful to shorten shelf-life testing time. New methods were also found for accurate a_w and pH measurement.

SUMMARY AND RECOMMENDATIONS

This study was a continuation of Contract NAS 9-12560 which was designed to determine methods for improvement of shelf-life stability of intermediate moisture foods. Areas under investigation were microbiological stability, nutritional losses, non-enzymatic browning, validity of accelerated shelf-life testing and design of improved techniques for measurement of physical properties of IMF. Based on the results obtained, several recommendations were made for industrial processors of IMF. These recommendations are as follows:

1. If nutritional claims are to be made with respect to vitamins, ascorbic acid will be the vitamin limiting shelf-life. To improve its stability, processors should:
   a. Keep the a_w as low as possible within IMF limits of palatability.
   b. Utilize a humectant to increase the aqueous phase viscosity (such as glycerol) as it will reduce destruction rate.
   c. Coat the ascorbic acid with an edible, non-water absorbing impermeable layer.
   d. Incorporate the ascorbic acid into a fat phase or icing.
   e. Utilize packaging methodology to keep the oxygen level as low as possible. The cost of extra packaging can be balanced out against the cost of the needed high overrun if no protection is used.

2. Microbiological growth is a problem in IMF, especially molds. With respect to microbes, to maximize shelf-life processors should:

   a. Utilize an adsorption process for water addition to IMF as this gives a high limiting a_w for growth of all potential problem organisms. The extra cost of the process must be balanced against the extra protection.

   b. Not rely on short term microbial challenge studies. They should be carried out for at least 6 months for IMF.

   c. Utilize a humectant/inhibitor system to protect against mold growth. The following systems are ideal at a_w 0.85.

      (1) Propylene glycol at 2% w/w

      (2) Glycerol at 2% w/w

      (3) The above at 1% with methyl paraben at 0.033% and propyl paraben at 0.007%.

      (4) Pimaricin at 0.002% if FDA approves

      (5) Butane diol at 2% if FDA approves

      These would all be effective at pH 4 to 6.

   d. Systems that work against mold growth do not necessarily inhibit Staphylococcus aureus, a potential pathogen. Specific challenge studies must be done using this organism.

   e. Although the pH is important in controlling growth, specific food acids themselves are not effective inhibitors. In combination with specific humectants, and growth inhibitors they reduce the amount needed to give protection.

Processors should keep the pH of the food as low as possible within palatability limits.

f. The heat resistance of vegetative cells, especially pathogens, is at a maximum in the IMF a_w range. Therefore, processors should precook--pasteurize any ingredient at high or low a_w before combining into the final product.

3. Non-enzymatic browning is a serious chemical problem during storage of IMF. To minimize deterioration:

   a. Reducing sugars should not be utilized in the formula unless absolutely dictated by cost and availability of other humectants.

   b. The ratio of sugar to protein is important. The upper and lower limits should be determined.

   c. Because of cost of proteins and availability many processors freely substitute proteins. Their relative effect on browning should be determined.

   d. Browning has a rate accelerating factor of about 5 x for each $10^{o}C$ rise. Thus, the processor should not heat treat (such as pasteurize, extrude, etc.) the product after combination of the sugar and proteins. They should be cooked separately, cooled, then combined.

   e. The temperature in the distribution chain should be maintained as low as possible.

   f. Although liquid humectants, such as glycerol, are useful from an antimicrobial standpoint, and increase the aqueous phase viscosity so as to slow down certain chemical reactions

such as vitamin C destruction, they also act as a dissolving phase for reducing sugars. This would then allow non-enzymatic browning to actually increase in rate as a_w decreases to near the monolayer. This means processors should keep the a_w as high as possible consistent with other reactions and microbial deterioration. Good moisture proof packaging is mandatory to prevent drying out and an increased rate of deterioration.

4. Measurement of the water activity of IMF is important. Many methods used today are inaccurate. A new simple low-cost technique using moisture exchange with a standard dry cellulose has been developed. It also allows use of large food sample sizes to reduce the error caused by heterogeneity.
5. The measurement of the pH of dry and IMF systems has had questionable methodology. In a comparison of various techniques, it was found that direct measurement with a non-aqueous type electrode was most reliable and accurate. In systems which are too dry, the special dilution technique should be used with the pH found by extrapolation to zero dilution on gran plot paper.
6. To prevent deterioration during storage, all ingredients should be precooked to destroy any enzymes present as these will react in the IMF a_w range.
7. To prevent rancidity, the product:
   a. Should have antioxidants incorporated, including BHA/BHT and the metal chelating agent, EDTA.

b. Should have as high quality ingredients as possible.

c. A low oxygen atmosphere is a necessity and must be used. The type of pouch and system used must be balanced out with the shelf-life improvement.

d. As low an a_w as possible should be used.

8. Textural changes which reduce palatability occur rapidly during storage of IMF. To prevent them:

   a. Deterioration from NEB must be prevented.

   b. Lipid oxidation should be prevented.

   c. A highly water-impermeable barrier should be used.

9. The use of accelerated shelf-life testing procedures is possible with IMF with respect to chemical stability. It is recommended that processors:

   a. Utilize high temperature (40-45^{o}C) to shorten shelf-life testing time.

   b. Several temperatures should be used (45, 40, 35^{o}C) so that accurate predictions of the rates of deterioration at room temperature are possible.

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Storage Stability and Improvement of Intermediate Moisture Foods

I. Introduction

A. Objectives

The purpose of the contract is to study the mechanisms of deterioration of intermediate moisture foods (IMF) and find methods to improve their shelf life. This is being conducted under Phase II of Contract NAS 9-12560. Previous studies supported by NASA have shown that the main problems in IMF stability were microbiological growth, lipid oxidation, non-enzymatic browning and loss of nutritional value. The basic premise of the present phase was to investigate each of these areas from the basis of how the reactions are controlled by the amount of water in the IMF as measured by both the moisture content and the thermodynamic availability of water. Based on this recommendations would be made to improve shelf life. The value of these results to the food processor would be immense, as present IMF products for human consumption have a relatively short shelf life in the marketplace. In fact, several have been withdrawn from the market after only a few months testing.

These results would be most important for the main objective of this study in the formulation for production of an IMF product to serve as a meal replacement and emergency ration in the space shuttle and sky lab programs. The intent is to have a single food item which is stable without heat processing, refrigerated storage or freezing. This food item should have a balanced nutrient composition supplying 25-35% of the daily needs, be high in energy and require minimal handling or preparation. The requirements are partially met by the same criteria used for IMF pet foods

and the several human foods on the market, but as noted, stability is a major problem. Through the study of the interaction of water with the food components, recommendations could be made to minimize deterioration.

B. Literature Review

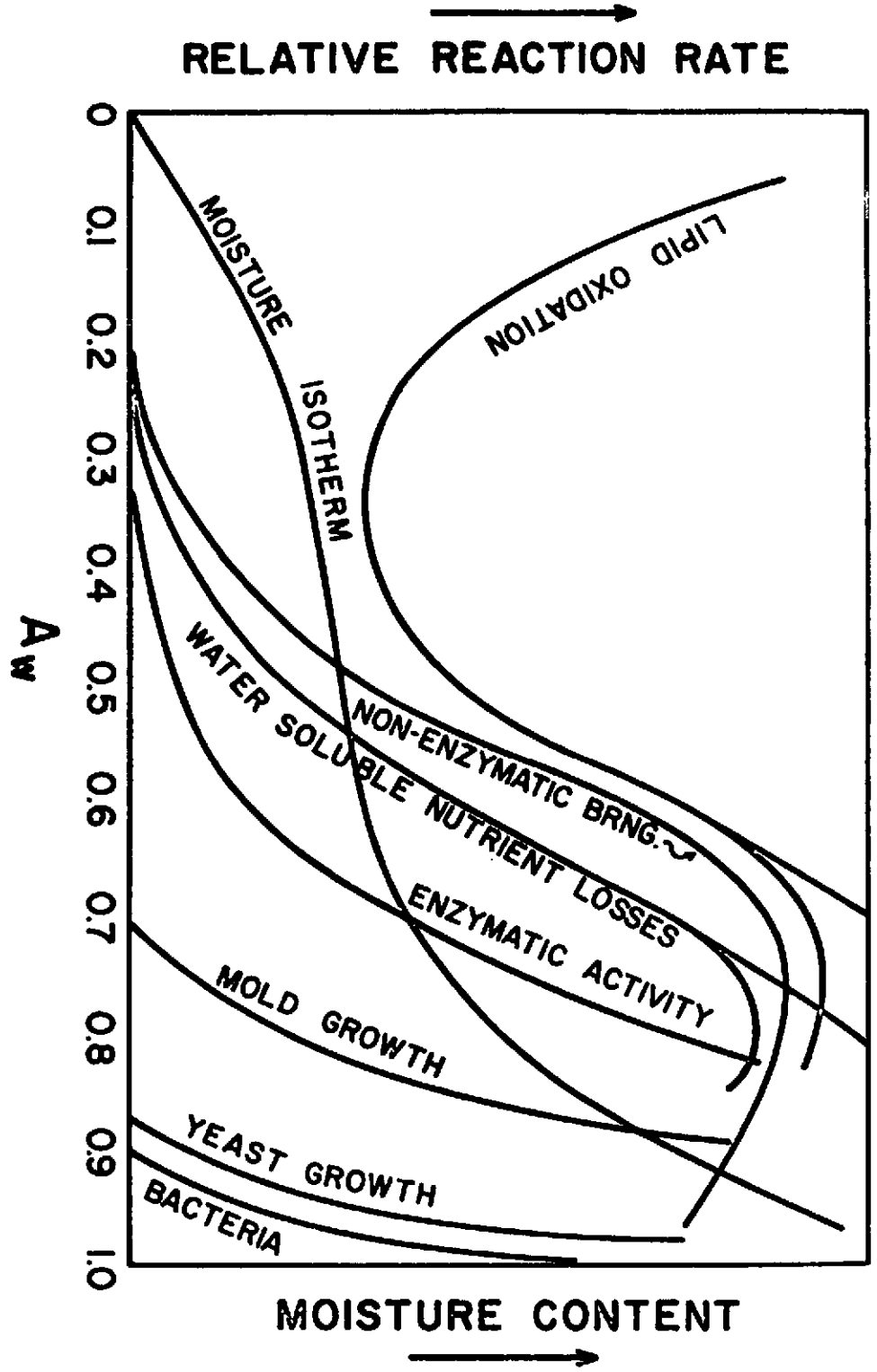
1. Water activity and moisture content: Effect on food quality

One of the primary attributes controlling the quality and acceptability of foods in general is the water content. This is because water is a major component of most foods.

With respect to reactions occurring in foods, water acts as a solvent by dissolving chemical species and allowing them to diffuse and react within it. The control of water content of food is thus a basic food processing technique. This can be done by either drying or other means of water removal. Water, however, does not have to be completely removed and in the new intermediate moisture food technology, chemical agents are added which bind the water to make it unavailable as a solvent. Thus, an important attribute of food quality is not only the absolute amount of water present, but also the physico-chemical state in which water exists.

In most foods after processing, microbiological growth is prevented during storage, however, chemical deterioration does occur affecting quality. Usually the higher the a_w, the faster the reaction as seen in Figure 1. As has been shown by Rockland (1969) and reviewed by Labuza (1971), the relationship between the loss of quality of the food and the moisture content of the food is best represented by the term "water activity" or "a_w" as represented by the X axis of Figure 1. Figure 1 shows the relative rates of deteriorative reactions as a function

Figure 1. Stability Map of Foods as a Function of a_w



of a_w. As seen, most reaction rates increase as a_w increases into the intermediate moisture range (a_w 0.6--0.9). At the higher end of this range, microbes begin to grow. This is usually prevented by the addition of some growth inhibitor.

Water activity, the availability of water for biological and chemical reaction, is determined by its vapor pressure in a system relative to that of pure water. It is defined by equation (1):

$$a_w = p/p_o = \frac{E.R.H.}{100} \qquad (1)$$

where a_w = water activity

p = partial pressure of water in food

p_o = saturation pressure of water at specified temperature

E.R.H.= equilibrium relative humidity (%)

The basis of food processing is to prevent the microbiological and chemical deterioration during processing and storage. Many methods have been utilized to lower water activity or remove water from foods. The basic principle of intermediate moisture foods is to lower a_w below where pathogenic organisms can grow but still have enough water present for palatability. Since not enough water is removed to prevent chemical deterioration, other means are needed to increase stability. It is the purpose of this study to determine these means.

The physical-chemical factors responsible for the lowering of water activity have been reviewed extensively by Van Arsdel and Copley (1963) and Labuza (1968, 1971). Many different factors are responsible for lowering water activities. First and most important is the effect described by Raoult's Law (Equation 2):

$$X_s = \frac{N_{solute}}{N_{solute} + N_{H_2O}} \quad (2)$$

$$X_{H_2O} = 1 - X_s \quad (3)$$

$$a_w = \gamma X_{H_2O} \quad (4)$$

where X_s = mole fraction of solute

X_{H_2O} = mole fraction of H_2O

N_{H_2O} = moles water

N_{solute} = moles solute

γ = activity coefficient

= 1 for ideal solutions

According to this law, water activity can be lowered either by adding solutes or by removing water. When a solute such as sodium chloride is dissolved in water, the availability of water is decreased. This is partially due to the creation of a hydration shell of water around a dissolved molecule. The water molecules are bound in one to several layers. The relative vapor pressure of the water is decreased as a function of the mole fraction of water to the total moles of water and solute in solution. However, the relative effectiveness of several edible agents used for lowering water activity appears to be different. Brockman (1973) and Bone (1969) determined the effectiveness of various compounds for adjusting water activity as seen in Table 1. An important aspect of IMF technology is to find an a_w lowering agent which will not impart a high degree of flavor to the food. Several laboratories are now investigating this.

The presence of small diameter pores is another factor in the lowering of water vapor pressure; the capillary effect. As found

TABLE 1

EFFECTIVENESS OF VARIOUS COMPOUNDS

FOR LOWERING WATER ACTIVITY

Solute \ a_w	0.75	0.80	0.85	0.90
Sodium chloride	274	332	422	600
Glycerol	72	96	132	203
Glucose			*	90
Sucrose		*	49	71
Gelatin	23.4	26.7	32.1	40.2
Serum albumin	18.0	20.6	23.9	28.7

unit : grams of water per 100 grams dry solute at a_w
* : solubility limiting

Reference - Brockman (1973)

in practice and as predicted from the Kelvin equation, as the size of a capillary is reduced, the vapor pressure of water is lowered. Bluestein and Labuza (1972) have shown most of the capillaries in a food are of greater than 10μ size but as water is removed, the water present in small capillaries (<100 Å) comprises about 7-10% of the total water. This is important only from the standpoint of finish drying and should not affect IMF technology.

A third factor responsible for lowering of water activity can account partially for the fact that hysteresis occurs. Labuza and Rutman (1968) and Gregg and Sing (1967) have reviewed some of the basic reasons for hysteresis. In general, hysteresis is based on the fact that the amount of water bound at a given a_w can be affected by the method of making the system. Thus, two systems of the same solids content at the same a_w can have a different amount of water present. Usually a food prepared by desorption or removal of the water has the higher water content at a given a_w. This method of preparation can also affect stability as shown in Phase I of this contract. Usually the higher moisture system deteriorates faster.

The last factor controlling a_w is the interaction of water with solid surfaces as well as with molecules of high molecular weight. Water interacts with the polar groups on surfaces and can be held very tightly to form a monolayer. Water above this monolayer is usually thought of as being the same as pure water in that it is totally available for reaction. However, some interactions cause the rate to decrease as the a_w decreases to the monolayer value even though in a food the water has a lower vapor pressure or a_w than pure water as illustrated in the general isotherm (Figure 2).

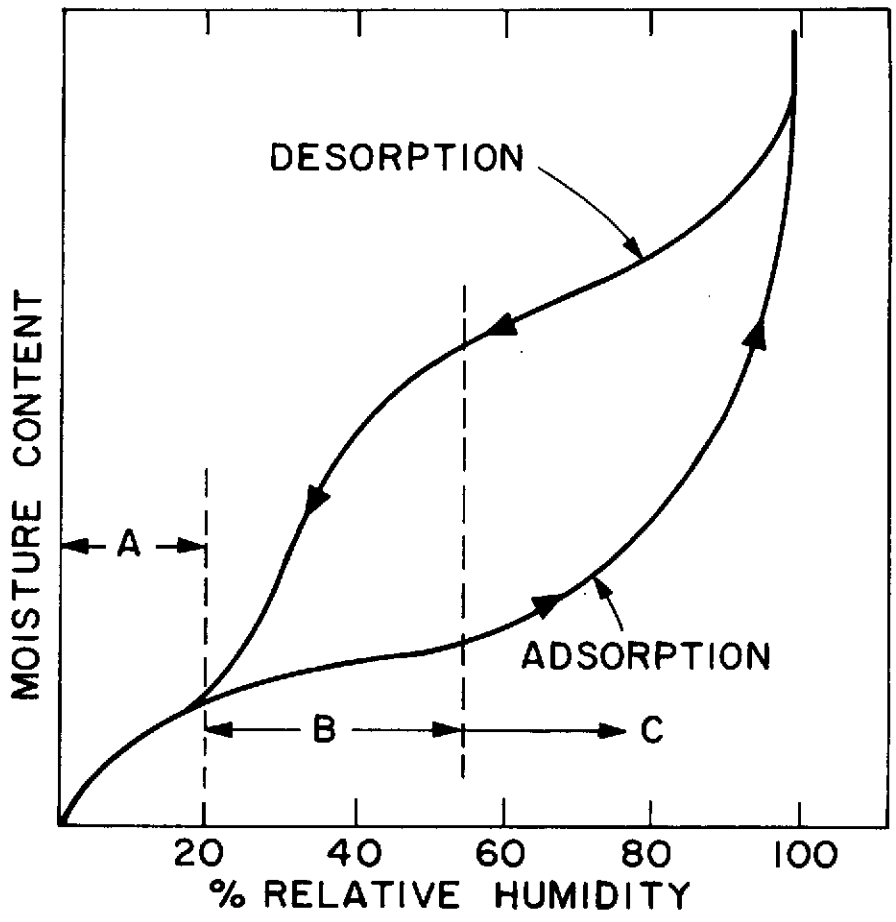


Figure 2. General Isotherm of a Food at Constant Temperature

This water plays an important role in the food since it still acts as bulk water as related to:

a. Water acts as a solvent permitting the dilution and mobilization of reactants and products.

b. Water may act as a reactant participating in specific reactions.

c. Water may modify properties of reactants by hydrogen bonding or hydration.

d. Water may modify the system by swelling the supporting matrix and decreasing the viscosity in the aqueous phase.

The effect of a_w on several deteriorative reactions will be reviewed below.

Labuza et al. (1970) and Labuza (1971) have discussed the chemical stability of foods as a function of both moisture content and water activity. These results are relative to the stability of IMF.

One of the major reactions limiting storage stability of dehydrated foods is non-enzymatic browning through the Maillard reaction. This is a reaction of reducing sugars under the influence of either free amino acids or protein side-chains leading to darkening, off-flavor and loss of solubility of proteins. Water plays an important role in the non-enzymatic browning reaction in which it serves to dissolve the substrates and mobilize them for reaction. Thus the reaction rate increases with increasing moisture content. However, Sharp (1963) showed that the reaction rate decreased at high water activity (>.60). Labuza et al. (1970) proposed that this was most likely due to dilution effects since the moisture content doubles or triples from a_w 0.6 to 0.75 and thus the concentration of the reactants is reduced. The real problem in IMF is that many of the

needed a_w lowering agents are reducing sugars and thus substrate is present to cause the reaction.

Another reaction controlling the stability of food is lipid oxidation. This is a complex reaction involving an unsaturated double bond of a lipid and oxygen by means of a free radical mechanism. Recent works showing the effect of a_w have been done by Maloney et al. (1966); Labuza et al. (1972); Chou et al. (1973); Chou and Labuza (1974) and Labuza and Chou (1974). They showed that there is a progressive antioxidant effect of water up to 0.5 a_w. Water hydrogen-bonds to the hydroperoxides produced during the free-radical reaction. Thus the water ties up the peroxides and slows the rate. In addition, water lowers the catalytic activity of trace metals. The antioxidant effect increases until the lipid interface is saturated with water.

As the water activity is increased into the intermediate moisture range, oxidation of lipids increases again. Labuza and Chou (1974) and Heidelbaugh and Karel (1970) found that because of increased mobility and solubility, trace metals were able to move more rapidly to oxidation sites and overcome the antioxidant properties of water. This implies a very short shelf life for IMF if unsaturated lipids are present. In addition, foods prepared by desorption methods oxidized at a faster rate (Chou and Labuza, 1974; Labuza and Chou, 1974; Chou et al., 1973). This was due to the greater mobility in the less viscous system.

A similar role of water occurs with respect to the stability of many of the essential vitamins in foods. Karel and Nickerson (1964) studied the effects of relative humidity, air and vacuum on browning and loss of ascorbic acid in dehydrated orange crystals. The samples

were divided into two groups, one stored in air and the other thoroughly deaerated and stored under vacuum. The water activity was maintained at several levels between 1% to 53.7% relative humidity. Their results indicated that, under all conditions of storage investigated, the ascorbic acid content of orange crystals decreased rapidly with time of storage. Oxygen had little effect on the rates of destruction of ascorbic acid but an increase in water content or a_w increased the rate of ascorbate destruction immensely.

Thompson and Fennema (1971) studied the rate of oxidation of ascorbic acid in acetate buffer solutions at various concentrations, and at temperatures ranging from +21 to $-23^{\circ}C$. The above-zero reaction rate constants of any given sample decreased linearly with temperature. The reaction rate constants of the samples which contained 85 mg ascorbic acid per 100 ml of solution at pH 4.6 declined greatly when passing from 0 to $-1^{\circ}C$ (partially frozen) and those of the samples which contained 0.074 mg ascorbic acid at pH 5.5 increased significantly. They explained the results by two factors: first, the solubility of oxygen in a solution decreases with increasing solute concentration; secondly, when a sample is brought to solid—liquid equilibrium at a given sub-freezing temperature, in initially dilute samples a greater amount of ice is formed and a greater increase in solute concentration occurs than in initially concentrated samples. Thus Karel and Nickerson's results (1964), the insignificant differences in rates of ascorbic acid destruction between air and vacuum stored samples, can be attributed to the poor solubility of oxygen in the aqueous phase of water in dehydrated orange juice which has a very low a_w corresponding to the a_w of frozen systems (Labuza, 1971).

Kapsalis (1973) studied the role of water with respect to thiamine and carotene. With respect to the thiamine, good retention at 43.3°C was exhibited between 0 and 0.23 a_w. Most likely not enough water was present to dissolve the vitamin. In contrast, the carotene content was adversely affected by dry conditions. Minimum retention was shown at 0% RH, while higher retention was evident between 0.11 to 0.5 a_w. This indicates the protective role of water towards lipid oxidation since vitamin A is a fat soluble vitamin which is oxidized in a manner similar to unsaturated fats. This is evidence that water may exercise either a protective or adverse role in food stability, depending on the particular component involved.

An important consideration with regard to moisture and a_w is the effect on the texture. Many dehydrated foods will become tough during storage. The higher the a_w of the food, the greater is the increase in hardness of the product during storage making it more unacceptable. Labuza (1973) explained that hardness may be caused by lipid oxidation and non-enzymatic browning acting on proteins causing irreversible aggregation and loss of water holding capacity.

The last area of concern, but a most important criterion for quality, is microbiological growth in the intermediate moisture food system. As seen in Figure 1, microorganism growth ceases at fairly high a_w's (Scott, 1957; Troller, 1973). This is probably due to the shut down of enzyme systems or the cessation of transport across the cell membrane. One problem with IMF is that to obtain stability with respect to molds, the a_w must be reduced below 0.7. This, however, creates a dry, unpalatable food. Thus, it is desirable to keep IMF products above this level; however,

they must also be kept below a_w 0.85. This is considered the upper limit since it is the point at which the pathogenic species Staphylococcus aureus can grow. To cope with this situation and produce an IMF of high a_w, processors have added various glycols which have antimycotic activity as well as adding specific antimetabolites. Little information is available, however, as to their relative effectiveness and their effect on flavor and quality. In addition, in formulating their product, processors might use the fact that the microorganisms present in foods prepared by an adsorption technique (the lower branch of the hysteresis curve in Figure 2) have a higher growth limiting a_w (Labuza et al., 1972a; Plitman et al., 1973).

Overall the safety factors, such as microbiological growth, as well as the quality characteristics, such as the flavor, texture, color and nutritional value of intermediate moisture foods, are markedly influenced by the water content and water activity as determined by the various reactions related above. The food industry must obtain information to determine the optimum moisture content and water activity in each food in order to use it in controlling the stability and acceptability of the food and to determine means to prevent or slow the deteriorative processes.

C. Specific Objectives

1. Determine the rate of ascorbic acid loss in IMF systems as a function of a_w and moisture content.

2. Examine the microbiological stability of IMF with respect to:

   a. Method of preparation — moisture hysteresis

   b. Influence of glycols and antimycotics on growth

   d. Influence of pH and various acids on growth

d. Influence of pasteurization temperatures on heat resistance as a function of a_w.

3. Study the mechanism and affect of a_w and water content on the kinetics of non-enzymatic browning in model systems in the IMF range.

4. Develop a simple system for a_w measurement in the high a_w range.

5. Develop a method for pH determination of IMF.

6. Study the stability of an IMF based on granola, peanut butter and sugar.

7. Determine the applicability of accelerated temperature on predicting the stability of an IMF at room temperature.

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II

DESTRUCTION OF ASCORBIC ACID AS A FUNCTION OF WATER ACTIVITY IN INTERMEDIATE MOISTURE MODEL SYSTEMS

II. Destruction of Ascorbic Acid as a Function of Water Activity in Intermediate Moisture Model Systems.

A. Cellulose-Glycerol Model System Study

1. Introduction

It is well-known that some important attributes which control quality of food in storage are both the absolute amount of water in the food and the physico-chemical state in which water exists. As has been shown by many investigators (Stitt, 1958; Labuza, 1968; Bone, 1969), the relationship between the loss of food quality and the moisture content is best represented by the term "water activity".

In studying intermediate moisture foods, Labuza (1971) and Labuza et al. (1972a) found that at the same water activity, the rate of several chemical reactions can be very different depending upon whether an adsorption or a desorption process was involved in preparing the system. They reported that unsaturated lipids oxidized faster for systems prepared on the desorption branch of the hysteresis loop which contain a higher moisture content than for adsorption systems at the same water activity. The effect was postulated to be due to the increased mobility of reactants and lower viscosity in the more dilute aqueous phase of the desorption system. Chou et al. (1973) confirmed the higher reaction rate for lipid oxidation in desorption systems with both high and low trace metal content. Labuza et al. (1972a) also found a difference in limiting a_w for growth of microorganisms depending on which branch of the isotherm the food was on.

With the advent of increased interest in nutrition on the part of both consumers and food processors, it is becoming increasingly important to understand what happens to the nutritional value of food products under

storage. Karel and Nickerson (1964), Jensen (1967) and Vojnovich and Pfeifer (1970) studied the stability of ascorbic acid in various dehydrated foods as a function of water content up to about a_w 0.5.

Karel and Nickerson (1964) studied the effects of relative humidity on loss of ascorbic acid in dehydrated orange juice. The samples were divided into two groups, one stored in air and the other thoroughly deaerated and stored under vacuum. The water activity was maintained at 1%—53.7% relative humidity. Their results indicated that, under all conditions of storage investigated, the ascorbic acid content of orange crystals decreased linearly with time of storage. Oxygen had little effect on the rates of destruction of ascorbic acid but an increase in water content or a_w increased the rate of ascorbate destruction. Jensen (1969) reported similar results on loss of ascorbic acid in seaweed and seaweed meal. The ascorbic acid was very sensitive to increase in moisture content. Even at 4^oC almost all the ascorbic acid was lost after four months of storage in the sample containing 25% moisture. With 10% moisture in the sample, approximately one-third of the ascorbic acid was still present in the meal after one year at 4^oC. The results of these studies are shown in Table 1.

Vojnovich and Pfeifer (1970) studied the stability of ascorbic acid in blends with wheat flour, corn-soya-milk, and infant cereals at various temperatures and moisture contents. Since data from all storage tests can be represented by straight lines on semilog plots, the kinetics covering the rate of destruction of ascorbic acid in all cereal blends are similar to those of a first order reaction. Destruction rates and activation energies were calculated as shown in Table 2. It is hard to

TABLE 1

DESTRUCTION RATES OF ASCORBIC ACID IN DEHYDRATED FOOD PRODUCTS

Product	H_2O content g H_2O/100g solids	T°C	Half life (days)	E_a (Kcal/mole)
Seaweed *1	11.1	25	105	
		10	182	7.65
		4	310	
	17.6	25	45	
		10	120	13.1
		4	240	
	33.3	25	9.6	
		10	10	30.3
		4	15	
Orange juice *2	11.0	37	20.7	
	7.4	37	28.4	
	3.2	37	85.7	
	1.1	37	174.6	

*1 from Jensen (1969)

*2 from Karel and Nickerson (1964)

TABLE 2

DESTRUCTION RATES OF ASCORBIC ACID

IN VARIOUS DRY CEREAL BLENDS

	Water Content	T (°C)	K (day^{-1})	E_a (Kcal)/mole
Corn soya milk	11.8%	45	8.74×10^{-2}	
		37	3.20×10^{-2}	34.61
		26	3.00×10^{-3}	
	10.4%	45	2.94×10^{-2}	
		37	8.29×10^{-3}	34.87
		26	1.29×10^{-3}	
	8.0%	45	1.86×10^{-3}	
		37	8.57×10^{-4}	18.82
		26	2.86×10^{-4}	
Wheat flour	14.6%	45	1.96×10^{-2}	
		37	1.86×10^{-3}	37.68
		26	4.29×10^{-4}	
	13.7%	45	7.00×10^{-3}	
		37	1.29×10^{-3}	30.92
		26	2.86×10^{-4}	
	12.9%	45	2.14×10^{-3}	
		37	5.71×10^{-4}	27.14
		26	1.43×10^{-4}	
Mixed cereal	10.7%	45	5.81×10^{-2}	
		37	4.59×10^{-2}	4.85
		26	3.59×10^{-2}	
	7.0%	45	4.94×10^{-2}	
		37	2.77×10^{-2}	12.47
		26	1.46×10^{-2}	
	5.0%	45	2.91×10^{-2}	
		37	1.53×10^{-2}	14.81
		26	6.71×10^{-3}	

From Vojnovich et al. (1970)

conclude the effect of water content on activation energy from their results. The table shows that in corn-soya-milk the activation energy increased with increasing water content. However, in mixed cereal, the activation energy decreased with increasing water content. Wanninger (1972) proposed a mathematical model for predicting stability of ascorbic acid and tested his model with the data of Vojnovich and Pfeifer. A model assuming that water affects the activation energy was tested and rejected. He concluded that water did not affect the activation energy for the data. However, the data in Table 2 show that it does. The problem lies in that the rates in some cases are so slow that the rate constant k can be in large error thus giving a larger error to E_a.

There is a marked similarity between the effect of water on the destruction of ascorbic acid and the effect on non-enzymatic browning. For example, Mizrahi et al. (1970) reported a decrease in activation energy with water content in dehydrated cabbage.

These studies show a lack of good data in the higher a_w range, especially with respect to intermediate moisture foods. This study was designed to obtain that data and to determine the effect of sorption hysteresis on destruction of ascorbate.

2. Materials and Methods

a. Ascorbic acid measurement (Horwitz, 1965)

(1) Reagents

(a) Metaphosphoric acid—acetate acid stabilizing extracting solution. Dissolve, with shaking, 15 g glacial HPO_3 pellets or freshly pulverized stick HPO_3 in 40 ml HOAc and 200 ml H_2O; dilute to 500 ml and filter rapidly through fluted paper into

glass stoppered bottle. (HPO_3 slowly changes to H_3PO_4, but if stored in a refrigerator solution remains satisfactory 7-10 days).

(b) Ascorbic acid standard. USP reference L-ascorbic acid; keep cool, dry, and out of direct sunlight in desiccator.

(c) Indophenol standard solution. Dissolve 50 mg 2,6-dichloroindophenol Na salt (Eastman No. 3463), that has been stored in desiccator over drierite, in 50 ml H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously and when dye dissolves dilute to 200 ml with H_2O. Filter through fluted paper into an amber glass stoppered bottle. Keep stoppered, out of direct sunlight and store in refrigerator. Decomposition products that make the end point indistinct occur in some batches of dry indophenol and also develop with time in stock solution. Add 5.0 ml of metaphosphoric acid—acetic acid extracting solution containing excess ascorbic acid to 15 ml dye reagent.

(2) Standardization

Weigh accurately 100 mg of the reference standard ascorbic acid, transfer to 100 ml glass stoppered volumetric flask and dilute to mark with the HPO_3-HOAc reagent. Standardize indophenol solution at once as follows: Transfer three 2.0 ml aliquots of the ascorbic acid solution to each of three 50 ml Erlenmeyers containing 5.0 ml of the HPO_3-HOAc reagent. Titrate rapidly with the indophenol solution from 10 ml buret until light but distinct rose-pink color persists at least 5 seconds. Similarly titrate 3 blanks composed of 7.0 ml of the HPO_3-HOAc reagent. After substracting average blanks from standardized titrations, calculate and express concentration of indophenol solution as mg ascorbic

acid equivalent to 1.0 ml reagent.

(3) Sample procedure

(a) Transfer the sample from storage into 100 ml volumetric flask.

(b) Add about 80 ml HPO_3-HOAc reagent and shake on a reciprocal shaker for 10 minutes.

(c) Filter under suction to remove solid residue.

(d) Wash with 60 ml HPO_3-HOAc and make up to 150 ml.

(e) Pipette out three 5 ml filtrate into each of three 50 ml Erlenmeyer flasks containing 5 ml of HPO_3-HOAc reagent.

(f) Titrate with the indophenol solution.

$$E = \frac{(\text{mg AA})}{100} \times \frac{2}{\text{ml dye}}$$

$$\text{mg AA/100g} = \frac{\text{ml dye} \times E}{\text{g sample}} \times D \times 100$$

where D = dilution factor

E = equivalent factor (mg AA/ ml dye)

AA = ascorbic acid

b. System preparation

The model system used is similar to that used for the lipid oxidation studies by Chou et al. (1973). The system composition is shown in Table 3. Several methods of preparation were used.

(1) Direct mixing (desorption process)

To prepare the model system, glycerol and corn oil were first mixed in a beaker using a glass stirring rod. To this the solid support (microcrystalline cellulose) was added and mixed thoroughly. The amount of water (buffer solution) necessary to achieve the desired

TABLE 3

COMPOSITION OF THE MODEL SYSTEMS USED FOR THE STUDY OF ASCORBIC ACID DESTRUCTION

Component a_w	0.32	0.33	0.51	0.52	0.67	0.75	0.82	0.84	0.88	0.93
Corn oil	10	10	10	10	10	10	10	10	10	10
Glycerol	40	40	40	40	40	40	40	40	40	40
Cellulose	50	50	50	50	50	50	50	50	50	50
Water * (DM)[a]	5.0	5.2	9.6	10	20	31	49	57	77	102
Water * (DH)[b]	4.7	4.9	8.3	9.4	18.2	26.0	36.1	43	56.1	73.1
Ascorbic acid **	300	300	300	300	300	300	300	300	300	300

* buffer system
** mg

a Direct mix system
b Dry humidified system

water activity was then added. The amount of water needed was obtained from Figure 1. The buffer system used is shown in Table 4. Both a citrate-phosphate buffer (A) and a phosphate buffer (B) were tested.

This system was designated as the direct mix system (DM) and corresponds to the upper branch in the sorption hysteresis isotherm loop. Although the water was added to the dry support, a previous work (Chou et al., 1973) showed this method to be no different than adding excess water and desorbing it in a desiccator. Zero time for the DM system was taken as the time at which the samples were put into desiccators at the water activity to which they were prepared.

(2) Humidified system (adsorption process)

(a) Freeze-dried and rehumidified system (FDR)

The direct mix samples were freeze-dried for 20 hours at room temperature and placed in desiccators containing saturated salt solutions giving the relative humidity desired. Table 5 shows the various salt solutions used to attain these humidities. The desiccators were evacuated until the saturated salt solutions boiled. However, precautions were taken to prevent the samples from becoming contaminated. The samples were held in the desiccators for 48 hours or until no weight changes were observed. Zero time for this system was taken as the time when the samples were equilibrated. This system was then designated as the freeze-dried and rehumidified system (FDR).

Chou et al. (1973) measured the glycerol content of the system after freeze-drying. The results showed that a small portion of the glycerol in the system was lost and thus sample composition

Figure 1. Sorption Isotherm for the Ascorbic Acid Model System at 23°C

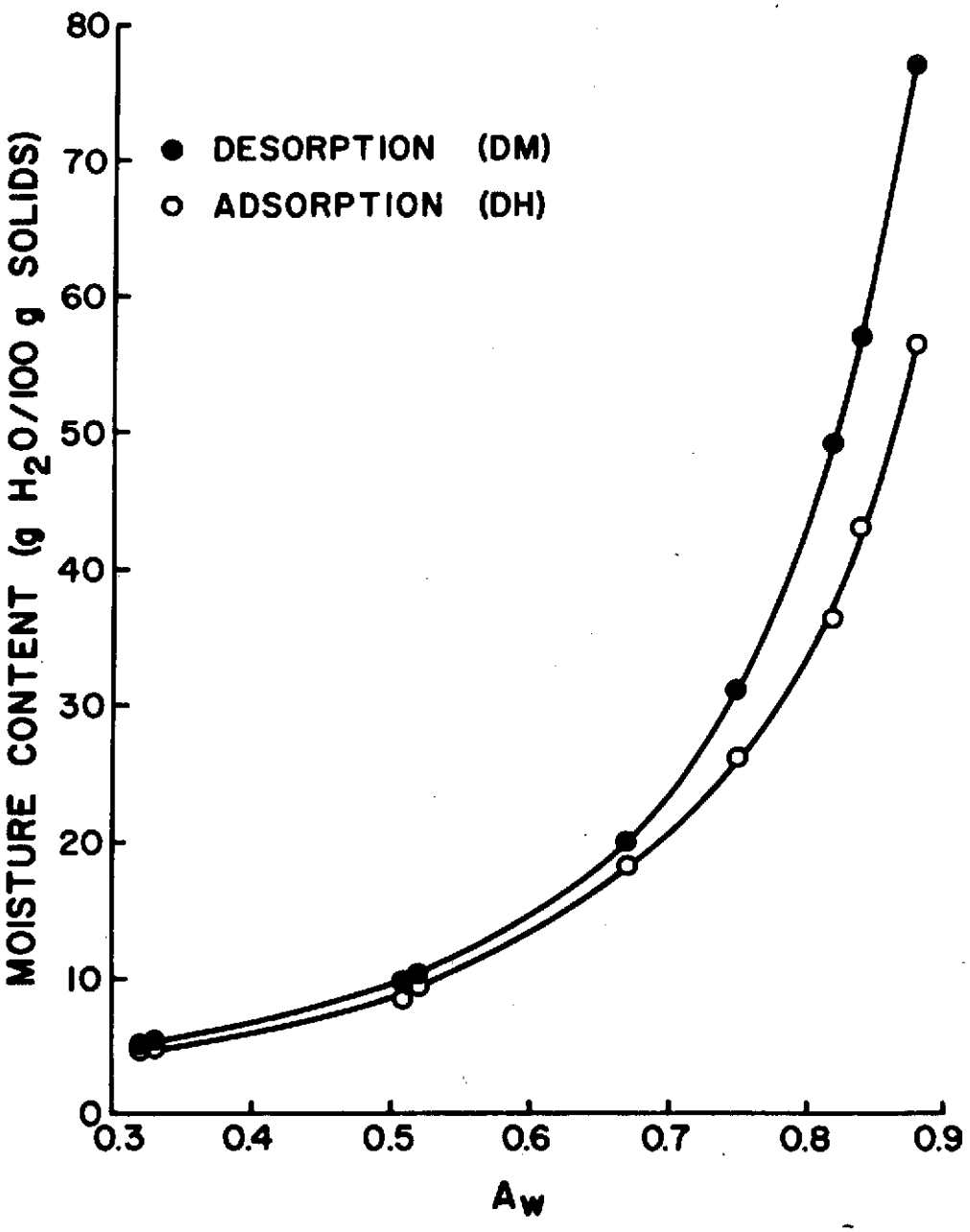

TABLE 4

BUFFER SYSTEM FOR ASCORBIC ACID STUDY

(A). Na_2HPO_4 -- Citric Acid Buffer

pH	ml of 0.2M Na_2HPO_4	ml of 0.1M Citrate
4.0	7.71	12.29
5.0	10.30	9.7
6.0	12.63	7.37
7.0	16.67	3.53

(B). Phosphate Buffer

pH	ml of 0.2M Na_2HPO_4	ml of 0.2M NaH_2PO_4	ml Water
6.0	12.3	87.7	100

TABLE 5

RELATIVE HUMIDITY VARIATION WITH

TEMPERATURES FOR SATURATED SALT SOLUTIONS

Salt	% Relative Humidity			
	25°C	35°C	40°C	45°C
$MgCl_2$	33	32	31	31
$Mg(NO_3)_2$	52	51	51	50
$CuCl_2$	67	67	67	67
NaCl	75	75	75	75
KCl	86	84	83	82
K_2CrO_4	87	84	82	81
$BaCl_2$	90	88	87	--
KNO_3	93	91	89	88
Na_2PO_4	97	93	91	--
$Pb(NO_3)_2$	97	96	95	94

was changed slightly.

(b) Dry-mixed and humidified system (DH)

To prevent the loss of the glycerol because of freeze-drying, water (buffer in which ascorbic acid was dissolved) and microcrystalline cellulose were first mixed and were then freeze-dried at room temperature for 20 hours. The dry samples were then mixed with the necessary glycerol and corn oil. The dry-mixed samples then were humidified to the same a_w as the FDR system.

c. A_w and moisture measurement

Water activities were determined using the manometric technique at the various water contents in both the desorption system and adsorption system at room temperature. The water content was measured by the methanol extraction-GLC technique reported in Phase I, NAS 9-12560. A 3.0 gram sample was weighed accurately in a 125 ml Erlenmeyer flask. 80 ml of anhydrous methanol was added and the moisture content was measured by a Hewlett-Packard thermal conductivity gas chromatograph. Separation of the methanol—water mixture was accomplished on a Poropak Q column operated isothermally at 110°C. The isotherm as shown in Figure 1 shows the hysteresis effect.

d. NMR measurement of adsorbed aqueous phase

An analysis of the T_1, spin-lattice relaxation time of water protons, was made on samples prepared by DM and DH methods. A pulsed Nuclear Magnetic Resonance Analyzer (Praxis Corp.) was used at the Pillsbury Research and Development Labs., Minneapolis, Minnesota.

The sample size used was approximately 35 g. Measurements were made at 30 MHz and 30°C. Amplitudes for the first 90°C pulse and

second 90°C pulse were made after delay time (τ) was set. Measurements were repeated for different values of τ.

To determine the T_1, Equation (1) which is recast from the Bloch equation can be used (Becker, 1969):

$$\ln (A_\infty - A_\tau) = \ln A_\infty - \tau/T_1 \qquad (1)$$

where A_τ = initial amplitude of the free induction delay following the 90° pulse at time τ

A_∞ = limiting value of A_τ for a very long interval between the first and second pulse.

The difference between the first pulse and second pulse was plotted against delay time on semilogarithmic paper and T_1 was determined from the slope of the plot.

3. Results and Discussion

a. Ascorbic acid destruction as a function of pH and trace metal catalysis at a_w 0.75

Experiments were prepared to determine the effects on the reaction of the pH and buffer system which would be used in the model system. Model systems were prepared to an a_w of 0.75 and held at 35°C. The results are shown in Figure 2 for Run 1 for the control and the various buffers and for Run 2 in Figure 3 where EDTA at 100 ppm total solids basis was added to the buffers. As seen in Figures 2 and 3, for buffer (A) the rate of destruction of ascorbic acid increased as pH increased up to pH 6.0 and then the rate decreased again. In both systems, buffer (B) at pH 6.0 was more protective than buffer (A). The rates of destruction determined from these data are shown in Table 6.

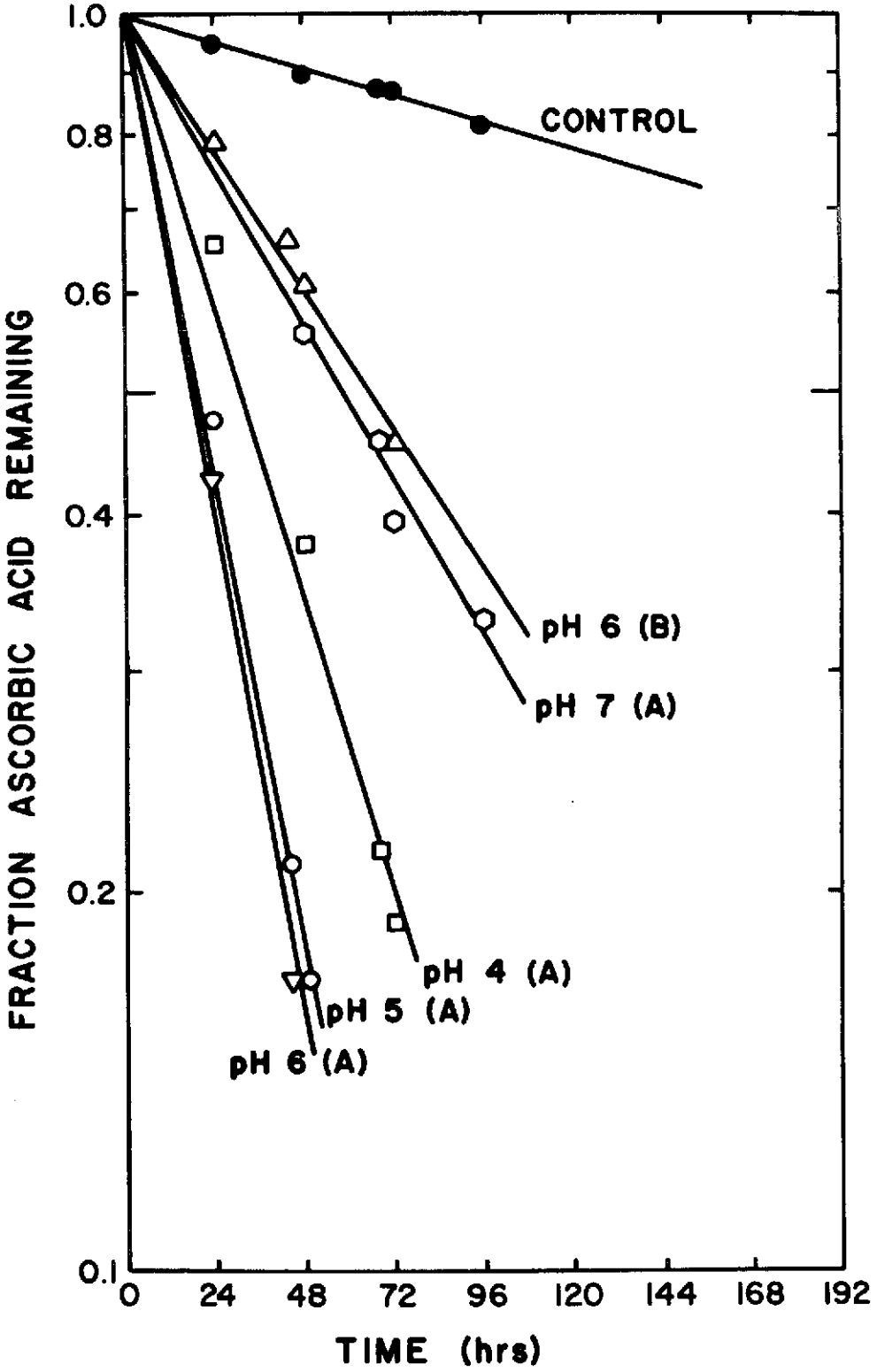

Figure 2. Run 1. Loss of Ascorbic Acid as a Function of pH and Buffer Type: Citrate-phosphate (A) or Phosphate at 35°C and a_w 0.75

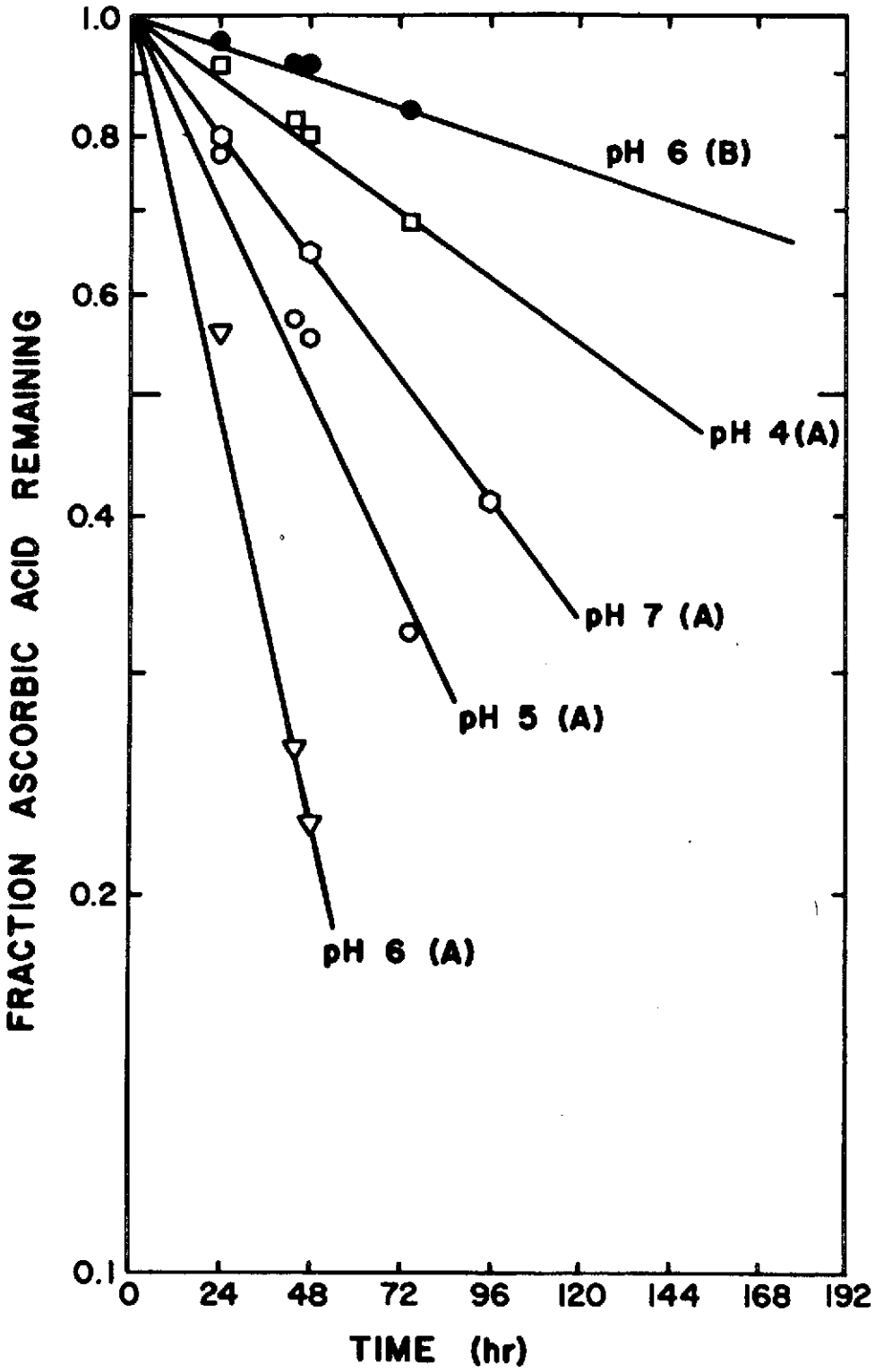

Figure 3. Run 2. Loss of Ascorbic Acid as a Function of pH and Buffer Type (A) or (B) at 35°C and a_w 0.75. EDTA Added at 100 ppm.

TABLE 6

RATE CONSTANT FOR ASCORBIC ACID

DEGRADATION AT 35°C AND a_w 0.75

pH	Run 1		Run 2 (EDTA added)	
	Buffer	$k(day^{-1})$	Buffer	$k(day^{-1})$
4	A	5.62×10^{-1}	A	1.43×10^{-1}
5	A	8.84×10^{-1}	A	3.64×10^{-1}
6	A	8.89×10^{-1}	A	8.47×10^{-1}
7	A	2.71×10^{-1}	A	2.21×10^{-1}
6	B	2.93×10^{-1}	B	4.89×10^{-2}
	no buffer	4.85×10^{-2}		

Buffer A - citrate-phosphate buffer
Buffer B - phosphate buffer

Weissberger et al. (1944) and Khan et al. (1967) reported that the oxidation rate of the ascorbic acid is dependent upon the concentration of monovalent ascorbate ion in the presence of metals since monovalent ascorbate ion is only subject to metals. On the assumption that the rate is governed by concentration of ascorbate ions (AH^-) and metals ions at pH values close to neutrality, the concentration of monovalent ascorbate ion would be increased ($pK_1 = 4.12$). Thus the rates should increase with increasing pH.

Finholt et al. (1963) and Flesh (1960) showed that the rate of degradation of ascorbic acid was also increased with an increase in total phosphate concentration. They assumed that trace metal impurities or the buffer components caused an enhanced autoxidation of ascorbic acid in phosphate buffer. It is also known that citric acid has an ability to complex metals.

According to the composition of buffer system (A), as shown in Table 4, the amount of phosphate increases and the amount of citric acid decreases with an increase in pH. The combination of these factors would then explain the increase in the rate of oxidation of ascorbic acid up to pH 6.0.

Over 50% of the citric acid is present as a trivalent ion at pH 7 because the pK_a of citrate is 6.4. Uprety and Revis (1964) showed that the protecting effect of citric acid was more profound at pH 6.5 than at lower pH. The decreased rate at pH 7 may then be due to the fact that trivalent ions may have much more ability to complex trace metals.

Table 6 shows that the rate of degradation between the two buffer systems and distilled water were significantly different. At the same pH the ascorbic acid in the phosphate buffer system was retained more than in the citrate-phosphate buffer system. In these particular buffers, the different rates at the same pH may be due to the concentration of phosphate or impurities, as Flesh (1960) suggested.

Table 6 and Figure 3 show the effect of EDTA, a chelating agent. The retention of ascorbic acid was improved by adding 100 ppm of EDTA at all pH values, as the trace metals present were chelated and thus were made unavailable for the reaction. Thus pH and buffer solutions are also important factors influencing the rate of the oxidation of ascorbic acid. From these results buffer (B) was chosen since it would not cause any unusual effects on the rate of reaction.

b. Effect of a_w and method of preparation on ascorbic acid destruction in model systems

Run 3

In order to study the effects of water activity on the rate of destruction of ascorbic acid and the activation energy of the reaction with respect to moisture hysteresis experiments were designed in a simplified model system utilizing the buffer (B) system as discussed previously to prevent any complex interactions. The ascorbic acid levels were measured as a function of time at 23, 35 and 40°C in the range of a_w 0.32--0.84. The results were calculated as the fraction of ascorbic acid remaining, as shown in Figures 4, 5 and 6. The calculated destruction rate constants are shown in Figure 7 as a function of a_w.

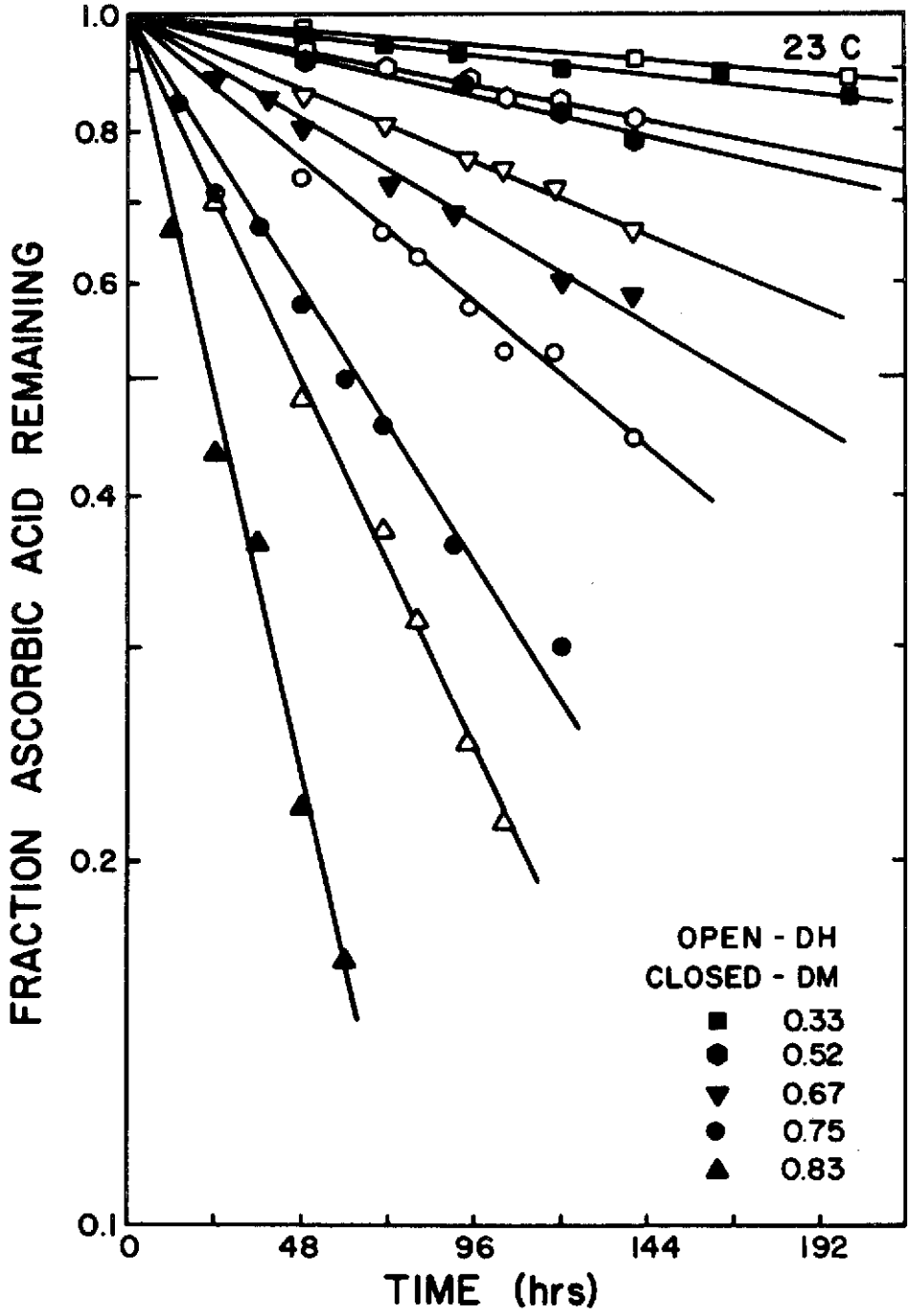

Figure 4. Loss of Ascorbic Acid as a Function of a_w with Respect to Sorption Hysteresis at 23°C. DM = desorption, DH = adsorption.

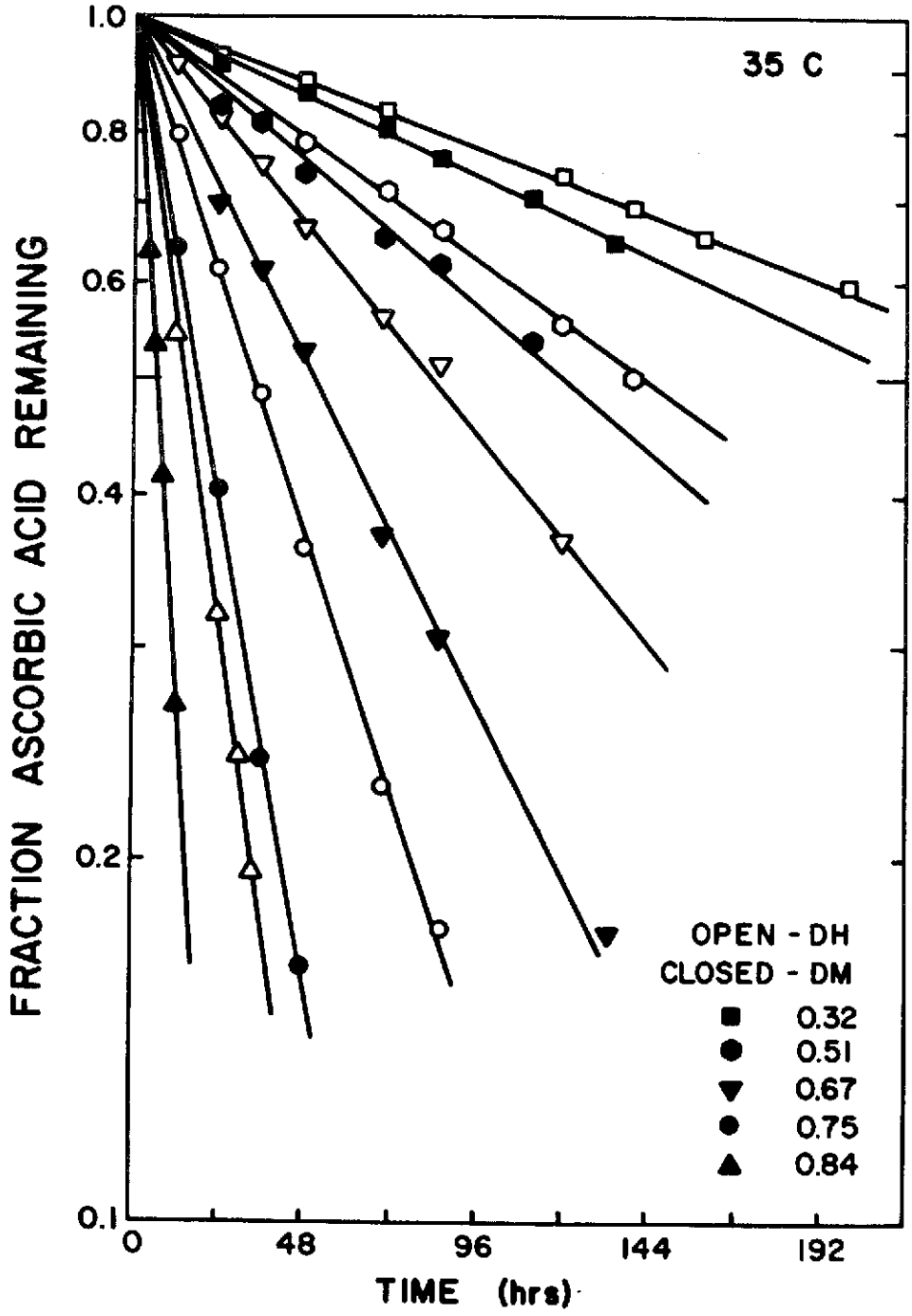

Figure 5. Loss os Ascorbic Acid as a Function of a_w with Respect to Sorption Hysteresis at 35°C. DM = desorption, DH = adsorption.

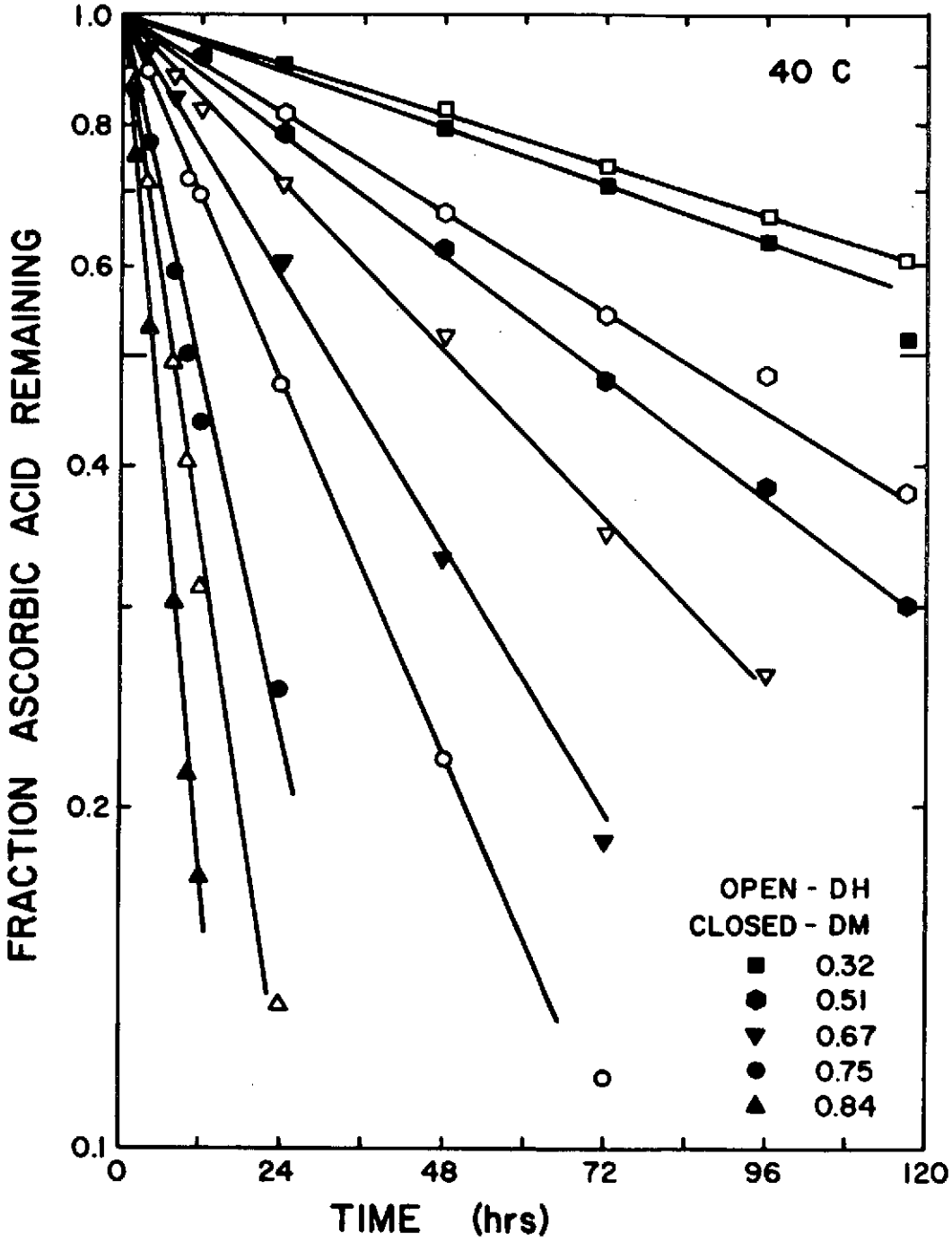

Figure 6. Loss of Ascorbic Acid as a Function of a_w with Respect to Hysteresis at 40°C. DM = desorption, DH = adsorption.

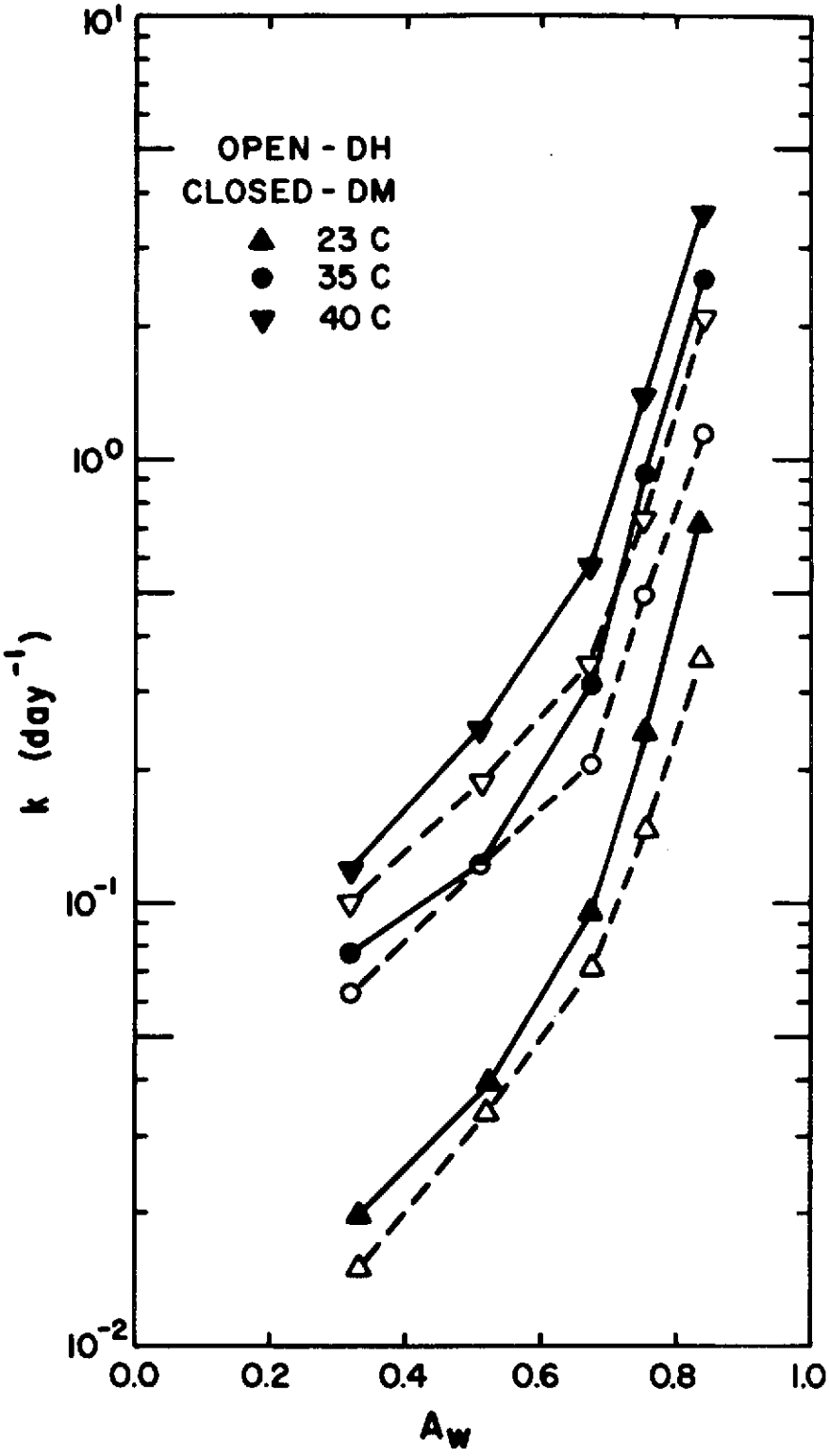

Figure 7. Rate Constants of Ascorbic Acid as a Function of Water Activity and Temperature. DM = desorption, DH = adsorption.

Figure 7 summarizes the results of the ascorbic acid destruction rate as a function of a_w, temperature and hysteresis. The results show the general increase in rate with a_w as in agreement with others (Gooding, 1963; Karel and Nickerson, 1964; Jensen, 1969; Vojnovich and Pfeifer, 1970). It is not surprising, however, that at similar a_w values for the foods the destruction rates of ascorbic acid in the model system do not fall in the same range. This could be due to various interactions with other components, especially trace metals present in foods. The results also show that the ascorbic acid was destroyed more rapidly in the direct mix system (DM) than in the humidified system (DH). This possibly may be explained by the affect of method of preparation. The sorption curve for ascorbic acid in Figure 8 indicates that it does not go into solution readily until an a_w of 0.85 during an adsorption test. The lower rate can possibly be attributed to the lower amount in solution. However, glycerol and cellulose in model system would take up moisture and dissolve ascorbic acid. The kinetics also follow a first order reaction based on the total ascorbic acid. The kinetics thus must be controlled by the amount of water available and the water activity, not the solubility of ascorbate.

Run 4

An additional study was made over a wider temperature range (23 to 45°C) to verify previous results and get an accurate measurement of the activation energy of the reaction.

Since the ascorbic acid would be rapidly destroyed at 45°C, the systems were equilibrated at 10°C initially, then transferred to the higher temperature. The results are plotted in Figures 9, 10 and 11

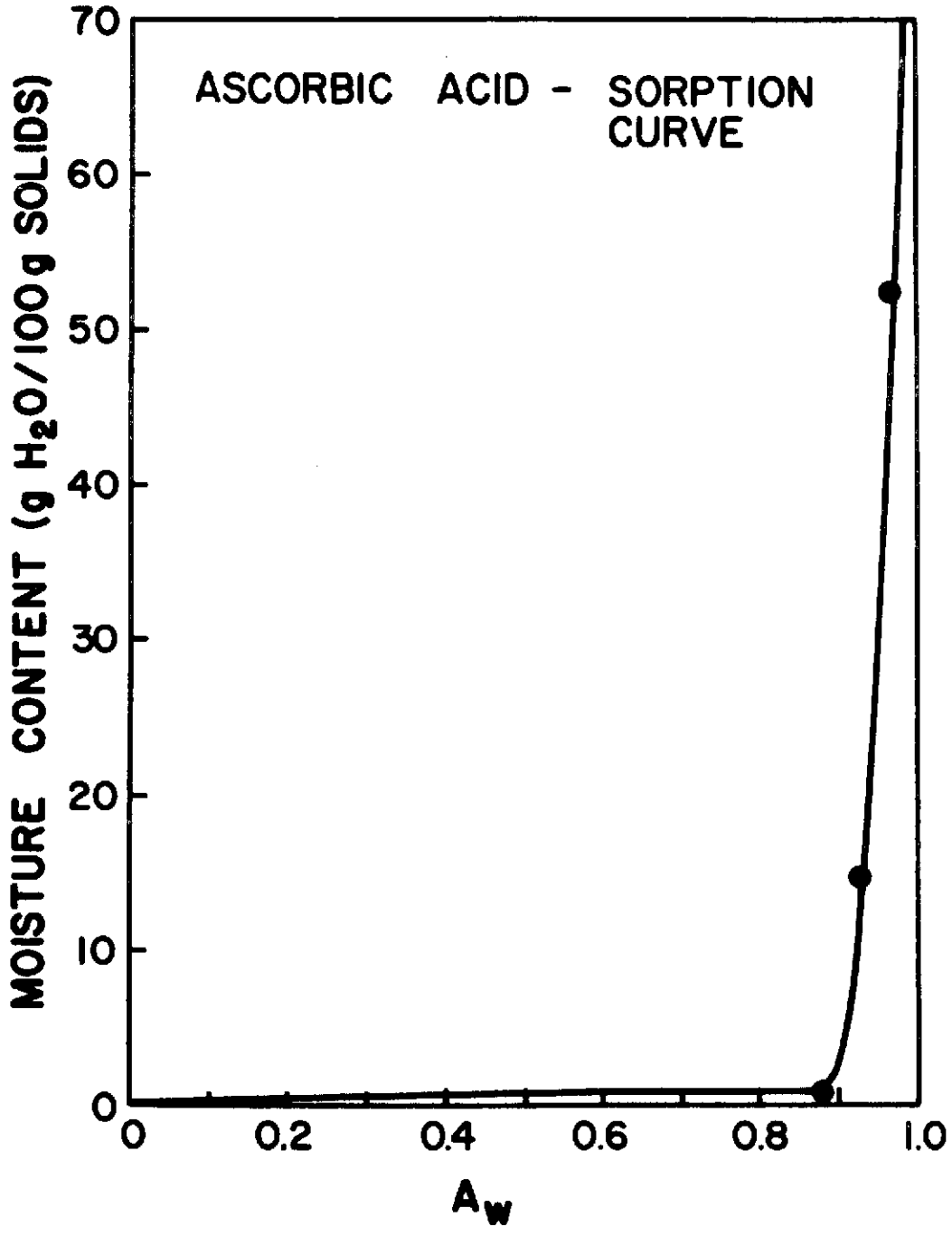

Figure 8. Sorption Curve of Ascorbic Acid at 23°C.

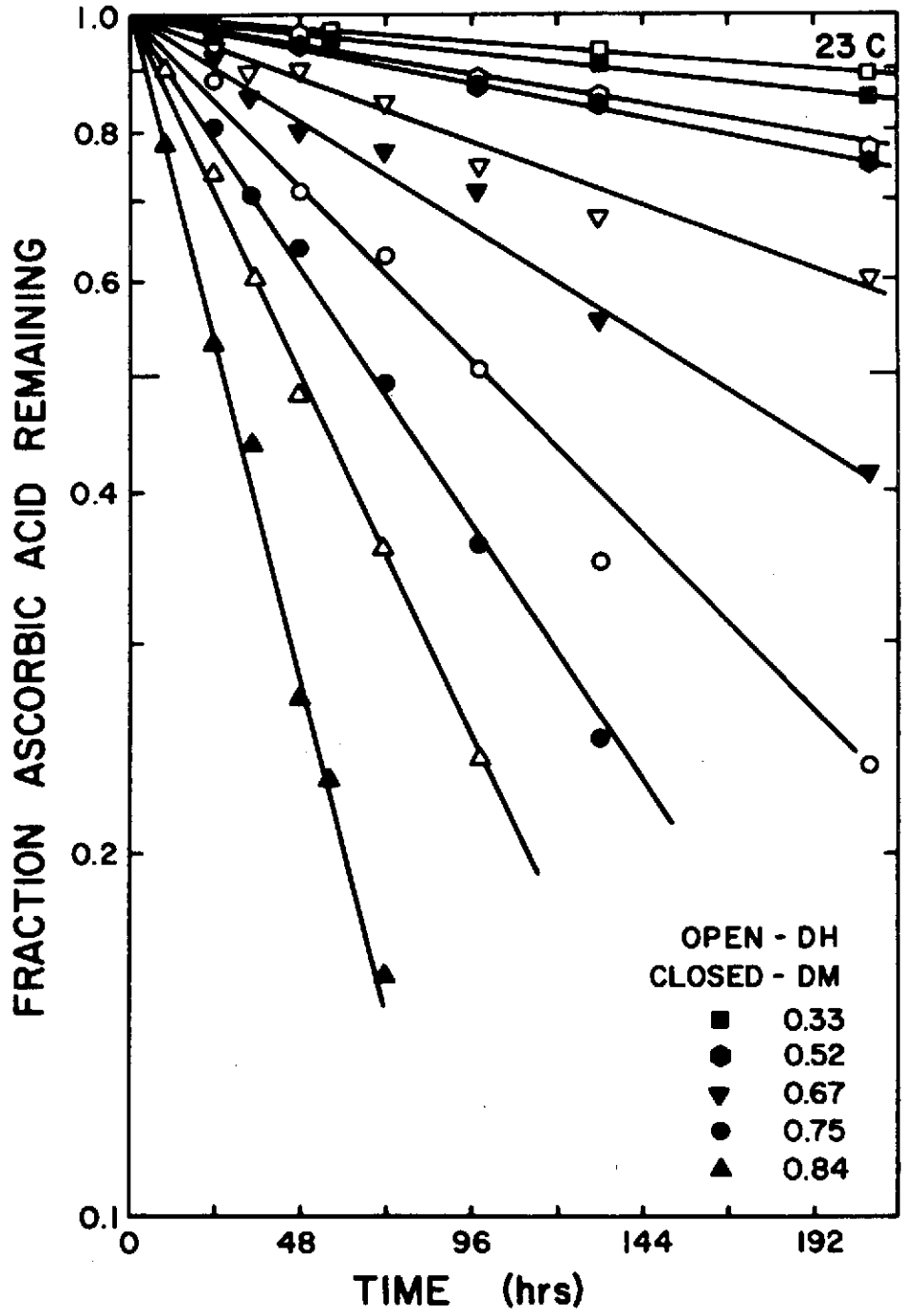

Figure 9. Loss of Ascorbic Acid as a Function of a_w with Respect to Sorption Hysteresis at 23°C. DM = desorption, DH = adsorption.

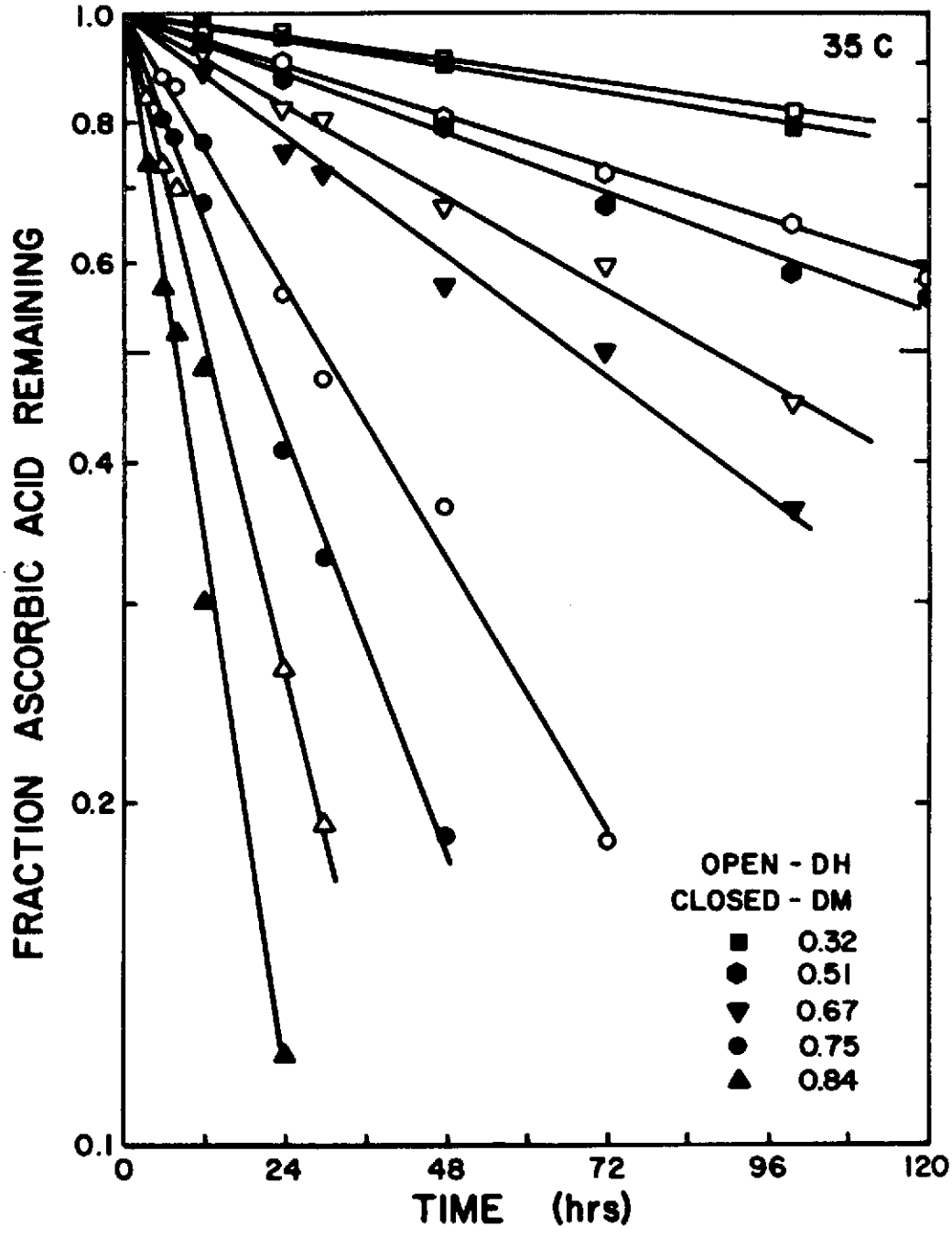

Figure 10. Loss of Ascorbic Acid as a Function of Water Activity with Respect to Sorption Hysteresis at 35°C. DM = desorption, DH = adsorption.

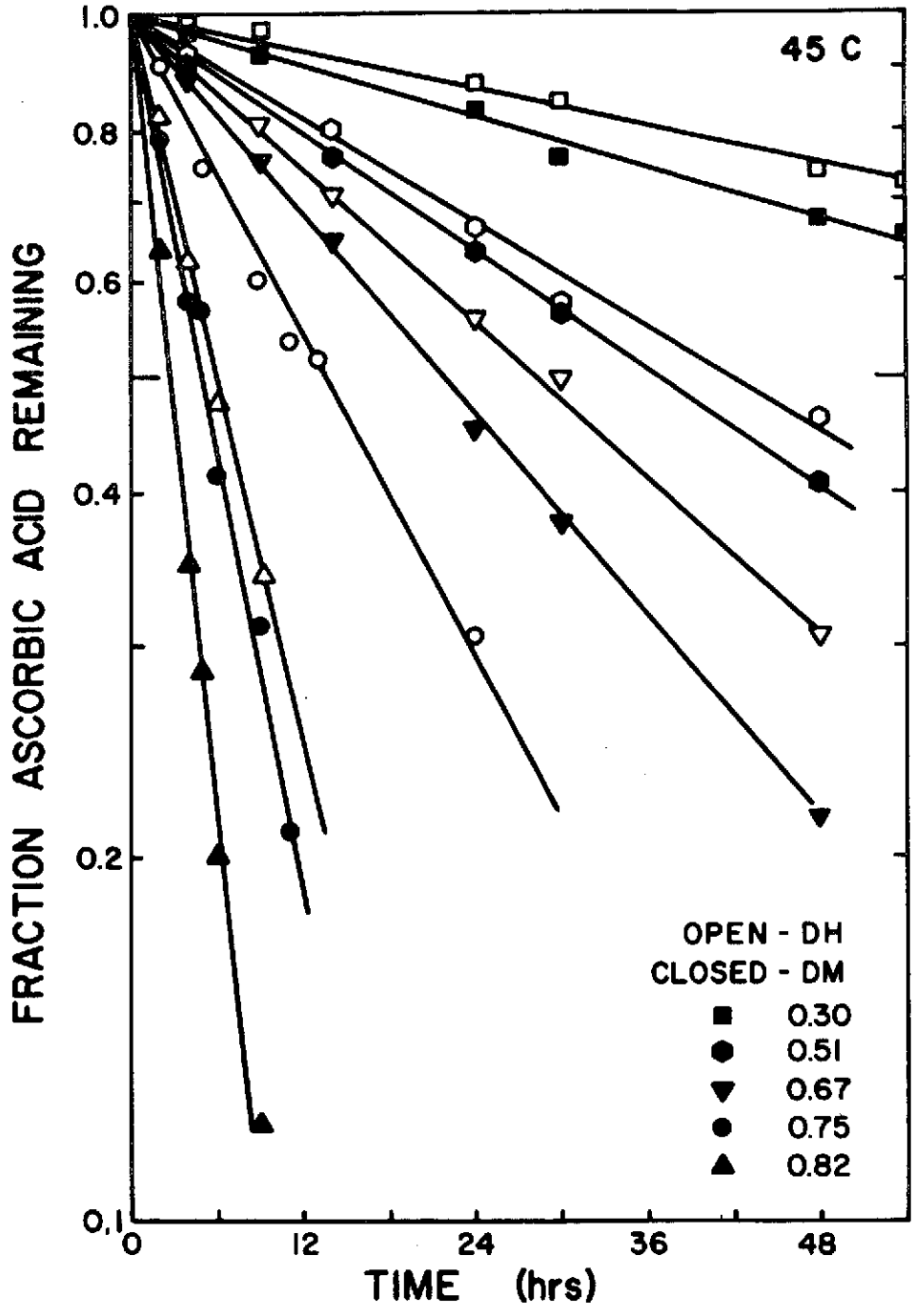

Figure 11. Loss of Ascorbic Acid as a Function of a_w with Respect to Hysteresis at 45°C. DM = desorption, DH = adsorption.

for the three temperatures. As seen, the rate of destruction closely follows a first order reaction as in Run 3. The rate constants are shown in Figure 12. The half lives calculated from these rate constants are presented in Table 7. The results compare very closely to those found in Run 3, the slight difference is probably due to slight differences in the amount of trace metals present. As seen, the rate increases as a_w increases.

Run 5

Previously it was shown that as moisture content increased in both the DM and DH systems, the rate of ascorbic acid loss increased proportionally. Miller et al. (1949) and Kyzlink et al. (1970) showed no dilution effect on the reaction rate at high water activities. From these studies, therefore, it was postulated that the increase in rate observed in the model systems was due to an increase in metal ion and ascorbic acid mobility. It was also postulated that most likely at very high moisture levels, no dilution effect would occur as was also found in the other studies.

Additional systems were prepared by both the DM and the DH methods to see the dilution effect on the destruction rates of ascorbic acid at a_w 0.88 and 0.93. The loss of ascorbic acid was measured at 23, 35 and 45°C. The results of ascorbic acid loss are shown in Figures 13 and 14. As seen, a first order reaction is still obeyed.

The overall half lives at these a_w's as compared to the lower ones are shown in Table 7. This shows a continuing decrease in half life with increasing a_w or water content at these a_w's. Based on a first order reaction, there should be no change in half life with a change in

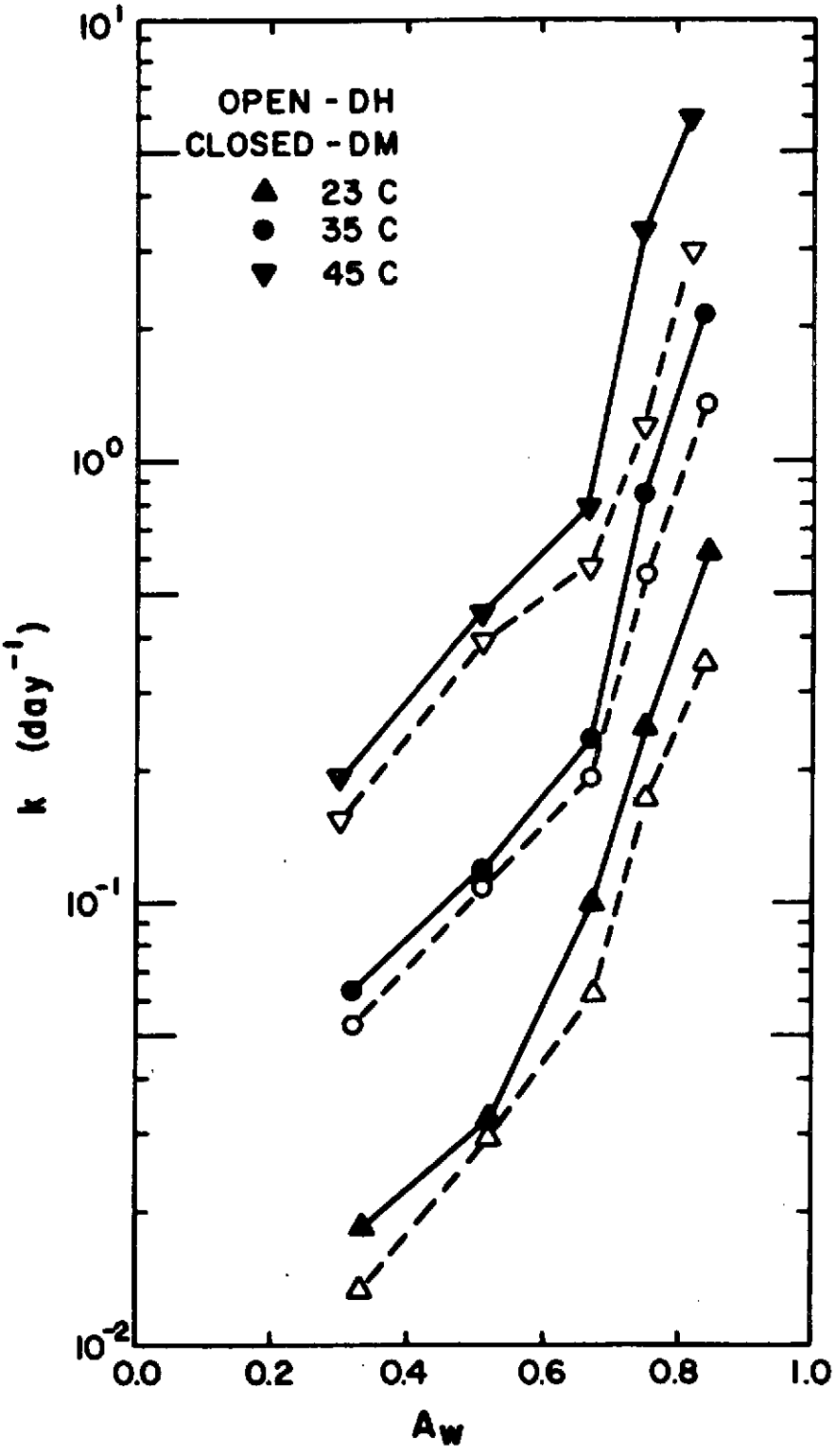

Figure 12. Rate Constants of Ascorbic Acid Degradation as a Function of a_w at 23, 35, 45°C.

TABLE 7

HALF-LIFE IN DAYS FOR ASCORBIC ACID DESTRUCTION IN MODEL SYSTEMS

a_w	Run	23°C			35°C			40°C		45°C		
		DM	DH	FDR	DM	DH	FDR	DM	DH	DM	DH	FDR
0.32	3	36.5	49.5	---	9.0	11.1	---	5.9	6.9	---	---	---
	4	38.9	55.4	---	11.2	13.3	---	---	---	3.5	4.2	---
0.51	3	18.2	21.3	---	5.6	5.6	---	2.8	3.8	---	---	---
	4	22.4	25.2	---	5.8	6.2	---	---	---	1.5	1.7	---
0.67	3	7.4	9.8	---	2.1	3.4	---	1.2	2.0	---	---	---
	4	6.9	11.2	---	2.9	3.6	---	---	---	0.9	1.2	---
	6	---	---	27.5	---	---	6.9	---	---	---	---	2.4
0.75	3	2.8	4.8	---	0.8	1.4	---	0.5	1.0	---	---	---
	4	2.8	4.0	---	0.8	1.3	---	---	---	0.2	0.6	---
	6	---	---	14.8	---	---	3.2	---	---	---	---	1.2
0.84	3	0.9	1.8	---	0.3	0.6	---	0.18	0.3	---	---	---
	4	1.1	1.9	---	0.3	0.5	---	---	---	0.1	0.2	---
	6	---	---	6.9	---	---	2.5	---	---	---	---	1.0
0.88	5	0.56	1.2	---	0.22	0.38	---	---	---	0.08	0.17	---
0.93	5	0.39	0.83	---	0.16	0.20	---	---	---	0.05	0.08	---

DM = direct mix - desorption
DH = dry mixed and humidified - adsorption
FDR = freeze-dried rehumidified - adsorption

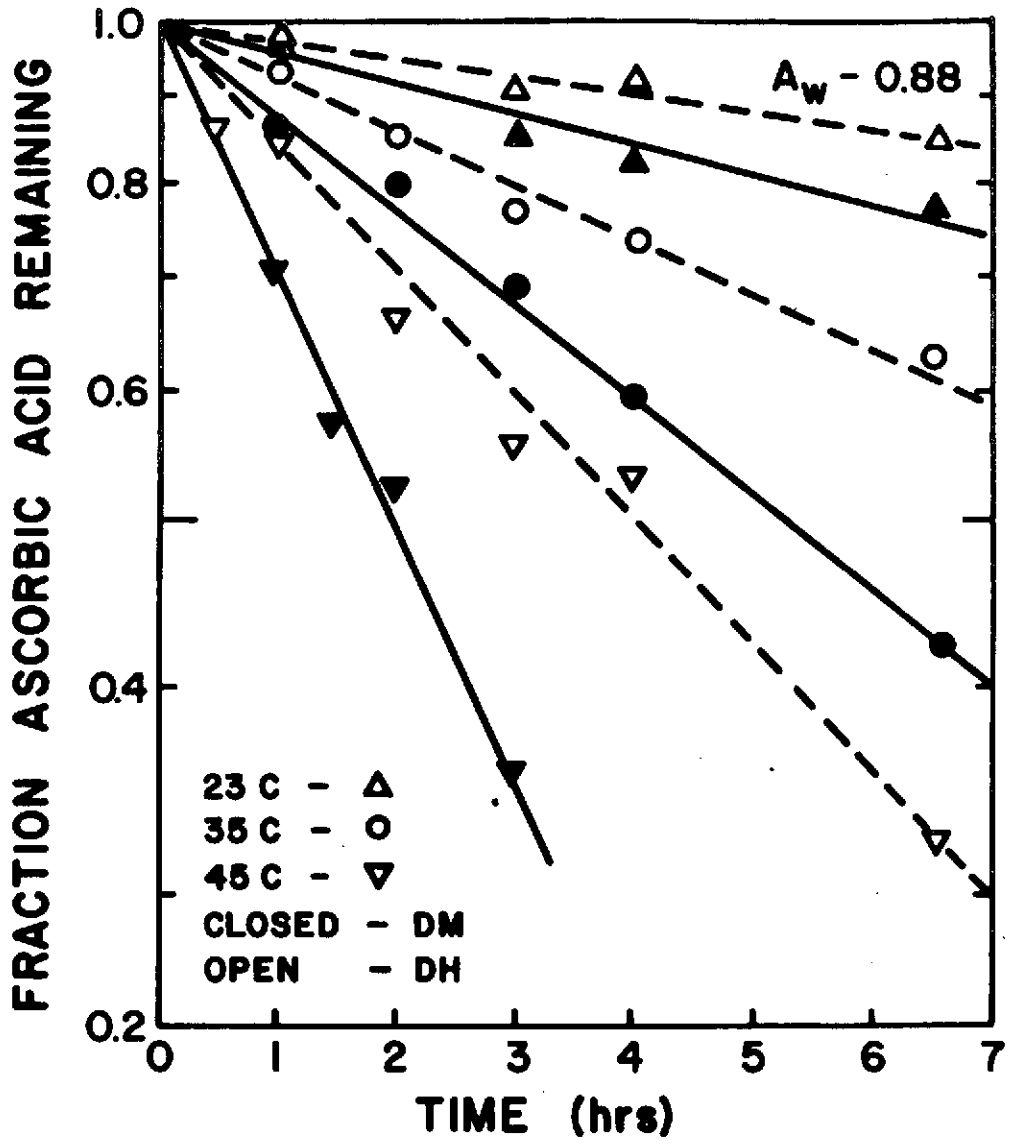

Figure 13. Loss of Ascorbic Acid at an a_w of 0.88 and 23, 35 and 45°C with Respect to Hysteresis. DM = desorption, DH = adsorption.

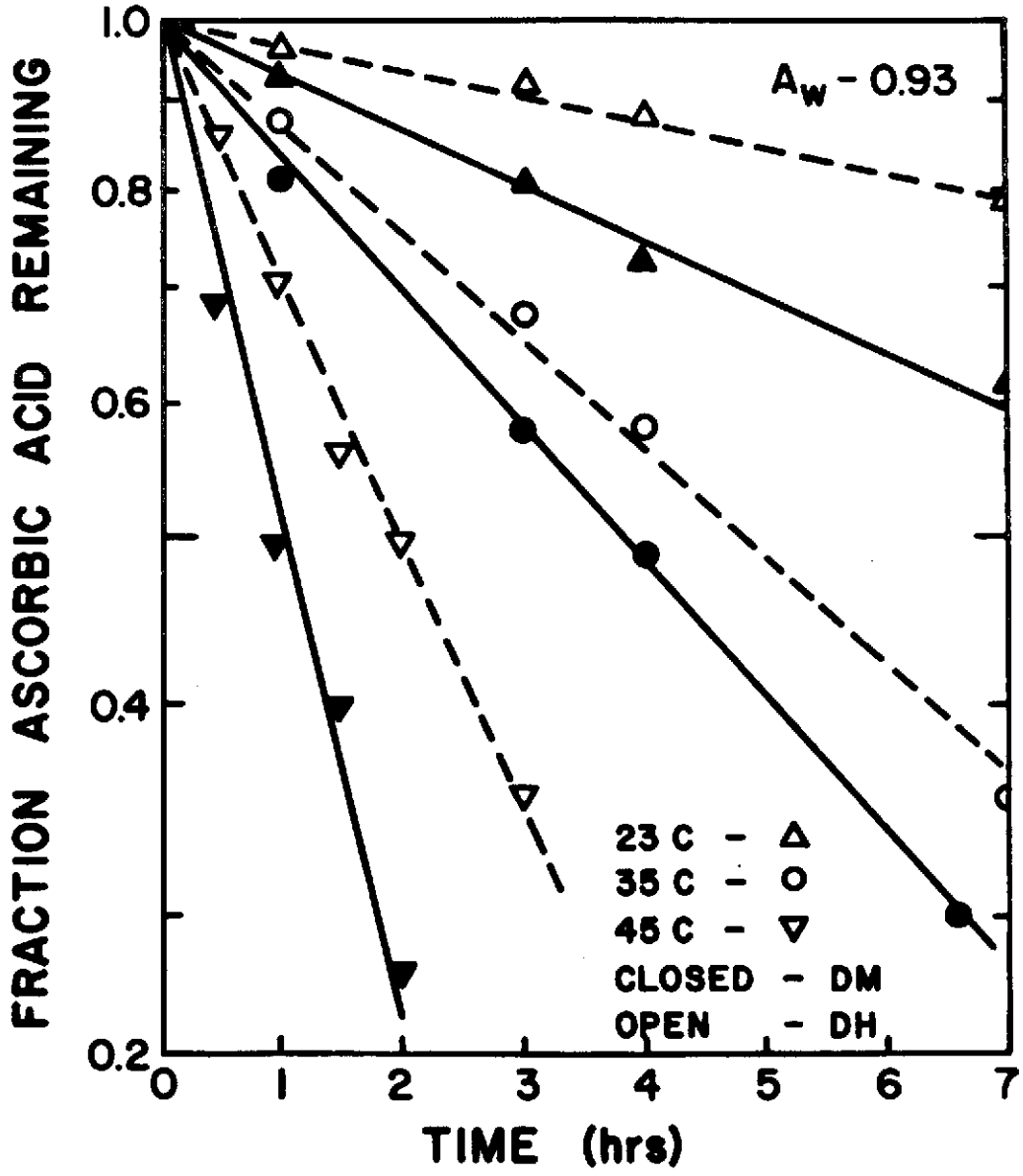

Figure 14. Loss of Ascorbic Acid at an a_w of 0.93 and 23, 35 and 45°C with Respect to Sorption Hysteresis. DM = desorption, DH = adsorption.

concentration. Thus as water content increases even if dilution occurred, all measured half life should remain constant, not decrease as seen. However, the decrease is very small at the high a_w. Thus either a dilution effect is not occurring up to these a_w's or some other mechanism is occurring which affects the rate more strongly. Under the conditions in this study, the rates were so rapid that higher moisture systems could not be studied.

Run 6

An additional system, the FDR, was prepared by mixing all the components together with glycerol and corn oil and freeze-drying for 24 hr. at room temperature in order to see another effect of the method of preparation. These were then humidified to equilibrium relative humidities. The results of moisture vs. a_w are compared to the DM and DH systems in Table 8. As seen, the FDR gives slightly higher moisture contents than the DH system. The reason is not certain. However, it was noticed that samples after freeze-drying were very porous and can possibly hold more water thus giving a slightly higher water content at the same a_w.

Samples were stored at 23, 35 and 45°C and destruction rates of ascorbic acid were measured. Figures 15 and 16 show the results for ascorbic acid loss at the three temperatures. The calculated half lives are shown in Table 7 as compared to the DM and DH systems. As seen, the half life for the FDR system is significantly higher than the other systems, yet the moisture is in between the DM and DH systems. The reason is not known except that some glycerol was lost in the preparation and may have an effect on the mobility, giving the system a higher aqueous phase viscosity.

TABLE 8

MOISTURE DATA (g H_2O/g solids)

Ascorbic Acid Systems

a_w	DM *	DH *	FDR *
0.32	3.4	3.0	---
0.33	3.6	3.2	---
0.51	9.6	8.3	---
0.52	10.0	9.4	---
0.67	20.0	18.2	19.6
0.75	31.0	26.0	29.6
0.82	49.0	36.1	43.5
0.84	57.0	43.0	---
0.88	77.0	56.1	---
0.93	102.0	73.1	---

* DM - direct mix
* DH - dry mixed & rehumidified
* FDR- freeze-dried rehumidified

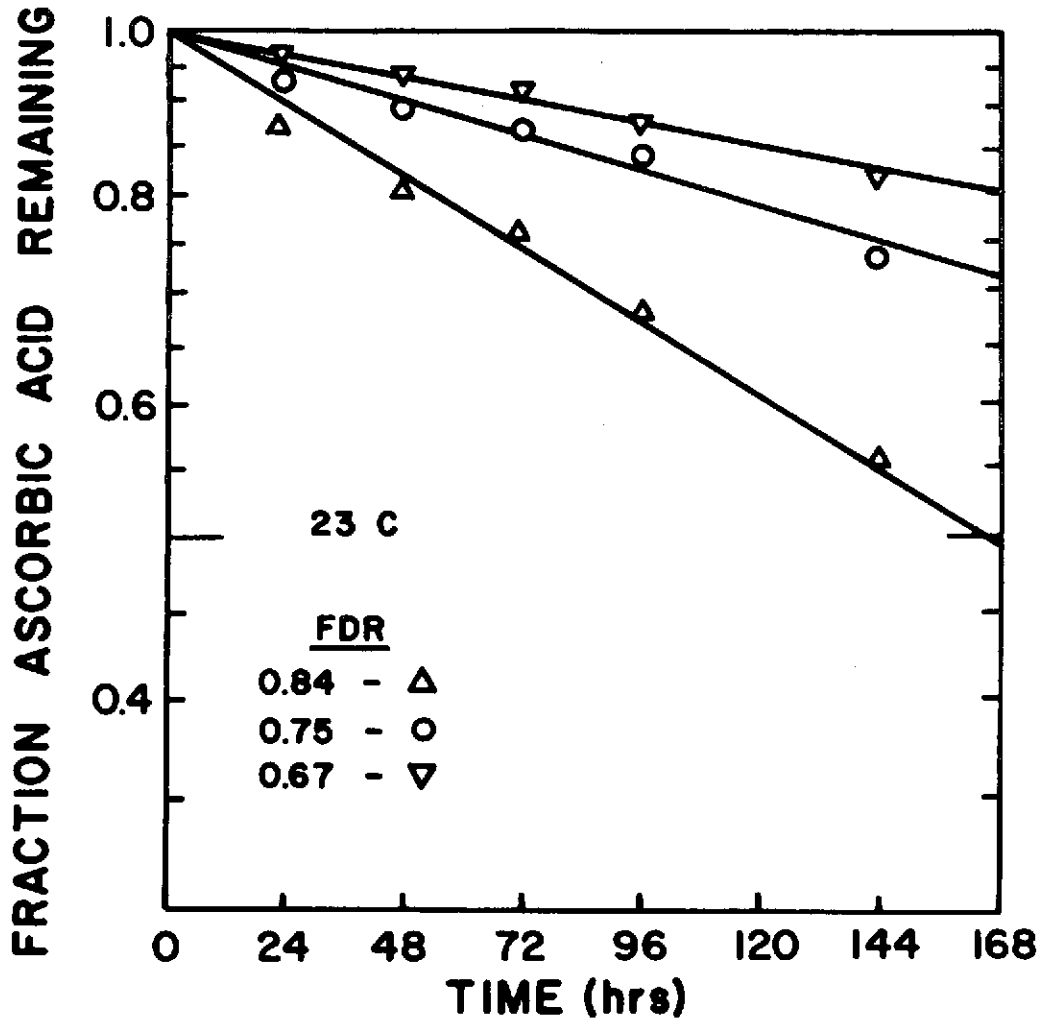

Figure 15. Loss of Ascorbic Acid as a Function of a_w for the FDR System at 23°C.

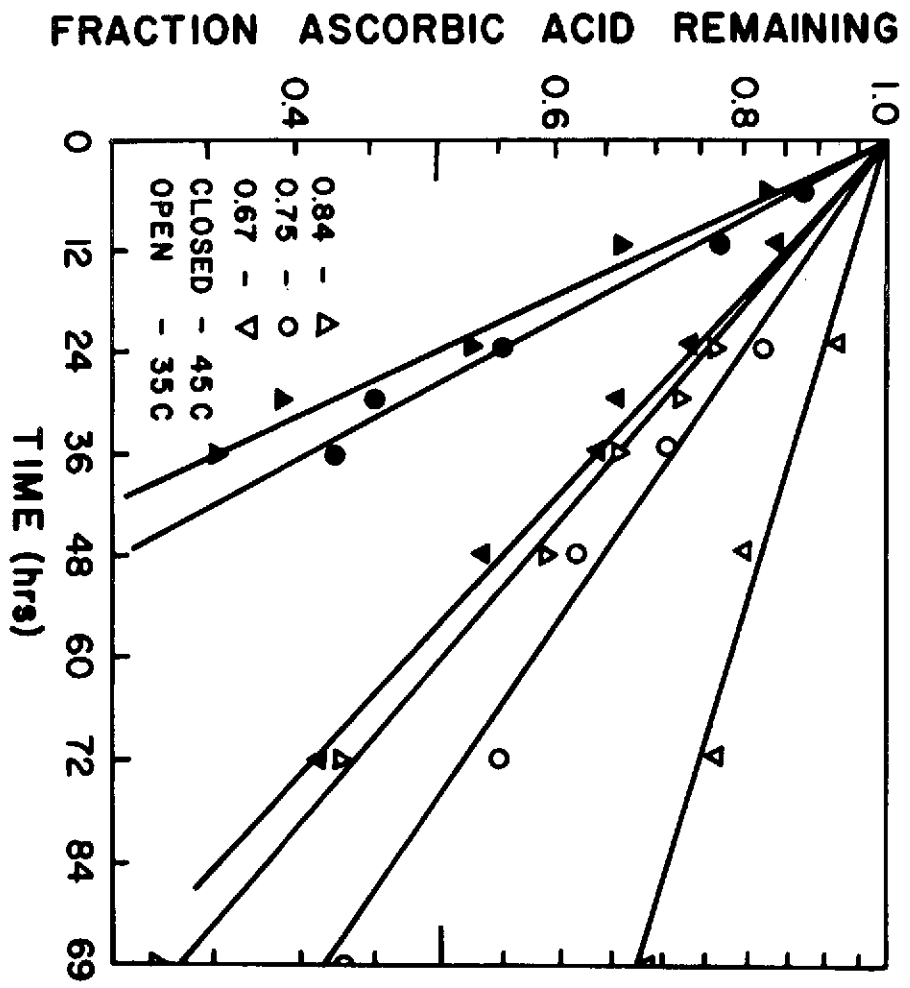

Figure 16. Loss of Ascorbic Acid as a Function of a_w for the FDR System at 35 and 45°C.

c. Mechanisms of action of a_w and moisture on ascorbic acid destruction

The previous results show that the mechanism of action of an increase in a_w is to increase the rate of degradation of ascorbic acid. The mode by which this occurs can be very complex. First, the mechanism of the oxidation of ascorbic acid might possibly be changed with respect to a_w. Secondly, water may act to dilute the concentration of ascorbic acid as a_w increases, thereby reducing the rate and thirdly, an increase in water content may make the reaction easier if the aqueous phase becomes significantly less viscous and diffusion is enhanced. If water plays a role in affecting the rate of oxidation, the effect of hysteresis can be used to answer these three questions.

To examine the possible mechanism change with increasing a_w, the rate constants were plotted against the reciprocal of absolute reaction temperature in a semilog plot. These are shown in Figures 17 and 18 for Runs 3 and 4. The slope of the line is related to the activation energy of the reaction. The measured activation energies are shown in Table 9. In order to test the hypothesis that the activation energy is the same at the various a_w's, a one-way classification test was used (Snedecor and Cochran, 1971). Since experiments were conducted at 23, 35 and 40°C for Run 3, and 23, 35 and 45°C for Run 4, the results from these two experiments can be considered duplicate. Table 10 shows the usual analysis of variance table with general computing instructions for a class with n observations per class. The symbol T denotes a typical class total, while $G = \Sigma T = \Sigma\Sigma X$ is the grand total.

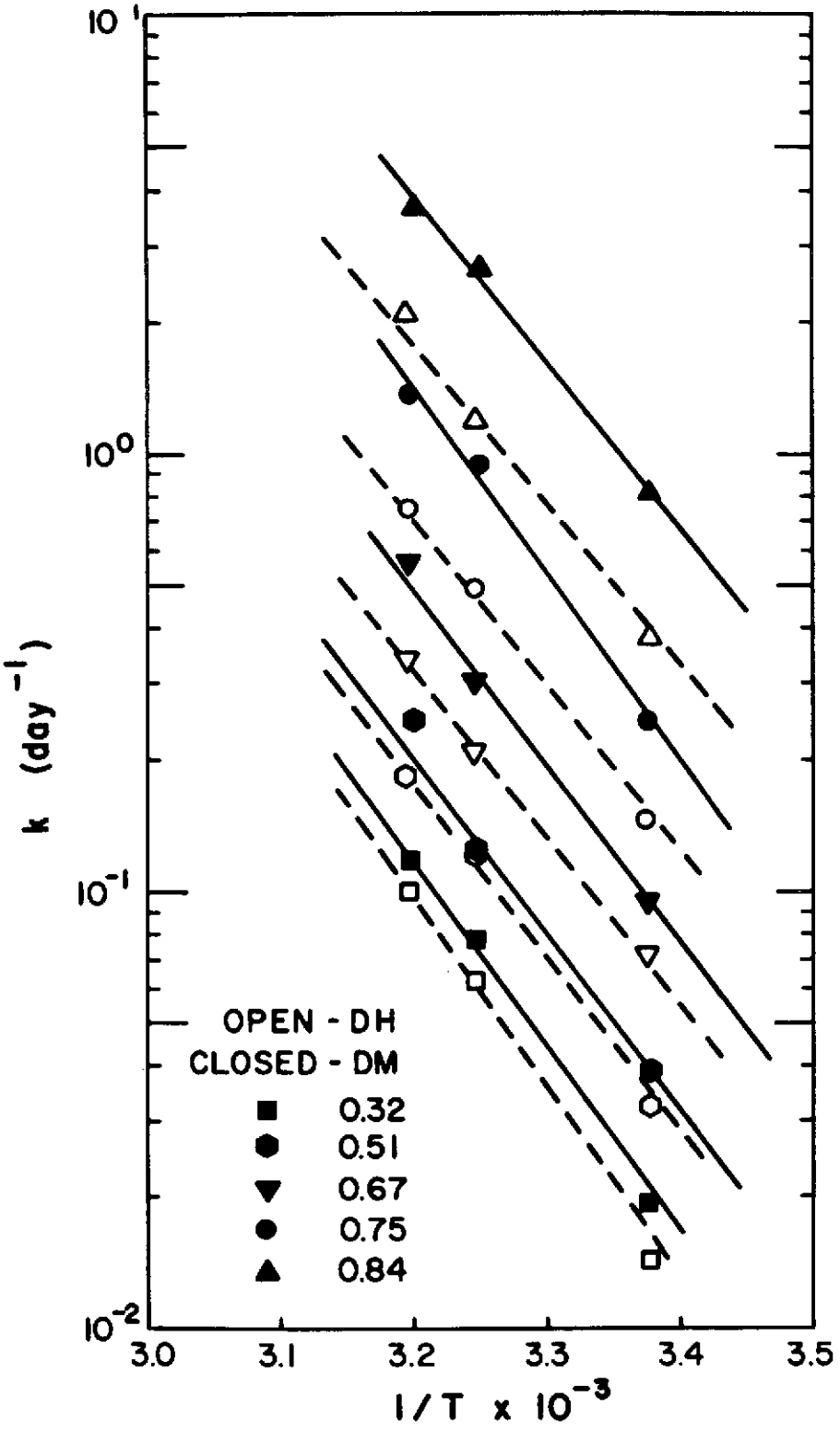

Figure 17. Arrhenius Relationship Between Rate Constants of Ascorbic Acid Destruction and Temperature. Run 3.

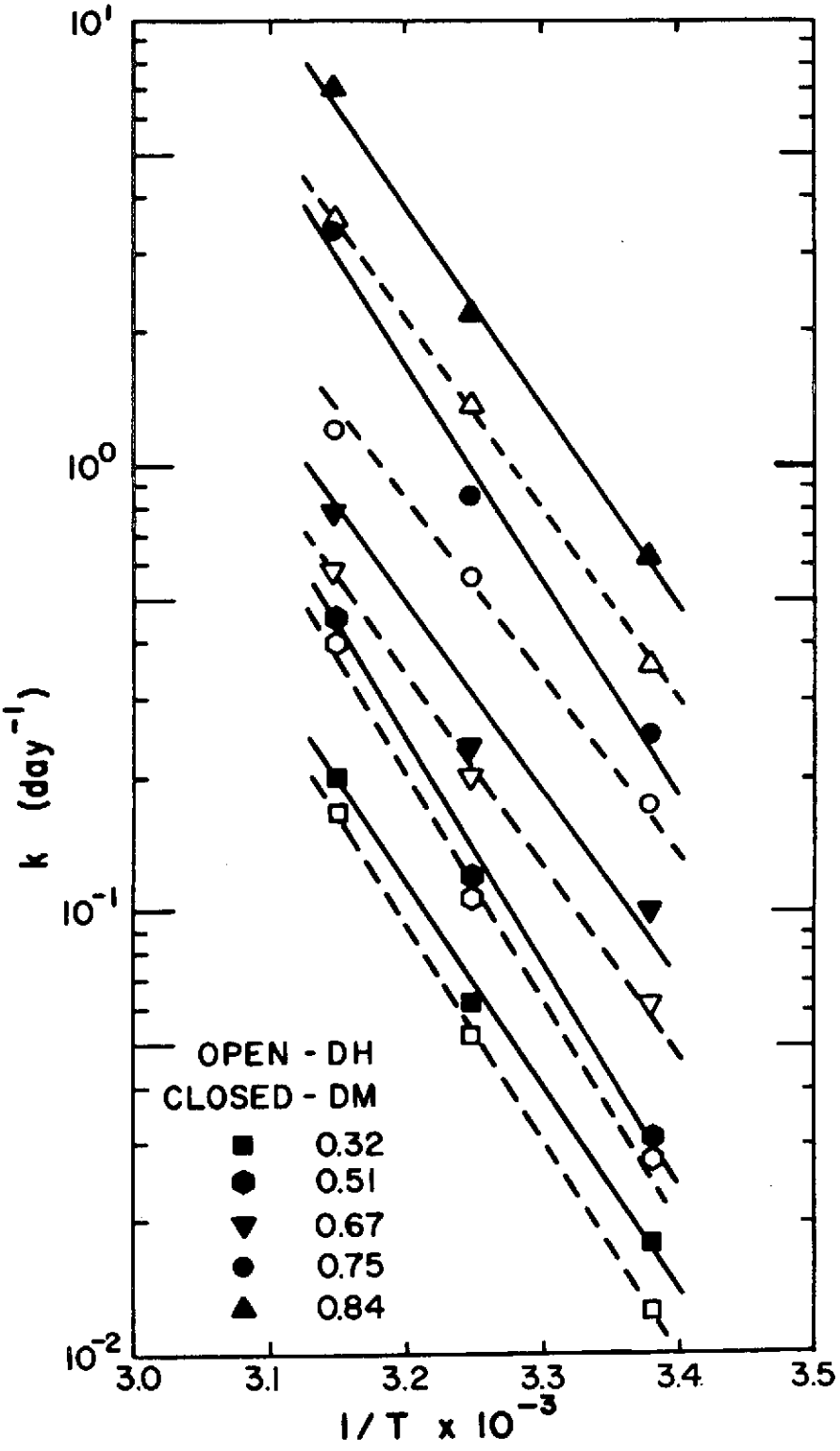

Figure 18. Arrhenius Relationship Between Rate Constants of Ascorbic Acid Destruction and Temperature. Run 4.

TABLE 9

ACTIVATION ENERGIES FOR ASCORBIC ACID DESTRUCTION

(Kcal/mole)

	Run 3		Run 4		Run 5		Run 6
a_w	DM	DH	DM	DH	DM	DH	FDR
0.32	20.0	21.5	20.5	21.9	---	---	---
0.51	19.2	19.0	22.6	22.6	---	---	---
0.67	19.0	16.7	17.3	19.0	---	---	19.5
0.75	19.1	17.8	21.9	16.4	---	---	20.5
0.84	16.4	18.2	20.4	18.4	---	---	17.5
0.88	---	---	---	---	17.2	17.6	---
0.93	---	---	---	---	18.4	19.9	---

TABLE 10

FORMULAS FOR CALCULATING THE ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Square	Mean Square
Between classes (a_w)	a - 1	$(\Sigma T^2/n) - C$	S.S/a-1
Within classes (a_w)	a (n-1)	Total S.S - S.S of between a_w	S.S/a(n-1)
Total	an - 1	$\Sigma\Sigma X^2 - C$	

where X = E_a (Kcal/mole)
n = observations per class
C = $G^2/an = (\Sigma\Sigma X)^2/an$
a = number of classes
S.S = sum of square
T = ΣX

$$F = \frac{\text{Treatment mean square}}{\text{Error mean square}} = \frac{\text{Mean square between classes}}{\text{Mean square within classes}} = \frac{\text{Mean square between } a_w}{\text{Mean square within } a_w}$$

The table provides a basis for the F test. This is a test of the hypothesis that the means for the various a_w's are identical. If the calculated F value is smaller than the F value from the Table for the distribution of F, the hypothesis can be accepted (Snedecor and Cochran, 1971).

The mean $\overline{X}$ and the sum of squares of deviations as calculated for the DM and the DH systems are shown in Tables 11 and 12. The calculated F value for the DM systems is 0.808, as shown in Table 11. The mean square between a_w's is less than that within a_w. Therefore, there is no evidence of differences among the means for the DM system. As shown in Table 12, the calculated F value for the DH system is 3.94. The value at the 5% level is 5.19 (the calculated $F < F_{0.05}$). Thus, from the distribution specified in the hypothesis (F distribution) there is less than 5 in 100 of a sample having a larger value of F. Thus, it can be concluded that the means for the DH system as well are the same; the activation energy would not be affected by changing the water activity.

This analysis shows that water in the model system does not have any real effect on the activation energy, while it does in foods, as was shown in Table 2. However, the amount of data collected for these foods is very small and thus may be suspect. The overall measured E_a for Runs 3 and 4 is 20.2 ± 2 Kcal/mole. The activation energies for Runs 5 and 6 as shown in Table 9 are also in the same range. Thus, it can be concluded that an increase in a_w does not change the reaction mechanism.

To show the effect of water content on the rate, the half lives were plotted against moisture content for both preparation methods from Runs 4 and 5 as shown in Figure 19. This shows a decrease

TABLE 11

ANALYSIS OF VARIANCE FOR THE DM SYSTEM IN RUN 3 and 4

	a_w					
	0.32	0.51	0.67	0.75	0.84	Total
E_a in Run 3	20.03	19.18	19.02	19.07	16.39	
E_a in Run 4	20.47	22.63	17.30	21.90	20.44	
ΣX	40.50	41.81	36.32	40.97	36.83	196.43
$(\Sigma X)^2/n$	820.13	874.04	659.57	839.27	678.23	3871.23
ΣX^2	820.22	879.99	661.05	843.28	686.43	3890.96
$\Sigma X^2 - (\Sigma X)^2/n$	0.097	5.95	1.48	4.0	8.20	19.73

Source of variation	Degree of Freedom	Sum of Square	Mean of Square
Between a_w	4	12.76	3.19
Within a_w	5	19.73	3.95
Total	9	32.49	

$$F = \frac{3.19}{3.95} = 0.808$$

TABLE 12

ANALYSIS OF VARIANCE FOR THE DH SYSTEM IN RUN 3 AND 4

	a_w					
	0.32	0.51	0.67	0.75	0.84	Total
E_a in Run 3	21.49	18.98	16.73	17.76	18.23	
E_a in Run 4	21.90	22.64	18.93	16.44	18.40	
ΣX	43.39	41.62	35.66	34.20	36.63	191.50
$(\Sigma X)^2/n$	941.35	866.11	635.24	584.69	670.88	3698.97
ΣX^2	941.43	872.81	638.24	585.69	670.89	3709.06
$\Sigma X^2 - (\Sigma X)^2/n$	0.08	6.69	2.42	0.87	0.01	10.08

Source of Variation	Degree of freedom	Sum of Square	Mean of Square
Between a_w	4	31.74	7.93
Within a_w	5	10.08	2.02
Total	9	41.83	

$$F = \frac{7.93}{2.02} = 3.95 \qquad F_{0.05} = 5.19 \text{ (from F distribution)}$$

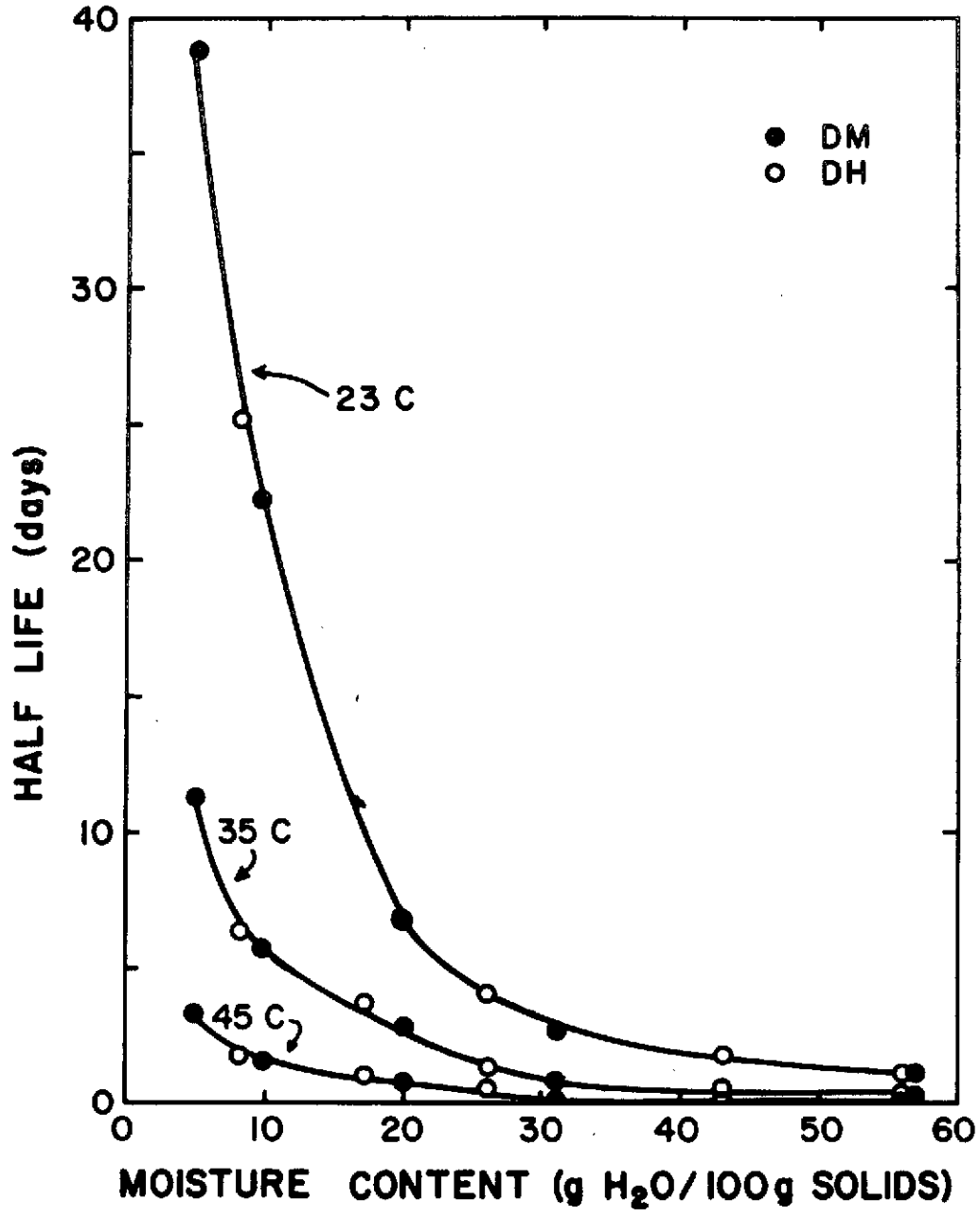

Figure 19. Relationship Between Half Life of Ascorbic Acid and Moisture Content with Respect to Hysteresis.

in half life as moisture content increases. The same pattern was shown for Run 3, however, the results from the FDR experiment did not fit on the same curve, indicating the glycerol loss. Based on a first order reaction, there should be no change in half life with a change in initial concentration of the reacting species. Thus, as water content increases, if dilution of the reactant species occurs, all measured half lives should remain constant not decrease as seen. However, a dilution effect may actually be occurring but could be masked by some other mechanism which would cause a rate increase. This is suspected since above a critical moisture value, the rate increase is very small and becomes almost constant. Miller and Joslyn (1949) showed that the half life of ascorbic acid in sucrose, glucose and fructose solutions decreased with an increase in water activity going from 0.96 to 1.0. The data of Kyzlink and Curda (1970) at pH 4 using sugar solutions (a_w 0.96 to 1.0) also show an increase in rate of destruction or decrease in half life. At 20°C, the half life went from 0.3 days at a_w 0.965 to 0.1 days at an a_w of 1.0. This would seem to fit in very well with what is shown by the present experiments in that the dilution effect is small or is masked by some other factor such as a change in the mobility of species in the adsorbed aqueous phase.

The remainder of the explanation for the effect shown is that the rate increase with a_w or moisture content must be related to mobility of the reaction species in the aqueous phase. A mobility factor can be empirically related to rate constants through the theory of reaction kinetics. The increased rate in this study could be due either to increased mobility of trace metal catalysts or of ascorbate itself. An inspection of Figure 19 shows that all the data fit on the same curve, that is, the

half lives for both preparation systems are not parallel, but are direct functions of the total water. Thus, it was postulated that the effect might be due to a decrease in viscosity and thus increased mobilization of the reaction species. The addition of water increased the rate constant because the ascorbic acid and metals were more easily mobilized. Above a certain moisture content, however, the aqueous phase viscosity would not be expected to change very much and thus, the half life could approach a constant value as seen.

In order to verify this postulation, an NMR study was made of each system for Run 3 at 30°C. Since the spin-lattice relaxation time of protons, T_1, is inversely related to the viscosity of the aqueous phase, the relaxation times were measured for each system. Figure 20 shows the plot of moisture content vs. $1/T_1$. As seen, viscosity is related inversely to the moisture content of the aqueous phase. The relaxation times for the different systems lie on the same curve with respect to hysteresis. This indicates that a decrease in half life with increasing a_w can be related to a viscosity phenomenon and the larger half life for the humidified system is due to a smaller amount of water and thus, increased viscosity as compared to the direct mix system at the same a_w.

However, viscosity is a linear function of the moisture content and the half life shows a hyperbolic function with the rate of destruction dropping off sharply as the moisture content decreases below a certain value. Thus, one must expect that several mechanisms by which water is controlling the reaction are taking place, the most likely being a control of diffusion rate and a dilution effect above a critical

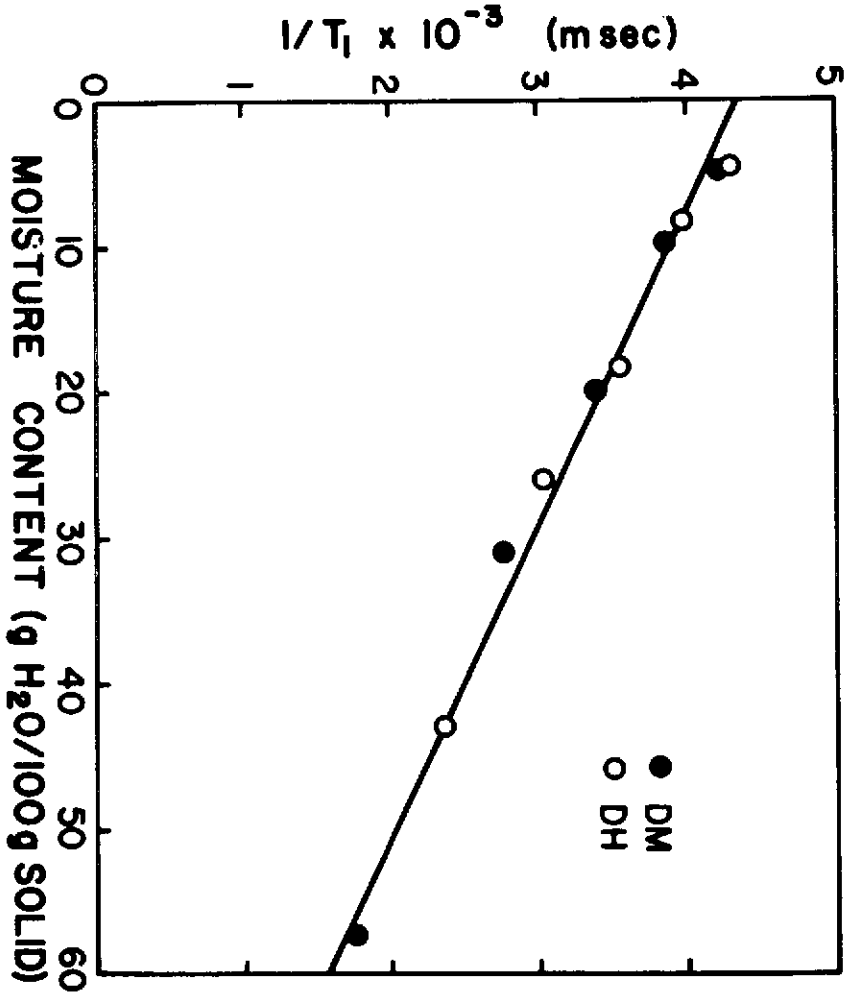

Figure 20. Viscosity of Model System ($1/T_1$) vs. Moisture Content as Measured by NMR.

phase volume. This would tend to flatten the half life out as seen in Figure 19.

In conclusion, ascorbic acid destruction rates were increased with increasing a_w, and ascorbic acid was more rapidly destroyed in the desorption system than in the adsorption system due to a decrease in viscosity and possible dilution in the aqueous phase. Thus, if food is prepared by a humidification method, the stability of ascorbic acid could be improved at the same a_w as compared to the direct mixing method. However, overall storage stability is very small and some type of coating of ascorbate would be necessary since the half lives are less than several days at the desired a_w.

4. Summary and Recommendations

The rate of destruction of ascorbic acid was determined in model systems as a function of a_w, temperature and method of preparation. Under all conditions, the loss of ascorbate followed a first order reaction with an increased rate as a_w and moisture increased. The half lives were in the range of 1 to 3 days at 35°C for the a_w range of 0.84 to 0.67. The increased rate was shown to be due to the decreasing viscosity of the aqueous phase as measured by NMR. However, the rate does not increase linearly with viscosity since a possible dilution effect is occurring which would cause the rate constant to be unchanged. Based on this, to insure ascorbic acid stability in IMF the following should be done:

a. Keep the a_w as low as possible.

b. Incorporate some agent to increase the aqueous phase viscosity which does not adsorb water to a high degree.

c. Coat the ascorbic acid with a digestible but water impermeable layer.

d. Incorporate the ascorbic acid into a fat phase.

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B. Vitamin C Stability in Hennican: An Intermediate Moisture Food

1. Introduction

In the previous section studies were reported on the destruction of ascorbic acid as a function of a_w in model systems at a pH of 6. This is close to the pH for Hennican (pH 5.2 to 5.5), an intermediate moisture food developed in Phase I of this contract. The pH was measured by dilution with water. An experiment was set up to determine the rate of destruction of ascorbic acid directly in the

intermediate moisture food and to compare it to the model system studies.

2. Materials and Methods

Some modifications of the extracting reagent previously used for the model system were necessary. Also since the official procedures were designed for either liquids or relatively dry solids, a different method of extraction was necessary as well because initial results showed poor extraction.

a. Extraction solutions

1. 6% HPO_3/8%HOAc/.005%EDTA solution

2. 3% HPO_3/4%HOAc/.0025%EDTA solution made by diluting number one above one to one with glass distilled water

The EDTA was necessary to complex the ferrous/ferric ions present in the product (mainly in the raisins) which would interfere in the titration. When preparing the above extraction solutions it is necessary to dissolve the EDTA in glass distilled water before adding the HOAc to prevent clouding of the solution. The solution also tends to cloud if refrigerated, so a fresh solution was prepared each time.

The standardization of the dye was carried out according to AOAC procedure previously reported with the exception that some of the ratios were changed (e.g. #mg.A.A./ml.A.A standard solution or #mg.3%HPO_3: #ml.A.A. standard solution) so that the standardization titrations would involve volumes comparable to the volume used in titrations of the Hennican samples.

b. Procedure for ascorbic acid extraction

Weigh into a tared one cup blender jar 10 g $\pm$ .01 of sample and make up to 100 g with solution #1 (this volume is the

minimum necessary for thorough blending of the sample) then blend at high speed for 60 to 90 seconds depending on the sample (drier samples take longer). Next transfer 5 g $\pm$ .01 of this slurry into a tared 100 ml volumetric flask and make up to 100 ml with solution #2. Pour this solution into a flask containing about 3 g of Celite, shake and then filter paper under vacuum into another flask. Immediately draw three 10 ml aliquots from the flask to suitable vessels and titrate to the endpoint (a light pink persists for 15 seconds).

c. Procedure for Hennican preparation

Hennican was prepared as previously reported in Phase I using the composition shown in Table 1 for two water activities. The product was divided into 10 g samples and vacuum sealed in Scotchpak #20 foil laminated film (3M Co.) and then stored at 25°C. Ascorbic acid was measured about every seven days. Initially 10 mg/g was added to each system so that analysis would be easier to perform.

3. Results and Discussion

The results are shown in Figure 1 for the loss of ascorbic acid with time at two a_w's. The calculated half lives are presented in the figure. It is obvious that at comparable pH the rate of loss of added ascorbic acid is much less than that in the model systems, which had a half life of 7 days at a_w 0.68 and about 1 day at a_w 0.86. This is due to the fact that the available metal catalyst concentrations must be different than those in the model system. Thus, the recommendations presented may not be as serious in reference to isolating the ascorbic acid; however, the measured half life of one to two months would mean that an excessive amount of ascorbic acid would need to be added if a

TABLE 1

HENNICAN-VITAMIN C STORAGE STUDY

COMPOSITION OF MODEL SYSTEMS

Ingredients	a_w 0.68		a_w 0.86	
	grams	% by weight	grams	% by weight
Dry roasted peanuts	66.50	18.23	66.50	15.59
Freeze-dried chicken	66.50	18.23	66.50	15.59
Raisins	133.00	36.46	133.00	31.18
Peanut butter	17.58	4.82	17.58	4.12
Honey	7.22	1.98	7.22	1.69
K-sorbistat	0.88	0.24	0.88	0.21
Non-fat dry milk	48.65	13.34	48.65	11.41
Glass distilled water	24.32	6.67	86.14	20.19
Total	364.74	100.00	426.56	100.00

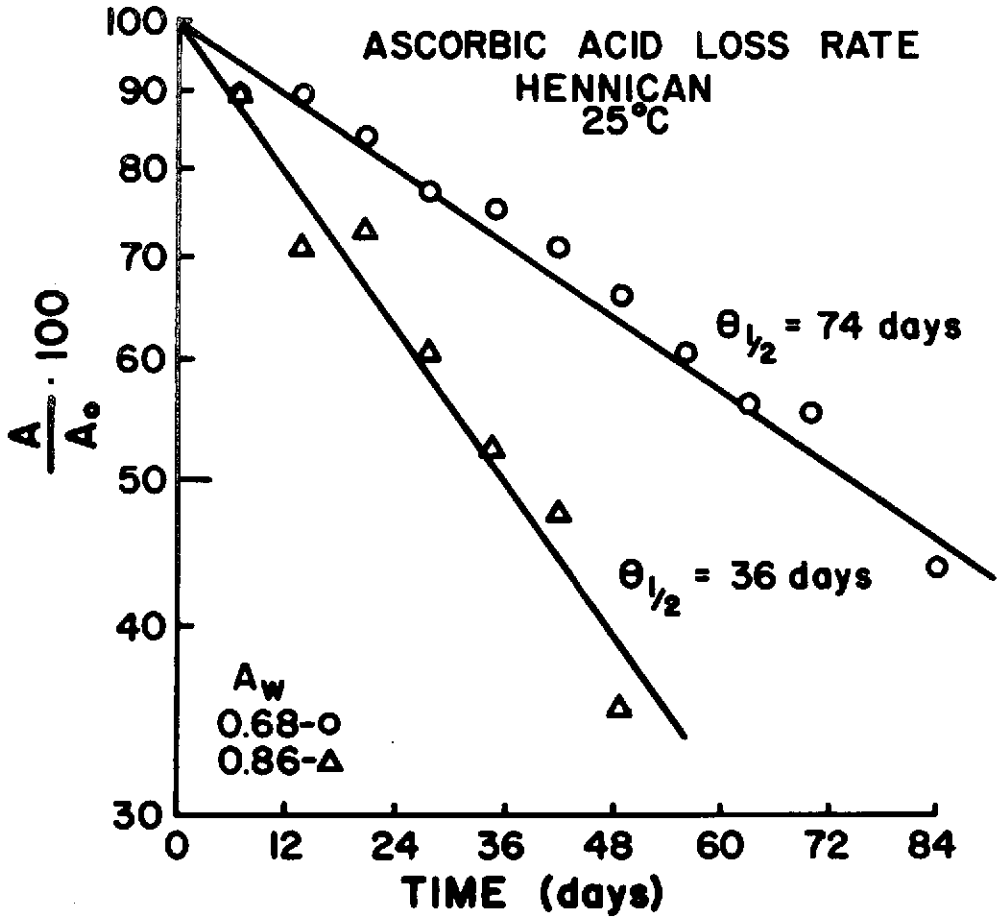

Figure 21. Loss of Ascorbic Acid as a Function of a_w in an Intermediate Moisture Food.

six month shelf life is required. Since this would make the product unacceptable from a flavor standpoint, vitamin C should not be considered in the formulation unless a stable coated form can be found. Of course, the a_w can be lowered. Assuming a fairly linear relationship as shown with the model systems for a six month half life, the product a_w would have to be close to zero thus changing the characteristics of the food. Thus the recommendations of the model system still hold.

4. Summary and Recommendations

In an intermediate moisture food the rate of ascorbic acid loss is much less than in a model system. This could be due to the difference in microenvironment. However, the rate is still very rapid and could lead to significant changes in the desired nutrient value of the food. Thus it is recommended that the processor:

a. Determine a method for coating ascorbic acid to slow its rate of destruction.

b. Keep the a_w of the food as low as possible, while still maintaining palatability.

III

MICROBIOLOGICAL STUDIES IN INTERMEDIATE MOISTURE FOOD SYSTEMS

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A. Effect of Sorption Hysteresis on Microbial Growth in Two Intermediate Moisture Food Systems

1. Introduction

A microorganism's requirement for water has been related to the water activity of its substrate. Troller (1973) has recently reviewed the work in this area. Scott (1957) determined that the minimum growth water activity may be identified for each group of microorganisms. However, this minimum may vary depending upon the solute used to adjust the water activity or the moisture content of the substrate (Marshall et al., 1971; Kushner, 1971). This minimum is also affected by the method of preparation of the food system. Foods prepared to a given water activity by desorption or adsorption, for example, exhibit different growth characteristics for microbes (Labuza et al., 1972b; Plitman et al., 1973).

Working with four microorganisms in model systems, Labuza et al. (1972b) determined that the minimum water activity allowing growth in a desorption system was below that defined in the literature. The minimum water activity allowing growth in a food of the same solids composition as that prepared by desorption, but prepared by adsorption, was not specifically defined. However, from these studies the growth minima were projected to be higher than in the desorption system exemplifying the moisture-hysteresis phenomenon as it affects microbial growth. This hysteresis effect causes some systems to have a higher moisture content at the same water activity if they are prepared by desorption as opposed to adsorption.

This phenomenon has practical importance in food processing and also raises basic questions concerning the properties of water and its interactions with food. This project was undertaken to re-examine the hysteresis phenomenon on the growth minima of four microorganisms; Pseudomonas fragi, Staphylococcus aureus (F265), Aspergillus niger and Candida cypolytica; in two intermediate moisture foods. The two model systems used were a pork system used by Labuza et al. (1972b) and an infusion soaked chicken cube system prepared by a modification of the method described by Hollis (1968). The systems were prepared, inoculated with a known population of test organism, held in storage at 22°C at the water activity of preparation and sampled to measure changes in the viable microbial population.

2. Materials and Methods

a. Basic food systems

The model system was based on Heinz pork baby food (3.88 g H_2O/g solids) to which a constant amount of glycerol to solids ratio was added to reach various a_w's for the direct mix (DM) system. Table 1 shows the model system composition. After mixing the direct mix systems, 5-6 g portions were transferred to sterile 2 oz. screw cap jars. Half of these were frozen at -20°C and then freeze-dried at 75°F, 200 μHg for 18 hr. No significant glycerol loss occurred under these conditions as determined by GLC. The dry samples were then rehumidified by adding an amount of water which was 5% less than that needed to reach the desired a_w's. The jars were then held in desiccators of appropriate a_w for equilibration before being capped, sealed with tape and put into storage at 22°C with the DM samples.

TABLE 1

PORK SLURRY MODEL SYSTEM COMPOSITION

a_w system *	Pork	Glycerol	g H_2O added	Moisture content g H_2O/g solids	
				DM**	FDR***
0.67	5 g	7 g	0.0 g	.50	.42
0.71	5 g	7 g	0.4 g	.58	.46
0.81	5 g	7 g	1.4 g	1.01	.87
0.86	5 g	7 g	3.0 g	1.39	1.11
0.92	5 g	7 g	7.0 g	2.28	1.27

* as measured after 4 weeks storage
** direct mix
*** freeze-dried rehumidified

The solid food system was based on infusion soaked chicken cubes. Sterile, cooked white chicken meat was cut into 1.3 cm cubes using aseptic technique. The chicken was soaked at 21°C for 18 hr. in the infusion solutions shown in Table 2 on a shaker at 100 rpm. The ratio of chicken to soak infusion was 1:10 (w/w). The infusion was drained from the chicken and half the meat was inoculated with the test organism and put into storage in sterile 2.0 oz. jars at the appropriate a_w. This constitutes the direct mix system. The other half was freeze-dried under the same conditions as the pork model system. When dry, the cubes were also partially rehumidified to 5% below the final moisture content. These samples were also stored in the appropriate desiccators.

b. Inoculation of systems and sampling procedures

The microbial suspensions were prepared in the same way for inoculation into both IMF systems. The bacteria were grown in 100 ml of TSY broth at 22°C for 18 hr. on a shaker. The mold was streaked onto a TSYA prescription bottle slant and grown for 5 days at 22°C. The spores were washed from the slant with 30 ml of sterile water. The population of each suspension was estimated by measuring the turbidity at 450 nm. Necessary dilutions were made to give the desired initial viable population in the food system.

The microbes were added to the direct mix systems during the mixing stage of the pork model food systems, and at the end of the infusion soak in the chicken cube systems. All adsorption (FDR) systems were inoculated via part of the water used in the partial rehumidification of the dried samples to prevent any death that may

TABLE 2

COMPOSITION OF INFUSION SYSTEMS USED TO

PREPARE THE SOLID CHICKEN SYSTEM

	% by weight						
a_w of Resulting Chicken Cube	0.75	0.79	0.82	0.86	0.90	0.93	0.97
H_2O	41.8	48.3	54.8	61.8	74.3	78.8	88.6
NaCl	2.3	2.1	1.8	1.6	1.0	0.8	0.4
Chicken bouillon	4.1	3.7	3.2	2.6	1.9	1.8	0.8
Glycerol	51.4	45.6	39.8	33.8	22.6	18.3	10.9
Moisture content (g H_2O/g solids)							
Direct mix (DM)	0.59	0.75	0.85	0.89	1.04	1.17	1.72
Freeze-dried rehumidified (FDR)	0.56	0.72	0.82	0.85	1.00	1.08	1.27

have occurred during freeze-drying. Specifically, they were inoculated by dropping 0.03 g of a suspension of the organism onto 5.0 g of the sample. To prevent possible loss of moisture during storage, the samples were then stored in the desiccators. The systems were inoculated to an initial viable population of 10^4—10^5 CFU/g IMF system.

Sampling was done by either diluting 1 g of pork model system with 9 ml of phosphate buffer (0.125%) or by blending 5 g of chicken with 45 ml sterile phosphate buffer. Further dilutions were made in phosphate buffer and duplicate 0.1 ml aliquots were spread on TSY agar plates. The plates were incubated at a temperature near optimum for each microorganism.

3. Results and Discussion

The study of the sorption hysteresis phenomena effect on growth limiting a_w in the IMF pork model system was done basically to further confirm work reported by Labuza et al. (1972b). The results of both studies show the same general effects for the four organisms tested. As an example, Figure 1 shows the change in viability of S. aureus in the pork model system vividly illustrating the effect of hysteresis. The direct mix (DM) system at a_w 0.92 shows growth, while the FDR system at the same a_w shows a rapid loss of viability. This confirms the previous work of Labuza et al. (1972b). It also should be noted that the inactivation rate decreased as the a_w decreased below the minimum for growth (0.92). The inactivation rate at a_w 0.86 was faster than at a_w 0.67 for the direct mix systems. The reason for this reversal is unknown because little is understood of the inactivation mechanisms involved.

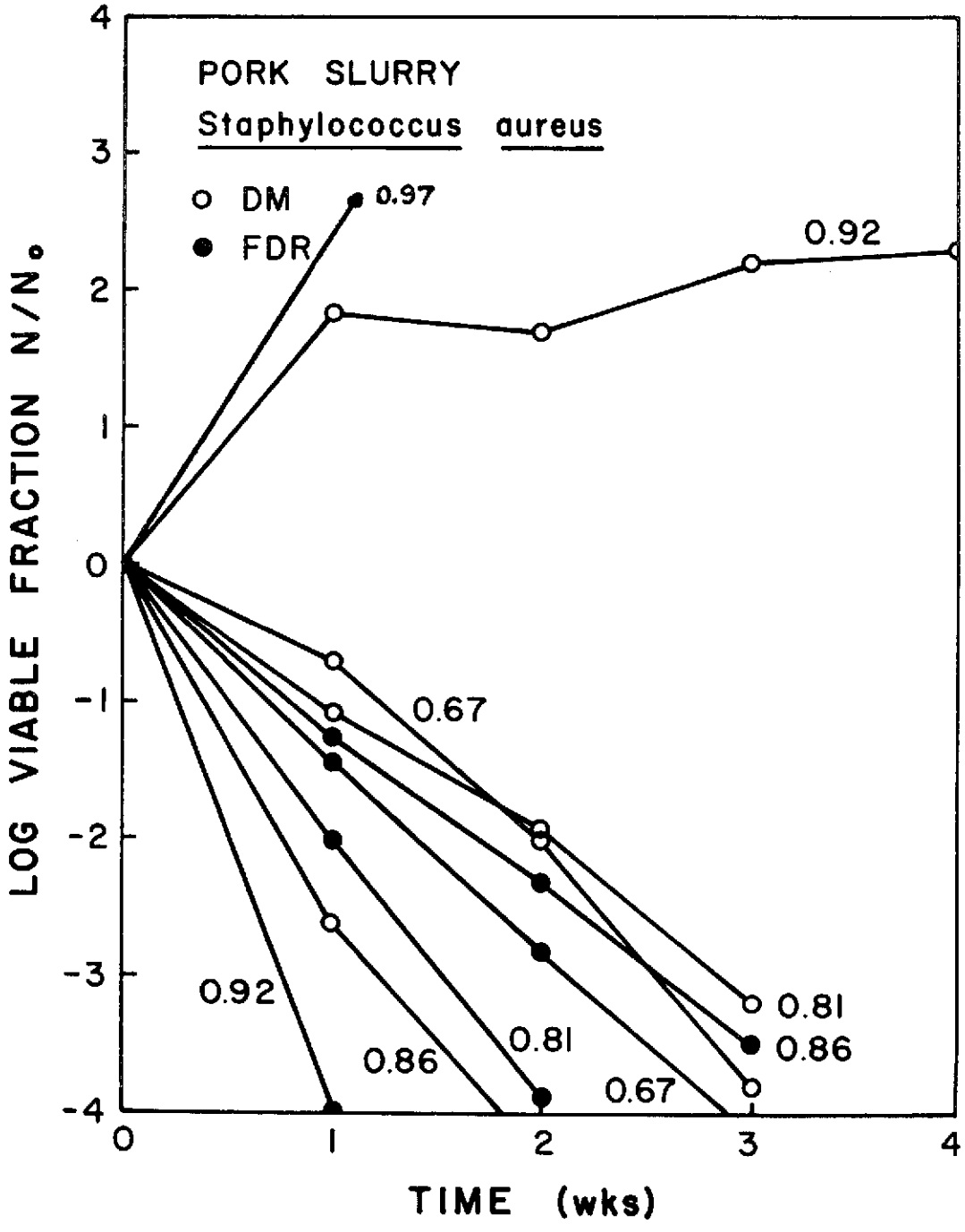

Figure 1. Viability of Staphylococcus aureus in a Pork Slurry Model System as a Function of a_w and Sorption Hysteresis.

The overall results are presented in Table 3. As seen, the new data extend the a_w range of the adsorption system beyond that of the previous work. In all cases the limiting a_w range for growth is higher in the adsorption systems than in the desorption (DM) systems. The limiting a_w for each organism tested in this study was higher in the DM systems than that reported previously by Labuza et al. (1972b). This is probably due to a change in the composition of the pork baby food. Salt and MSG (mono sodium glutamate) were excluded from the pork baby food used in this study. Although the moisture contents were similar (3.6 and 3.9 g H_2O/g solids) the a_w of the baby food in the 1972 study was 0.90 as compared to 0.99 in this study. Therefore, more glycerol was needed in the present system to achieve a comparable a_w. As shown by Plitman et al. (1973) and Acott and Labuza (1974), glycerol itself may have an inhibitory effect on microbes beyond that of lowering the a_w. The high glycerol to solids ratio in this case may have resulted in the inhibition of growth at a higher a_w.

The chicken cube food system represents a typical IM food posing a practical challenge to the hysteresis phenomenon as it affects microbial behavior. The results of the present chicken cube study are shown in Figures 2 through 5 and summarized in Table 3. P. fragi grew at a_w 0.93 in the DM system but was rapidly inactivated in the FDR system at an a_w of 0.93, again demonstrating the hysteresis effect. This species is very sensitive to stress. Leistner (1970) lists the limiting growth a_w as being 0.96, which is higher than found here. This difference may be due to the method of a_w determination employed by the various investigators or the specific effect of glycerol.

TABLE 3

LIMITING A_w GROWTH RANGE FOR MICROORGANISMS

System of preparation	Pork Model System		Chicken Cube	Literature
	1972 **	Present work	Present work	Minimum growth a_w *
Pseudomonas fragi				
DM [a]	0.75—0.84	0.86—0.92	0.86—0.93	0.96
FDR [b]	> 0.92	0.92—0.97	0.93—0.97	
Candida cypolitica				
DM	0.75—0.84	0.81—0.86	0.90—0.93	0.88
FDR	> 0.92	0.86—0.92	0.88—0.93	
Staphylococcus aureus				
DM	0.75—0.84	0.86—0.92	0.86—0.90	0.86
FDR	> 0.92	> 0.92	0.90—0.93	
Aspergillus niger				
DM	0.68—0.75	0.81—0.86	0.75—0.79	0.64
FDR	> 0.92	0.86—0.92	0.79—0.83	

* Leistner (1970)
** Labuza et al. (1972b)

[a] direct mix
[b] freeze-dried rehumidified

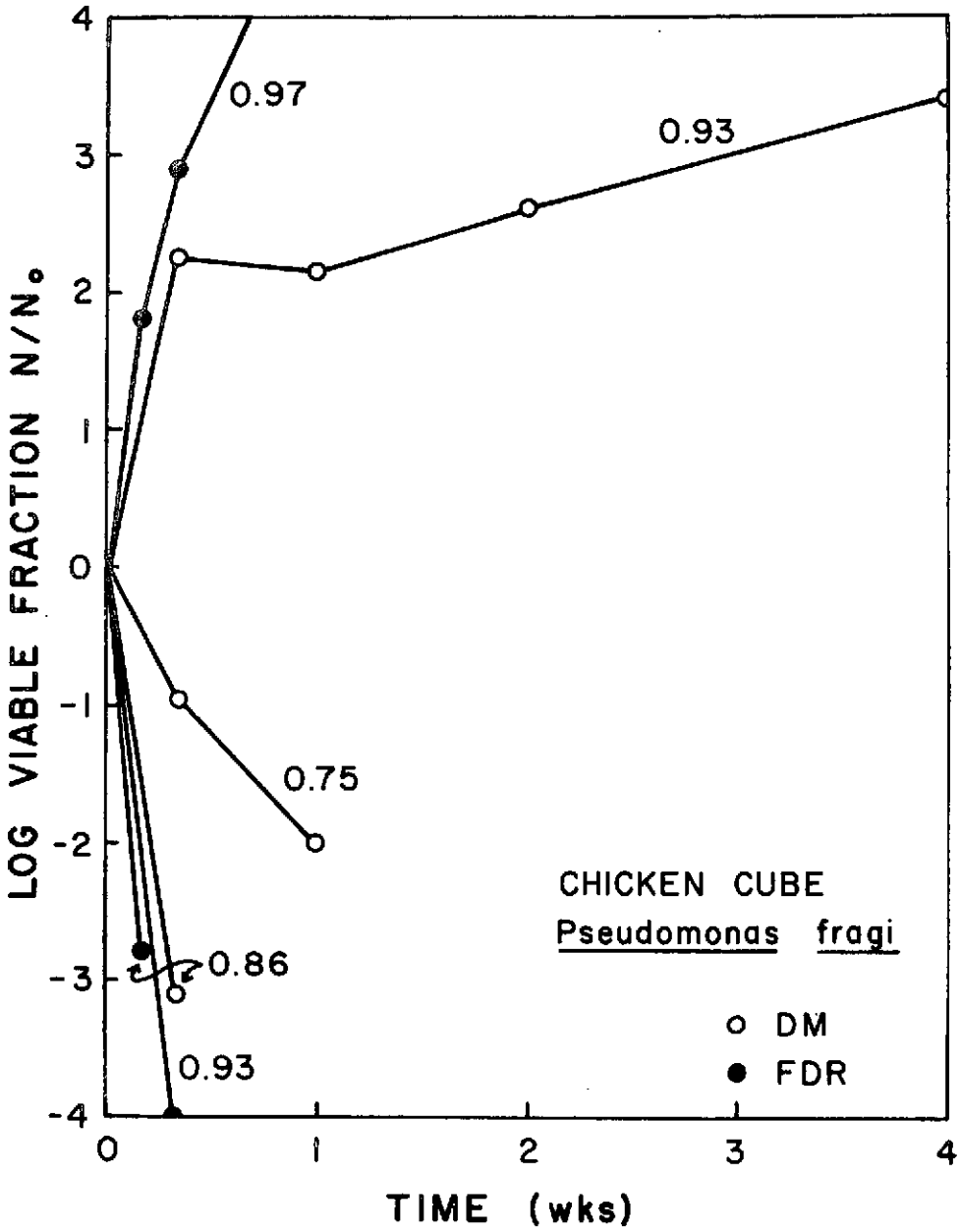

Figure 2. Viability of Pseudomonas fragi on a Soak Infusion Chicken Cube System as a Function of a_w and Sorption Hysteresis at 21°C.

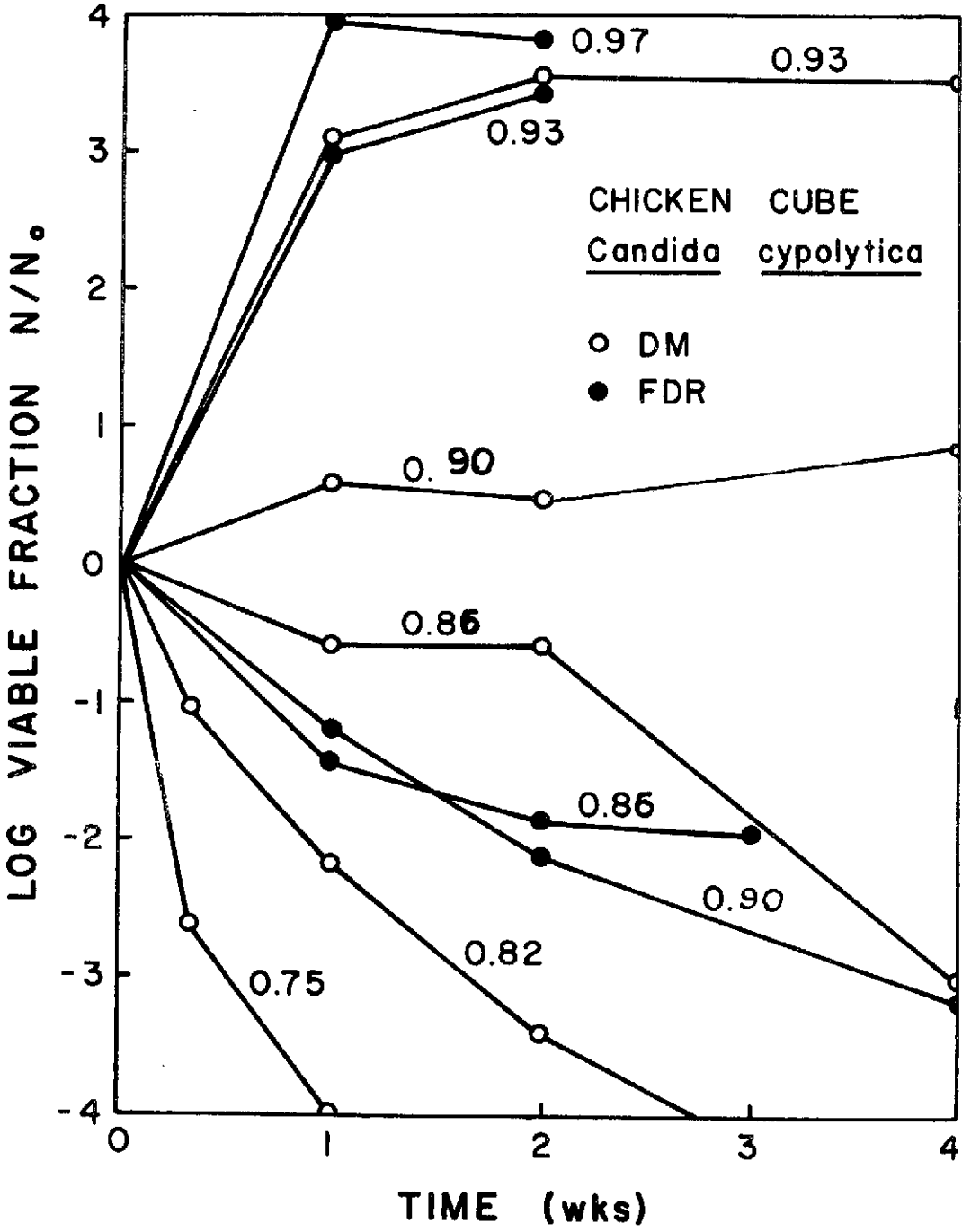

Figure 3. Viability of Candida cypolytica on a Soak Infusion Chicken Cube System as a Function of a_w and Sorption Hysteresis.

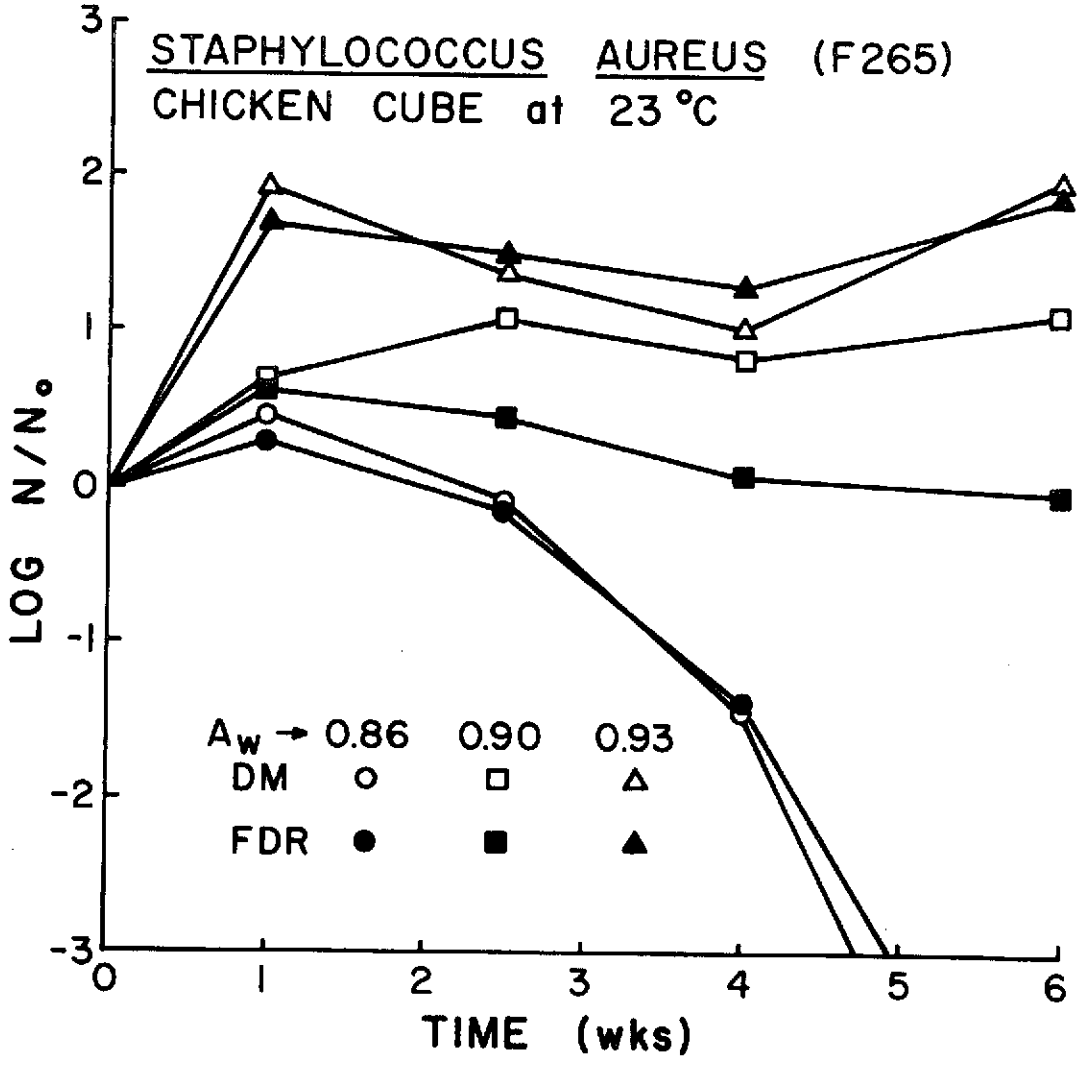

Figure 4. Viability of Staphylococcus aureus on a Soak Infusion Chicken Cube System as a Function of a_w and Sorption Hysteresis.

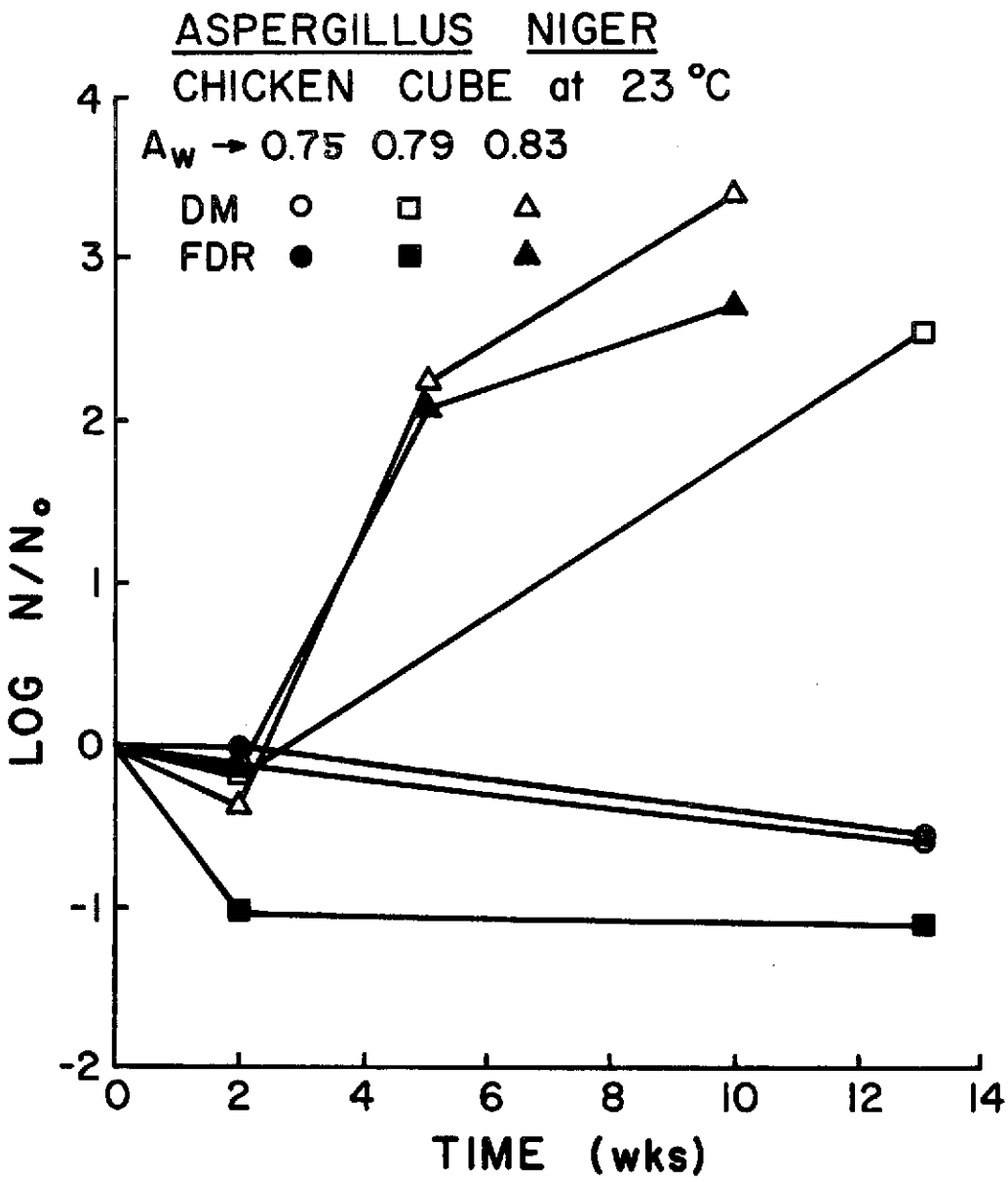

Figure 5. Viability of Aspergillus niger on a Soak Infusion Chicken Cube System as a Function of a_w and Sorption Hysteresis.

The yeast (Figure 3) shows a growth minimum very close to that found in the literature (0.88). The hysteresis effect was not as pronounced in this case as it was with Pseudomonas fragi. As seen in Figure 4, the effect of hysteresis on Staphylococcus aureus in the solid food was not as pronounced as in the liquid food system, however, it is greatest at the a_w which seems to be at the borderline of growth. Possibly if samples were taken for a more extended period at an a_w of 0.90 the effect would have been more obvious. The results for Aspergillus niger show a very obvious hysteresis effect at an a_w of 0.79. It is possible that spores of the FDR system may have died or may have eventually grown after an extended lag phase since the experiment was only carried out for 13 weeks. The frequency of plating was determined by the times for visual appearance of the mold on the chicken, a criterion for consumer acceptance. This explains the scarcity of data points for the mold systems. The overall results are reported in Table 3. It is obvious that the growth range changes slightly with the system, especially for the mold.

4. Summary and Recommendations

This study was done to confirm the fact that the method of preparation of an IM food can affect the possible growth of microorganisms. Specifically, a food prepared by a desorption technique has a higher moisture content at a given a_w than does one prepared by adsorption. In a liquid slurry system, studies with four organisms showed that the organisms had a higher a_w requirement for growth in the adsorption system, confirming previous work. The same phenomenon was studied in a solid food system and the results were repeated.

This suggests that the total amount of water present as well as the availability as defined by water activity is important for biological reactions, as has been shown for chemical deterioration reactions (Labuza et al., 1972a). Thus, one must know something of the history of the system. These results also suggest why literature values may be variable since different compositions, humectants and preparation techniques are usually used. Based on this, it is recommended that, if there is no problem with palatability, manufacturers should use an adsorption process to prepare IMF since there is an advantage of increased microbial inhibition.

5. References

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B. Inhibition of Aspergillus niger in an Intermediate Moisture Food System

1. Introduction

The effect of water activity (a_w) on the growth of microorganisms has been reviewed by Scott (1957) and Troller (1973). It is known that as the water activity decreases, the growth of microbes is slowed or prevented. Recently, intermediate moisture foods (IMF) having a moisture content of 15-40% H_2O and an a_w of 0.65—0.85 have been examined in terms of microbial stability (Hollis et al., 1969; Labuza et al., 1972; Plitman et al., 1973). Microbial spoilage in foods of this type is due primarily to mold or yeast growth since most bacteria do not grow at the lower a_w.

Pet foods are examples of commercial IMF meat products. During the processing of these semi-moist foods, which includes extrusion cooking, the incident yeast and pathogenic bacteria are presumably killed, but the mold spores survive. Since these foods have a relatively high pH (pH 5-6) and are stored at room temperature, the mold spores are a potential spoilage problem. Thus, mycostatic agents are incorporated into the food product to prevent growth and thus extend the shelf-life of the product.

The growth of mold (as well as other microbes) is dependent on a_w, temperature, atmosphere and substrate, i.e. pH, nutrients and inhibitors. At a given a_w, the growth response will change if any of the above factors is suboptimal (Christian, 1963; Tatini, 1973). The effectiveness of a mycostatic agent should be greater in the IMF a_w range than in high moisture foods. The mycostatic system used in most

IM meats is a combination of 2.0% propylene glycol and 0.3% potassium sorbate (w/w) as described by Kaplow (1970). Plitman et al. (1973) studied glycerol, butane diol and propylene glycol in aqueous brain heart infusion and found the butane diol to be most inhibitory to growth. The effect was greater than just an a_w lowering action. The inhibiting concentrations were 40% for glycerol (a_w 0.865); 15% for butane diol (a_w 0.97) and 18% for glycol (a_w 0.95). A synergistic effect was also observed among the inhibitors.

Frankenfeld et al. (1973) made a similar study of the effectiveness of glycerol, 1,3- butane diol, propylene glycol, potassium sorbate (among others) against mold growth. Two of the systems tested were near the pH of the Hennican system used in this study. One was an apple flake system (1.94 g H_2O/g solids) at pH 4.4 and the second was a chicken baby food system (3.54 g H_2O/g solids) at pH 6.5 These were inoculated with spores and stored at 37°C. Table 1 lists their results. It should be noted that the a_w's were not measured but, based on the high moisture levels, the a_w's are greater than 0.90 in both systems. A comparison of these results with Plitman et al. (1973) shows similar results in that fairly high levels of glycerol and butane diol are required when starting with high moisture systems. However, some inhibitory effect occurs between a_w and the inhibitors as evidenced by the growth inhibition at different a_w's. The comparison for propylene glycol cannot be made since in the work of Frankenfeld et al. (1973) growth occurred at the maximum level tested. In any case, it would be probable that as a_w is lowered the level of additive can be lowered.

The purpose of this storage study was to test a variety of common food additives for their ability to inhibit the growth of

TABLE 1

EFFECTIVE MOLD INHIBITOR CONCENTRATIONS[(a)]

Inhibitor	Effective level*	
	Apple flakes	Chicken
Glycerol	>20%	>25%
1,3- Butane diol	10%	25%
Propylene glycol	>0.3%	N.T.**
Potassium sorbate	0.3%	N.T.

(a) Frankenfeld et al. (1973)

* % by weight of total system
** N.T. = not tested

Aspergillus niger on food at an a_w in the intermediate moisture food range and as a function of pH. Foods can be classified as low acid (pH > 5), medium acid (pH 4.5—5), acid (pH 3.7—4.5) and high acid (pH < 3.7) (Cameron and Estey, 1940). In this study, the Hennican was prepared to pH 5.7 and pH 4.2 as estimated using the gran plot technique. Thus a low acid and an acid food were tested. Many inhibitors only work under acid conditions. A. niger can grow in the pH range of 2.8 to 8.8. Webb (1919) found that germination increases as the acidity rises with a maximum at pH 3. Increased acid decreases this rapidly. A. niger is one of the most active citric acid producers itself which at high pH get metabolized to oxalic acid and CO_2. As citric acid increases, the mold produces very few if any spores.

With respect to a_w (as seen in the previous study, Section III, A.) the mold used can grow down to a_w 0.81 in a pork--glycerol slurry. However, when inoculated on a solid surface the mold can grow as low as a_w 0.79. When inhibitors are added, however, this can increase the minimum growth a_w, as was found by Plitman et al. (1973) for Staphylococcus aureus.

2. Materials and Methods

a. Food systems

The intermediate moisture food used was an adaptation of Pemmican, an old Indian trail and winter storage food made of buffalo meat and berries. Chicken was chosen as the base for the IMF used in this study. The composition of the basic system is shown in Table 2.

TABLE 2

COMPOSITION OF IM FOOD: HENNICAN

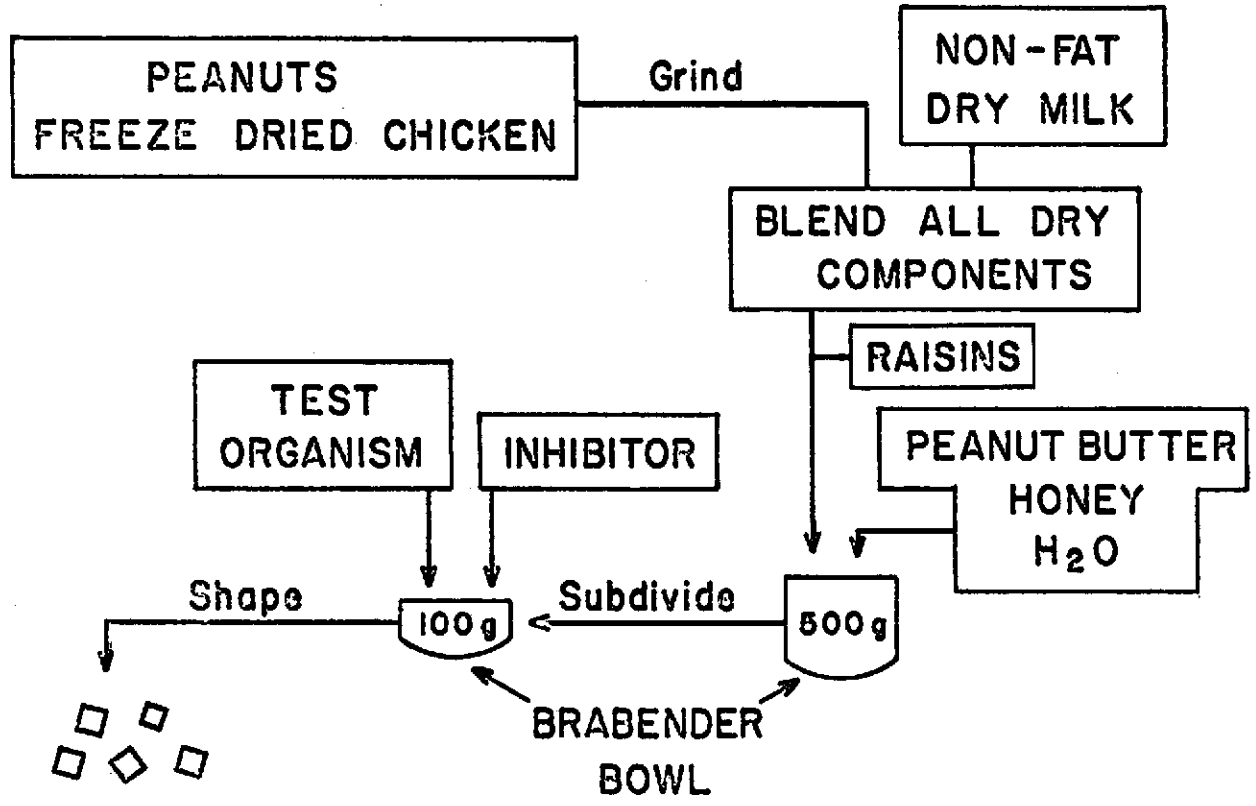
Raisins	30%
H_2O	23
Peanuts	15
Chicken (freeze-dried)	15
Non-fat dry milk	11
Peanut butter	4
Honey	2

Moisture content = 41 g H_2O/g solids

a_w = 0.85

The a_w of 0.85 allowed the growth of the mold but inhibited the growth of the few natural bacterial contaminants in the raw materials. No yeast contaminants were found. A cold-mixing procedure, as shown in Table 3, was used to prepare the Hennican (the name given to the chicken based IMF). The unsalted, hulled peanuts (Skippy Co., Mpls, MN) and the chicken (Aslesen's Banquet Table, canned deluxe boned chicken meat, #3022), which was freeze-dried, were finely ground separately in one-half pint glass blender jars on an osterizer blender. The non-fat dry milk and all other dry ingredients were mixed together. The raisins (dried seedless) were ground in a Hobart food chopper and then blanched in a microwave oven for 1.5 min. to destroy the enzymes responsible for enzymatic browning. The dry components, ground raisins, peanut butter (creamy style), honey and sterile distilled water were kneaded together in a 500 ml Brabender Farinograph bowl at fast speed for 5 min. to achieve a workable paste. This mixture was divided into two equal parts and citric acid, 2% (w/w), was mixed into one part for 3 min. in the Farinograph bowl. The food additives were added via the appropriate carriers to the systems with and without citric acid, and a spore suspension of Aspergillus niger was added to all systems. Three min. of mixing was found adequate for each addition. All systems were then shaped into squares weighing approximately 2.5 g each by rolling out the paste and cutting the squares to 2 cm x 1.5 cm x 0.5 cm. Two samples were placed into sterile plastic petri dishes (60 x 15 mm) and then all samples were stored in desiccators without vacuum at 22°C over a saturated solution of $LiSO_4$ (a_w 0.85 at 22°C). No weight change occurred during storage, showing equilibrium.

TABLE 3. Hennican Preparation Method



The cover was removed periodically so that oxygen was not limiting.

b. Additives

The additives tested fall into four main categories: acid-type, parabens, an antibiotic and polyhydric alcohols. Their FDA status is listed in Table 4. Of the acid-type microbial inhibitors, potassium sorbate is commonly used in cheeses, breads and intermediate moisture foods to prevent mold and yeast growth. Calcium propionate is used in cheeses and yeast leavened breads to inhibit molds and bacteria. It does not interfere with the fermentation activity of yeast. Benzoic acid inhibits molds and yeast and is used in foods of low pH where bacteria normally do not grow.

The acid-type inhibitors must be in the undissociated form to be inhibitory to microbes (Sauer, 1972; Ingram et al., 1956). Chichester et al. (1968) suggest that the reason these preservatives are effective only in the undissociated form is because in that form it is highly lipid soluble and accumulates in the lipid structures of cells. There it somehow inhibits normal cell metabolism. However, the mode of action of these preservatives is still not known.

The effect of pH on dissociation of the three additives tested is shown in Table 5. The relationship of pH and dissociation is described by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{\{A^-\}}{\{HA\}}$$

where $pH = -\log \{H^+\}$
$\{HA\}$ = conc. of undissociated acid
$\{A^-\}$ = conc. of dissociated acid

As the pH decreases, more of the acid inhibitor is in its active, undissociated form. When the pH of the food is equal to the pK_a of

TABLE 4

FDA STATUS OF "MICROBIAL INHIBITORS"

Food additive	Cleared under Section	Final Ruling on Food additive as of Feb. 4, 1974	Max. % allowed
Benzoic acid	121.101	GRAS as chemical preservative	0.1
Calcium propionate	121.101	GRAS as chemical preservative	0.32 dry white flour basis
			0.38 dry whole wheat basis
Potassium sorbate	121.101	GRAS as chemical preservative	----
		GRAS by FEMA	----
		Cleared by Meat Inspection Div.	2.5 to dip casings
1,3 Butane diol	121.2566	Cleared as antioxidant and/or stabilizer in polymers used in manufacture of articles intended for use in, ie. preparing food	----
Glycerol	121.101	GRAS as miscellaneous--general purpose food additive	----
		GRAS by FEMA	----
	121.2552	Cleared for use in packaging materials	----
Synthetic glycerol	121.1111	Should be produced by the hydrolysis of carbohydrates	
		a. it should have no more than 0.2% (w/w) Butanetriols	----
		b. should be used not to an excess of that reasonably required to produce the intended effect	----
Methyl paraben	121.2001	Cleared as antimycotic in food packaging materials	----
	121.101	As a chemical preservative	0.1
	121.249	For use in treatment of bovine mastitis	----
	121.104 (G) (1)	Tolerance in milk	0.00

Table 4 continued (2)

Food additive	Cleared under Section	Final ruling on Food Additive as of Feb. 4, 1974	Max. % allowed
Mannitol	121.101	GRAS as a nutrient and/or dietary supplement	5.0
	121.1115	Cleared for use in foods, in amount not to exceed that reasonably required to accomplish tech. effect	---
	121.104	GRAS as sweetener, formulating aid, stabilizer & thickener, surface-finishing agent at levels not to exceed manuf. practices	40.0 soft candy 8.0 frosting confections <2.5 all other food
		When food consumption may result in daily ingestion of 20 grams of Mannitol, label statement required, "Excess consumption may have a laxative effect."	
Propylene glycol	121.101	GRAS as an emulsifying agent	---
		GRAS as miscellaneous and/or general purpose food additive	---
Propyl paraben	121.2001	Cleared as an antimycotic in manuf. of food pckg. materials	---
	121.101	GRAS as chemical preservative	0.1
	121.249	For use in treatment of bovine mastitis	---
	135G.42	Tolerance in milk	0.00
		Cleared by Meat Inspection Div. to retard mold growth in dry sausage	3.5
Sorbitol	121.101	GRAS as a nutrient and/or dietary supplement	7.0

Table 4 continued (3)

Food additive	Cleared under Section	Final ruling on Food Additive as of Feb. 4, 1974	Max. % allowed
Sorbitol	121.1053	Food additive - levels not to exceed manuf. practices which NAS survey indicates in a max. conc. of:	
		Hard candy	97.0
		Chewing gum	62.0
		Soft candy	98.0
		Frozen desserts & mixes	17.0
		Baked goods & mixes	30.0
		All other foods	8.0
		When food consumption may result in daily ingestion of >50g/day of Sorbitol, label statement must say, "Excess consumption may have a laxative effect."	
Citric acid	121.101	GRAS as a sequestrant	----
	121.101	Cleared as miscellaneous and/or general purpose additive	----
	------	Cleared by Meat Inspection Div. to protect flavor of oleomargarine & to flavor chili con carne at levels sufficient for purpose	
		Cleared by Meat Inspection Div. to increase effectiveness of antioxidants in lard, shortening & dry sausage as follows:	
		--alone or in combination with antioxidant in lard, short.	0.01
		--in dry sausage in combination with 0.003% antioxidant	0.001
		--in combination with antioxidant in fresh pork sausage	0.01

TABLE 5

EFFECT OF pH ON DISSOCIATION OF ACID INHIBITORS

pH	% Active - Undissociated Acid		
	Sorbic	Benzoic	Propionic
3	98	94	99
4	86	60	88
5	37	13	42
6	6	1.5	6.7
7	0.6	0.15	0.7
pK_a	4.76	4.19	4.87

the inhibitor, $\{HA\} = \{A^-\}$, i.e. only 50% of the inhibitor is in the active form. From Table 5, for example, if 0.5% of sorbic acid is effective at pH 2, then to achieve the same effectiveness at pH 5, 1.35% sorbic acid would be required. At pH 5, 3.8% benzoic acid is needed for equivalent activity to 0.5% at pH 2. This high level of acid, however, is not allowed by FDA specifications. At the pH's used in this study (pH 5.5 and 4.2) the effect of water activity in creating an additional stress can be determined.

The parabens, which are esters of parabenzoic acid, are effective as growth inhibitors in pH ranges up to neutrality and are used widely in cosmetics. Their solubility is not as good as acid type inhibitors, however, the effectiveness is a function of the ester chain length. The longer chain is less soluble but more effective. They are also used in beverages, baked goods and dressings. Because of the substitution they are better inhibitors. According to Furia (1968) the parabens are very effective against many microbes, especially mold and Gram-positive bacteria. The recommended levels for inhibition against several microbes is listed in Table 6. In fact, the parabens should be useful since they are more effective at high pH than either propionate or sorbate. The maximum allowable concentration is 0.1%. Methyl paraben is less effective, but more soluble in water while the opposite is true for propyl paraben. To achieve the best effect the esters are often used in combination (Chichester et al., 1968).

The antibiotic studied was pimaricin (natamycin) which is an effective inhibitor of mold and yeast and is used in very low concentrations as a dip solution for cheeses and sausages in some

TABLE 6

ANTIMICROBIAL ACTIVITY OF PARABENS

Microorganisms	% Required for Inhibition	
	Methyl	Propyl
Aspergillus niger	0.1	0.02
Salmonella typhosa	0.2	0.1
Staphylococcus aureus	0.4	0.05
Candida albicans	0.1	0.0125
Saccharomyces cerevisiae	0.1	0.0125
Bacillus subtilis	0.2	0.025

European countries (Clark et al., 1964). On agar, pimaricin inhibits A. niger at 5 ppm (Klis et al., 1959).

1,3 butane diol (Celanese Chem. Co.), a polyhydric alcohol, is used in foods as a flavor carrier. Mannitol, sorbitol and glycerol are used in foods as humectants, plasticizers or sweeteners. The additives were mixed into the food via a water or acetone carrier, depending on the solubility of the additive. When water was used, water had been omitted from the formulation of that particular system to prevent an increase in a_w. When acetone was used, the food system was kneaded until the solvent odor disappeared (an additional 5 min.).

c. Mold inoculation

The mold used in this study was chosen since it is a frequent contaminant of commercial intermediate moisture foods. Aspergillus niger (Plant Pathology, University of Minnesota) was streaked onto a sterile cotton plugged 150 ml prescription bottle slant containing TSYA (Trypticase soy agar and 0.5% yeast extract, BBL brand). The culture was grown at 22°C for 5 days. The mold spores were washed from the slant with 30 ml of sterile phosphate buffer (0.125%) and the turbidity of the suspension was measured to estimate the mold spore concentration. The spore suspension was subsequently diluted and 1 ml was inoculated into the mixing bowl to give an initial mold count of 1×10^4 CFU/g food. The water used as a vehicle for the mold spores was omitted from the formulation of the systems to maintain the desired a_w of 0.85.

After 3 min. mixing in the Brabender bowl, samples of the systems were plated to determine the initial viable mold count. 5 g of the food was blended with 45 ml of sterile deionized water for 1 min. and TSY agar plates were used in duplicate at 22°C for 3 days.

d. pH of systems

The pH of the systems was determined by two methods. A direct reading was taken by pressing a non-aqueous Beckman electrode (#39142) into the squares of food. The gran plot method was also used. To 3.0 g of food either 1, 2 or 3 ml of distilled deionized water was added and stirred in to make a slurry. The pH was read after 5 min. equilibration. The pH was plotted against the grams of H_2O added on gran plot paper (100% volume-corrected, Orion cat. no. 900093). The value at zero addition is the pH. This method is useful for IMF systems and was found more reliable than the method recommended in the A.O.A.C. book of standard methods (A.O.A.C., 1970). The two methods used in this study were found to give the same pH value within $\pm$ 0.05 pH units which is the probable variation in composition. The pH of each system is shown in Table 7.

e. A_w and moisture content

The water activity (a_w) was measured by the manometer technique. The technique has an accuracy of $\pm$ 0.005 at an a_w of 0.85. Storage of the samples over the saturated salt solution made certain that this a_w was constant throughout storage.

The moisture content of representative duplicate samples of the systems with and without citric acid was determined by the vacuum oven method at 29" Hg and 60°C for 24 hr.

3. Results and Discussion

The parameters and results of this study are shown in Table 8. The criteria for no inhibition was when mold became visible. This would indicate a consumer acceptance criterion. As should be expected,

TABLE 7

pH OF HENNICAN WITH MICROBIAL INHIBITORS

Inhibitor	% Level	pH	
		Plain Hennican	Hennican w/2% citric acid
Methyl paraben	0.03	5.53	4.23
	0.05	5.52	4.23
	0.10	5.50	4.34
Propyl paraben	0.01	5.48	4.26
	0.03	5.49	4.24
	0.04	5.51	4.24
Parabens Methyl:Propyl (2:1)	0.05	5.48	4.29
	0.10	5.50	4.30
Mannitol	1.0	5.47	4.25
	2.0	5.45	4.22
	3.0	5.45	4.22
Sorbitol	1.0	5.34	4.23
	2.0	5.47	4.23
	3.0	5.52	4.22
Glycerol	1.0	5.60	4.27
	2.0	5.53	4.20
	3.0	5.52	4.20
Propylene glycol	1.0	5.39	4.18
	2.0	5.50	4.24
	4.0	5.63	4.25
Pimaricin	0.002	5.53	4.25
	0.005	5.53	4.30
Control	0	5.60	4.20

TABLE 8

EFFECTIVENESS OF MICROBIAL INHIBITORS IN HENNICAN, a_w 0.85

Inhibitor	%w/w	Time for 1st Appearance of A. niger* (wks)	
		pH 5.7	pH 4.2
Potassium sorbate**	0.15	2	ng (no growth)
	0.30	ng	ng
Calcium propionate**	0.1	2	ng
	0.2	19	ng
	0.3	ng	ng
Benzoic acid**	0.2	7	ng
	0.3	ng	ng
Methyl paraben*	0.03	ng	ng
	0.05	ng	ng
	0.10	ng	ng
Propyl paraben*	0.01	ng	ng
	0.03	ng	ng
	0.04	ng	ng
Parabens Me/Pro* (2:1)	0.05	ng	ng
	0.10	ng	ng
Pimaricin*	0.001	1	4.5
	0.002	ng	ng
	0.005	ng	ng
1,3 Butane diol**	1.0	1	22
	2.0	ng	ng
	4.0	ng	ng
Propylene glycol*	1.0	ng	ng
	2.0	ng	ng
	4.0	ng	ng
Mannitol*	1.0	ng	ng
	2.0	ng	ng
Sorbitol*	1.0	ng	ng
	2.0	ng	ng
Glycerol*	1.0	ng	ng
	1.0	ng	ng
Control**		1	4.5

* 6 months storage; ** 9 months storage 22°C

all the acid-type inhibitors were completely effective at pH 4.2 showing no growth for over 9 months in this intermediate moisture food. With a pH in the normal range for meat products, 0.3%, K-sorbate is an effective mold inhibitor without the added effect of propylene glycol. If the food were higher in pH, more K-sorbate than the FDA allowance would be necessary. A similar trend is found for the propionate. Benzoic acid is not effective in the amount allowed by FDA restriction (0.1%) at the higher pH.

The parabens inhibited the mold at all levels tested. As seen, a lower concentration than found for the acid-type inhibitors is effective. The antibiotic, pimaricin, is effective at 0.002% (or 20 ppm) at both pH 5.7 and pH 4.2. Klis et al. (1959) found inhibition at 5 ppm in agar at pH 5.6. However, they only incubated for two weeks. It is possible growth might have occurred after that time, showing from a practical standpoint that a longer time is needed. This study found 10 ppm to be ineffective. Most likely the antibiotic was not distributed as well in the heterogeneous food of this study.

The polyhydric alcohols, including propylene glycol, were inhibitory at lower concentrations compared to the amount allowed for various uses by the FDA. The minimum inhibitory concentration determined for 1,3 Butane diol, 2.0%, is below the inhibitory concentration found by Frankenfeld et al. (1973) in studies of Aspergillus niger on food systems of higher a_w, and similar pH.

The interaction of a_w, solute used and pH in their effect on microorganisms has been reported by many workers (Troller, 1973). The solutes used to lower a_w are often polyols, such as glycerol, propylene

glycol, 1,3 butane diol, and it is certain that their inhibitory effect is not entirely related to their water binding capacity; however, the reason for their toxicity is not known. Karmas suggests that it may be due to water structuring by the polyols (personal communication). Working with Neurospora sp., Charlang and Horowitz (1970) found that glycerol was less inhibitory as compared to NaCl or sucrose at the same a_w. They suggest that the difference is due to the solute's electrolytic properties. They found that at low a_w, a substance essential for spore germination was lost to the medium and when the substance was isolated and supplied to the spores germination occurred. They suggest that the release of this substance is due to plasmolysis which is a function of the permeability of the cell to a solute.

Webb (1960) suggested that death at lowered a_w was due to the dehydration of an essential macromolecule. He suggested that if the solute had a hydrogen bonding ability, it may bind on the macromolecule and prevent denaturation from loss of the hydration shell as a_w decreases. This could explain why glycerol was less toxic than NaCl in the Charlang and Horowitz (1970) study, however, it does not explain the toxicity in this study.

Horner and Anagnostopoulos (1973) studied the growth rate of several molds as a function of pH, a_w, temperature and the solute used to adjust a_w. They found glycerol to be more inhibitory to Aspergillus niger than sucrose at the same pH and a_w. On agar at a_w 0.86 and pH 3.7, growth of Aspergillus niger was visible on media containing glycerol as the humectant after 5 days at 25°C. This is a very short lag time compared to the present study in which the control

Hennican (no glycerol added) at a_w 0.85, pH 4.2 didn't show growth of the mold for 4.5 weeks. Under the stress presented by this real food system as compared to nutrient agar, the additional adverse effects of only 1% of glycerol was enough to completely inhibit the mold for over 6 months. That stresses on the cells are taking place can be seen in Table 9 which shows the change in morphology of the mold under the different conditions.

After six months, a sample of each of the systems which showed no growth was plated to determine if viable mold spores were present. Table 10 shows these results. Total count is reported showing that some of the low number of natural contaminants originally in the system have survived but haven't increased in numbers. A gram stain was done on various representative colonies from the TSYA plates. Most of the persisting bacteria were Gram (-) rods. This is unusual as Gram (-) microbes are usually less stress resistant. Gram (+) cocci were found in the propyl and methyl paraben systems. Note that there were no viable mold spores detected in any of the systems, with the exception of the glycerol 1, 2 and 3% systems at pH 4.2. This shows that most conditions were actually lethal to the mold.

4. Summary and Recommendations

The interaction of pH with several proposed antimycotic food additives was studied in an intermediate moisture food (IMF) to determine their efficacy against mold growth. An IMF chicken based product was prepared to a water activity (a_w) of 0.85. To one system citric acid was added to change the pH from 5.7 to 4.2. These systems were then blended with the antimycotics tested including potassium

TABLE 9

VARIATIONS IN MORPHOLOGY AFTER 6 MONTHS

AT 22°C IN HENNICAN AT A_w 0.85

Antimycotic (% added)	Mycellium characteristics		Spore characteristics	
	pH 5.7	pH 4.2	pH 5.7	pH 4.2
K-sorbate				
0.15	gray--long	ng	black	ng
0.30	ng	ng	ng	ng
Calcium propionate				
0.1	tan	ng	black	ng
0.2	tan	ng	black	ng
0.3	ng	ng	ng	ng
1-3 Butylene glycol				
1.0	gray	white	black-brown	black
2.0	ng	ng	ng	ng
4.0	ng	ng	ng	ng
Benzoic acid				
0.2	white	ng	black	ng
0.3	ng	ng	ng	ng
Pimaricin				
0.0005	tan	white (long myc)	black	black
0.0010	light tan	white (long myc)	black	black
Control				
0.0000	white	white	black	black

ng = no growth
myc = mycellium

TABLE 10

TOTAL VIABLE COUNT AFTER 6 MONTHS @ 22°C IN HENNICAN AT A_w 0.85

	%	Gram reaction* and (count/g of Hennican) pH 5.7	pH 4.2
Methyl paraben	0.03	$<10^3$ g^+	$<10^2$ g^-
	0.05	$<10^2$ g^-	$<10^2$ g^-
	0.10	$<10^2$ g^-	$<10^2$ g^-
Propyl paraben	0.01	$<10^2$ g^+	$<10^2$ g^-
	0.03	$<10^2$ g^+	$<10^2$ g^+
	0.04	$<10^2$ g^+	$<10^2$ g^+
Combo paraben	0.05	$<10^2$ g^-	$<10^2$ g^-
	0.10	$<10^2$ g^-	$<10^2$ g^-
Mannitol	1.0	$<10^2$ g^-	$<10^2$ g^-
	2.0	$<10^2$ g^-	$<10^2$ g^-
	3.0	$<10^2$ g^-	$<10^2$ g^-
Sorbitol	1.0	$<10^1$ g^-	$<10^1$ g^-
	2.0	$<10^1$ g^-	$<10^1$ g^-
	3.0	$<10^1$ g^-	$<10^1$ g^-
Glycerol	1.0	$<10^2$ g^-	2.3×10^4 mold
	2.0	$<10^2$ g^-	1.2×10^3 mold
	3.0	$<10^2$ g^-	$<10^2$ mold
Propylene glycol	1.0	$<10^2$ g^-	$<10^2$ g^-
	2.0	$<10^2$ g^-	$<10^2$ g^-
	3.0	$<10^2$ g^-	$<10^2$ g^-
Pimaricin	0.002	$<10^1$ g^-	$<10^2$ g^-
	0.005	$<10^1$ g^-	$<10^2$ g^-

* g^- = g^- rods; g^+ = g^+ cocci

mold was typical A. niger

sorbate, calcium propionate, methyl and propyl paraben, 1-3 butane diol, glycerol, mannitol, sorbitol, propylene glycol, benzoic acid and pimaricin at 2-3 levels. To each system spores of Aspergillus niger were added to give a count of 10^4/gram. The systems were stored in desiccators at a_w 0.85 at 22°C and time for mycelia to appear was measured over a nine month period. At least 10 separate samples were used. The results showed that most of the growth inhibitors were as effective as potassium sorbate below the accepted FDA approved levels of addition. For example, calcium propionate at pH 5.7 and 0.3% w/w was as effective as the sorbate. Glycerol at 1.0% was effective at both pH levels. This study showed that many approved food additives not used in IMF systems are as effective as potassium sorbate in preventing the growth of a mold. From a processors standpoint based on price and availability, the following should be used in preparation of an intermediate moisture food at a pH around 5 to 6:

(1) Propylene glycol or glycerol at 1-2% w/w.

(2) The above recommendation at 1% in combination with methyl paraben at 0.033% and propylparaben at 0.007%.

(3) Pimaricin at 0.002% if approved by the FDA.

(4) Butane diol would be applicable if it receives FDA approval.

Although the other glycols were as effective at pH 5.7, their poorer water sorption capacity and flavor would make them unacceptable.

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C. Antimicrobial Action of Polyhydric Alcohols on Microorganisms

1. Introduction

Olitzky and Maitly (1967) suggested as a method for the detection of Gram (+) bacteria the addition of 6 or 12% propylene glycol to plating medium. 6% propylene glycol allowed the growth of Staphylococcus sp. and Streptococcus sp., but inhibited Proteus sp. and other Gram (-) bacteria. 12% propylene glycol in agar plates supported the growth of coagulase (+) Staphylococcus sp., but not other species. It is of interest to know whether this inhibition is due to a specific action of the propylene glycol or if it is due to a water activity lowering effect. An experiment was designed to test the glycols used in the mold inhibition experiment (Section III, B.) against S. aureus (F265) and A. niger, it was also proposed to measure the a_w of the agar with 12% propylene glycol medium, to better understand the mode of action of the propylene glycol. The a_w growth minima for most of the Gram (-) bacteria tested by Olitzky and Maitly is 0.95, however, for Staphylococcus aureus it is 0.86 (Leistner, 1970). The minimum growth a_w for A. niger is between 0.79 to 0.82 (Section III, A.).

2. Methods

Five polyhydric alcohols were tested to determine their effectiveness in inhibiting the growth of Staphylococcus aureus F265 and Aspergillus niger. Each alcohol was added to trypticase soy yeast extract agar in two concentrations: 6% and 12%. The polyols were added to 50 ml of liquified TSY agar in 3 and 6 ml aliquots to obtain these concentrations. After mixing by swirling the bottles, each 50 ml portion of agar was poured into 5 plastic petri dishes. The agar was allowed

to solidify and one plate from each group was divided in half and streaked with each of the two organisms to be tested. The inoculated plates were incubated at 37°C for 24 hr and the growth which occurred was recorded. The water activity of the TSY agar plus 12% propylene glycol was measured using the method described by Vos and Labuza (1974).

3. Results and Discussion

The measured a_w of the 12% propylene glycol — TSYA medium is 0.96. This is slightly higher than the minimum growth a_w in the literature for the Gram (-) bacteria tested by Olitzky and Maitly. It is hard to be certain of literature values for a_w since many methods used in the past for a_w measurement are not reliable. The a_w's of the other systems were not measured but would be higher than the propylene glycol. It is possible therefore that inhibition was due to a_w lowering, since Gram (-) bacteria are more sensitive to lowered a_w than the Gram (+) bacteria. On the other hand, the marginal a_w and the inhibitory property of propylene glycol could have prevented growth. The a_w of the 6% propylene glycol in TSYA was 0.98 which should support growth of the Gram (-) bacteria tested. Inhibition therefore appears to be due to some specific action of the glycol and not due to a_w lowering.

As seen in Table 1, the S. aureus grew at both concentrations of all the polyols. 12% propylene glycol and 1,3 butane diol offered more stress than the others tested. The mold grew typically in the 12% sorbitol and mannitol systems which have relatively high a_w's, while in the Hennican (0.85), 1% of these were inhibitory as shown in Section III, B. On 6% propylene glycol and 1,3 butane diol the mold grew weakly

TABLE 1

GROWTH OF MICROORGANISMS ON POLYOL-TSYAGAR STREAK PLATES

WITH VARIOUS POLYOLS

Polyol	*S. aureus* (F265) 6%	*S. aureus* (F265) 12%	*A. niger* 6%	*A. niger* 12%
Glycerol	+++	+++	+++ green spores	+ green spores
Propylene glycol	+++	+	+	ng
1,3 Butane diol	+++	+	+	ng
Sorbitol	+++	++	+++	+++
Mannitol	+++	+++	+++	+++

+++ - heavy growth
++ - moderate growth
+ - light growth
ng - no growth

and no growth occurred at the 12% levels, although the staphylococci (which are less tolerant of low a_w than mold) did grow.

Frankenfeld (1974) found 2% propylene glycol plus 20% glycerol in nutrient broth, and 5% 1,3 butane diol and 20% glycerol in nutrient broth to be ineffective in inhibiting A. niger. In light of the present results, the failure of these high concentrations to inhibit the mold growth is surprising. The difference could be related to the type of media used and growth of mold which occurs in liquid vs. semi-solid media. The pH of both studies was 6.8—7.0.

The growth morphology of the mold on the glycerol TSYA plates was unusual. The spores that were produced in both cases were green, a physiological response of A. niger which is indicative of poor nutrition, specifically, a lack of Cu^{++}. This color reaction is so fundamental that A. niger may be used for bioassay for Cu^{++} (Cochrane, 1958).

4. Summary and Recommendations

The results of this study suggest that in liquid medium, the response of a pathogen namely Staphylococcus aureus to the presence of humectants is different than that of a mold. The organism in this study grew in a basal medium with up to 12% addition of either glycerol, propylene glycol, 1,3 butane diol, sorbitol or mannitol whereas the glycol and butane diol at 1% inhibited the mold growth. However, this was at a lower a_w. These latter were suggested as being good antimycotic humectants from the previous study. This study therefore leads to the recommendation that processors should challenge their finished IMF product with Staphylococcus aureus to be certain that it prevents growth,

especially if the a_w is above 0.86. It is possible that at a_w 0.86, the same levels of humectants (about 1%) that inhibit mold will also be effective against staphylococcal growth.

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D. The Effect of pH on the Growth of Aspergillus niger in an Intermediate Moisture Food

1. Introduction

The stability of an intermediate moisture food is determined by the aqueous environment. With respect to microbial growth, the basic factors are the amount of nutrient available, the availability of water as determined by the water activity (Section III, A.), the presence of growth inhibitors (Section III, B.) and the affect of pH. Many organisms especially pathogens are inhibited as pH decreases, with a sharp decrease coming below pH 4.5. This study was designed to test the inhibitory effects of various food acids on the growth of a mold in Hennican, an intermediate moisture food.

2. Methods

a. System preparation

Hennican was prepared according to the procedure outlined in Phase I, NAS 9-12560. Table 1 shows the composition, moisture and a_w. After mixing, the system was divided and a calculated amount of various acids was mixed into the different portions. Levels of 1 and 2% were used for the acidulants. The mold spore suspension was added at this time, as done previously, giving an initial count of 10^4 CFU/g of Hennican. It was then cut into squares and stored in desiccators at a_w 0.85 and 22°C.

b. Acids used

The properties and GRAS status of the acidulants used are listed in Table 2. Fumaric, citric, lactic, adipic and phosphoric acid are used in food items to adjust pH, act as antioxidants (chelating

TABLE 1

COMPOSITION OF HENNICAN SYSTEM

Component	%
Skippy peanuts	15.06
Freeze-dried chicken	15.06
Raisins	30.12
Skippy peanut butter	3.99
Honey	1.63
Non-fat dry milk	11.01
H_2O	23.13

	Value
Moisture content[a] (g H_2O/g solids)	40.82
a_w[b]	0.85

[a] - vacuum drying oven (18 hr, 27 Hg, 64°C)
[b] - VPM @ 71°F

TABLE 2

ACIDULANT PROPERTIES FOR pH STUDY

	Adipic	Citric	Fumaric	Lactic
K_{a1}	3.7×10^{-5}	8.2×10^{-4}	1.0×10^{-3}	1.4×10^{-4}
K_{a2}	2.4×10^{-6}	8.8×10^{-5}	3.0×10^{-5}	----------
K_{a3}	----------	3.9×10^{-6}	----------	----------
Properties:	Non-hygroscopic	Very H_2O soluble	Non-hygroscopic	Non-volatile liquid
	Buffer in pH range 2.5-3.0	Buffer index 2.5	Buffer index 3.46	Self esterification on heating
	Low acidity	Metal chelater	-------	-------
FDA status:	GRAS	GRAS	GRAS	GRAS
Uses:	Grape flavored products	Dairy prod. (pH & flavor)	Supplements grap flavor	Spanish olives
	Dry food powders, e.g. gelatin	Prevents staling in frying oils	Dry foods prevent caking e.g. pudding	Liquid shortening
	Fruit drinks	Fruit juices	Prevent rancidity in lard, butter sausage, nuts bacon	Cheeses
	Processed cheeses	Jellies, dressings		Jelly, jam
	Candy	Honey-prevents crystallization		Wine, juices
	w/Na meta-phosphate as preservative in sausage & meat products	Antioxidant in fish	w/Na Benzoate in green foods & fish	
			Stabilizer for suspended matter in flash-sterilized or frozen concentrates	

agents) and produce tartness in accordance with the particular properties of the acid and its acid strength. Individual acids may have flavor and physical properties other than acid strength which are especially advantageous in particular food items. Table 1 lists some properties and uses of the acidulants in foods. For instance, adipic and fumaric acid have a flavor quality which complement grape flavor. Their non-hygroscopicity makes their presence in dry products better than citric acid, which would encourage caking. Citric acid will chelate metals so would aide in slowing lipid oxidation. Use of certain acidulants may have a synergistic effect when used with the pH-dependent acid type inhibitors. This synergism may be even more evident in IM foods where the conditions for survival and growth of microbes are already adverse because of low moisture content and water activity. The amount of preservative needed may be minute enough so that undesirable acid flavor would not be noticed, i.e. in a meat or other product when tartness is not a natural flavor characteristic. For instance, adipic acid and sodium metaphosphate, or fumaric acid and benzoate have been found to be antimicrobial as well as antioxidant in meat products.

Two systems were studied. In the first study, the acids were added at 1 and 2% for each of them. In addition, a citric acid system at 4% and a 10% adipic acid system were used. The pH as measured by the gran plot technique is shown with the results in Table 3. In the second study, the acids were compared at similar pH as determined by the Beckman method. The pH vs. acid concentration curve is shown in Section V, C. The levels tested are shown in Table 4 along with the results.

3. Results and Discussion

The results are presented as the time until the first sign of the white mold mycellium and also the amount of time required for production of the black spores. This means of presentation does not depict the morphological variations but does give an indication of the difference in the extent of inhibition as a function of pH and of acidulant.

The results are presented in Tables 3 and 4. There is a slight difference in the time required for sporulation between the two batches of Hennican. The reason for this is not known, but may indicate a variable in the nutrients of the Hennican components, as the other variables were the same for both batches. The study presented in Table 3 was designed to compare acid strength in terms of the pH attained after adding 1 or 2% of the acidulant. The control Hennican which has had no acidulant added is a low acid food (pH 5.6). 1% level of all acidulants put the Hennican in the medium acid range (pH 4.5 to 5.0). 2% fumaric and citric acid make Hennican an acid food (pH 3.7 to 4.5). The 10% level of adipic acid was needed to make an acid Hennican system.

The pH of the control (5.6) is optimum for growth of A. niger and mold appeared there after 9 days. The addition of 1% citric acid didn't affect the time for first appearance but the time for spore formation was greatly affected. 1% of the other acids increased the lag phase and as expected, as the concentration of acid increased, the time for appearance increased.

TABLE 3

EFFECT OF ACIDS ON THE GROWTH OF A. NIGER ON HENNICAN

(a_w - 0.85 -- 22°C)

Acid	% Added	pH	Time for 1st Sign of Mold (days)	Time for Sporulation (days)
Control	0	5.6	9	12
Adipic	1.0	5.0	11	--
	2.0	4.6	19	--
	10.0	4.3	--	--
Citric	1.0	4.7	9	49
	2.0	4.3	11	42
	4.0	3.9	16	28
Fumaric	1.0	4.5	11	39
	2.0	4.0	14	44
Lactic	1.0	4.9	13	--
	2.0	4.5	21	--

-- = none in 63 days

Lactic acid was more inhibitory at the 2% level (pH 4.5) than the other acids at that level although the pH wasn't as low as in the 2% fumaric acid system. This indicates some specific inhibitory effect. Adipic acid at 10% gives the same pH as the 2% citric acid system. It completely inhibited mold growth for 63 days whereas the citrate did not, again a specific acid effect. The type of acid is as important as the pH attained. The second study, reported in Table 4, was made to determine the effects of the acidulants at similar pH levels. The time for first appearance of mold varies over a period of only 3 days with all the samples showing mold in that time. This was less than occurred in the previous test and the reason is unknown.

Adjusting the pH to 5.0 with adipic, citric or lactic acid gave the same results as the control at pH 5.6. Samples with citric acid or phosphoric acid of pH from 5.0 to 4.2 all showed mold on the same day suggesting that lowering the pH to 4.2 had no effect on growth. However, an effect is evident in the difference in morphology of the mold that appeared. This indicated that some stress occurred on the organism. The growth of mycellium at the lower pH's is much less dense than at high pH's.

An interesting effect was noted in the first study with citric acid. Mold appeared first at the higher pH but the black spores actually appeared first at the lower pH. A. niger is one of the most active citric acid producers. At high pH, this citric acid gets metabolized to oxalic acid and CO_2. When a lot of citric acid is being produced, spores are not produced.

TABLE 4

EFFECT OF pH AND ACID TYPE ON GROWTH OF A. NIGER ON HENNICAN

(a_w - 0.85 -- 22°C)

Acid	% Added	pH	Time for 1st Sign of Mold (days)	Time for Sporulation (days)
Control	0	5.5	7	9
Adipic	1.01	5.0	7	11
	3.03	4.6	8	12
	16.0	4.2	9	13
Citric	0.65	5.0	7	11
	1.24	4.6	7	11
	2.03	4.2	7	11
Fumaric	0.52	5.0	8	11
	0.83	4.6	8	12
	1.68	4.2	9	13
Lactic	0.72	5.0	7	11
	1.10	4.6	8	12
	2.48	4.2	8	13
Phosphoric	0.52	5.0	8	11
	0.80	4.6	8	11
	1.56	4.2	8	11

As mentioned previously, the morphological differences show unusual effects. Table 5 shows the color variations noted in the first batch of samples put into storage. Table 6 gives color variations noted in the second batch. Why a difference between the color variations in Tables 5 and 6 occurs is unknown.

4. Summary and Recommendations

This study did not determine the lower pH limits of growth for A. niger. However, as seen, the acid pH where growth still occurred may be too low for human consumption. The results also indicate that the acid used for pH adjustment may affect the metabolism and growth of mold. The acids tested in this food over the range of pH 4.2--5.0 slow the growth of mold at a_w 0.85, however, the inhibition is not as significant as with the antimycotics. Thus, the acidulants should not be considered as microbial inhibitors in themselves. When used in combination with microbial inhibitors such as glycerol, propylene glycol or potassium sorbate as seen in Section III, B., the antimycotic effect of acid is significant. It is recommended that acids themselves cannot be used as antimycotics in intermediate moisture foods.

TABLE 5

MORPHOLOGICAL APPEARANCE OF A. NIGER IN HENNICAN

AFTER 7 WEEKS AT 22°C (a_w 0.85)

Acid	(Mycellium/spore color) 1%	2%	4%
Adipic	white/none	white/none	-----
Citric	white/black (few)	white yellow/black	yellow/black
Fumaric	yellow/black	white yellow/black	-----
Lactic	white/none	white/none	-----
Control	white/black		

TABLE 6

MORPHOLOGICAL APPEARANCE OF A. NIGER ON HENNICAN

AFTER 2 WEEKS AT 22°C (a_w 0.85)

Acid	(Mycellium/spore color) pH 4.2	pH 4.6	pH 5.0
Adipic	white/black	white/black	white/black
Citric	white/black	white/black	white/black
Fumaric	white/black	white/black	white/black
Lactic	white/black	white/black	white/black
Phosphoric	white/black	white/black	white/black
Control	white/black	white/black	white/black

E. The Effect of a_w on the Heat Resistance of Vegetative Cells in the IMF Range

1. Introduction

The lowering of moisture content and water activity has a pronounced effect on the heat resistance of microbes. There is much evidence in the literature to support this. Troller (1973) showed this in a review on bacterial pathogens. Murrell and Scott (1966) found that the heat resistance of Cl. botulinum spores in the dry state was greater than when held in water vapor at an a_w of 1. However, the maximum resistance to heat occurred in the a_w range of 0.2 to 0.4. No theoretical basis could be found for this maximum, however, it must be related to the monolayer value. Harnulv and Snygg (1972) found that above a concentration of 55.8% glucose solution, spores of B. subtilis had an increase in heat resistance. This would correspond to a_w 0.85.

Salmonella species, because of their pathogenicity, have been the subject of much research with respect to a_w. Goepfert et al. (1970) found that for seven Salmonella serotypes and E. coli the heat resistance increased with a decrease in a_w to 0.75. On the other hand, Baird-Parker et al. (1970) showed a decreased resistance for certain strains at about a_w 0.94. At lower a_w, however, the heat resistance increased. The slight decrease in heat resistance is opposite of the effect on the spores reported above, but occurred only in the very high a_w range. Kadan et al. (1963) have also shown this reversal in the heat resistance of S. aureus in sugar solutions. Heat resistance decreased as sucrose increased to 14%; above that (up to 57%) sucrose gave a protective effect. Gibson (1973) also showed a decrease in heat resistance in vegetative cells down to about a_w 0.84 with a slight

reversal. The reasons for the change in heat resistance have never been explained and make it difficult to predict death of microbes as a function of a_w, especially during spray drying and extrusion processing of foods in which a_w and temperature can be continuously changing. The purpose of this study was to determine the effect of a_w on the death of vegetative cells over a wide range of water activities.

Studies were made on the effect of glycerol as an a_w lowering agent on the heat resistance of Saccharomyces cerevisiae, Salmonella anatum and Staphylococcus aureus. This was done to determine, over a wider range of a_w, the effect of a_w on heat resistance of vegetative cells. These results could then be used to verify the predictions made by Labuza et al. (1970) for death during processing of foods containing pathogenic vegetative cells. In addition, if pathogens have a heat resistance similar to yeast, the effect of simultaneous drying and heating on death could be studied without the need of a sealed pathogen room.

2. Materials and Methods

a. Test procedure

Saccharomyces cerevisiae ATCC 7754 was grown in nutrient broth at 70°F on a shaker at 300 rpm for 48 hr. The cells were concentrated by centrifugation, washed and suspended to give a count of about 10^9 CFU/ml. The test medium (99.9 ml) comprised of a mixture of glycerol and sterile skim milk at the desired a_w was transferred into a sterile 125 ml flask in a model FK2 Haake water bath. A sterile magnetic stirring bar was put into the flask to facilitate mixing. Four temperatures were used (25, 55, 57 and 60°C

± 0.05). After temperature equilibration, 0.1 ml of the suspension was introduced directly into the test solution by means of a 100 µl liquid syringe to give an initial count of 10^4 to 10^5 CFU/ml. At selected times 0.1 ml was removed, diluted in phosphate buffer and plated by the surface spread technique on potato dextrose agar and counted after 48 hours at 35°C. The plate counts were plotted on semilog paper vs. time. The death rate constant k (min^{-1}) was calculated as the slope of the straight line found. This is theoretically equal to 2.3 times the reciprocal of the decimal reduction time.

The same technique was used for the two pathogens, Staphylococcus aureus (196E) and Salmonella anatum (NF_3). These were grown for 24 hr at 37°C in 100 ml TSYB, centrifuged, suspended to give a population of around 10^9 CFU/ml. The thermal inactivation study was carried out in glycerol—BHI broth with an initial population of 10^6/ml. Samples were taken at 5 min. intervals, diluted in peptone water and the organisms were plated by the surface spread technique on TSYA. The data were treated the same way to get D, the decimal reduction time, and k, the death rate constant.

b. Test solutions

Sterile skim milk (9% solids Mid America Dairy) or Brain Heart Infusion (BHI) was mixed with glycerol in the combinations shown in Table 1. The a_w was measured directly in the vapor pressure manometer. A water activity below about 0.24 could not be used in the heat resistance studies because the high viscosity of the solution prevented rapid mixing with the magnetic stirrer.

TABLE 1

DEPRESSION OF A_w IN TEST SYSTEMS BY GLYCEROL

Glycerol Percent by Weight	Glycerol BHI -- Measured a_w	Glycerol-- Milk Mixture Measured a_w
0	0.999	0.999
20	0.95	----
30	----	0.87
40	0.87	----
50	0.83	0.76
60	0.77	----
63	----	0.67
70	0.68	----
75	----	0.50
80	0.53	----
82	----	0.47
90	0.35	0.29
95	0.24	----

c. Effect of dilution stress on cell death

Preliminary studies on death of vegetative cells indicated that the dilution blank used might have some effect on the viable counts of dehydrated cells from spray drying. Supposedly, to detect the effect of a stress treatment, no further stress should be imposed on the cells after the treatment in the medium. Thus, the dilution blank should be cooled for heat-treated cells. Following this, it seems in order that some osmotic stress or shock might occur in plating cells that are dehydrated or heated at various a_w's. In fact, it was frequently found that the viable count in direct plating without any dilution was more than 10 times higher than found by plating of the first dilution. In some cases, the viable count from direct plating was too numerous to count while that from the first dilution was less than ten for the same sample size. It was hypothesized that osmotic shock of the cells heated in glycerol at various a_w's might occur in the dilution blank if the water activity of the dilution blank was different from that of the heating medium. With this in mind, an experiment was conducted to determine the significance of the "dilution blank effect."

A set of glycerol-peptone water dilution blanks were made. It was assumed that the same weight percentage of glycerol in both BHI and peptone water would give the same water activity. Table 2 shows the composition of the 9 ml glycerol-peptone water dilution blanks at various water activities.

Salmonella anatum NF_3 was chosen for this study as it is the least resistant to a_w stress. The organism was introduced at

TABLE 2

COMPOSITION OF GLYCEROL-PEPTONE WATER

DILUTION BLANKS AT DIFFERENT A_w LEVELS

a_w	ml of glycerol	ml of peptone water
1.00	0	9.0
0.95	1.5	7.5
0.87	2.4	6.6
0.83	4.0	5.0
0.77	4.91	4.09
0.68	5.86	3.14
0.53	6.86	2.14
0.35	7.90	1.10
0.24	8.44	0.56
0.01	9.0	0

10^6 CFU/ml into the BHI-glycerol solutions at 52°C and an initial sample and samples for every five min heating time were taken. Appropriate dilutions were made in both peptone water and glycerol-peptone water using a dilution blank at the same a_w as the heating medium and plated immediately by the surface-spread method on TSYA plates. The plates were inverted and enumerated after incubating 18 hr at 37°C.

3. Results and Discussion

Table 3 shows that the initial count decreases steadily with the increase in weight percentage of glycerol in the glycerol-BHI heating solution based on a decrease in water activity. As seen, when glycerol is not used in the dilution blank, at low a_w's there is an immediate two- to six-fold increase in death. This indicates the sum of all the stresses including osmotic shock and suggests the need for use of proper dilution blanks. This was not done with the yeast study which follows. The data also indicate that stress occurs without heating, since the data are from zero time at 52°C.

Table 4 shows the ratio of the D values (decimal reduction times) at 52°C as a result of using the two different dilution blanks. As seen, at high a_w the difference is small with more death in the non-glycerol system as expected. The difference becomes more pronounced at lower water activity, but is always less than one logarithm cycle. Thus, even though a dilution stress exists, its effect is not as large as first thought. Data on the other cells have not been collected. Studies on death were thus made without the use of a dilution blank at the same a_w.

TABLE 3

EFFECT OF DILUTION BLANK ON DEATH OF SALMONELLA ANATUM

INTRODUCED INTO DIFFERENT A_w SOLUTIONS

52°C at zero time

Heating BHI solution a_w	Relative Count *		Ratio of counts in glycerol-pep. to pep. H_2O dil. blank
	Peptone water dilution blank	Glycerol-peptone water dil. blank	
1.00	1	1	1
0.95	0.833	1	1.20
0.87	1.083	1.083	1
0.83	0.708	0.691	0.976
0.77	0.60	0.817	1.361
0.68	0.342	0.517	1.512
0.53	0.275	0.508	1.848
0.35	0.175	0.342	1.952
0.24	0.0667	0.242	3.625
0.01	0.0342	0.208	6.098

* - ratio to count at 0% glycerol

TABLE 4

D Values for Different Dilution Blanks at 52°C for Salmonella anatum NF_3 *

a_w at 23°C	D_1 (min) Peptone water dilution blank	D_2 (min) Glycerol-sterile H_2O silution blank	D_2/D_1
0	6.8	8.8	1.79
0.35	11.8	22.0	1.86
0.53	14.6	39.0	2.67
0.77	25.7	36.3	1.41
0.87	20.7	28.0	1.35
0.95	10.4	10.4	1.0
1.00	3.10	3.10	1.0

* (based on one run)

The results of the thermal inactivation studies on Saccharomyces cerevisiae, Salmonella anatum and Staphylococcus aureus are shown in Figures 1, 2 and 3. An increase in k means a greater death rate (smaller decimal reduction time). From the pure skim milk or BHI medium, as glycerol is added (decrease in a_w to 0.76-0.85) the heat resistance increases for all the test organisms. This is as would be expected for spores and was also found for the pathogenic organism studies cited in the introduction. However, at lower a_w the heat resistance decreases.

Acker (1963) has shown that the rate of enzymatic reactions decreases as a_w is decreased and Labuza (1971) has shown this for other reactions. Possibly the binding of water reduces the rate of the reaction causing death, or the glycerol itself stabilizes any labile macromolecules since it helps to structure water. Glycerol itself does not cause death of the cells in this water activity range (0.99-0.75) as seen in Table 3 at 52°C and in Table 5 at 25°C. The diffusion of glycerol into the cells should not be much greater at 50-60°C than at 25°C since diffusion rate increases as a function of the square root of temperature in absolute degrees.

Below a_w 0.75, the heat resistance decreases significantly becoming close to that at the high a_w (0.99). In this region, the glycerol itself may be having an effect on the cell. This is obvious for the Salmonellae at high temperature (52°C), as seen in Table 3, since the counts immediately began dropping rapidly. Table 5 shows these results for the yeast at 25°C. However, the effect does not become obvious until an a_w below 0.5 is reached after 3 hrs holding time, a

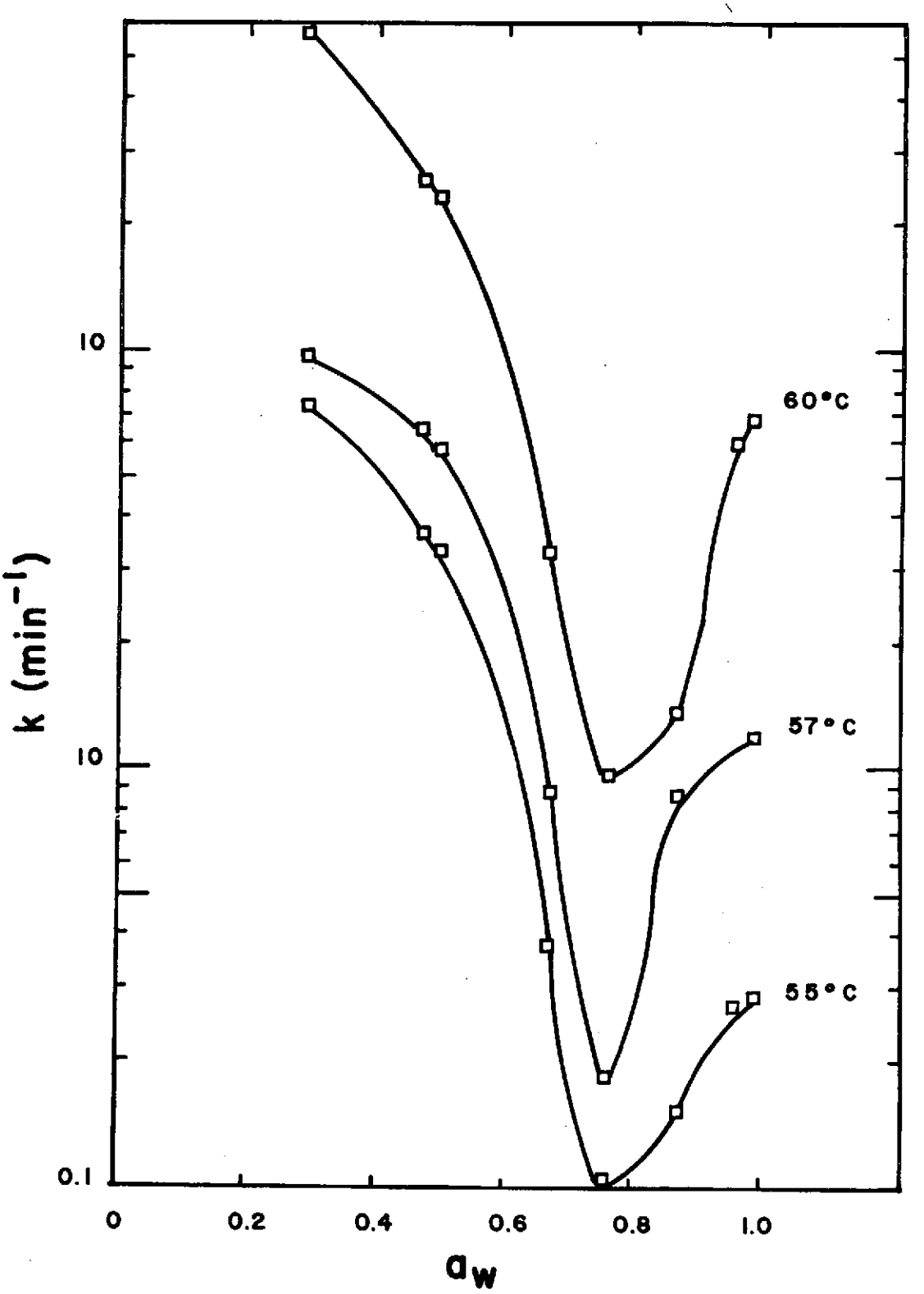

Figure 1. Death Rate Constant for Saccharomyces cerevisiae as a Function of a_w (Glycerol Solutions) and Temperature.

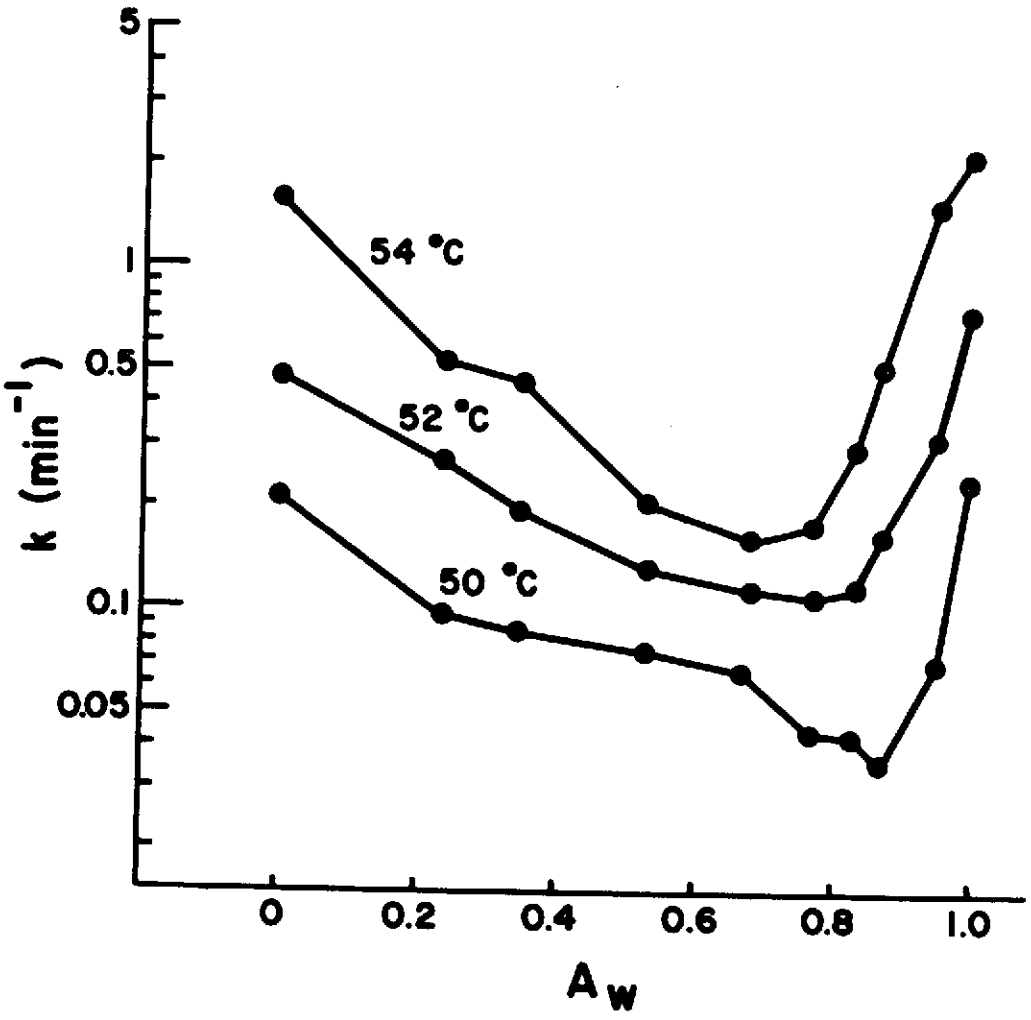

Figure 2. Death Rate Constant k as a Function of Temperature and Water Activity (S. anatum NF_3 in BHI—Glycerol Systems)

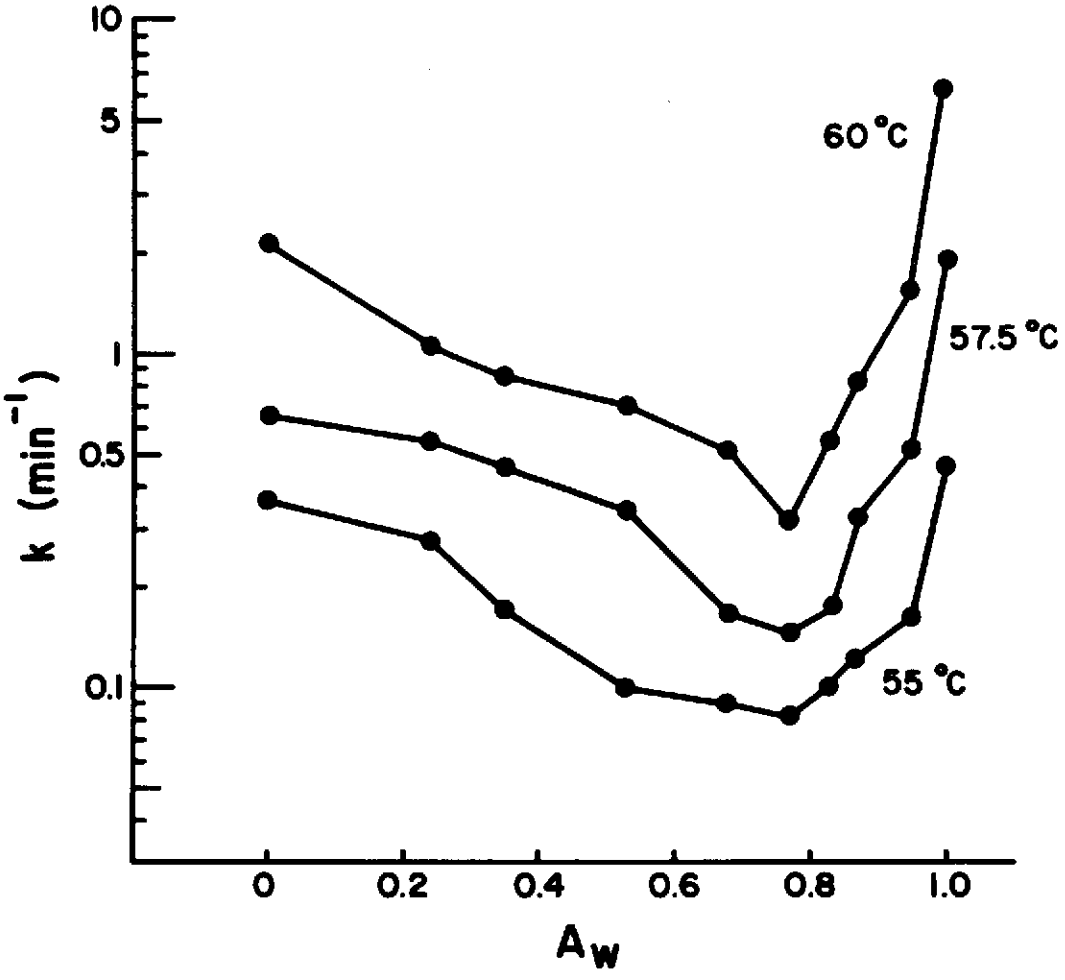

Figure 3. Death Rate Constant k as a Function of Temperature and Water Activity (S. aureus 296E in BHI—Glycerol Systems)

TABLE 5

EFFECT OF GLYCEROL ON SACCHAROMYCES CERERVISIAE AT 25°C (VIABLE CELLS PER ML)

Incubation time	Control skim milk	Glycerol-Milk Test 0.87	Solutions 0.76	a_w 0.67	0.50	0.47	0.29
Zero	5.5×10^4	6.0×10^4	5.2×10^4	3.5×10^4	3.9×10^4	3.1×10^4	2.7×10^4
2 min.	-----	6.0×10^4	5.0×10^4	3.4×10^4	3.9×10^4	3.0×10^4	2.7×10^4
3 hour	5.5×10^4	6.2×10^4	4.6×10^4	1.5×10^4	5.1×10^3	6.4×10^2	4.0×10^1
24 hour	6.6×10^5	3.8×10^4	6.8×10^3	2.3×10^2	<30	0	0

time much longer than that used in the heating studies. Thus, some other factor such as osmotic stress in combination with the heat stress decreases the cell resistance.

As noted, Murrell and Scott (1966) found a maximum in heat resistance for spores at 0.2—0.4 a_w. Other work with vegetative cells never covered the full range, so only the increase in resistance was shown down to an a_w of about 0.74—0.84 where the maximum was found in this study. Possibly the difference in the maxima between the studies could be due to a sorption hysteresis effect. The spore study was done with dried spores re-equilibrated to different a_w's on the adsorption branch of the isotherm whereas the yeast study would constitute a desorption isotherm. Labuza et al. (1972) and the work in Section III, A. show that sorption hysteresis can have an effect on the limiting a_w for microorganisms. This could partially account for the difference found. Further work is needed to clarify this. Another important factor as seen in the figures is that the yeast used dies at a rate about ten times faster than the pathogens. This suggests, therefore, that yeast cannot be used in extrusion or drying studies as an indicator as had been hoped.

With respect to cell death, Schmidt (1954) stated that the only single practical criterion is the failure to reproduce when, as far as is known, suitable conditions for reproduction are provided. Pflug and Schmidt (1968), in discussing the death of microorganisms, recognized that a lethal agent can prevent the cell from reproducing either by a direct effect on the reproductive mechanism or by disrupting cellular metabolic systems that provide energy and chemical intermediates

for reproduction. Although in their discussion, the lethal agent is heat, there is little ground to suspect that this cannot be extended to osmotic dehydration or hydration stresses as seen in this study.

While adequate conditions for handling or recovering heat-treated cells are well-known, this is not the case for cells after an osmotic or a_w stress. As shown, the dilution blank probably is a dilemma for those who want to study the effect of water activity on the cells. What is needed is an osmotic stabilizer, namely, an agent which will neither impose further dehydration stress nor cause the bursting of the cells during the transfer of dehydrated cells to a higher water activity environment. The same problem apparently can exist in the study of the change in cell morphology in preparation for electron microscopy studies since dehydration is one of the preparative steps leading to the fixation of cells for freeze-etching.

However, this study reveals that the real situation is not entirely pessimistic with respect to the interaction between a_w and death rate constant. Ideally, it can be felt that the death rate will be within one order of magnitude no matter which dilution blank is used. This is equivalent to saying that survivor curves obtained from different dilution blanks will be parallel to each other. The parallel property of survivor curves from different dilution blanks is based on the assumption that the death of cells due to dehydration and/or hydration stress (osmotic) is, again, first order. Thus, the same fraction of cells will be destroyed in the dilution blank at one water activity over different time periods of heating.

Since the main problem is the dilution blank, this problem can be eliminated by not using the dilution blank at all. One possibility is by using multiple sets of heating menstruum with the same water activity in which different initial populations are injected such that survivors from each heating menstruum at different time periods will be able to be counted without dilution. This is not only laborious but the error induced probably will outweigh that caused from using dilution blanks.

With no tenable method to recover dehydrated cells, the present peptone water dilution blank probably furnishes a good, reasonable estimation of survivors. This is further justified by the fact that in real food systems, thermal death studies of microorganisms are always completed by using common dilution blanks such as phosphate buffer or peptone water. Since the water activity is usually changing during processing of foods, not like the constant water activity in this study, a pertinent selection of a dilution blank, of course, will be a common one before any "osmotic stabilizer" is found.

4. Summary and Recommendations

This study shows that for vegetative cells including the pathogenic species Salmonella anatum and Staphylococcus aureus, the heat resistance is at a maximum value in the intermediate moisture range of liquid medium. Although this has not been studied in a solid food system, it would be expected that the same conditions would exist. Thus, it is recommended that components such as meat, eggs, etc. which are at high a_w should be heat pasteurized prior to combination with the a_w lowering agents and other dry food components. This would insure maximum kill and minimum use of energy during final processing.

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IV. Non-Enzymatic Browning in Model Food Systems

A. Introduction

Non-enzymatic browning is a common reaction occurring in many foods. Its qualitative results are either beneficial or deleterious. Browning may be beneficial during the commercial production of syrups or caramel colorings, the browning of bakery products, or the roasting of coffee or meat. Here desirable color, flavor and aroma changes are realized. Conversely, browning of foods may be deleterious. The oxidation of vitamin C during the processing or storage of concentrated citrus juices, the degradation of sugars when they are exposed to excessive heat, or the flavor, texture, visual and nutritional changes observed in a proteinaceous carbohydrate-containing food undergoing Maillard browning are all examples of non-beneficial changes induced by the browning reactions.

Naturally, the beneficial browning reactions are not a problem for the food industry, however, the deleterious browning reactions are cause for concern. The control of the deleterious reaction is desirable, but such control is not always attained. Of the various types of non-enzymatic browning reactions, the Maillard reaction presently appears to be the most complicated and hence the least understood and controlled. This is particularly true in low moisture food systems and is especially true for the ever-expanding market of intermediate moisture foods.

Qualitatively, Maillard browning has been indicated as the primary mechanism for causing excessive brown discoloration with a concomitant loss of protein quality. The degree of these qualitative

IV

NON-ENZYMATIC BROWNING

changes as a function of food composition, method of food preparation and processing and storage conditions has not been characterized for true intermediate moisture foods. As the market for intermediate moisture foods grows, so will the need for the characterization of the Maillard browning reaction as it affects the quality and overall acceptance of intermediate moisture foods. Specifically, what is needed is the kinetics of non-enzymatic browning in intermediate moisture foods. The kinetics of substrate utilization or melanoidan pigment production as a function of moisture content or water activity would prove useful to the intermediate moisture food manufacturer. A model system would facilitate the collection of such kinetics data. This kinetics data could then be applied to real food systems. From this suggestions for the control of undesirable Maillard browning in intermediate moisture foods could be made.

B. Materials and Methods

1. Model system preparation

A casein-glucose model system was developed to study the kinetics of non-enzymatic browning in intermediate moisture food systems. The formulation is shown in Table 1. Casein is used as the protein source and glucose is added as the source of reducing sugar. Potassium sorbate is added to prevent microbial growth. A range of a_w from 0.32 to 0.90 was chosen so that a possible maximum in browning rate would fall in between. For example, Loncin et al. (1968) found a maximum in browning at about 0.65 for non-fat dry milk, however, they did not measure the kinetics of the reaction. Unless noted otherwise, components were mixed together in descending order of listing in Table 1. Water

TABLE 1

MODEL SYSTEM COMPOSITION

Component	Grams
K-sorbate	0.3
Glucose	10.0
Glycerol	20.0
Casein	30.0
Apiezon B Oil	20.0
Microcrystalline cellulose	20.0
Water	Variable

was added in two ways. A predetermined amount of liquid water was blended into the mixed non-aqueous components for the direct mix (DM) system. The DM systems were then stored in desiccators in vacuo for one day at room temperature over appropriate saturated salt solutions for final moisture equilibration. The humidified mix (HM) systems were brought to proper moisture content by storing the blended dry components in desiccators in vacuo over appropriate saturated salt solutions held at room temperature (23°C). These systems were humidified for 3 to 6 days until proper moisture content or a_w was obtained.

Following the HM or DM equilibration time, 25 gram quantities were placed in 202 x 214 epoxy lined tin cans, the cans were sealed and the newly sealed end was dipped in Glyptal to retard sample moisture loss should the can seal not be perfect. The cans were then placed in an incubator at 25, 35 or 45°C. Samples were then periodically analyzed for extent of Maillard browning by measuring melanoidan pigment production and glucose disappearance.

2. Non-enzymatic browning pigment production (procedure modified from Choi et al., 1949)

a. To 2.00 g sample, add 2.5 ml of 10% (w/w) Trypsin solution and 20 ml phosphate buffer (pH 7.8).

b. Incubate system for 2 hr at 45°C under 120 CPM agitation.

c. Following the incubation period, trypsin is denatured by adding to it 2 ml of 50% (w/v) Trichloroacetic acid (TCA).

d. Add 0.1 g Celite (filter aid).

e. System is filtered through Watman #1 filter paper.

f. Absorbancy of filtrate is read at 420 nm against a reagent (sample-free) blank.

g. Browning is reported as absorbancy at 420 nm per 1.0 g solid.

3. Glucose content (utilization)

Glucose content is measured with a Glucose Oxidase (GOD) Blood Sugar test kit (Boehringer Mannheim Corp., New York, Cat. No. 15756). {Method: Werner, W., H.G. Rey and H. Wielinger. 1970. Z. Anal Chem. 252: 224}.

a. Extract glucose from model system by shaking 3 g sample in 100 g water for one hour (room temperature, 180 CPM). Filter contents through Watman #1 filter paper. Analyze filtrate.

b. To 10 µl extracted filtrate, add 5 ml buffered glucose oxidase—peroxide test solution. Mix well.

c. Incubate system for 25-40 minutes at room temperature.

d. Read absorbancy at 600 nm against reagent blank.

e. Measure absorbancy of test-kit standard.

f. Glucose content is calculated thusly:

$$\frac{\text{Abs sample}}{\text{Abs standard}} \times 176 = \text{mg glucose/3 g sample}$$

Glucose content is expressed as g glucose per 100 g solid.

4. Water content and activity

Moisture content was measured with the methanol extraction GLC technique. Water activity (a_w) was measured by the vapor pressure manometric technique.

5. Available lysine and reducing value

The method of Booth (1971) was utilized for determining FDNB-available lysine. The method of Lund (1968) was used for Fehling's reducing value test. Unfortunately, neither the available lysine or reducing value data gave meaningful results. Therefore, available lysine and reducing value results will not be presented in this report.

C. Results and Discussion

1. Moisture hysteresis data

The moisture content—a_w data is shown in Table 2. As shown in Figure 1, significant moisture hysteresis does not occur between the DM and HM systems. This was found at all temperatures. Thus, the method of moisture addition as employed in these runs does not cause moisture hysteresis.

Because the model systems employed in these runs do not exhibit moisture hysteresis, the influence of moisture hysteresis on the kinetics of non-enzymatic browning could not be studied. However, it was still possible to study the effect of method of moisture addition, that is DM vs. HM, on the kinetics of Maillard browning in intermediate moisture foods.

2. Non-enzymatic browning pigment production

a. NEB pigment production at 45°C

The results of browning as measured by production of pigment at 45°C are shown in Figures 2 and 3. Following a short induction time of less than three days, pigment production is linearly related to storage time. The browning rates are tabulated in Table 3 and, as

TABLE 2

NEB STUDY MODEL SYSTEM

A_w - MOISTURE DATA

System	a_w of salt solution (humidification)	45°C System a_w	45°C System g H_2O/100g solids	35°C System a_w	35°C System g H_2O/100g solids	25°C System a_w	25°C System g H_2O/100g solids
DM	---	--	-----	.32	6.31	--	-----
DM	---	--	-----	.41	7.67	--	-----
DM	---	.56	10.62	.51	11.20	.67	13.81
DM	---	--	-----	.62	16.62	--	-----
DM	---	.73	20.82	.73	23.53	.74	20.48
DM	---	.88	44.37	.87	43.89	.84	31.87
DM	---	--	-----	--	-----	.90	45.70
HM	.33	--	-----	.32	6.50	--	-----
HM	.43	--	-----	.43	8.38	--	-----
HM	.52	.55	10.41	.53	11.80	--	-----
HM	.67	--	-----	.65	17.10	.71	17.37
HM	.75	.75	21.06	.72	22.82	.75	21.06
HM	.85	--	-----	--	-----	.82	30.60
HM	.90	.83	34.83	.80	31.13	.86	39.66

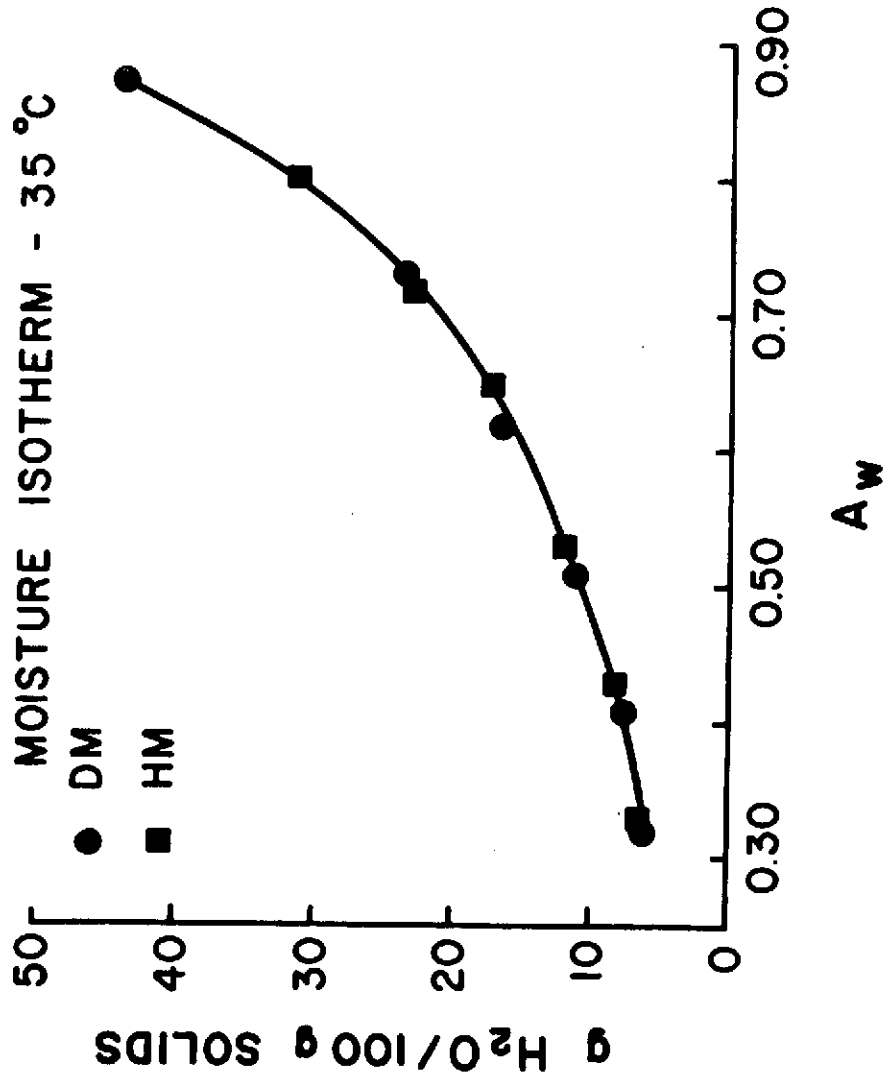


Figure 1. Moisture Sorption Isotherm of NEB Model System. DM = desorption, HM = adsorption.

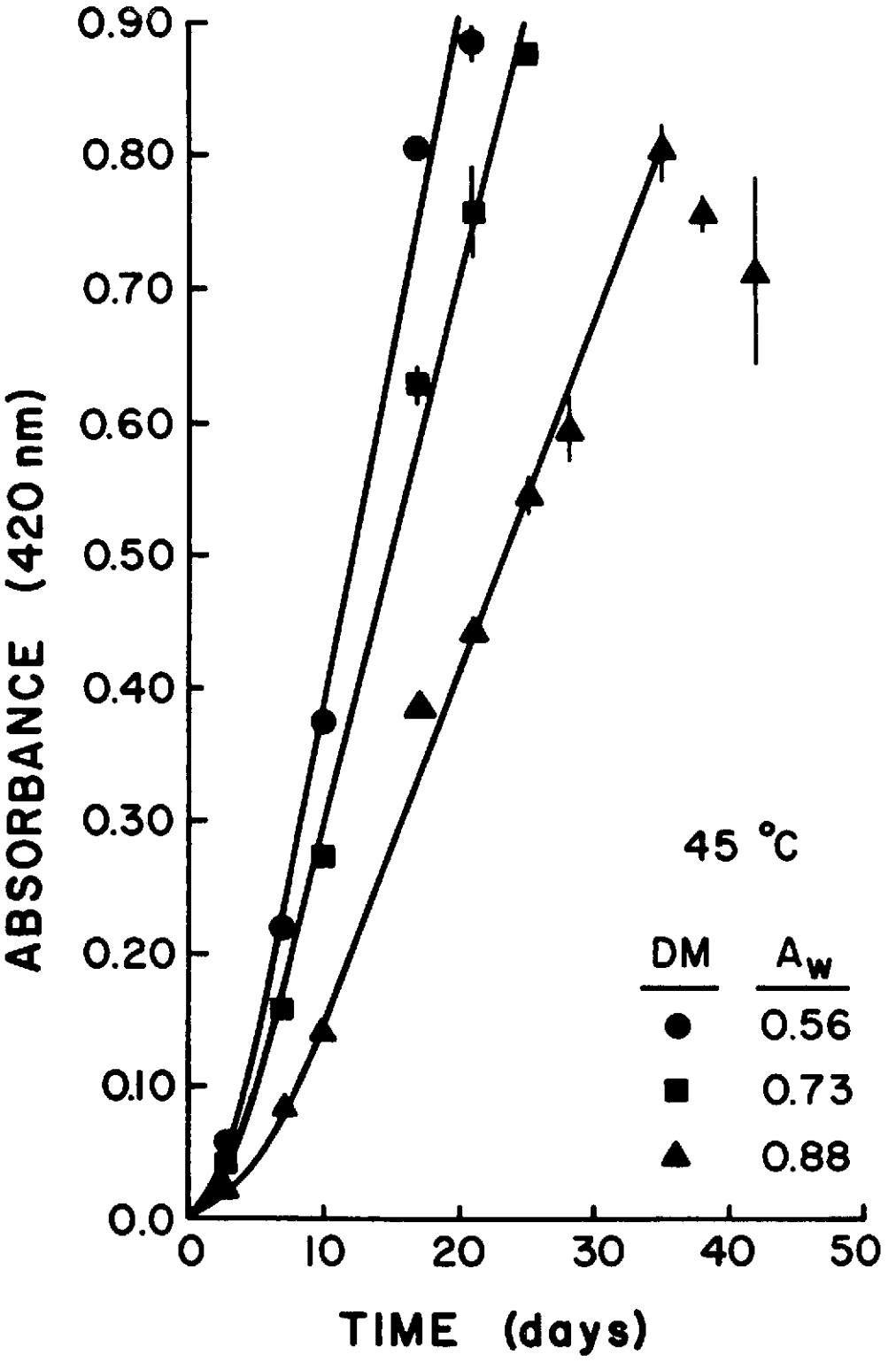


Figure 2. Increase in Non-Enzymatic Browning as a Function of a_w at 45°C - Desorption System (DM).

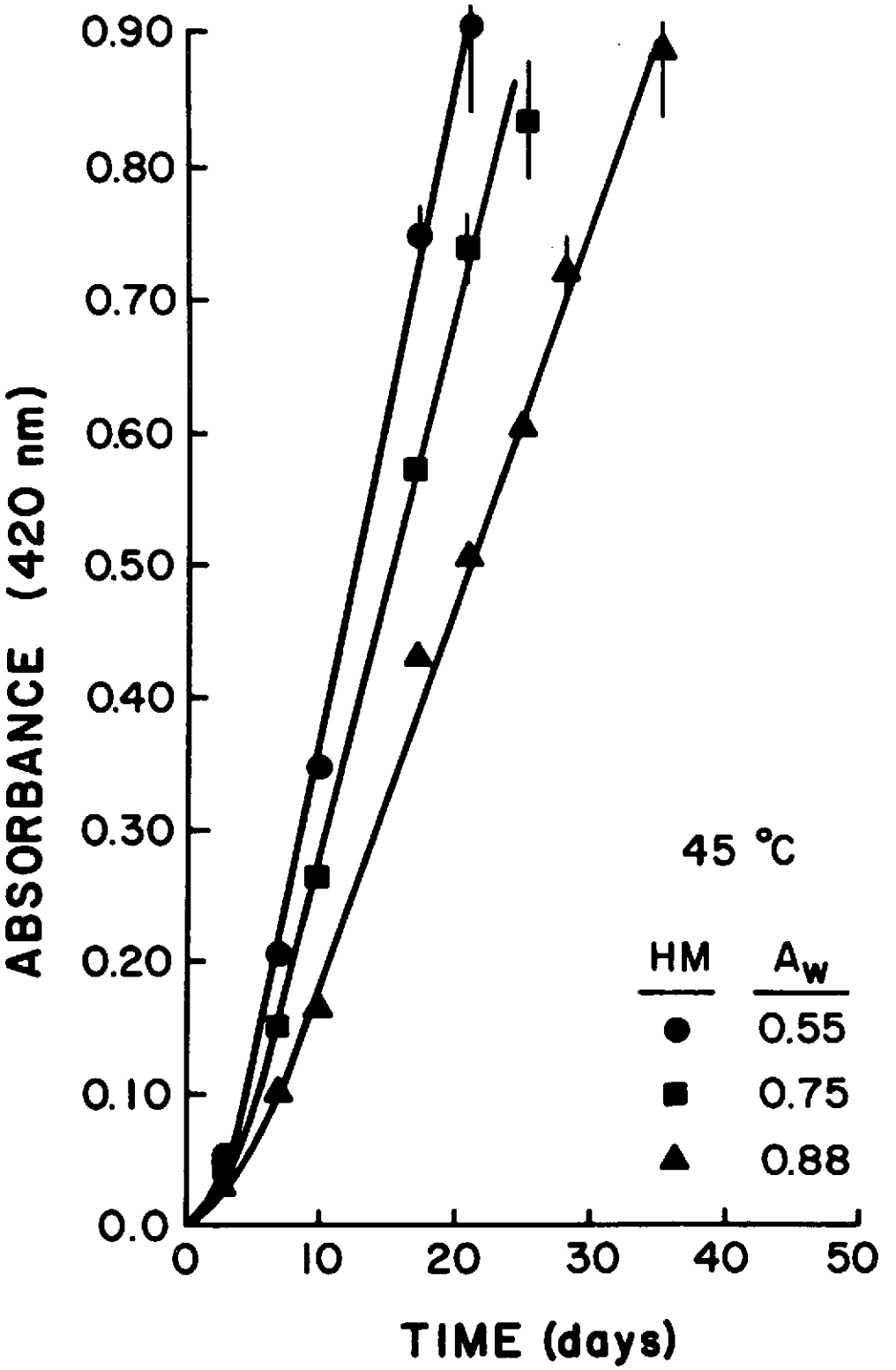


Figure 3. Increase in Non-Enzymatic Browning as a Function of a_w at 45°C.- Adsorption System (DH).

TABLE 3

RATES OF NEB PIGMENT PRODUCTION

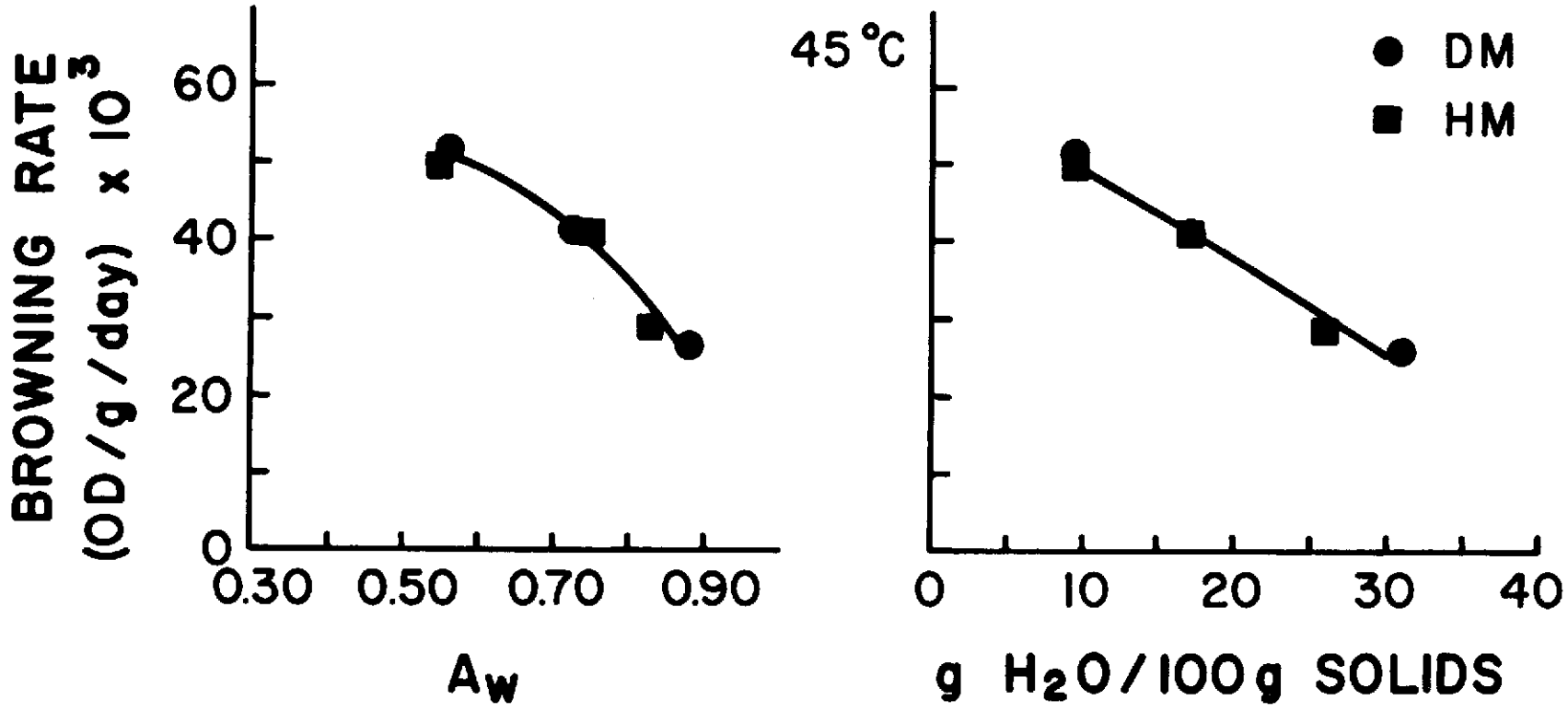
45°C			35°C		
System	a_w	Rate*	System	a_w	Rate*
DM	.56	51.7	DM	.32	9.48
			DM	.41	11.58
DM	.73	41.2	DM	.51	10.80
			DM	.62	10.83
			DM	.73	8.38
DM	.88	26.4	DM	.87	4.42
HM	.55	50.0	HM	.33	9.33
			HM	.43	11.67
			HM	.53	11.00
HM	.75	41.2	HM	.65	9.00
			HM	.72	8.23
HM	.83	28.7	HM	.80	6.67

* Rate = $\frac{\Delta \text{ Abs. } 420 \text{ nm} \times 10^{+3}}{\text{g solid} - \text{day}}$

seen within the range of a_w's tested, the rate decreases with increasing a_w. This could be due to the fact that the water may dilute the reactants of non-enzymatic browning such that as a_w increases, reactant dilution occurs. This observation, in this range, is opposite of what is observed in many food systems. For many foods, browning rate usually reaches a maximum approximately at an a_w of 0.70—0.75 and then decreases. This shows that system components may completely change the interaction with water. This is further illustrated in Figure 4 where the rate is plotted vs. moisture and vs. a_w. Figure 4 indicates that over the moisture contents studied, an approximately linear relationship exists between browning rate and moisture content. Both direct mix (DM) and humidified mix (HM) systems brown at approximately equal rates when contrasted at equivalent moisture contents. Therefore, it appears that the method of moisture addition, that is DM vs. HM, does not alter the rate of pigment production. The moisture content per se controls the rate of browning under the conditions of this test. The same pattern exists with respect to a_w, although a linear relationship does not exist.

These results rule out a viscosity effect. A reduction in aqueous phase viscosity occurs as a_w or moisture increases. If diffusion of the reactants were limiting, then the rate should increase with an increase in moisture content. In other studies utilizing model systems in the same a_w range, both lipid oxidation (Chou and Labuza, 1974) and ascorbic acid oxidation (Lee and Labuza, 1974; Section II, A.) have been shown to be controlled by the viscosity of the aqueous phase since the rates increased with a_w above the monolayer. Obviously, this is not the case for browning in this a_w range. One final observation

Figure 4. Rate of Browning as a Function of a_w and Moisture Content (g H_2O/100 g solids) for desorption (DM) and adsorption (HM) systems.



drawn from the results at 45°C is that the variability of browning values increases as pigment accumulation increases, especially at a_w 0.88. As pigment accumulation proceeds to high levels, the reactive amino groups undergo polymerization to the extent that enzymatic digestion and/or aqueous extraction of the pigments from the product matrix is impaired. Falk (1956) and Tu and Eskin (1973) have shown that trypsin, which is the digestive enzyme used in the above procedure for determining pigment production, has impaired activity when Maillard browning is excessive. Reynolds (1965) also states that initially the malanoidan pigments are water soluble but become water insoluble as the Maillard browning reaction proceeds. This can be seen in the present results.

b. NEB pigment production at 35°C

Non-enzymatic browning pigment production results for the 35°C system are shown in Figures 5 and 6. As before, after an initial induction period the rate of browning is constant. The rates are tabulated in Table 3 and follow a similar pattern as found at 45°C. As a_w decreases, browning rate increases over the same a_w range between both temperatures. However, as a_w is lowered beyond the lowest a_w studied at 45°C, a true maximum browning rate is observed. Figure 7 shows that the browning rate at 35°C reaches a maximum at about a_w 0.43. What is quite unusual is that this is close to the monolayer a_w of about 0.25. Based on these results, water at low a_w could be controlling the reaction rate by controlling substrate concentration, mobility and solubility. A lowering of moisture content can cause increased substrate concentration and therefore, increased

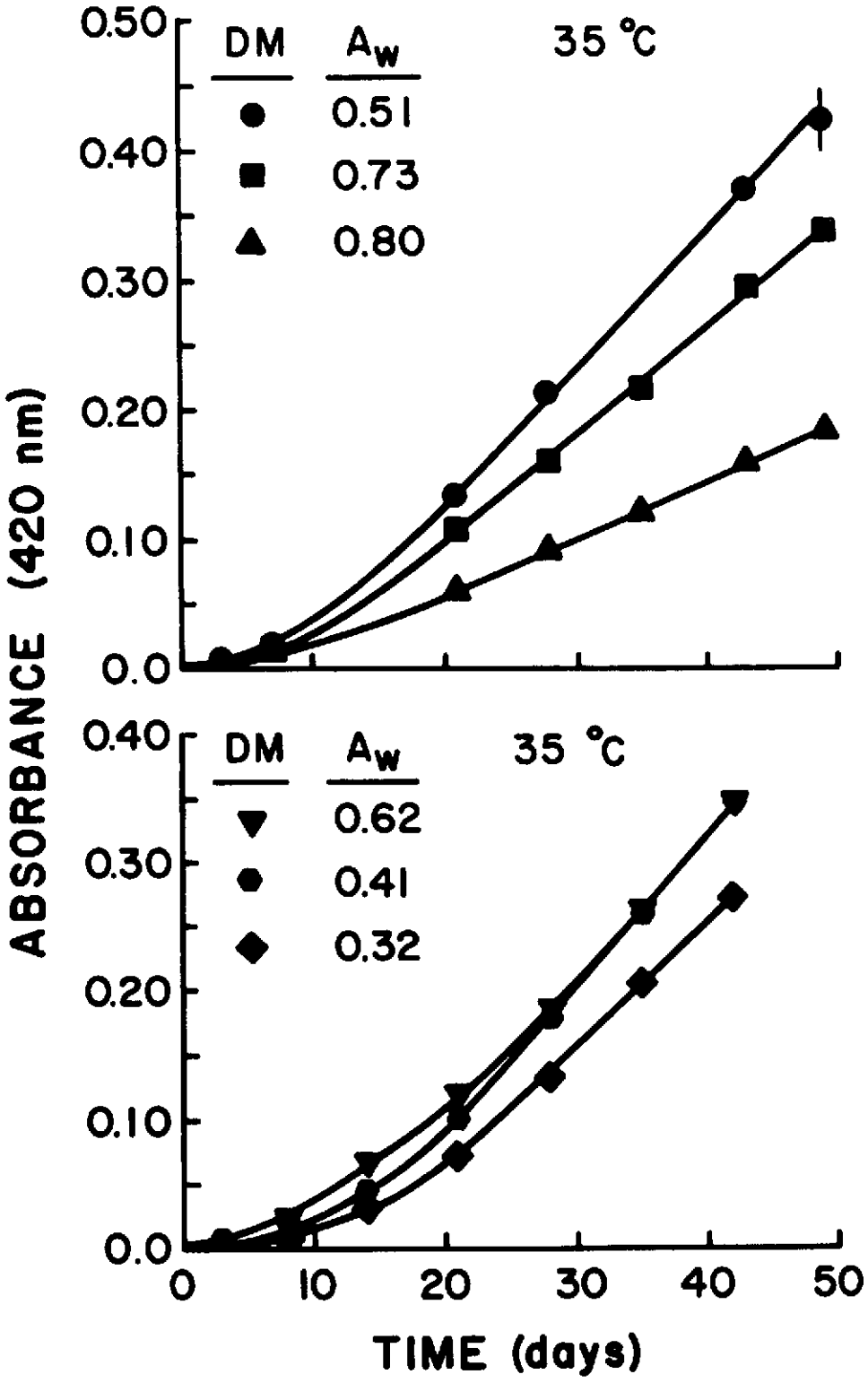


Figure 5. Increase in Browning (absorbance) as a Function of a_w at 35°C in the desorption system (DM)

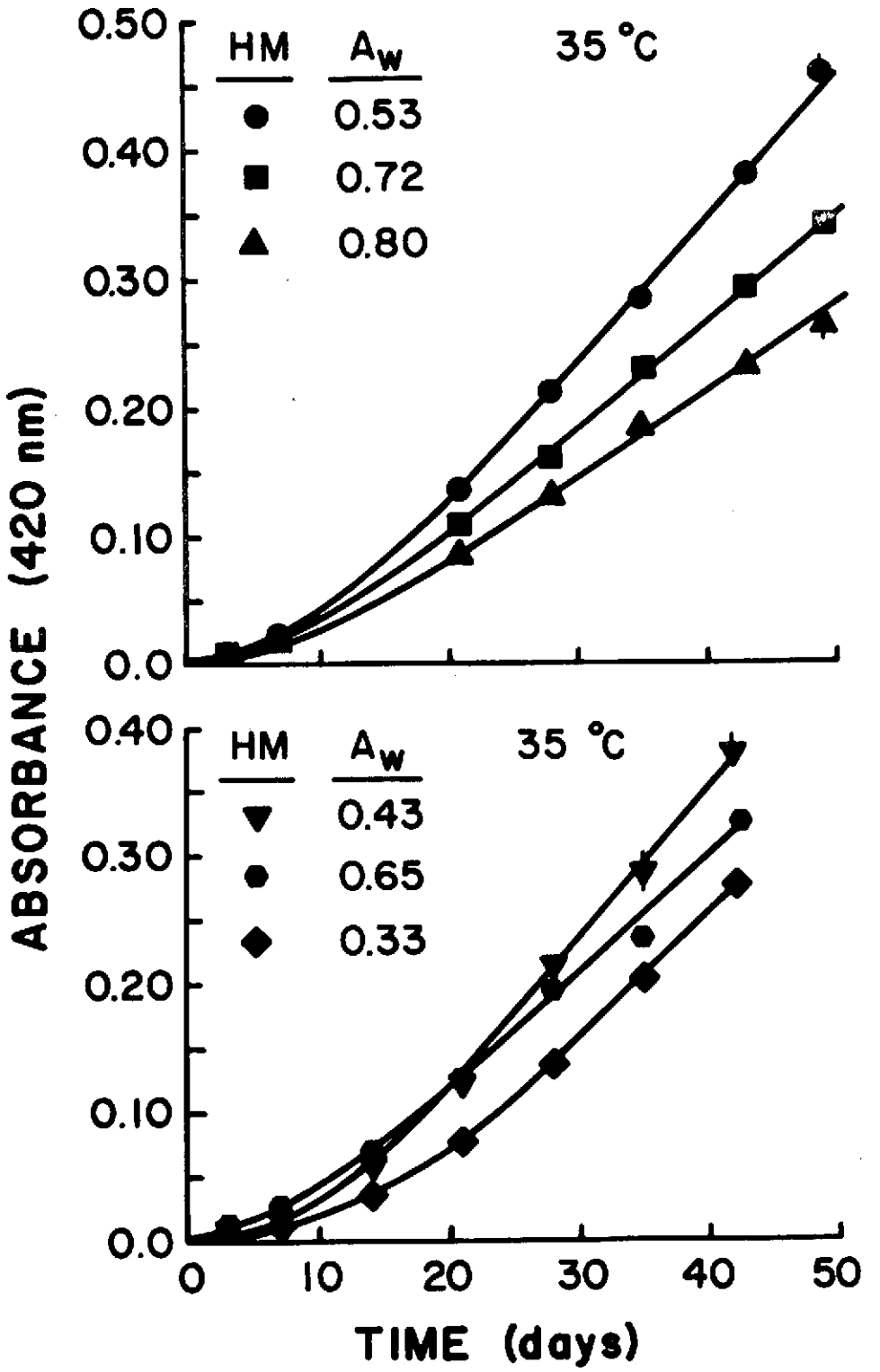


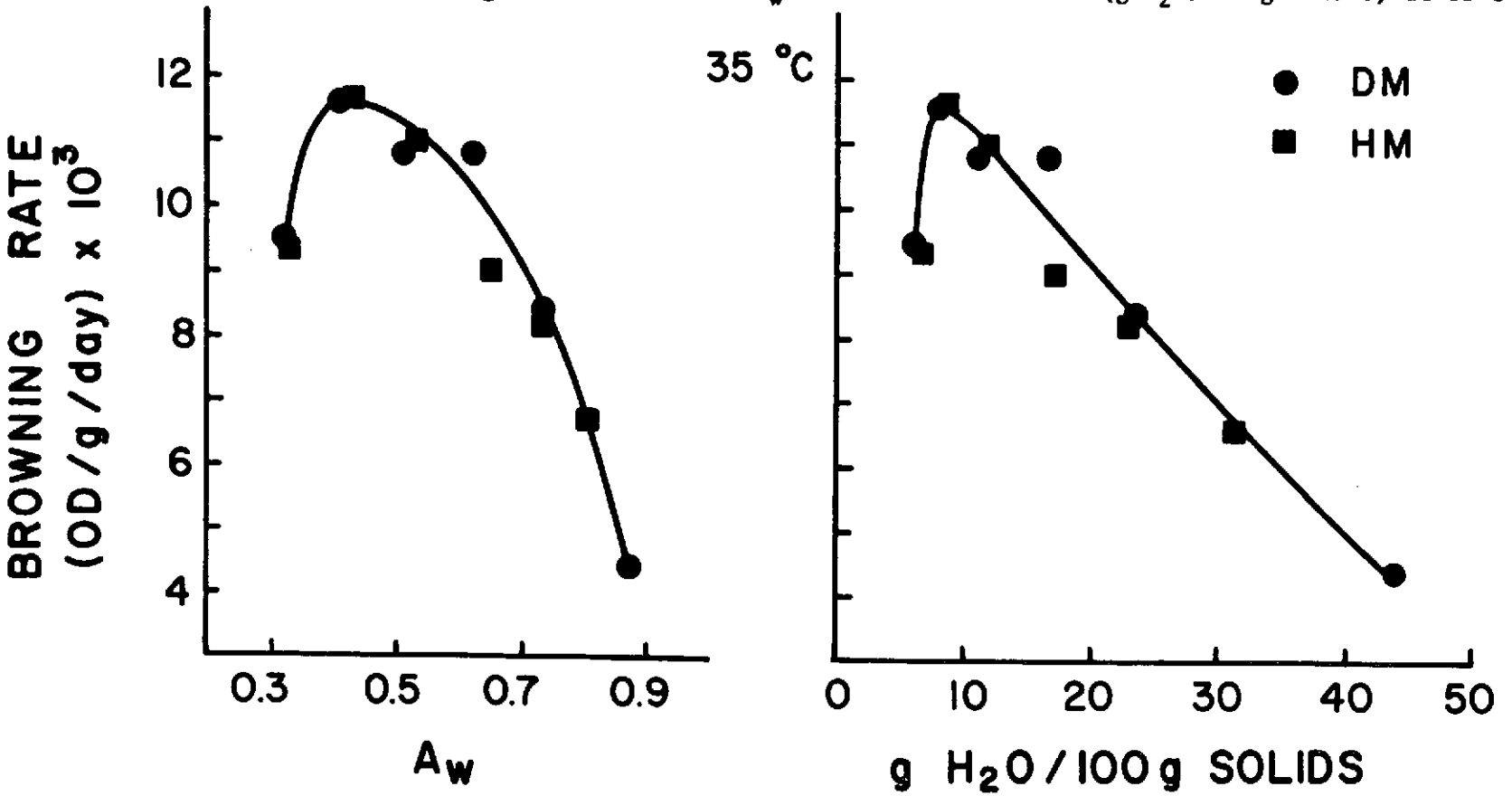
Figure 6. Increase in Browning (absorbance) as a Function of a_w at 35°C in the adsorption system (HM)

browning rate. However, as moisture content is lowered beyond a critical level, substrate mobility probably becomes impaired and the substrate can precipitate out. As the effect of limited substrate mobility and solubility exceeds the effect of substrate concentration, browning rate will decrease as moisture content is further lowered. At the point where the effects of substrate mobility, solubility and substrate concentration are equal, a maximum browning rate should be observed. The data at 35°C indicate that this occurs at a_w 0.43, which is a moisture content of approximately 8 g H_2O/100 g solids. The 0.43 a_w value is considerably lower than the 0.70 a_w value considered to be the usual level at which a browning rate maximum is observed in dehydrated foods. This unusual effect can be attributed to the uniqueness in composition of the system. Glycerol can solubilize glucose and thus must be acting as part of the aqueous phase. This could be the case with any intermediate moisture food to which a high level of humectant is added. The browning rate at 35°C, as seen in Figure 7, is approximately linearly related to moisture content above the rate maximum. This indicates that moisture content thus controls the browning rate. It also appears that the method of moisture addition, be it through the humidified mix or the direct mix method, does not significantly influence browning reaction rate.

c. NEB pigment production at 25°C

Browning pigment results for the systems stored at 25°C are shown in Figure 8. The data indicate that the Maillard non-enzymatic browning reaction has not progressed much beyond the induction phase. The browning data have not yet become differentiated

Figure 7. Rate of Browning as a Function of a_w and Moisture Content (g H_2O/100 g solids) at 35°C



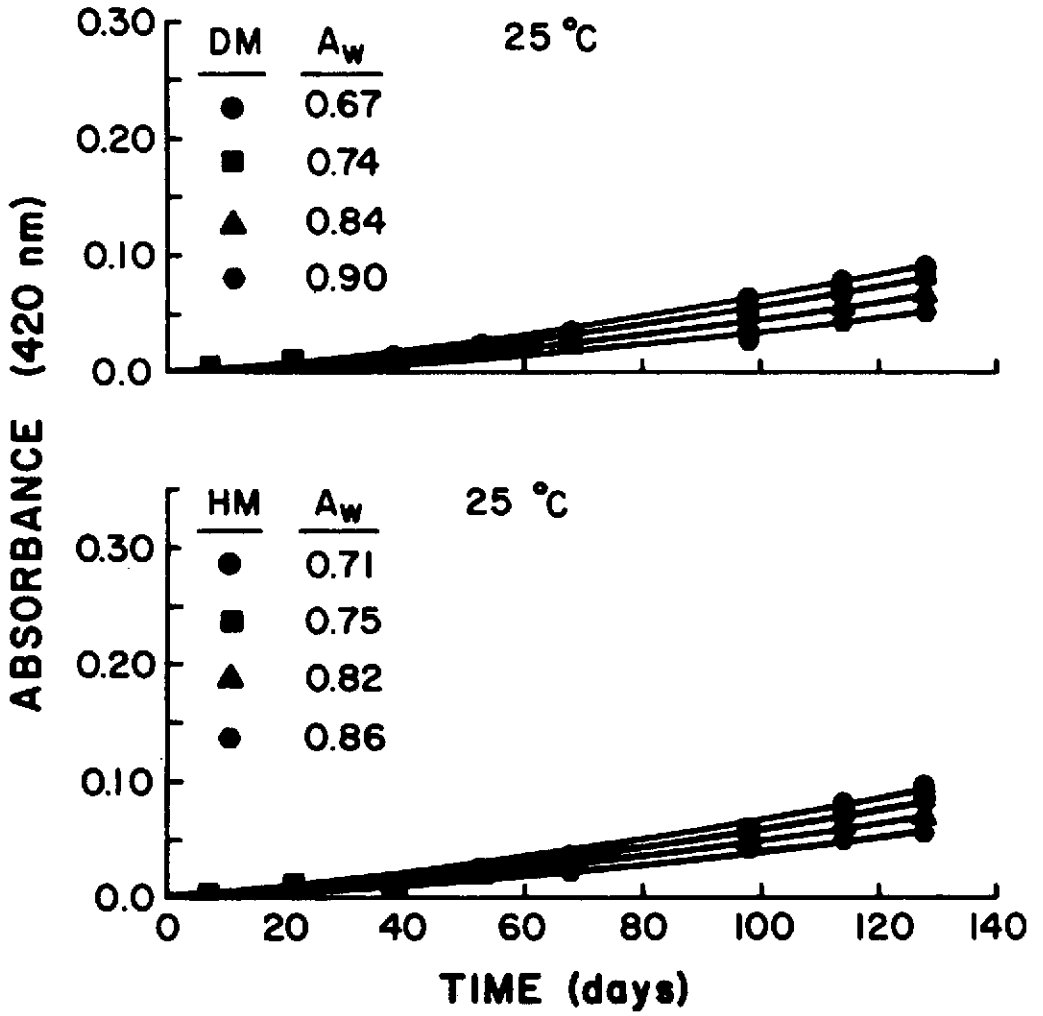


Figure 8. Increase in Browning (absrobance) as a Function of a_w and System Preparation at 25°C. DM - desorption, HM - adsorption.

enough so reliable rate constants can be determined. However, the trend of increased browning rate with decreased a_w is starting to be established. A longer storage time at 25°C is needed to establish accurate conclusions.

d. Effect of temperature

The rates of 35 and 45°C were plotted in Figure 9 to get a measure of the activation energy at constant moisture contents. Since only two temperatures are available, the results are not accurate; however, they give some indication of the relative activation energy. A value of 28.9 Kcal/mole was found which gives a Q_{10} of about 5x. Thus storage at 45°C accelerates the reaction 25 times faster than at 25°C.

3. Glucose reaction

To gain further insight into the kinetics of non-enzymatic browning, it would be useful to also observe the kinetics of reactant utilization. As glucose is the only reducing sugar used in the model system in this study, glucose content can be monitored as an indicator of reactant utilization as storage time and browning progress. Results of glucose destruction for the samples stored at 25, 35 and 45°C are shown in Figures 10, 11 and 12 where glucose concentration is plotted as a first order reaction. Since browning occurred rapidly at 45°C with an almost negligible induction time, glucose content should rapidly decrease as glucose is used from the start of the browning reaction. Figure 10 shows that an appreciable amount of glucose is reacted after only a few days of storage at 45°C. The expected less rapid loss of glucose at 25 and 35°C is also seen as compared to 45°C. The calculated

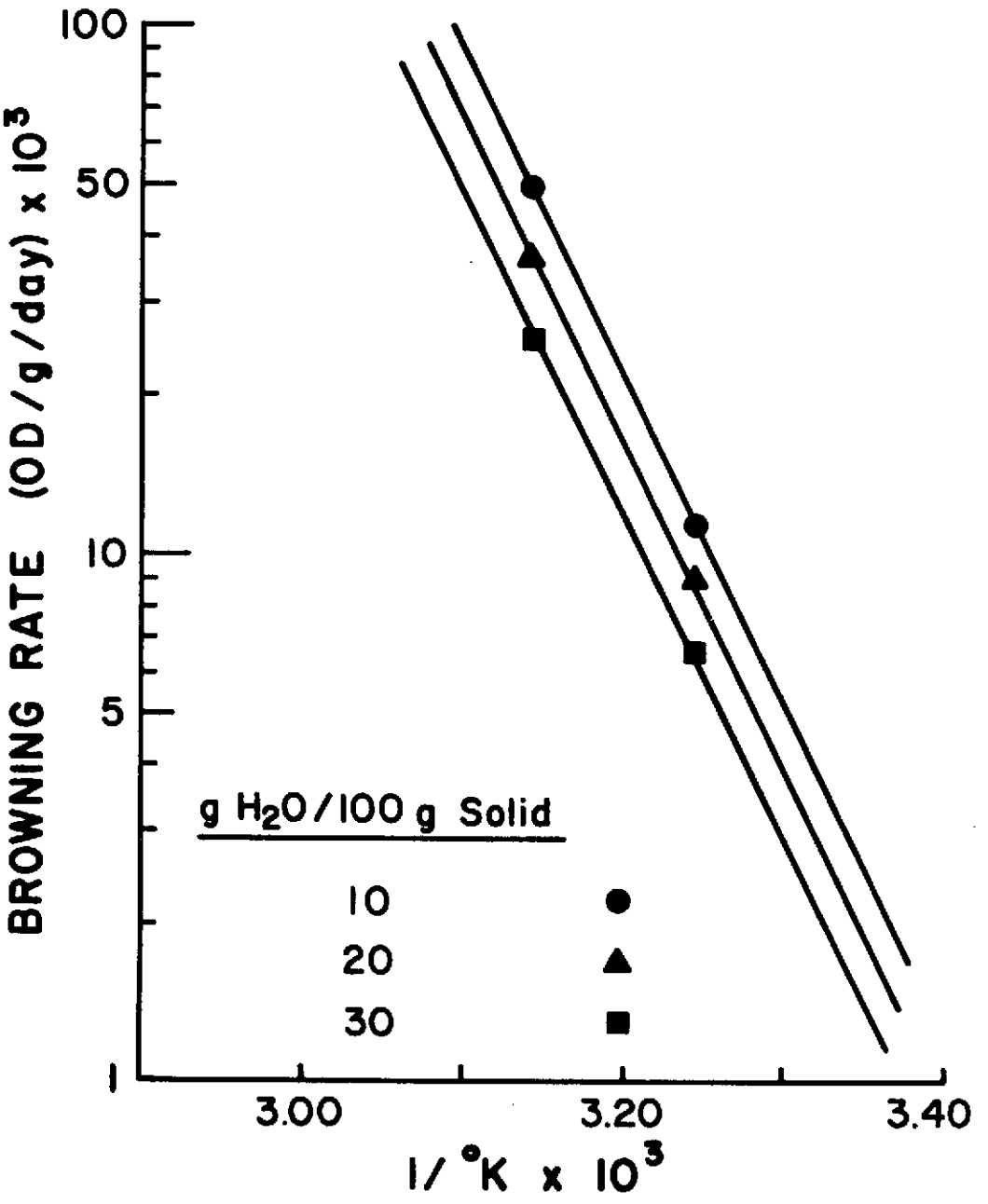


Figure 9. Arrhenius Rate Plot for Non-Enzymatic Browning at Three Moisture Levels.

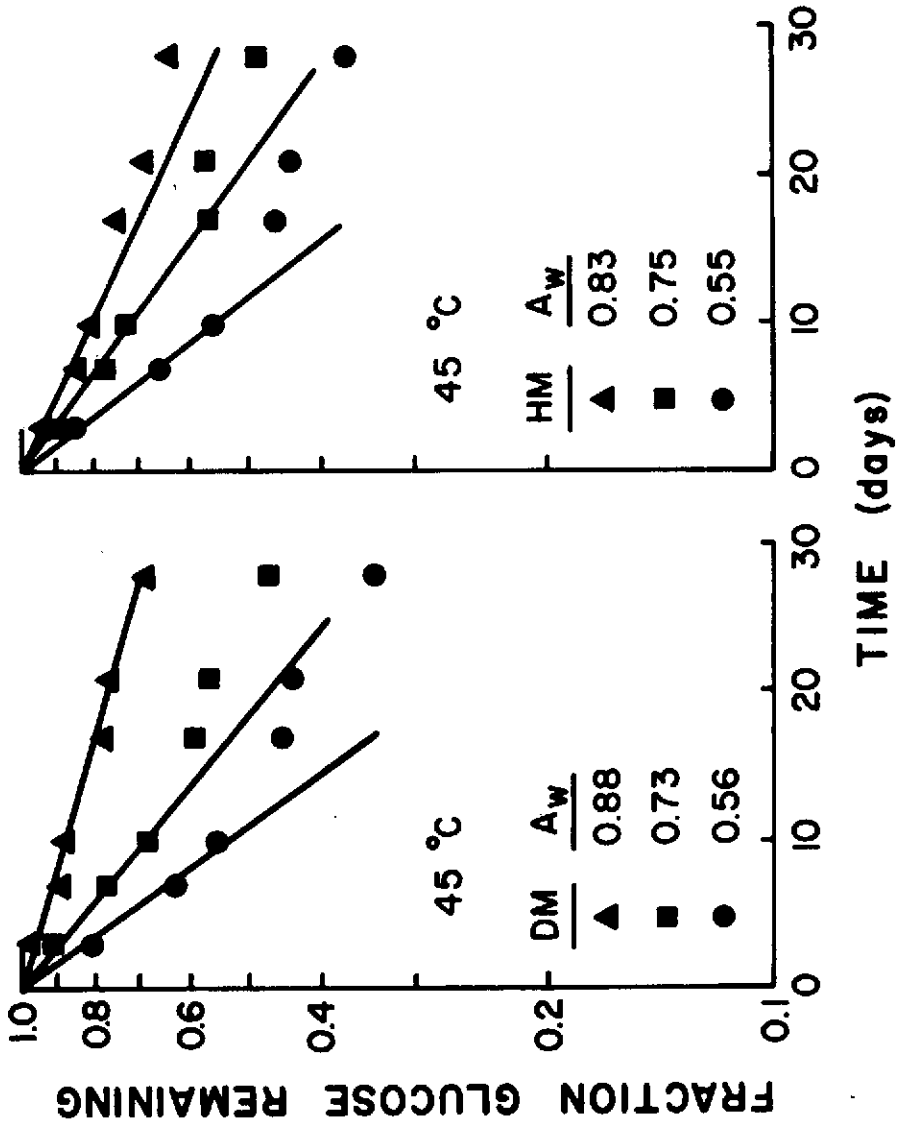


Figure 10. Loss of Glucose as a Function of a_w and System Preparation at 45°C. DM - desorption, HM - adsorption.

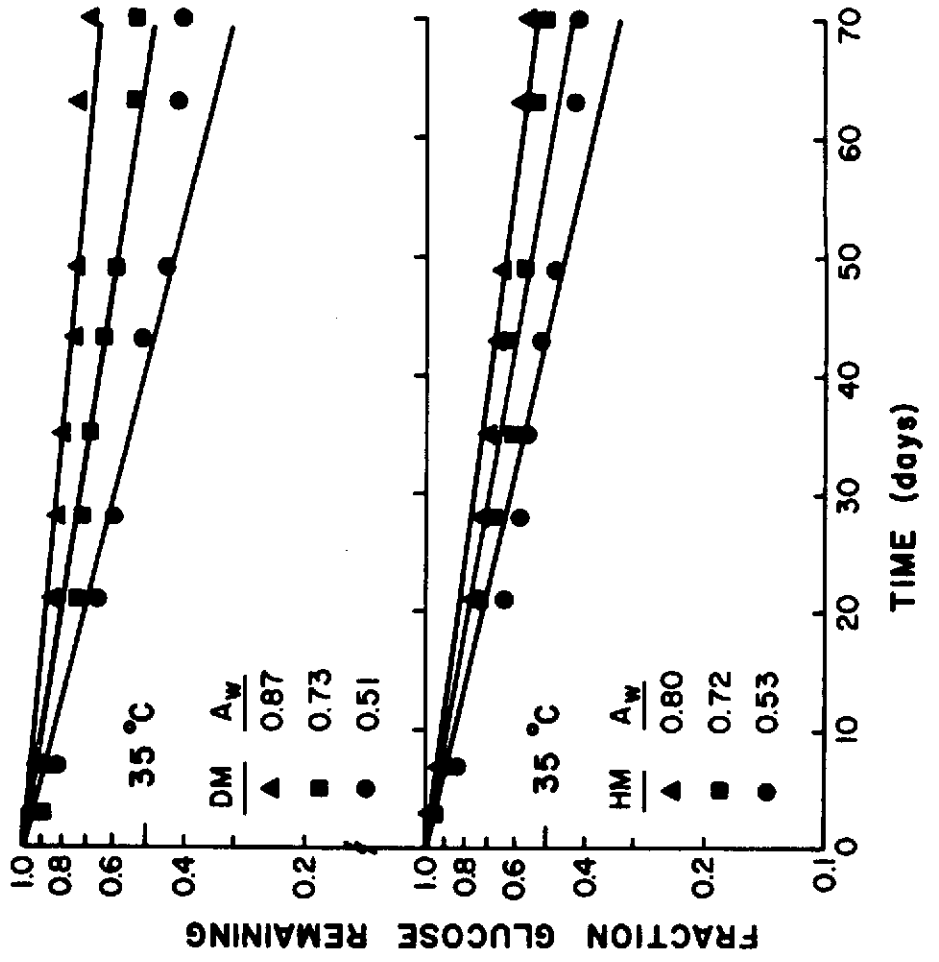


Figure 11. Loss of Glucose as a Function of a_w and System Preparation at 35°C. DM - desorption, HM - adsorption.

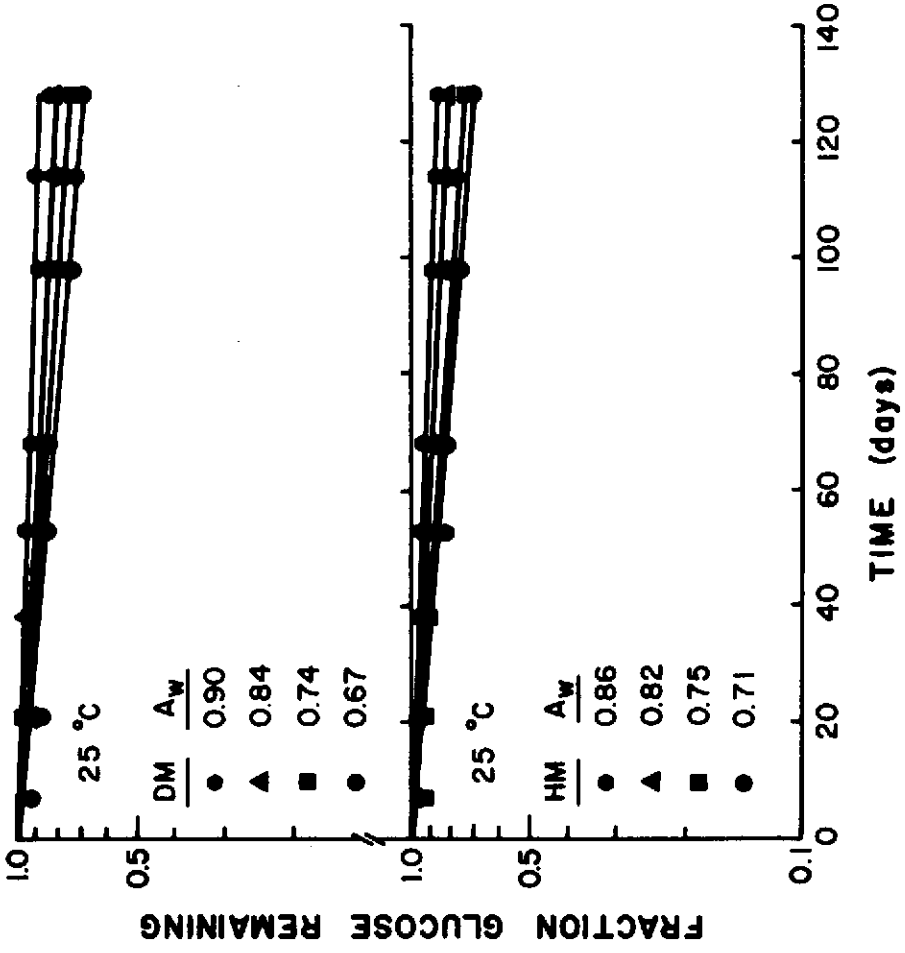


Figure 12. Loss of Glucose as a Function of a_w and System Preparation at 25°C. DM - desorption, HM - adsorption.

destruction rate constants for glucose loss are shown in Table 4. The rate constants were derived from regression analyses (least square method).

Glucose loss follows the expected first order rate during the initial period of pigment production but thereafter deviates to a lower rate. Because of this, rate constants were derived from the initial days of storage. The storage time utilized for the regression analyses of glucose loss were days 0 through 10 for 45°C, days 0 through 49 for 35°C, and days 0 through 114 for 25°C. The Arrhenius activation energy values of initial glucose destruction were obtained from regression analyses of the log k_G vs. 1/T plot (Figure 13, where k_G is equal to 0.693 divided by the half life). The values are reported in Table 4. As seen, the average E_a is 25.54 Kcal/mole, a value near that for non-enzymatic pigment production as would be expected if the first step is the controlling rate limiting step of non-enzymatic browning. Since accurate data at only two temperatures are available for browning, a direct comparison could not be expected.

The results of glucose loss follow the same pattern as observed for pigment production with respect to a_w. A greater loss occurs at the lower a_w or moisture content as seen in Figure 14. Since the drier systems react more rapidly than do the high a_w systems, glucose solubility is not an influencing factor on the rate of Maillard browning at a_w's above the rate maximum. If solubility were a factor, one would expect a greater concentration of reactable soluble glucose to be present as moisture is increased. What is unusual is that a saturated solution of glucose has an a_w of 0.915. Thus, sugar must

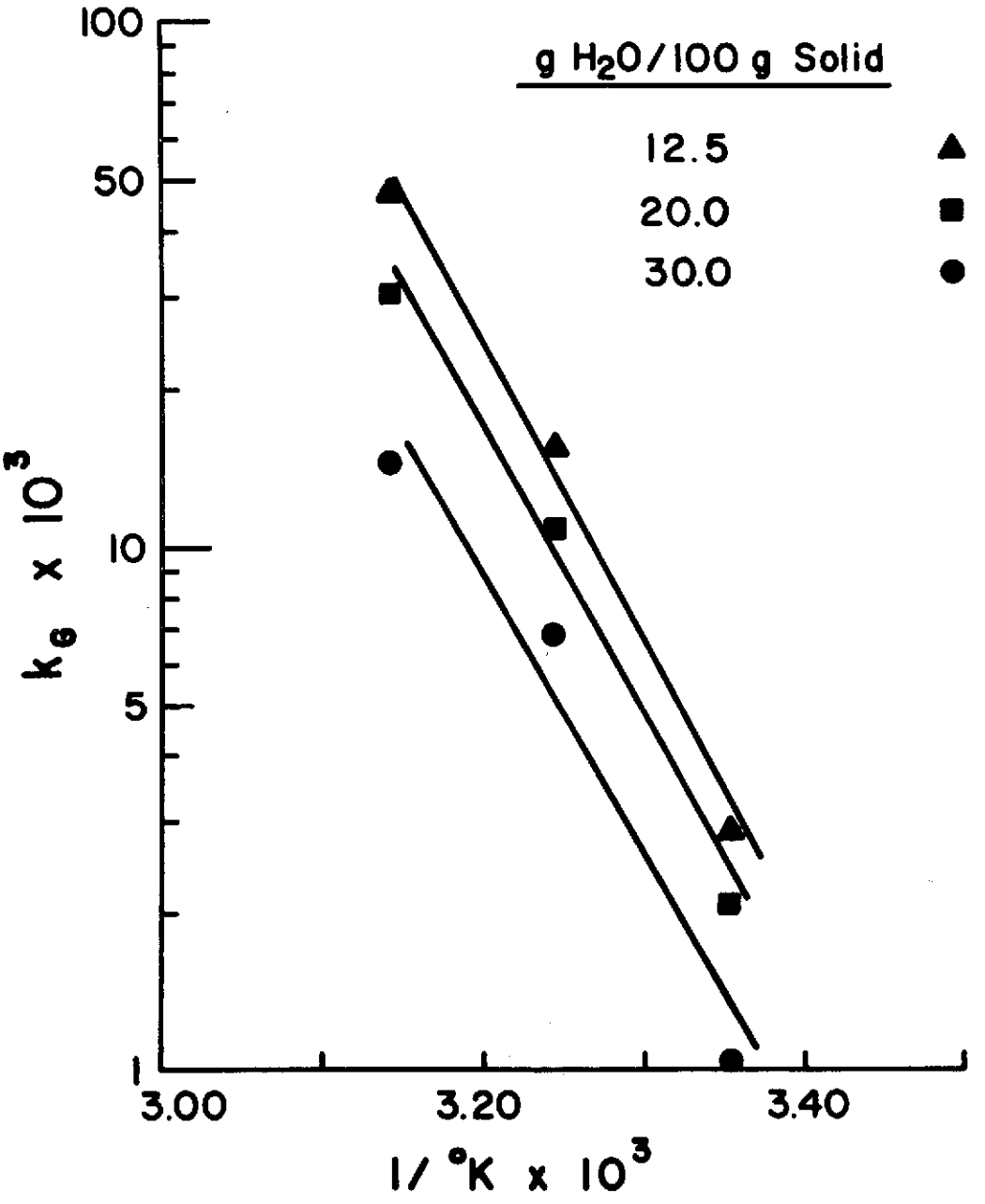


Figure 13. Arrhenius Plot of Glucose Destruction Rate as a Function of Moisture Content.

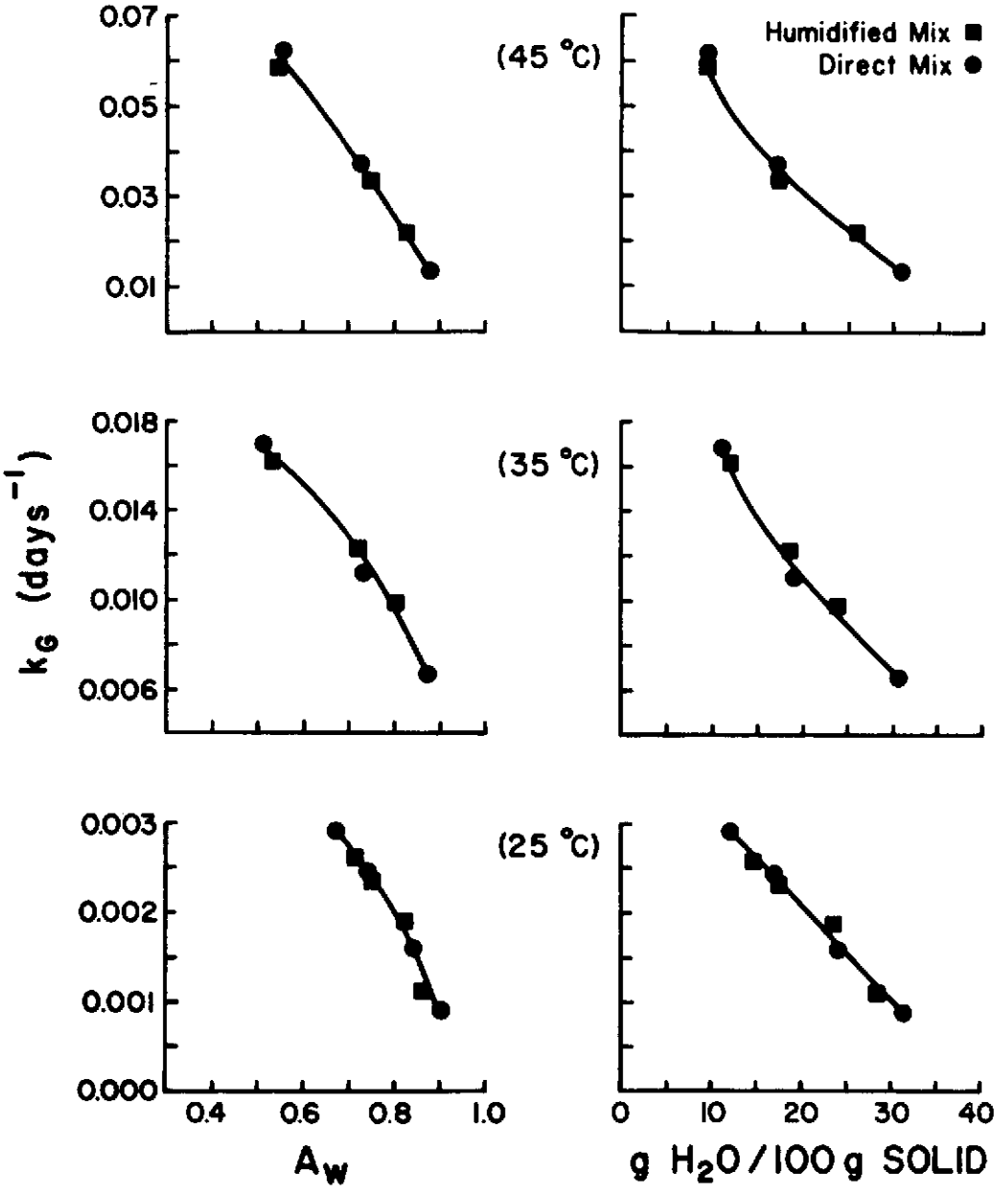


Figure 14. First Order Rate Constant for Glucose Destruction as a Function of a_w, Moisture Content and Temperature for the DM (desorption) and HM (adsorption) systems.

TABLE 4

INITIAL GLUCOSE DESTRUCTION RATE CONSTANTS

System	45°C		35°C		25°C	
	a_w	Kg (day^{-1})	a_w	Kg (day^{-1})	a_w	Kg (day^{-1})
DM	.56	.0618	.51	.0168	.67	.0029
DM	.73	.0370	.73	.0111	.74	.0024
DM	.88	.0131	.87	.0066	.84	.0016
DM	--	--	--	--	.90	.0009
HM	.55	.0583	.53	.0161	.71	.0026
HM	.75	.0334	.72	.0123	.75	.0023
HM	.83	.0217	.80	.0098	.82	.0019
HM	--	--	--	--	.90	.0011

Activation energy (Kcal/mole:

12.5 g H_2O/100 g solids	E_a = 26.3
20 g H_2O/100 g solids	E_a = 25.3
30 g H_2O/100 g solids	E_a = 25.0

be dissolved in the glycerol as well. The fact that there is no break in the activation energy plot (Figure 13) also indicates solubility is not a problem. Table 5 shows that temperature strongly affects glucose solubility but this does not affect the rate. Thus, water must have some other effect.

Eichner and Karel (1972) have shown that the rate of browning in glycerol-containing systems can be limited by the feed-back inhibitory effect of water that is produced during the browning reaction. Therefore, the browning rate, or glucose destruction rate, would decrease with a_w. Such is the observation in this study; however, if the feed-back inhibitory factor of water is to be considered as a major factor controlling browning rate, the rate of browning at any given a_w would not be constant but should decrease slowly with reaction time. Browning rates as measured by pigment production do not decrease with reaction time. However, following the initial first order reaction period, the glucose destruction rate at any given a_w does tend to decrease with reaction time. This decreasing rate of glucose destruction could be due to the feed-back inhibitory effect of the produced water since water is produced in the first step involving glucose. What this seems to suggest is that there is an excess of reactable glucose to amine. Thus, water is a product of the first condensation reaction and hence slows that rate, however, enough reducing compounds are being formed to maintain a constant rate of pigment production.

The kinetics of the reaction according to the results found can then be established. The rate of browning is a constant which decreases as moisture content increases thus:

TABLE 5

GLUCOSE SOLUBILITY

Temperature	g Glucose/ml H_2O
25	.91
30	1.25
50	2.44
70	3.57
90	5.55

$$\frac{dB}{d\theta} = \frac{k(R^*)}{M_c} = k_B$$

where B = browning value
k_B = overall rate constant
k = pseudo-rate constant
M_c = moisture content
R^* = reactable reducing sugar
θ = time

The first steps in browning involve the formation and subsequent degradation of a Schiff's base which in this case is a glucose-amine reaction product. This then reacts to produce R* which is the reactable reducing sugar content. The glucose to primary amine ratio is 4.8:1.0, high enough that the rate of browning is unaffected by glucose loss. Thus, there must be a maximum amount of R* present at all times. Within the limits of error of glucose measurement ($\pm$ 1 g glucose/100 g solids), the results indicate that glucose is being initially destroyed by a first order reaction. The amine should be given up initially, but would later be tied up by some mechanism as it is incorporated into the pigment. It could thus be considered in excess. Over the initial period then, the glucose is destroyed by a reaction as follows:

$$-\frac{dG}{d\theta} = k_G (G)\ (P) - k'_G\ (S)\ (M)$$

where (G) = glucose concentration in aqueous phase
k_G = reaction rate constant
(P) = primary amine content
k'_G = backward reaction rate constant
(S) = Schiff's base
(M) = water content

Under the conditions studied, if P does not change appreciably and the amount of (S) is small, then the loss of glucose can be treated as a first order reaction as:

$$-\frac{dG}{d\theta} = k_G (G)$$

The results show that glucose is initially disappearing by a first order reaction. The rate of browning over this time period has remained constant.

Thus, both dilution and inhibition by water are occurring. Glucose is breaking down to initially form reactable reducing compounds (R*) in the intermediate steps which lead directly to pigment formation. After an initial induction period, the (R*) builds up to a level which leads to a constant browning rate. This R* is dependent both on the rate of glucose destruction and the total volume of the aqueous phase. If the phase volume is very high, as occurs with an increased a_w, the concentration of R* that is produced in terms of moles per liter is less and thus the absolute browning rate is less. The same occurs with glucose destruction if we assume it is dissolved in the glycerol-water phase. Above the a_w point where a solubility or viscosity problem occurs, increasing the phase volume by increasing the moisture content reduces the initial glucose destruction rate. As the Schiff's base builds up, there is feed-back inhibition and the glucose rate drops. However, the amount of R* produced from the Schiff's base remains constant so the browning rate follows a zero order reaction after an initial induction time.

To further complicate this one could assume that not all the glucose is in solution. Thus, as some glucose reacts and disappears, more glucose would dissolve, but at a slower rate. Thus, the first order would not be followed. Schobel et al. (1969) found this to be the case for sucrose hydrolysis at a_w 0.75 where excess sugar was added. Karel and Labuza (1968) also had constant browning rates in a

sucrose-acid-protein system and suggested that the aqueous phase was being replenished by reducing sugar. According to this hypothesis, at higher a_w more total glucose would be dissolved, however, the present results show that the browning reaction rate decreases with a_w or moisture. Thus, this theory does not apply.

D. Summary and Recommendations

This study has yielded information on the kinetics of Maillard non-enzymatic browning in a casein-glucose intermediate moisture model system. Kinetics of both reactant utilization (glucose destruction) and end-product accumulation (melanoidan pigment production) have been presented. In general, the factors that control reactant utilization also control end-product accumulation.

Specifically, the extent of Maillard browning is directly proportional to storage temperature and time. The rate of pigment production, after an initial induction period, is constant with storage time under the conditions of this study. This rate would be expected to decrease if storage time was extended for an excessively long length of time. The data also indicate the usefulness of using an accelerated reaction temperature. The rate at 45°C is over 20 times faster than at room temperature. Glucose destruction initially follows first order kinetics. This implies that either there is an excess of reactable reducing compounds to amine or that, less likely, more than one glucose moiety combines with a free primary amine group when glucose content is excessive. Reynolds (1965) gives a mechanism whereby more than one glucose moiety may combine with one free primary amine group. The rate of Maillard browning as measured by both pigment

production and reducing sugar disappearance increases as water activity or moisture content decreases down to a_w 0.43. This implies that the reactant concentration is controlling the reaction rate influenced by the available liquid phase. In this region mobility and solubility are not important.

Below the maximum in browning rate, any further decrease in moisture content lowered the reaction rate. This rate decrease would be expected to continue until the BET monolayer of moisture is reached. That the system of this study showed maximum browning at an a_w less than the a_w range (0.70—0.75) that is thought to produce maximum browning in most dehydrated foods, implies that a_w or moisture content alone may not be controlling browning rate. In this case, reactant concentration as expressed per total phase (water plus glycerol) is controlling the reaction rate. Thus, the addition of glycerol to a food system, in addition to increasing the product's microbial stability by lowering the a_w and increasing the textural appeal of the product by "plasticizing" the product, increases the total effective "solvent" volume and thereby decreases reactant concentration. Naturally, a decrease in effective reactant concentration should decrease reaction rate. Hence it could be theorized that the addition of glycerol decreases reactant concentration such that the above glycerol containing model system at an a_w of approximately 0.45 probably possesses the same reactant concentration as do humidified non-glycerol containing foods that show an a_w maximum for browning at 0.70—0.75 a_w. However, this would not be desirable since the glucose remains in the liquid phase to a lower a_w than in a dehydrated system. One would

have to compare the effective browning rates in various systems.

Another general conclusion realized from this study is that the methods of preparation with respect to water addition do not significantly alter the browning rate. That is, if moisture addition to the blended "solid" components was by the humidification process or by direct liquid water addition, the resulting browning rates are not altered. However, the effect of adding the reducing sugar to the water prior to adding the solution to the remainder of the dry solids is not known. This might affect reactant concentration. If this sample preparation parameter does affect browning rate, shelf life of foods as determined by non-enzymatic browning could be controlled not only by environmental storage conditions and food composition, but also by the method of ingredient incorporation into the food.

A final suggestion for future study is that of investigating the influence of protein source on overall non-enzymatic browning rate. As economics or supply might dictate, the protein source for a food product may have to be changed and could affect the browning rate. Protein substitution may change the reducing sugar to primary amine content ratio even though absolute protein concentration is not altered. Also, the reducing sugar to primary amine ratio might be constant, however, the degree to which the primary amine groups are "free" and hence available for condensation with a reducing sugar may change with protein substitution. Finally, the total parameter of protein substitution as it affects non-enzymatic browning and product acceptability could be affected by final moisture content or a_w of the product.

With respect to processing recommendations, since the rate of non-enzymatic browning in an intermediate moisture content food system increases as a_w decreases, as high a moisture content as is possible while yet keeping the food stable against other reactions should be used. The method of moisture addition does not affect the rate. The degree of reducing sugar solubility, reducing sugar to primary amine content ratio, and protein source substitution as they affect the kinetics of non-enzymatic browning should be studied further however. The use of a liquid humectant such as glycerol, although useful for other reasons, may be detrimental because it maintains the reactants in the liquid phase below their water solubility limit. Work on the optimum ratio must be done to minimize reaction rates. Finally, since browning increases as a_w decreases a good moisture barrier is necessary. These results would be useful towards extending the shelf-life of intermediate moisture foods that are susceptible to non-enzymatic browning.

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V

MEASUREMENT OF THE PHYSICAL PROPERTIES OF INTERMEDIATE MOISTURE FOODS

V. Measurement of the Physical Properties of Intermediate Moisture Foods

A. Technique for Measurement of Water Activity in the High a_w Range at 35°C.

1. Introduction

Reprinted on the next pages is a copy of the article concerning this technique that was published in the Journal of Agriculture and Food Chemistry 22: 326-327. (1974).

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Technique for Measurement of Water Activity in the High A_w Range

Patricia T. Vos and Theodore P. Labuza*

A method was developed to determine the water activity (A_w) of food systems in the range of 0.85 to 0.98 A_w. The method is based on the equilibrium moisture absorption of microcrystalline cellulose at a given temperature. Sulfuric acid solutions of known concentration and A_w were used to prepare a standard curve of equilibrium moisture absorption *vs.* A_w at 35°. A known amount of the standard microcrystalline cellulose was placed in desiccators containing about 50- to 100-g food sample and evacuated for 1.5 min. After 24 hr, the weight gain of the cellulose was measured and the moisture content calculated. Results show that the method is comparable to that of the electric hygrometer and considerably better than the manometric technique.

The control of water activity (A_w) in the processing of foods is of major importance in relation to microbial spoilage and growth of pathogens. It is only at the higher A_w range, 0.90 to 0.99, that microorganisms usually grow in foods, and the rate of growth of most microorganisms is greatly accelerated at the higher A_w's.

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In this high range of A_w's, measurement by the electric hygrometer of A_w based on the electrical resistance of a salt-coated probe is inaccurate and sometimes misleading (Troller, 1973). Hygrometer probes are accurate to within ±0.005 A_w when new, but with age become less accurate so they must be recalibrated constantly. They are also subject to errors due to absorption of volatiles, such as glycerol, from the food (Block *et al.*, 1961). Measurement by a manometric technique as described by Labuza (1974) based on the design of Karel and Nickerson (1964) of the

Table I. A_w of Food Samples Obtained by Three Methods

Method	Bread	Salami	Whey concentrate	Pancake batter[a]
Microcrystalline cellulose method	0.951	0.969	0.820	0.961
	0.948	0.968	0.821	0.960
	0.948	0.969	0.820	0.960
Av	0.949	0.969	0.820	0.960
Manometric technique	0.94	1.00	0.89	0.97
	0.92	0.98	0.87	1.00
Av	0.93	0.99	0.88	0.99
Hygrometer	0.945	0.970	0.805	0.895
	0.955	0.965	0.820	0.871
Av	0.950	0.968	0.815	0.883

[a] One-month old probe used.

vapor space surrounding the food is also inaccurate at A_w's greater than 0.90 because of temperature control problems. An accuracy of ±0.005 below 0.85 is expected; above that, it falls to ±0.02. Also, if temperature is not controlled accurately, condensation of water vapor occurs and the results become meaningless.

Fett (1973) devised a method to measure A_w greater than 0.80 in foods based on the equilibrium moisture absorption of standard proteins at a known A_w and a given temperature. At A_w's greater than 0.95, more than 24 hr was necessary to achieve moisture absorption equilibrium. Also, a standard curve had to be made for each new batch of protein.

The present study was conducted to devise a simple technique that would give an accurate measurement of A_w's greater than 0.90 within 24 hr, based on the equilibrium moisture absorption of microcrystalline cellulose at a given temperature. In addition, cellulose would be better to use since proteins oxidize with age and change their absorption slightly whereas the crystalline cellulose is extremely stable (Bluestein and Labuza, 1972). In their work, they showed the same B.E.T. monolayer for the cellulose as was found by Maloney *et al.* (1966) working six years previously.

EXPERIMENTAL SECTION

The A_w of several food products was measured using three methods: (1) Hygrodynamics electric hygrometer (model 15-3001); (2) manometric technique; (3) equilibrium moisture absorption of microcrystalline cellulose (Avicel FMC Corp., Marcus Hook, Pa.). For measurement by the hygrometer, approximately 5 to 10 g of the food was placed in jars which contain the sensor in the cover. They were allowed to equilibrate at 35° for 24 hr prior to reading. The same amount of food was used for A_w measurement by the manometric device described by Labuza (1974). For the new method, the microcrystalline cellulose was dried in a vacuum oven for 48 hr, 100°, 29 mm Hg. Samples of 2 g (to 0.0001 g) of the standard dried microcrystalline cellulose were weighed into 35-ml weighing bottles. The cap was removed from the weighing bottle and triplicate samples were placed on the plate in a 214.9-cm vacuum desiccator containing 50 to 100 g of the food sample. The desiccators were evacuated for 1.5 min and were placed at 35° for 24 hr. No measurable loss of water occurs in this evacuation time. After 24 hr, air was gradually let into the desiccators over a period of 5 min (at this high temperature, there was no condensation apparent). The weighing bottles were capped and wiped dry prior to weighing. The moisture content was calculated from the weight gain.

The A_w of the food product was determined by referring to the standard curve (Figure 1) in which A_w is plotted *vs.* moisture content for the microcrystalline cellulose at equilibrium. This isotherm was found by measuring the adsorption isotherm of Avicel over standard sulfuric acid-water solutions (Wilson, 1940). The exact composition of the sulfuric acid solutions was determined by titration with base. Quadruplicate samples were used in preparation of the curve. The accuracy is about ±0.002 A_w as has been found by Fett (1973).

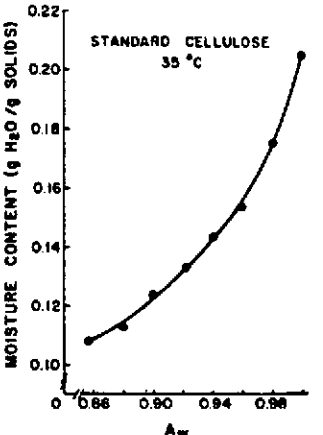

Figure 1. Standard sorption isotherm curve for microcrystalline cellulose at 35°.

RESULTS AND DISCUSSION

A comparison of results obtained by the 3 methods (Table I) demonstrates that the microcrystalline cellulose method is comparable to the measurements using a hygrometer with a new sensor. The manometric technique gave results very different from the other two methods. This was due to the difficulty in preventing condensation at the high A_w.

The microcrystalline cellulose method may be used for measurement of A_w's greater than 0.90 and should be more accurate, especially if new hygrometer probes are not available. This method is more valuable than the use of the hygrometer or manometric device since it does not involve the expense of a special instrument. The hygrometer probes lose their accuracy with time, as is seen in the data for the pancake batter. The probe used was 1-month old, and was recalibrated over saturated sodium chloride but still gave a different value than the cellulose method. This could be due to the volatiles in the batter causing interference. The larger food sample size (about 10 times that used in other techniques) is also advantageous in that a more representative sample of the food product may be used for the A_w measurement.

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B. Standard curve for Isopeistic Technique of a_w Measurement at 23°C

1. Introduction

The a_w of various foods may be determined by the isopeistic method from an established standard curve of moisture content vs. a_w for a standardized material. Preparation of such a curve involves:

a. The set up of desiccator systems with varying concentrations of the solution to be used to given a_w's.

b. A pre-dried powder to be weighed and stored in weighing dishes within the desiccators under vacuum for a standardized period of time. Moisture gained by the powder over a designated period of time at a specific temperature may then be determined and plotted against the a_w of the desiccator. In Section V, A. such a system was presented for a_w measurement above 0.86 and at 35°C.

In this study, H_2SO_4 was used due to the fact that concentrations of the acid exhibit a consistent relative humidity. Because of convenience and availability, microcrystalline cellulose was used as the powder. This study was carried out at 23°C and over a wider range than previously reported.

2. Procedures

a. Twelve solutions of H_2SO_4 and distilled water were prepared and titrated with sodium hydroxide.

b. The H_2SO_4 solutions were placed in desiccators. Two weighed samples of pre-dried cellulose were placed in dishes

directly above the solution in each desiccator. A vacuum was pulled for 1 min and the system was left standing for 24 hr at 23°C.

c. The samples were reweighed and the moisture content of the cellulose was calculated.

d. To find the a_w at 23°C, each solution was titrated and the a_w found from the standard curve of % H_2SO_4 vs. the relative vapor pressure of pure water (R. Wilson, 1921) as shown in Figure 1.

3. Results and Discussion

The data of the moisture content of duplicate samples at the various sulfuric acid contents are shown in Table 1. As can be seen, variations were not greater than 0.0006 in moisture content between duplicates and $\pm$ 0.2 ml for the titration. This gives an error in a_w of $\pm$ 0.001 in the high a_w range (>0.85) and of $\pm$ 0.0025 below a_w 0.85 if the same procedure is used. Below this range, the method becomes too insensitive since the isotherm is very flat at this point, as seen in Figure 2 in comparison with the 35° isotherm. As seen and as should be expected for this high a_w range, the isotherms are similar at both temperatures.

4. Summary and Recommendations

A new and accurate method has been developed for determining a_w in the high intermediate moisture range of foods. Because of the large sample size of food used, heterogeneity of the food does not cause errors. It is recommended that where cost is a problem this method be used.

Figure 1. Effect of Sulfuric Acid Concentration on Water Activity of the Solution.

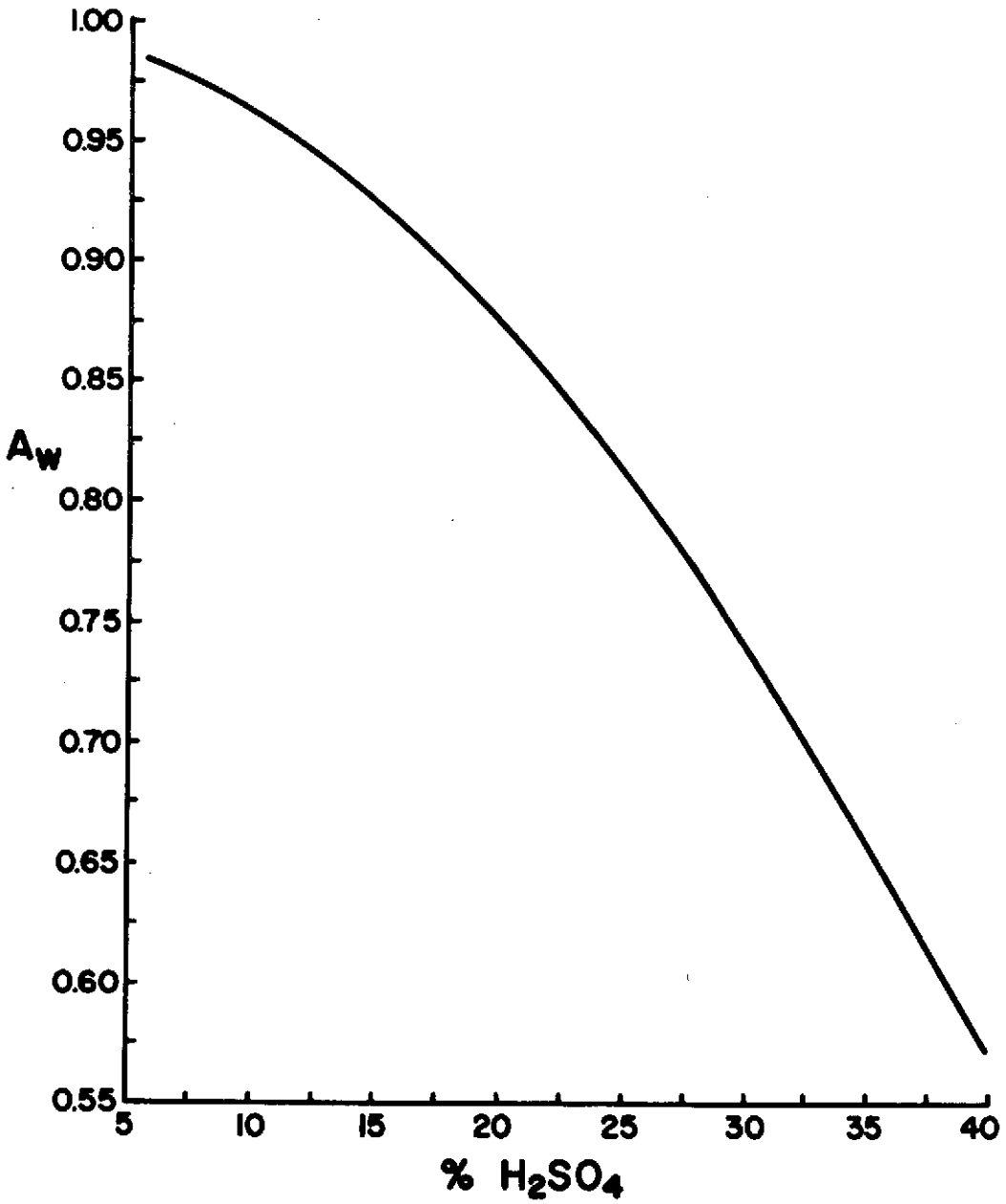

TABLE 1

ISOPEISTIC—CELLULOSE METHOD RESULTS AT 23°C

Initial % H_2SO_4	Titrations*	Moisture content g H_2O/100 g solids	%RH	Average moisture content	Average % RH
6.0	5.60 5.64	0.177 0.178	98.5 98.4	0.1775	98.45
15.0	13.57 13.66	0.141 0.142	93.8 93.5	0.1415	93.65
18.4	17.57 17.64	0.124 0.124	89.9 89.8	0.124	89.85
20.5	19.65 19.52	0.117 0.115	88.5 87.8	0.116	88.15
25.0	23.68 23.73	0.094 0.093	82.9 82.8	0.0935	82.85
28.5	27.38 27.63	0.086 0.086	78.0 77.65	0.086	77.325
34.5	32.73 32.72	0.075 0.075	69.75 69.76	0.075	69.755
35.6	34.81 34.71	0.069 0.069	66.4 66.5	0.069	66.45
37.0	35.69 35.97	0.074 0.074	64.8 64.4	0.074	64.6
38.3	37.13 37.00	0.074 0.072	62.4 62.5	0.073	62.45
40.4	38.40 38.56	0.060 0.061	60.2 59.9	0.0605	60.1
42.0	40.03 40.38	0.062 0.060	57.0 56.5	0.061	56.75

* ml NaOH (1.0 N)

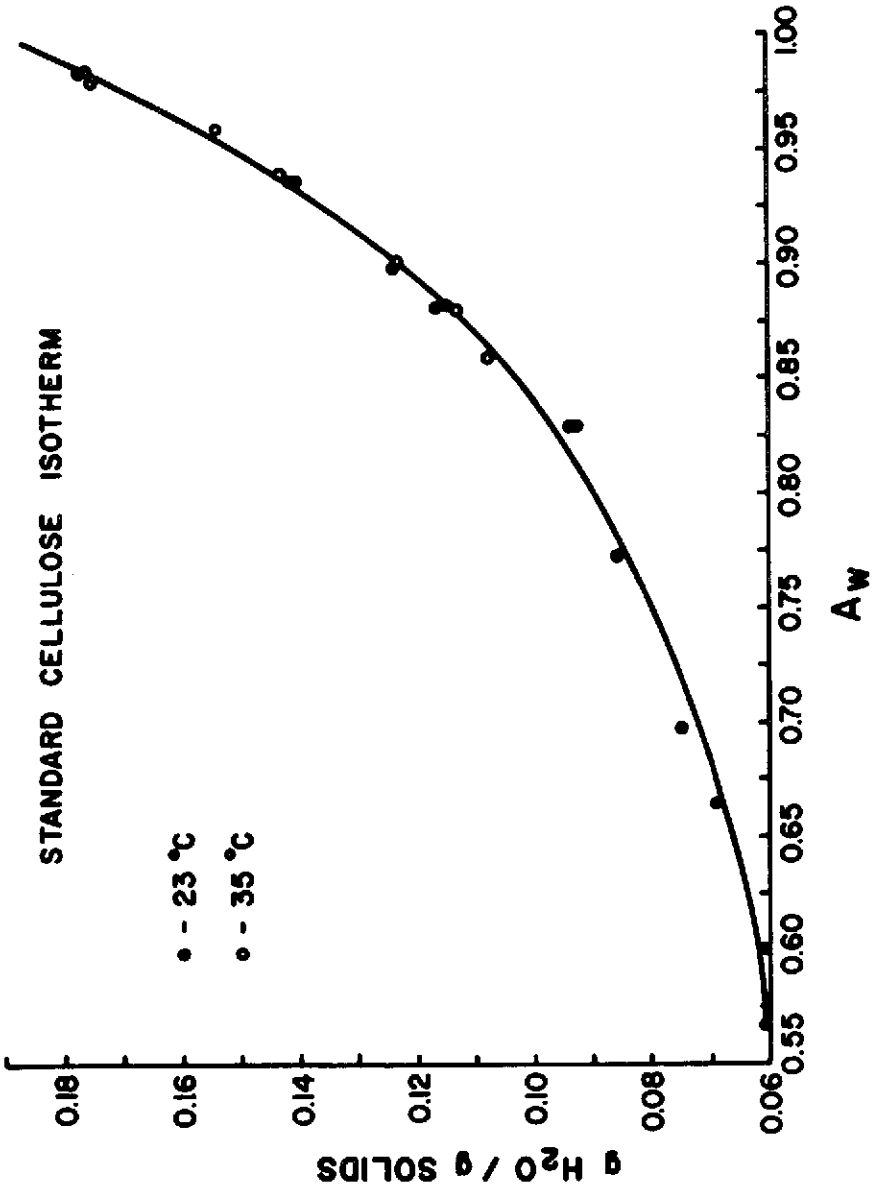

Figure 2. Standard Cellulose Isotherm - Isopeistic Method

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C. Measurement of the pH of Intermediate Moisture Foods

1. Introduction

The overall purpose for this experiment was to determine the effect of acidulant concentration on the pH attained in the Hennican in preparation for microbiological studies using pH as a parameter. It was first necessary to determine a reliable procedure for determination of pH in intermediate moisture foods.

One method that can be used is the gran plot method in which serial dilutions are made and the measured pH is plotted on special paper. By extrapolation to zero dilution, the pH is estimated to one decimal place. This method is used by several food companies for dry foods.

A question arises when different components are added to a food system followed by serial dilutions with water. It is possible that the time for equilibration and dissolution of acid in an IM food could vary and thus some equilibration time would be needed. In addition, the amount of water added could change the amount of dissociation of the acid and thus affect pH as well. Preliminary results showed that the pH changed slowly over 24-48 hr. This time requirement is too long to be useful. Thus, another method was needed for estimation of pH. Some alternate methods were tested.

2. Methods

a. Food system

For the preparation of the standard acidulant curves, Hennican was prepared as reported in Section III, A. Table 1 gives the composition, moisture content and a_w of the systems used. After mixing, the Hennican was divided up and various amounts of five acidulants were added into the food by mixing for 3 minutes. Acidulants were adipic, citric, fumaric, lactic and phosphoric acid. All systems were equilibrated at 4°C for 24 hr. The pH was then determined by four different methods.

b. pH measurement

The methods used for pH estimation were as follows:

(1) AOAC Method #14.022

The IM food in this study is not in any category described in the A.O.A.C. book of Standard Methods (1970), but most food companies producing IM foods use method #14.022. 10 g of Hennican was blended with 100 ml of recently boiled, distilled and deionized

TABLE 1

COMPOSITION FOR HENNICAN USED FOR STANDARD pH CURVE DETERMINATIONS

Component	% weight of component	
	a_w 0.86*	a_w 0.88*
Peanuts	14.66	13.59
Freeze-dried chicken	14.66	13.59
Raisins	29.20	27.18
Peanut butter	3.86	3.60
Honey	1.58	1.47
Non-fat dry milk	10.63	9.90
H_2O	25.53	30.67
Moisture content g H_2O/g solids	0.43	0.56

*a_w (measured by VPM)

water after cooling to 25°C. After standing for 30 minutes, the pH was measured with a standardized normal pH electrode such as an Orion #910200. This method was only tested for the citric acid system.

(2) Direct measurement

Two types of pH electrodes were compared by pushing them directly into the Hennican with no H_2O added. The pH was read after 3 min equilibration. The Orion electrode (#910200) used was a typical semi-micro combination electrode. The second type of electrode was a Beckman non-aqueous electrode (#39142) specially designed to correct for osmotic gradients.

(3) Slurry dilution method

The measurement of pH in meat is described as blending the meat with an equal weight of distilled H_2O and the pH is read on the slurry (Salisbury and Crampton, 1960). 5 g of H_2O was added to 5 g Hennican. It was mixed to a slurry, then allowed to equilibrate 24 hr at 4°C. The pH was measured with the Orion electrode after equilibrating for 2 hr at 23°C.

(4) Gran plot extrapolation

To 3 g of food, 1, 2 or 3 ml of distilled, deionized water was added and stirred into the food to make a slurry. The pH was read after 5 min equilibration. The pH was plotted against the grams of H_2O added on gran plot paper (100% volume-corrected, Orion cat. #900093). The value at zero addition, which is found by extrapolation from the measured pH values, is the pH of the food. The pH electrode used was the Orion electrode.

3. Results and Discussion

The results of the pH measurements are shown in Table 2. The value obtained by direct measurement with the Beckman non-aqueous electrode is considered the most reasonable, since the electrode is designed to function under conditions similar to those defined by an IMF system, namely a reduced water content. As can be seen from Table 2, the variation of the other methods from the Beckman electrode values is $\pm$ 0.2 pH units and in most cases the range is $\pm$ 0.1. This shows that any of these methods could be used to measure the pH of Hennican. The major consideration is that the standardized electrode be given time to equilibrate in the sample prior to reading the pH.

When using the gran plot method the pH value is limited to one decimal place of certainty because of the inaccuracy of extrapolation. The other methods could also be limited to $\pm$ 0.1 units if the foods are a heterogeneous mixture which could vary in acid composition. Table 2 shows the variation between batches of different systems at a_w 0.86. It was also found that the pH measurements with the Beckman electrode on the same sample taken from different places would give different values. For example, for one sample pH readings of 4.78, 4.80 and 4.81 were found. This variation reflects the problem of microenvironments which may cause unrealistic representation of microbial activity or chemical reactivity.

It should be noted that the AOAC recommended method compares very closely with other methods tested. This is probably due to the buffering capacity of the ingredients in the food. This may not be true in all foods, however. For instance, pH determinations of an

TABLE 2

pH MEASUREMENT IN HENNICAN

System	% Acid	a_w:	Gran plot			AOAC	Slurry	Direct - Orion			Direct-Beckman	
			0.86	0.86	0.88	0.86	0.86	0.86	0.86	0.88	0.86	0.88
Control	0.00			5.5			5.5	5.3	5.5	5.6	5.5	5.5
Adipic acid	0.10								5.4	5.5	5.3	5.5
	0.25								5.3		5.3	5.3
	0.50			5.0			5.0	4.9	5.2		5.1	5.1
	1.00			4.7			4.7	4.9	4.9	4.9	4.9	4.8
	2.50								4.7	4.6	4.6	4.5
Citric acid	0.10								5.4		5.3	5.4
	0.25		5.3	5.2	5.3	5.2	5.2		5.3		5.1	5.2
	0.50		5.0	4.9	5.1	4.9	4.9		5.1		4.9	4.9
	1.00		4.8	4.6	4.7	4.6	4.6		4.7	4.6	4.5	4.5
	2.50		4.2		4.1	4.1			4.3		4.1	4.0
Fumaric acid	0.10										5.2	5.3
	0.25			5.0			5.0	5.0			5.1	5.1
	0.50			4.7			4.7	4.7			4.8	4.8
	1.00			4.3			4.3	4.3	4.4	4.4	4.3	4.3
	2.50										3.7	3.7
Lactic acid	0.10										5.2	5.2
	0.25			5.2			5.2	5.4			5.1	5.2
	0.50			5.0			5.0	5.2			5.0	5.0
	1.00			4.7			4.7	4.9	4.7	4.8	4.6	4.7
	2.50										4.0	4.0
Phosphoric acid	0.10										5.2	5.3
	0.25										5.1	5.1
	0.50										4.9	4.9
	1.00										4.5	4.4
	2.00								4.2	4.2	3.8	4.0

TABLE 2

pH MEASUREMENT IN HENNICAN

System	% Acid	a_w:	Gran plot 0.86	Gran plot 0.86	Gran plot 0.88	AOAC 0.86	Slurry 0.86	Direct - Orion 0.86	Direct - Orion 0.86	Direct - Orion 0.88	Direct-Beckman 0.86	Direct-Beckman 0.88
Control	0.00			5.5			5.5	5.3	5.5	5.6	5.5	5.5
Adipic acid	0.10								5.4	5.5	5.3	5.5
	0.25								5.3		5.3	5.3
	0.50			5.0			5.0	4.9	5.2		5.1	5.1
	1.00			4.7			4.7	4.9	4.9	4.9	4.9	4.8
	2.50								4.7	4.6	4.6	4.5
Citric acid	0.10								5.4		5.3	5.4
	0.25		5.3	5.2	5.3	5.2	5.2		5.3		5.1	5.2
	0.50		5.0	4.9	5.1	4.9	4.9		5.1		4.9	4.9
	1.00		4.8	4.6	4.7	4.6	4.6		4.7	4.6	4.5	4.5
	2.50		4.2		4.1	4.1			4.3		4.1	4.0
Fumaric acid	0.10										5.2	5.3
	0.25			5.0			5.0	5.0			5.1	5.1
	0.50			4.7			4.7	4.7			4.8	4.8
	1.00			4.3			4.3	4.3	4.4	4.4	4.3	4.3
	2.50										3.7	3.7
Lactic acid	0.10										5.2	5.2
	0.25			5.2			5.2	5.4			5.1	5.2
	0.50			5.0			5.0	5.2			5.0	5.0
	1.00			4.7			4.7	4.9	4.7	4.8	4.6	4.7
	2.50										4.0	4.0
Phosphoric acid	0.10										5.2	5.3
	0.25										5.1	5.1
	0.50										4.9	4.9
	1.00										4.5	4.4
	2.00								4.2	4.2	3.8	4.0

IM dog food done in this laboratory show differences of close to one pH unit with the AOAC giving the highest pH as would be expected if the buffering capacity is low.

The standard curves of acid concentration vs. pH are plotted in Figure 1 using the values obtained with the Beckman non-aqueous electrode.

4. Summary and Recommendations

Various methods can be used for determining the pH of intermediate moisture foods. Four methods were investigated and all gave similar results. These included the standard AOAC procedure for dry foods, a dilution test, the gran plot technique and a direct reading with a non-aqueous electrode. It is recommended that the use of a direct reading electrode would be as reliable as any other technique and would take less than five minutes. Of course, the food must be plastic enough to allow the penetration of the electrode. If the food is drier, then the gran plot technique would be best since the buffering capacity may not be known.

5. References

Official Methods of Analysis of the Association of Official Analytical Chemists. 1970. Horowitz, editor. 11th edition. Washington, DC. pg. 214.

Salisbury, G.W. and E.W. Crampton, editors. 1960. The Science of Meat and Meat Products. W.H. Freeman & Co. pg. 234.

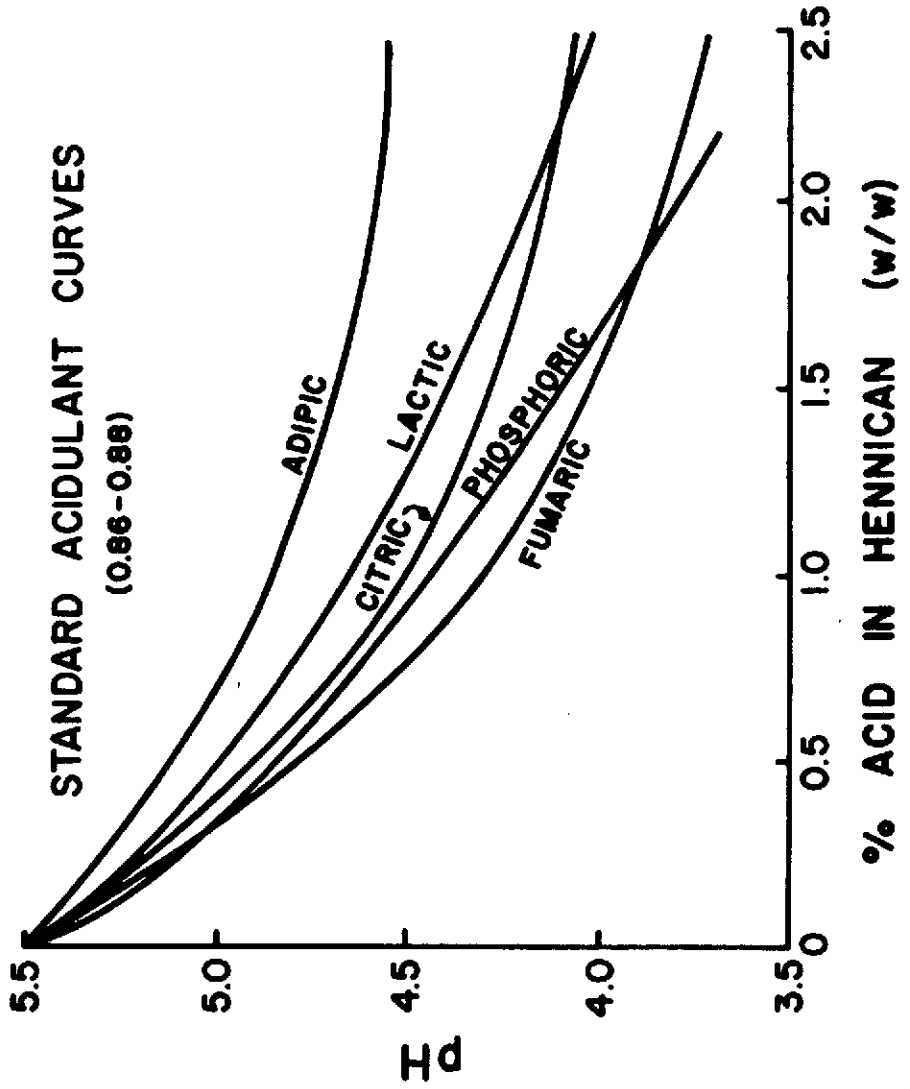


Figure 1. Affect of Acid Concentration on pH in Hennican.

VI

STUDY OF THE STORAGE STABILITY OF AN IM HUMAN FOOD SYSTEM: GRANOLA BARS

VI. Study of the Storage Stability of an IM Human Food System: Granola Bars.

A. Granola Bar Storage Study

1. Introduction

In Phase I of this contract a new IMF was developed based on Granola cereal, marshmallows and peanut butter. A storage study of the system at three a_w's: 0.68, 0.75 and 0.85 in both direct mix (DM) and freeze-dried rehumidified (FDR) systems was made to determine the shelf-life of this product. No antioxidants were added since the mechanism of deterioration was not known. The following tests were proposed to be done on the Granola Bars:

a. Peroxide values

b. Warburgs

c. Non-enzymatic browning

d. Texture (Instron)

e. Organoleptic (compared to frozen taste control)

The use of the two methods of preparation would allow for determination of the effect of sorption hysteresis on chemical deterioration.

2. Experimental procedures

a. System preparation

Granola bars were prepared by two methods, desorption (direct mix - DM) and adsorption (freeze-dried rehumidified - FDR), to obtain an isotherm and to determine the extent of hysteresis.

b. Method of Granola Bar preparation

(1) Weigh components into separate beakers.

(2) Add marshmallows into the bowl of the Brabender 300 g mixer, knead on fast speed until the marshmallows become taffy-like.

(3) Add non-fat dry milk.

(4) If water is added, add using a volumetric pipette, continue mixing until a uniform mixture is obtained.

(5) Add the peanut butter and mix until uniform (base mixture).

(6) Add the granola, mix until the granola is uniformly distributed throughout the base (do not over-mix).

(7) Pat into a sheet approximately 3/4 - 1 cm in thickness and cut into approximately 1.5 cm square pieces.

The composition of the direct mix systems are shown in Table 1, with the measured moisture by GLC. The isotherm for the DM is plotted in Figure 1. The a_w's were determined by the manometric technique.

To prepare the FDR systems, granola bars were made at a_w 0.70 and freeze-dried for 46.5 hr (21 hr at room temperature, 30°C, and then for the remainder of the time at a platen temperature of 100°F).

Two samples were weighed in glass weighing dishes to record the weight lost during drying. After freeze-drying, duplicate samples (2 squares each) were weighed in glass weighing dishes and placed in desiccators (under vacuum) over a range of a_w's from 0 to 0.98. The weight change was recorded and the average moisture content of the two samples was determined. The samples were held in the desiccators for 6 days. The moisture content was

TABLE 1

Composition Granola Bars

IMF System

Direct Mix

	Basic	Theoretical A_w		
Components	%	0.68	0.75	0.82
Granola *	29.4	154.35	154.35	154.35
Marshmallows	29.4	154.35	154.35	154.35
Peanut butter **	31.4	164.85	164.85	164.85
Non-fat dry milk	9.8	51.45	51.45	51.45
K-sorbate	---	1.58	1.58	1.58
ml H_2O	---	30.87	51.45	82.32
% Fat	---	15.28	14.56	11.73
% Protein	---	16.19	15.50	14.85
Salt solution for rehumidification	---	$CuCl_2$	NaCl	$CdCl_2$

* Pillsbury Co., Minneapolis

** Skippy Peanut Butter Co., Minneapolis

then calculated correcting for the weight loss in the samples placed in the dry desiccator using Equation 1.

$$\text{moisture content} = \frac{A + B \cdot C}{B - B \cdot C}$$

where A = weight gain
B = initial sample weight
C = g H_2O/g solids residual water lost in drierite desiccator

The weight change was determined periodically and is shown in Figure 1. The samples at a_w 0.88 and below came to equilibrium in about 100 hr. The data used for the isotherm is the six-day reading (144 hr). At 11 days the sample at a_w 0.98 was high in surface bacterial growth and showed leaking of water. The samples at 0.88 had some green mold growth.

As can be seen in Figure 2, there is sufficient hysteresis in the granola bar to incorporate that as a parameter in storage studies. Table 2 shows the overall nutrient composition for a granola bar at a_w 0.74. Systems were prepared to three a_w's and were stored at 35°C in 2 oz. jars (approximately 20 to 30 g per jar), covered with two layers of Reylon PVC film, capped and sealed with 3M black electrician's tape. Beakers of H_2O were placed in the surrounding chamber to prevent moisture loss. Samples were analyzed over a period of 56 days.

c. Storage test procedures

The test procedures used are reported in detail in Phase I, Final Contract Report, NAS 9-12560 except for the following tests:

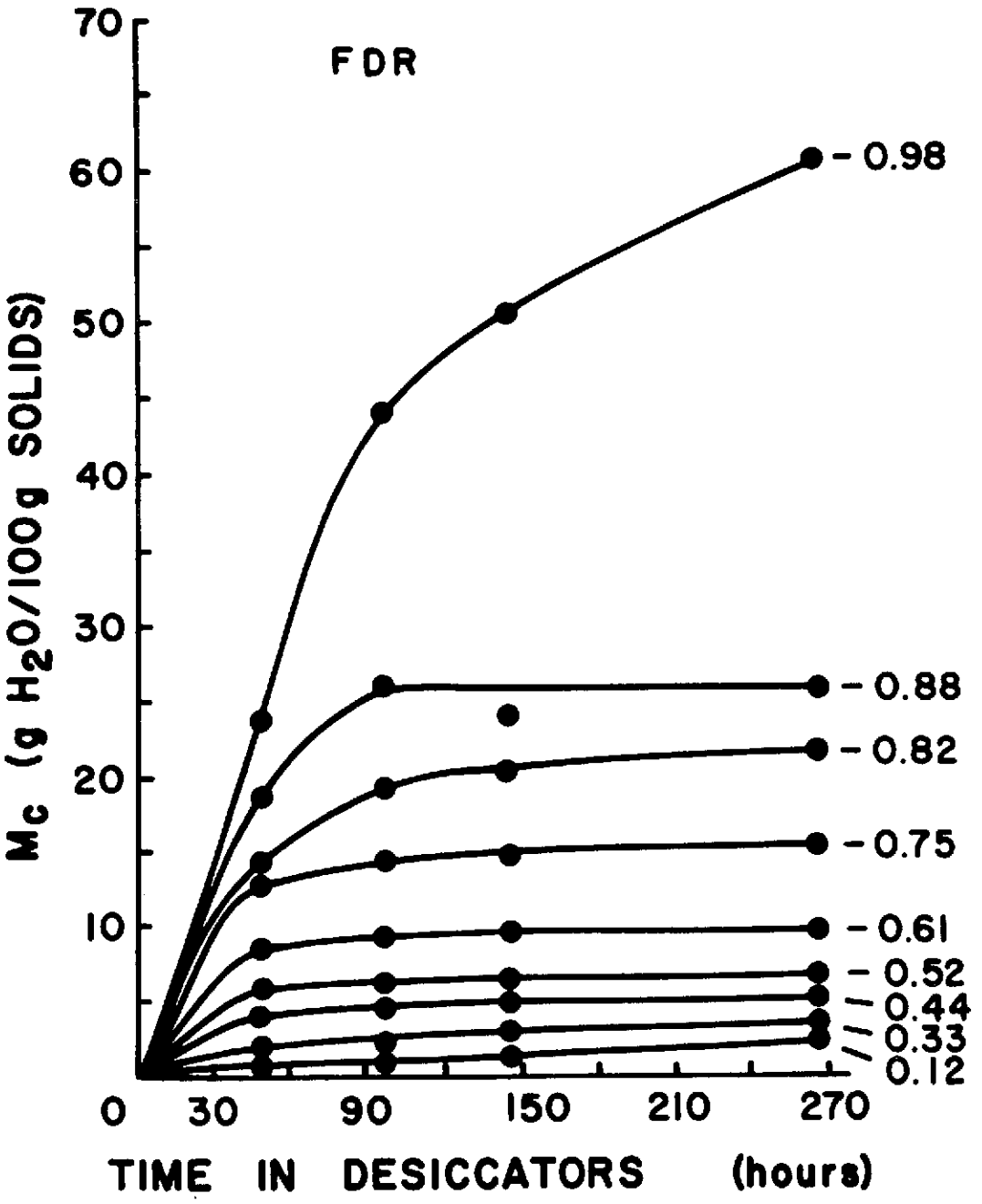


Figure 1. Equilibration Time for Moisture Adsorption of Granola Bars at Various a_w's.

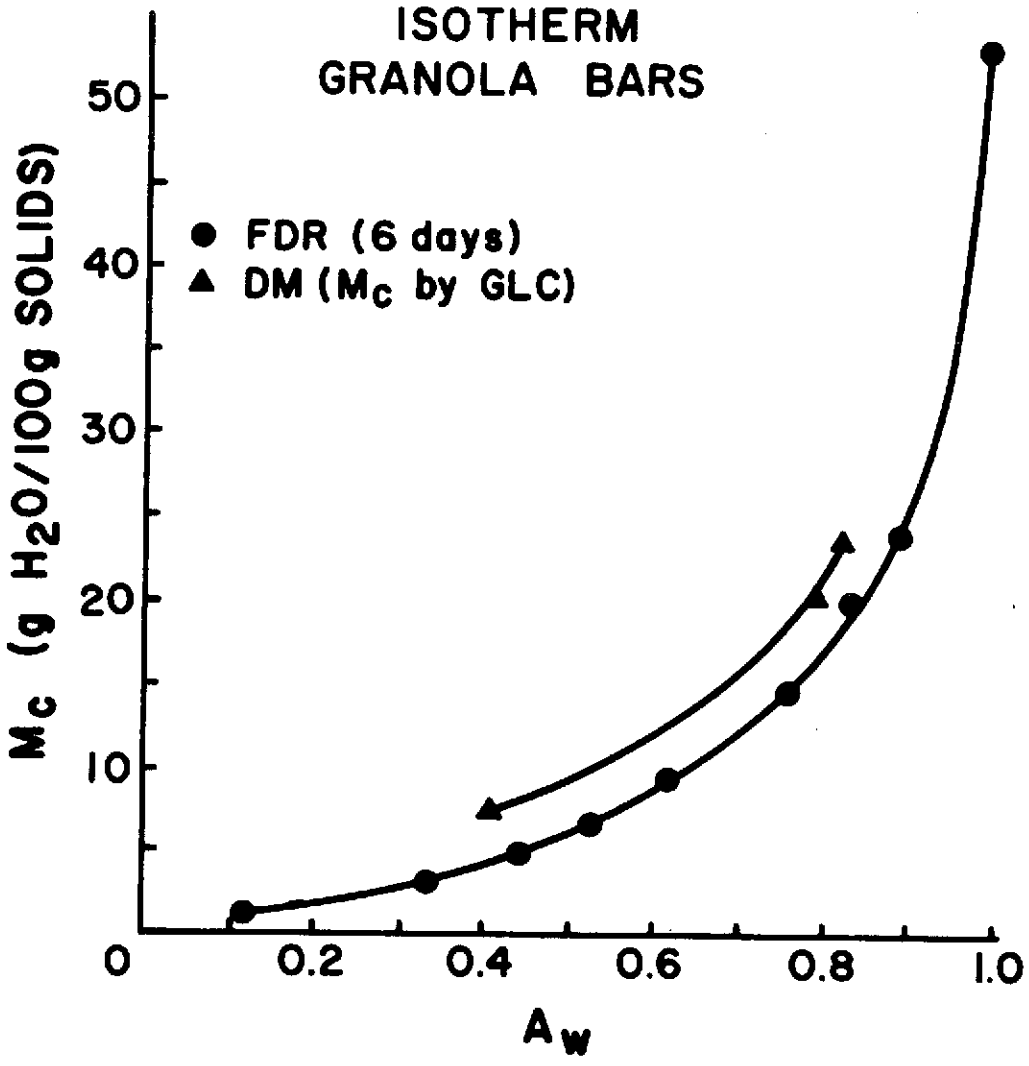


Figure 2. Moisture Sorption Isotherm for Granola Bars at 35°C. DM - desorption, FDR - adsorption

TABLE 2

Nutrient Composition

Values for 100 g of Granola Bars (A_w 0.74)

Component	IU Vitamin A	mg Vitamin C	mg Thiamine	mg Riboflavin	mg Niacin	mg Iron
Granola	--	--	.07	.04	--	.48
Marshmallows	--	--	--	trace	trace	.44
Peanut butter	--	--	.04	.04	4.45	.58
Non-fat dry milk	2.73	.64	.03	.16	.82	.06
Total	2.73	.64	.14	.24	5.27	1.56

moisture	8.99%
fat	26.50%
protein	14.13%
carbohydrate*	50.38%
cal/gram	5.0

*by difference

(1) Non-enzymatic browning

(a) 0.5 g sample suspended in 2.5 ml 10% trypsin + 20 ml pH 7.8 phosphate buffer

(b) Incubate system for 2 hr at 45°C, 120 CPM

(c) Following 2 hr incubation, trypsin is denaturated by adding 2 ml 50% (w/v) Trichloroacetic acid

(d) Add 0.1 g Celite (filter aid)

(e) System is filtered through Whatman #1 filter paper

(f) O.D. 420 nm is measured on Coleman Jr. II against 0.00 OD blank (blank has no sample added to trypsin solution, but treated otherwise as regular samples)

(2) Instron texture test

(a) Samples were shaped into the dimensions of 1.7 x 1.7 x 6 cm $\pm$ .15 cm

(b) Samples were compressed on a Universal Instron tester through an 8-wire grid to within 0.1 cm of the original sample thickness at a rate of 0.5 cm/min

(c) A plot of force vs. distance was recorded and 3 slopes ($F\ (kg) / \Delta L/L$) were calculated at total ΔL, 2/3 ΔL, and 1/3 ΔL. From this an average slope was determined.

3. Results and Discussion

Tables 3 and 4 show the results of changes in a_w and moisture content during storage of the granola bar IM food product. As seen, the a_w of all the products decreased during storage. This could be expected if the initial equilibrium was not complete and

TABLE 3

Measured A_w - Granola Bars during Storage at 35°C

Measured A_w	Theoretical A_w		
	0.68	0.75	0.82
Direct Mix			
Initial	0.70	0.77	0.82
35 days storage[(a)]	0.64	0.71	0.81
56 days storage	0.65	0.73	0.78
76 days storage	0.64	0.70	0.76
FDR			
Before FD	0.69	0.78	0.83
After 8 days rehumidification	0.69	0.75	0.85
27 days storage	0.64	0.70	0.79
39 days storage	0.65	0.72	0.81
59 days storage	0.66	0.70	0.80

(a) storage at 35°C

TABLE 4

Moisture Content Changes

Granola Bar Storage Study

	M_c = g H_2O/100 g solids		
A_w	0.68	0.75	0.82
Direct Mix - Initial			
VO	11.01	14.65	20.01
GLC	9.64	16.07	23.02
56 days storage (VO)	11.55	15.45	18.72
77 days storage (VO)	9.30	11.73	15.29
81 days storage (GLC)	9.86	13.73	15.83
FDR - Initial			
GLC	11.83	15.61	19.76
39 days storage (VO)	10.71	14.57	20.68
50 days storage (VO)	10.30	12.62	19.82
54 days storage (GLC)	9.96	14.13	19.02

VO = vacuum oven 18 hr. @ 65°C

GLC= gas liquid chromatography

there was a transfer of water from the aqueous phase into the low moisture ingredients such as the cereal and marshmallows. This was accompanied by a decrease in moisture content for all samples which could be due to water loss into the storage cabinet suggesting that the jars were leaking. The data also show that hysteresis is negligible, thereby negating the effect of method of preparation.

The redistribution and loss of moisture led to all products becoming unacceptable within 30 days, as seen in Table 5. All panelists commented on the loss of crispiness during storage which in turn led to the product becoming dry-tasting or almost rubbery. Eventually, all samples developed a bitter taste and had a slightly rancid odor due to some reaction during storage. The increase in toughness as measured by the Instron can be seen in Figure 3 for the lowest a_w systems (0.68), however, the other systems showed no significant increase. The large change for the a_w 0.68 samples may be because of an accelerated chemical reaction or that at a moisture content of around 9-10 g/100 g solids the plasticizing effect of water becomes a minimum. With respect to the sorption isotherm this is the region where capillary effects decrease and the isotherm flattens out.

Several tests were made to determine the cause of the off-flavors developed in the product. Table 6 shows the results of the oxygen absorption rate for up to 30 days. As seen, except for the direct mix system at a_w 0.82, all systems oxidized at a similar rate of about 10 µl O_2/gram per day. In addition, as expected the oxidation rate increased as a_w increased. In contrast, the Hennican systems studied in Phase I oxidized at close to 100 µl O_2/g per day.

TABLE 5

Granola Bars @ 35°C

Organoleptic Score Summary *

Average of 5 Scores

Day	Taste control	0.68		0.75		0.82	
		DM	FDR	DM	FDR	DM	FDR
0	7	7	5	7	5	6	5
6	8	7		8		7	
10	9		4		4		3
13	7	6		7		8	
20	9	5		5		4	
21	9		2		2		2
31	9	4		3		5	
34	9	4		4		4	

* A value of 4 or below for two successive periods indicates unacceptability

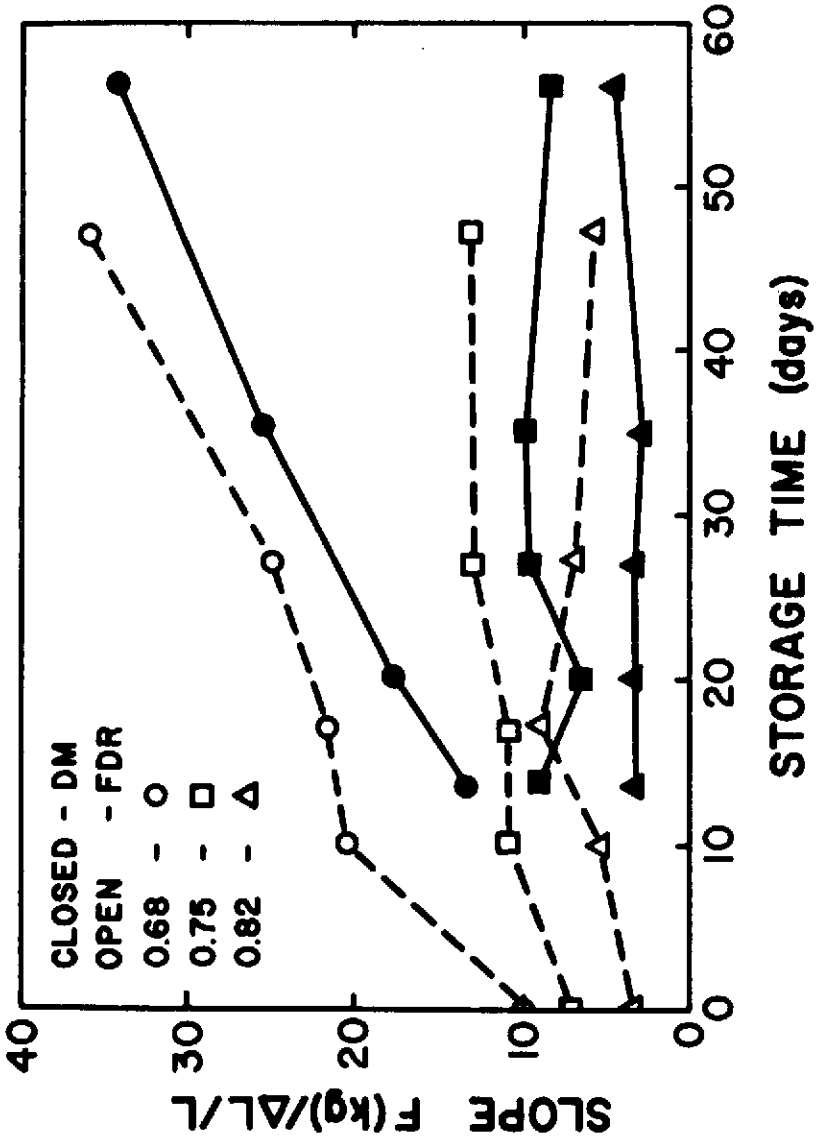


Figure 3. Increase in Toughness of Granola Bars during Storage at 35°C.

TABLE 6

GRANOLA BAR STORAGE STABILITY STUDY

OXYGEN UPTAKE RATE * - 35°C

System	Direct mix	Freeze-dried rehumidified
a_w 0.68	8.16	6.72
a_w 0.75	10.91	10.4
a_w 0.82	15.84	10.4

* As measured by Warburg O_2 uptake

This suggests that rancidity of the fats may not be responsible for the off-flavor. However, this slow oxidation does not mean that rancidity cannot occur. The peroxide values for all the test systems is shown in Table 7. As seen, after about 10-20 days the peroxide values rise from a value of 1-2 to about a value of 5-6 and then decrease again, which is typical of lipid oxidation. Again, this is a small change as compared to the large increases found for Hennican but only a small quantity of unsaturated fat has to oxidize to produce off-flavors. This is most likely responsible for the poor flavor and odor found in all the products after 10-20 days.

The slight bitter taste developed during storage may be due to free fatty acids being formed from enzymatic hydrolysis of the lipids. As seen in Table 8, there was an increase in free fatty acids as compared to the frozen control. Also as would be expected, there were more free fatty acids produced at the higher a_w. This is similar to what Acker (1969) has found for enzymatic hydrolysis in cereals with respect to a_w. This could account for the bitter flavor found, although not enough samples were available to test this during the whole storage period.

A bitter flavor can also be developed in an IMF product if non-enzymatic browning occurs. The results of the NEB determination are shown in Table 9. As seen, an increase in browning did occur, however, in the first 20 days when the product became organoleptically unacceptable only the direct mix at a_w 0.82 had a large increase and this was the most acceptable product. Thus browning should be ruled out as a major deterioration reaction.

TABLE 7

Granola Bar Storage Study 35°C

Run 1

Peroxide Value (meq/Kg fat) *

Theoretical A_w

	0.68		0.75		0.82	
Day	DM	FDR	DM	FDR	DM	FDR
0	0.77	--[a]	1.05	1.32	1.40	--
3	--		--		1.07	
6	--		--		1.95	
10		1.33		1.85		--
13	--		--		1.99	
17		1.79		2.75		3.36
20	1.77		0.45		1.99	
27	2.55	5.64	2.67	6.64	1.90	
31		4.53		5.09		6.87
35	2.76		6.76		4.83	
43	3.67		4.17		6.36	
50		6.31		3.75		3.11
60	4.12		3.65		4.40	

a) No color change when 1% starch solution added as an indicator

* Average of two values

TABLE 8

IMF Storage Study

Granola Bars 35°C

Free Fatty Acids

Theoretical A_w	DM 51 days	FDR 39 days	Frozen control
0.68	91.01	95.85	---
0.75	95.70	118.70	82.42
0.82	105.16	136.17	---

Note: Samples not done in duplicate

TABLE 9

GRANOLA BARS AT 35°C

NON-ENZYMATIC BROWNING

Absorbance at 420 nm

Days in storage	DM 0.68	FDR 0.68	DM 0.75	FDR 0.75	DM 0.82	FDR 0.82
0	0.130	0.155	0.127	0.170	0.105	0.160
10	0.145	0.180	0.140	0.182	0.150	0.157
13	0.135		0.164		0.135	
17		0.155		0.210		0.142
20	0.182		0.200		0.170	
27	0.153	0.205	0.188	0.195	0.175	0.205
32		0.225		0.205		0.188
35	0.180		0.240		0.170	
59	0.248		0.220		0.195	

Lastly, the resulting unacceptability could be due to microbial growth. Table 10 shows that the counts were very low initially and did not increase during storage.

4. Summary and Recommendations

Overall, the granola bar, although initially highly acceptable, is extremely unstable. In 10-20 days all panelists rated it undesirable due to a dry—rubbery texture, a bitter flavor and a metallic, rancid odor. This is most likely due to enzymatic reactions causing free fatty acid production and oxidation of lipids. No antioxidants were used which could inhibit the latter reaction, but to inhibit the lipase, a heat treatment would be needed. The real problem is moisture redistribution which makes the product dry and rubbery tasting. No solution for this is possible unless a highly impermeable barrier to water could be sprayed on the cereal. None are available that can be used on foods.

The recommendations for further work on this product as well as for IMF in general are:

a. Make sure that all components are blanched to prevent enzymatic activity.

b. Incorporate antioxidants to prevent oxidation of lipids or use a low oxygen atmosphere.

c. Keep the a_w as low as possible to minimize oxidation.

d. Use a highly impermeable moisture barrier for the package as moisture loss will cause the product to become hard and unacceptable.

5. References

Acker, L.W. 1969. Water activity and enzyme activity. Food Technol. 23: 27.

TABLE 10

TOTAL PLATE COUNT ON GRANOLA BAR AT 35°C

Initial a_w	counts/gram
Direct mix (27 days)	
0.70	2×10^2
0.77	2×10^2
0.82	3×10^2
Freeze-dried rehumidified (21 days)	
0.69	5×10^2
0.75	2×10^2
0.85	2×10^2
Initial control	3×10^2

VII

THE ACCELERATED SHELF-LIFE TESTING OF AN INTERMEDIATE MOISTURE FOOD

VII. The Accelerated Shelf-Life Testing of an Intermediate Moisture Food

A. Introduction

In the production of any new food one important aspect is the knowledge of the shelf-life. This life must at least exceed the minimum distribution time required from processor to the consumer. The shelf-life is controlled by:

1. The components of the system.
2. The process used.
3. The package permeability to light, moisture and gases.
4. The time—temperature—relative humidity distribution during transportation and storage.

The processor must have a knowledge of all these factors as well as a knowledge of the critical modes of failure of the food. With this information, the processor can then choose the best systems to maximize shelf-life or even put an open date on the product indicating the maximum high quality life of the product.

Unfortunately, little or no information is available for the processor since much product development work has not considered the physical-chemical laws which could be used to predict the modes of deterioration and even the shelf life. Oswin (1945) was the first to publish methodology which could be used to predict the shelf-life of a product. He developed an equation to predict the rate of loss of moisture from cigarettes in various packaging films. Charie et al. (1963) extended some of this work to food products. In a major effort,

Mizrahi et al. (1970a) utilized these schemes to develop a simple mathematical model to predict the change in moisture of a dehydrated food during storage as well as the extent of a chemical reaction, namely non-enzymatic browning. Their equations could be used to determine what packaging film should be used for a given desired shelf-life. What was needed was the rate of chemical deterioration of the product as a function of environmental conditions, the maximum extent of reaction that could be tolerated and the external conditions. This work was further extended to other systems (Labuza et al., 1972; Karel et al., 1971). Davis (1970) has also done similar work for foods and Harrington (1973) extended it to storage of seed.

Simon et al. (1971) introduced the same concepts in the prediction of the shelf-life of a product which undergoes oxidation (freeze-dried shrimp). Quast and Karel (1972a, 1972b) extended this further to the study of potato chips which both adsorb water leading to sogginess and oxidize leading to rancidity. These latter studies showed the complexity of the problem but indicated short cuts for the solution.

Salwin and Slawson (1959), Hokoji et al. (1969) and Charie et al. (1963) showed how to calculate moisture transfer between ingredients in a dehydrated food mix and use it to predict stability. The major problem was that the ingredients must all be kept at or near the monolayer to prevent deterioration. Labuza (1968, 1971) has reviewed the area of the amount of moisture in a food in terms of stability.

Much work has been done in predicting the shelf-life of frozen foods during distribution (Gaudagni, 1968). Olley and Ratkowsky (1973) have studied the time—temperature distribution during transport using special indicators similar to defrost indicators (Schoen and Byrne, 1972). In order to predict shelf-life, however, knowledge of the mode of deterioration as a function of temperature is needed. Some data has been collected by Dyer (1968) for fish, Jul (1968) for meat and Kramer (1974) and Olson (1968) for various frozen foods. Rutgers (1970) published a major survey of the shelf-life of various foods including refrigerated, frozen and dried, which also gives an idea of the modes of deterioration.

Even if all this data can be collected, it is usually a requirement that the shelf-life be verified before the product goes to market. This imposes a burden if the product has a long shelf-life so that some form of acceleration of deterioration would be desirable. With dehydrated foods, as was shown by Mizrahi et al. (1970b), the water content can be used to control the reaction. Since most reactions increase rapidly above the monolayer moisture value, holding the food in the IMF range should increase the rate of reaction. However, as shown by Chou and Labuza (1974), above a certain a_w the rate of rancidity decreases in certain systems. In Section IV it was shown that for IMF systems the maximum rate of browning occurs below the optimum moisture value. With respect to vitamin stability, however (Section II, A.), an increase in a_w increases the rate significantly. What is needed is data to show the rate of reaction as a function of a_w so that it can be applied for shelf-life

predictions.

Many researchers have used the oxygen bomb technique which uses high oxygen pressure to accelerate rancidity. However, as shown by Labuza (1971), the oxidation rate does not change significantly at any level above about 5% oxygen. The major factor that accelerates the reaction is the high temperature used during the experiment. Mizrahi et al. (1970b) used high temperature in conjunction with high moisture to accelerate browning of cabbage. Different reactions are affected to different extents by an increase in temperature. This is usually measured by the Q_{10} of a reaction, the increase in rate for a 10°C increase, or the activation energy which is the true function since Q_{10} is related to E_a by:

$$\log Q_{10} = \frac{2.3\ E_a}{(T)\ (T + 10)}$$

where E_a = activation energy in Kcal/mole
T = temperature in °K

Table 1 lists typical activation energies for food deteriorative reactions and illustrates the Q_{10} over two temperature ranges. This data could be useful in shortening shelf-life experiments.

Even though high temperatures do accelerate chemical reactions, one must be careful since at high temperature the mode of deterioration may change if one reaction is accelerated more than another as seen in Table 1. In addition, fats may melt in the product and cause other changes. However, use of high temperature is the easiest way to accelerate shelf-life testing. Labuza (unpublished) has proposed that if a plot of 1/LOG (shelf-life) vs. 1/T°K gives a straight line, then the mode of deterioration is not changing. At least 3 points

TABLE 1

EFFECT OF ACTIVATION ENERGY ON Q_{10}

E_a Kcal/mole	80°F to 98°F Q_{10}	212°F to 230°F Q_{10}
5	1.31	1.19
10	1.72	1.42
15	2.26	1.70
20	2.97	2.03
30	5.11	2.88
40	8.74	4.10
50	15.03	5.83

TYPICAL ACTIVATION ENERGIES

	Kcal/mole
Diffusion controlled	0 - 8
Enzyme reactions	10 - 15
Hydrolysis	15
Lipid oxidation	10 - 25
Non-enzymatic browning	25 - 50
Spore destruction	60 - 80
Vegetative cell destruction	50 -150
Protein denaturation	80 -120

are needed, about 5 to 10° apart. Thus, 45 to 30°C would be a useful range for accelerating most reactions and also can be used to determine the major mode of deterioration. However, these presumptions have not been adequately tested.

The purpose of this study is to test the usefullness of using high temperature to accelerate the deterioration of an IMF product, Hennican. By using three temperatures, it should be possible to determine whether the mode of deterioration changes with temperature. In addition, it was proposed to test the usefullness of a special packaging film (American Can Co., Maraflex 7 Oxygen Scavenger Web) in preventing rancidity.

2. Methods

a. Systems preparation

Hennican, a_w 0.85 with the composition as shown in Table 2, was prepared following the procedure reported in the final report NAS 9-12560, Phase I, March 27, 1972--March 17, 1973, pg. 125, 239. Additives were added at the following levels:

(1) BHA - 100 ppm (based on % fat)

(2) BHT - 100 ppm (based on % fat)

(3) Citric acid - 0.1% (based on solids)

(4) Ascorbic acid - 2 mg/g Hennican

(5) K-sorbate - 0.3% (based on solid weight excluding H_2O and non-fat dry milk)

The Hennican was rolled flat and cut into pieces with the dimensions of 1.7 x 1.7 x 0.6 cm.

TABLE 2

COMPOSITION OF HENNICAN a_w 0.85

FOR SHELF LIFE STUDY

Component	% (a)
Peanuts	15.44
Freeze-dried chicken	15.44
Raisins	30.87
Skippy peanut butter	4.08
Honey	1.67
Non-fat dry milk	11.24
H_2O	21.26

(a) Final Report, NAS 9-12560, March 27, 1972—March 17, 1973. Phase I. pg. 125, 239.

b. System storage parameters

The samples were prepared and stored at 25, 35 and 45°C. One-half were sealed into cans with air as the environment, while the other half were sealed in the special oxygen scavenger web described in the next section.

c. Oxygen scavenger packaging system

(1) Film description

A new film developed by American Can Company, Maraflex 7F Oxygen Scavenger film, was used to store Hennican in an "oxygen-free" environment. The laminated film is composed of the following layers (outside to inside surface); polyester, adhesive, foil, surlyn, catalyst (palladium), surlyn. The pouches containing the product are flushed with a gas mixture of 8% hydrogen and 92% nitrogen to minimize the residual oxygen in the pouches. The oxygen reduction activity works on the basis of one molecule of O_2 combining with two molecules of H_2. The O_2 and H_2 travel through the inner layer of surlyn and react in the presence of the catalyst to form H_2O. The H_2O remains trapped between the two surlyn layers. Therefore, the system can handle 4.0% O_2 when there is 8% initial H_2 in the pouch. This total of 4% O_2 includes O_2 in the package headspace immediately after packaging, O_2 dissolved in the product which may shift to the headspace after packaging, and O_2 which may permeate the packaging material during storage. A low initial residual amount of O_2 (approximately 1%) in the package headspace is the most favorable condition for the packaging to be most effective.

(2) Sealing conditions

To test the effectiveness of the sealing conditions, samples were sealed in pouches 9 x 17 cm. The pouches were sealed on a PAC Vac model 12V sealer using the following settings:

(a) Sealing selector switch low

(b) Dwell timer set at 4 on 0-10 scale (seal bar is down for a total of 7 seconds)

(c) Seal timer set at 3 on 0-10 scale (equal to 3 seconds with impulse heat on)

(d) Vacuum timer at 7 on 0-10 scale (3 sec.)

Only one flushing of the pouches with the special gas mixture was done on the sealer. To check the initial % O_2 in the sealed pouches, a gas chromatograph analysis of the pouch headspace was done. A standard for 1% and 2% O_2 was made by calculating the volume of the pouches filled with only N_2 by using the H_2O displacement method. The required volume of O_2 was injected into the pouch using a syringe and the injector site immediately sealed with 3M black electricians tape. The injector site was a crossmark of the tape to minimize leakage. The ratio of the O_2 to N_2 peak areas was calculated using the values printed by the Perkin Elmer computerized Electronic Integrator. The sealing condition of vacuum setting at 7 and gas setting at 3 yielded a residual % O_2 level of 1.15% to 1.20% in the test samples. Since a lower value is desired, a preflush technique was found to be needed.

The following conditions were used as reported in Table 3:

(a) M = one flushing with special gas mixture (92% N_2, 8% H_2) on sealer only

(b) F = pouches containing samples were placed in a desiccator, evacuated, flushed with special gas mixture, evacuated, flushed and final flushing done on the sealer

(c) H = pouches were flushed for approximately 20 seconds with the gas mixture by means of a piece of tubing connected to the gas tank and the tubing used as a "hose" to flush the interior of the pouches. A final gas flushing was done on the sealer.

The results are shown in Table 3. As can be seen, using method F gives the lowest residual oxygen levels in the pouches. These settings were used in the Hennican storage study for the low oxygen system.

d. Test methods

The methods for the food deterioration tests used are stated in the Final Report, Phase I, NAS 9-12560, March 27, 1972—March 17, 1973. The following tests were run:

(1) Peroxide value (AOCS Method Cd 8: 53)

(2) Lysine (Section IV)

(3) Non-enzymatic browning (modified Choi et al. (1949) by Labuza (Contract NAS 9-10658).

(4) Organoleptic - score sheet on Table 4 of this report (a panel of eight people was used)

(5) Ascorbic acid. The procedure in Section II, B. was used with the following modification in sample preparation:

TABLE 3

EFFECT OF GAS FLUSHING AND MACHINE SETTING ON RESIDUAL O_2

Method of Flushing *	Setting on Machine *	Residual % O_2
M	V 1, G 3	1.15
M	V 1, G 3	1.20
M	V 1, G 3	1.05
M	V 1½, G 3	0.60
M	V 1½, G 1½	0.70
F	V 1, G 3	0.03
F	V 1, G 3	0.05
H	V 1, G 3	0.57
H	V 1, G 3	0.55

* See text

TABLE 4

Sample # _____

Product _____

Date _____

	Appearance	Aroma	Flavor	Texture	Overall
Excellent					
Good					
Fair					
Poor					
Unacceptable					

Comments:

(a) Weigh 15 g Hennican into tared 8 oz. blender jar

(b) Bring up to 100 g with extracting solution #1

(c) Blend at high speed for 1 min using an Osterizer blender

(d) Pour approximately 50 ml of the blended sample into a 125 ml Erlenmeyer flask containing 1 g Celite

(e) Filter, using vacuum, through Whatman #1 filter paper, approximately 10 min

(f) Using a volumetric pipette take 10 ml of the filtrate and add 10 ml of extracting solution #2 and titrate with the standardized 2,6 Dichloroindophenol dye

(6) Texture measurement

A new method was devised for toughness determination as follows:

(a) Instron Universal Tester Model TM

(b) OT MS - Ottowa Texture Measuring System wire grid

(c) Compression rate 0.5 cm/min with a chart speed of 5 cm/min

(d) Duplicate samples of uniform size (1.7 x 1.7 x 0.6 cm)

(e) Sample compressed to 1 mm distance from grid

(f) Data collected is Kg force necessary for compression.

3. Results and Discussion

The results of each individual test will be discussed separately. Overall, however, as would be expected deterioration proceeded faster in air than in the low O_2 system and the rates of deterioration increased significantly with temperature. Moisture content checks of the samples at 25 and 35°C showed the packages to be adequate (Table 5). Therefore, no interference with a change in moisture occurred during storage.

a. Peroxide value: rancidity

As seen in Table 6, the data illustrate that the pouches with the oxygen-free environment have maintained a protective action against lipid oxidation. This protection was maintained even after 3 months storage at 35°C. Lipid oxidation is occurring in the systems stored in air (cans) but as indicated by the low peroxide values, the rate is very slow. Up to the times indicated, there is no apparent difference in peroxide values among the samples stored in air at the three different temperatures, implying that the anti-oxidants added are effective in this study. Figure 1 shows the data for peroxide value in Hennican stored in vacuum sealed foil pouches at 35°C from the previous contract Final Report, NAS 9-12560, Phase I. As seen, at about 50 days the PV increased rapidly and the product became rancid. This confirms the effectiveness of the antioxidants. However, as will be discussed later, rancidity was not the mode of deterioration at 35 and 45°C. Thus, samples are only being retained at 25°C to see if there is a change in deterioration mode. It should be noted, however, that if rancidity were a problem this new pouch

TABLE 5

MOISTURE CONTENT CHANGE OF HENNICAN DURING STORAGE

M_c = g H_2O/g solids

Storage (days)	25°C can	25°C pouch	35°C can	35°C pouch
Initial	0.38	0.38	0.38	0.38
29			0.38	0.39
31		0.38		
32				
33	0.38			
34				
50			0.35	0.35
54		0.38		
55				
56	0.38			

Method: vacuum, 18 hr, 70°C
samples done in duplicate in glass weighing dishes

Figure 1. Effect of Storage Time at 35°C on the Peroxide Value of Hennican Storage in Vacuum Pouches.

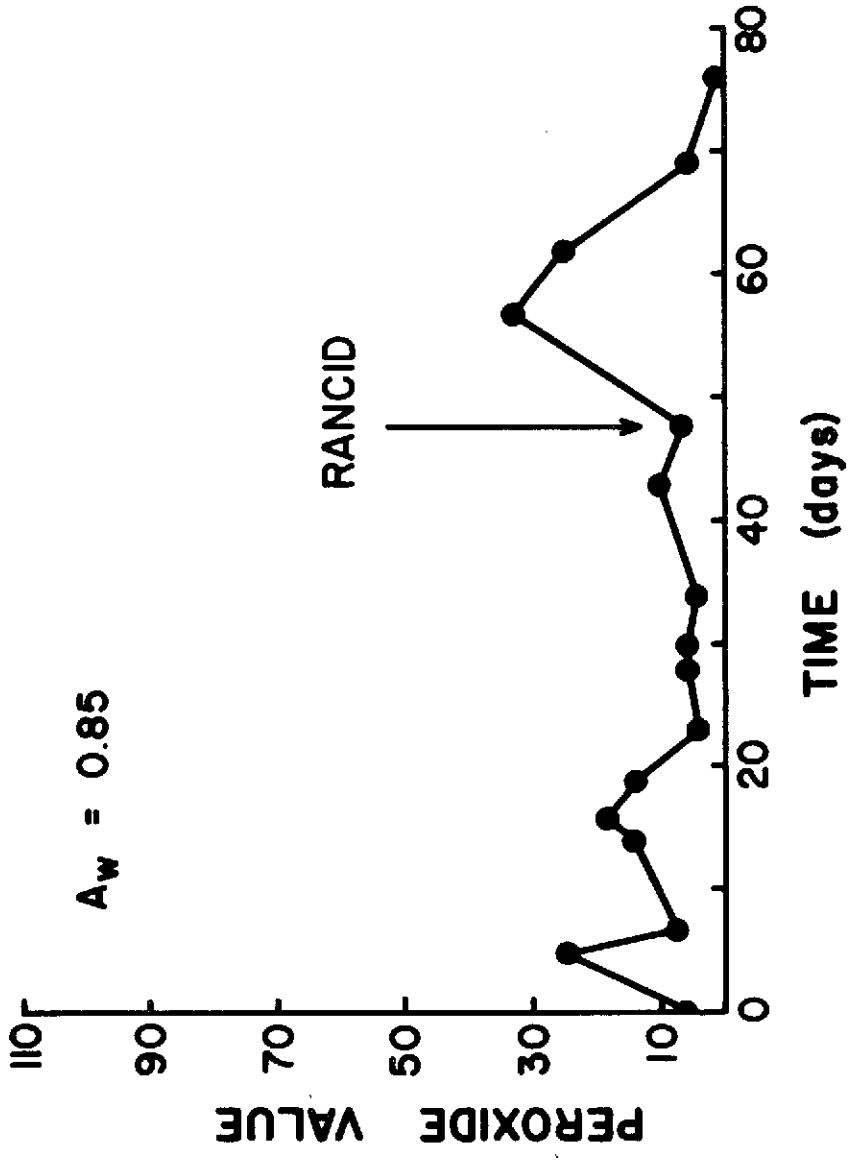


TABLE 6

HENNICAN SHELF-LIFE STUDY
PEROXIDE VALUE

meq/Kg fat

	25C		35C		45C	
DAY	AIR	N_2/H_2	AIR	N_2/H_2	AIR	N_2/H_2
0	N*	N	N	N	N	N
2	0.39	N				
5	0.88		2.08	N	3.12	
10	4.06	N			3.56	
15			3.80	N	3.72	
20	2.62	N	2.27	N	2.48	
25	4.34	N			5.05	
30	4.52	N	2.93	N	4.38	N
35	3.54	N				
40	3.09	N				
45	3.00	N	2.40	N		
50	4.03	N	3.28	N		
60	4.03	N				
65	4.24	N	4.09	N		
70			1.67	N		
75	3.03					
80	3.79		3.31			
85			3.93			
90	3.23					

* no detectable peroxides

system would be an adequate way to prevent the reaction.

b. Ascorbic acid

As illustrated in Figure 2, ascorbic acid degraded rapidly during storage of Hennican. It can also be seen that ascorbic acid is less stable in air than in the N_2/H_2 pouch. Also the rate of destruction of ascorbic acid in both air and the N_2/H_2 systems was accelerated by an increase in temperature.

Table 7 compares the half-lives and the activation energies of ascorbic acid loss under the different storage conditions studied. At 25°C, the rate is approximately seven times less in the N_2/H_2 system as compared to the air system. At both 35 and 45°C, there is about a three-fold decrease in the rate of destruction of ascorbic acid in the N_2/H_2 systems as compared to air. Even though the special pouch (Maraflex 7F Scavenger Web) is more protective against ascorbic acid degradation than the air system, its effectiveness is reduced with this model food at the higher temperatures. When the data was plotted on an Arrhenius plot as seen in Figure 3, the activation energies calculated were not the same indicating that some mechanism change of ascorbate destruction is occurring in the N_2/H_2 system. This may indicate that the residual oxygen which is being used up in the reaction may have become limited or is diffusion limited. The activation energy for ascorbic acid loss of about 18-20 Kcal is comparable to the values obtained in the model system study (Section II, A.), however, the half-life was longer. The half-life was also almost twice that found for the Hennican prepared for Section II, B. at the same a_w. In that study a half-life of 36 days was

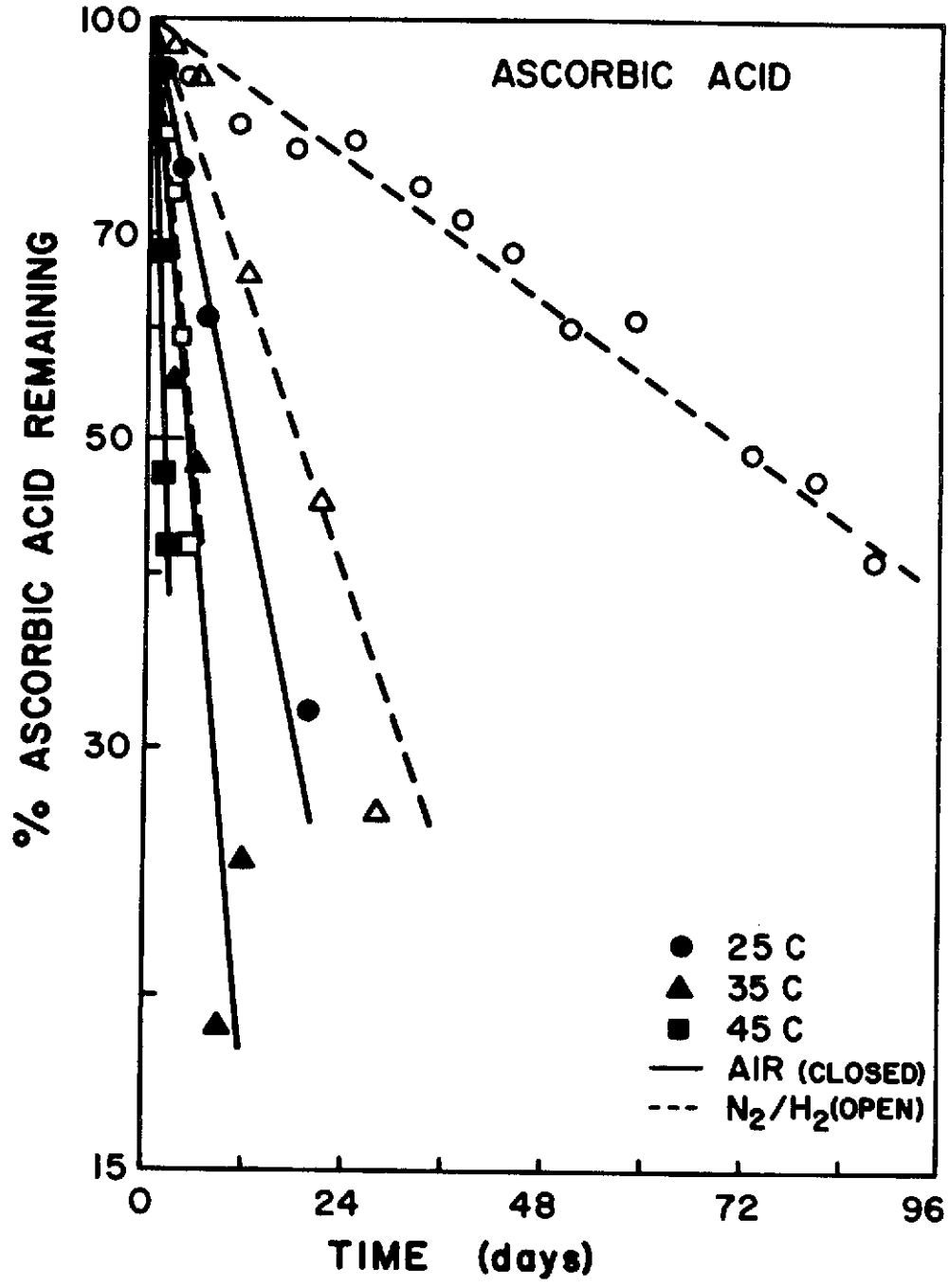


Figure 2. Destruction of Ascorbic Acid in Hennican as a Function of Temperature Stored in Two Atmospheres.

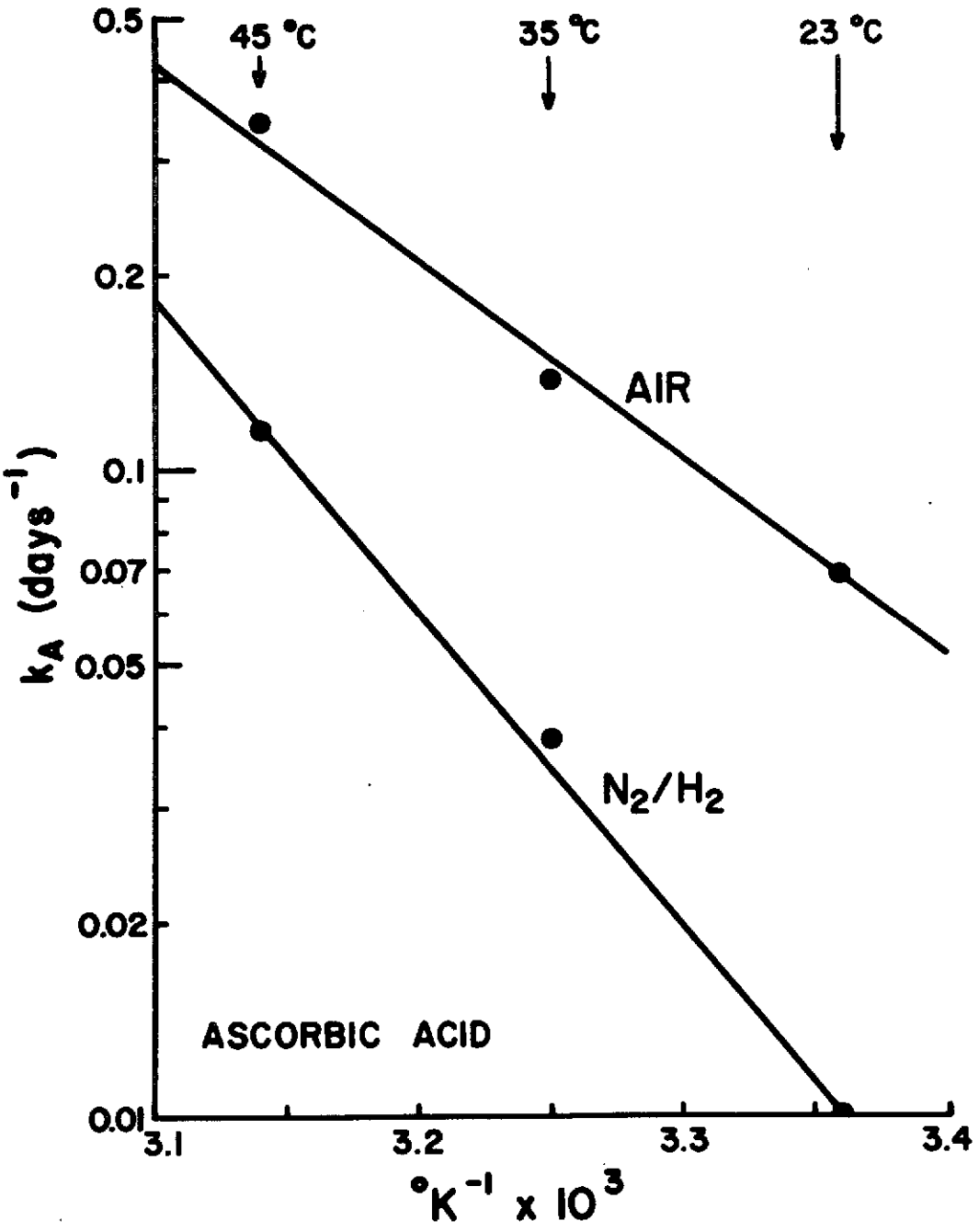


Figure 3. Arrhenius Plot for Ascorbic Acid Destruction in Hennican Stored in Air and in the N_2/H_2 Atmosphere.

TABLE 7

ASCORBIC ACID RESULTS

HENNICAN SHELF LIFE STUDY

Temp (°C)	System	Days $\theta^{1}/_{2}$	Q_{10} (from E_a plot)
25	AIR	10	
			1.70
35	AIR	5	
			1.67
45	AIR	2	
25	N_2/H_2	70	
			3.74
35	N_2/H_2	18	
			3.44
45	N_2/H_2	6	

E_a = 9.93 Kcal/mole in air

E_a = 24.04 Kcal/mole in N_2/H_2

E_a = 18 $\pm$ 2 Kcal/mole (literature)

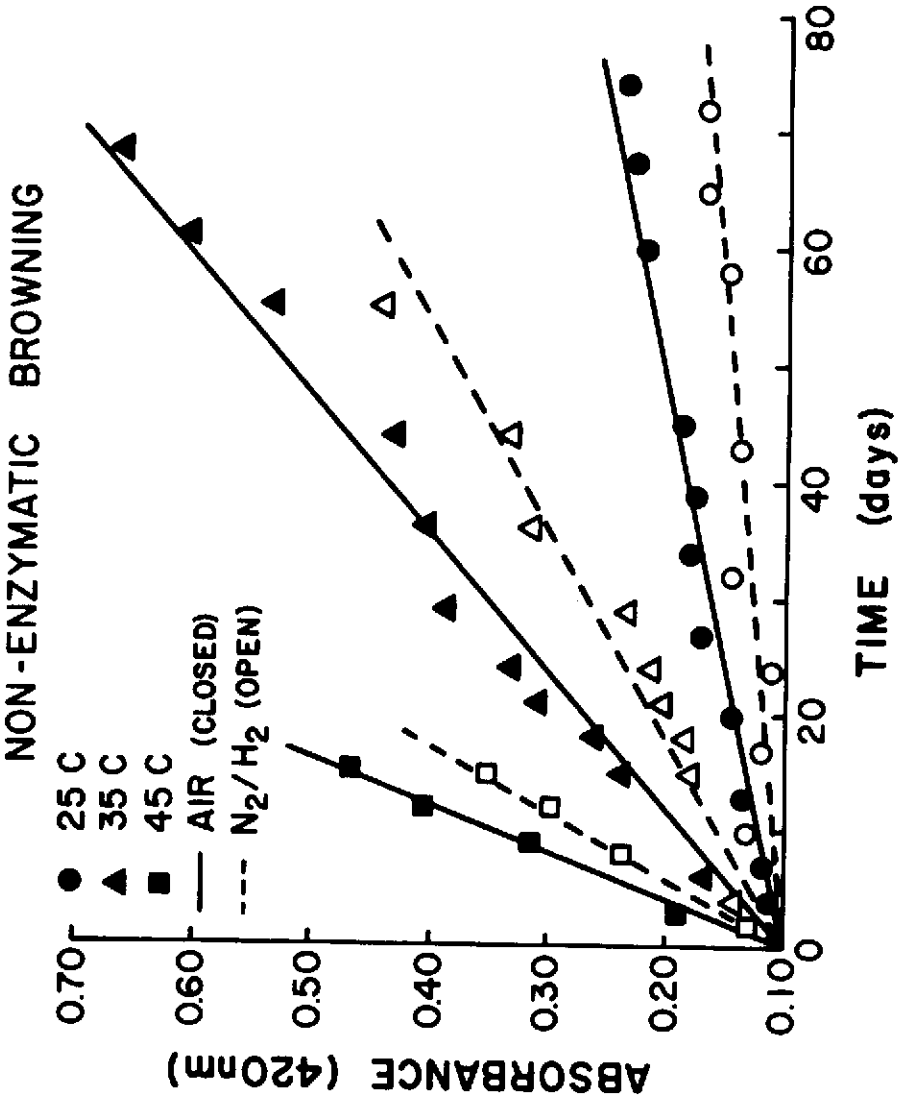
found at 25°C for the product sealed under vacuum in foil pouches. This indicates that at least with vitamin C destruction, the extra protection of a catalyst-type pouch was necessary. S aling the product in air alone is totally inadequate.

Overall, the data in air indicate that significant overruns of ascorbate would be necessary if the product is to supply a significant part of the RDA (60 mg). If 100 g of product has 200 mg of ascorbate, then at 25°C in one month the product is at 1/3 the RDA for an average man and in two months the product has less than 5% of the RDA. The pouch system at low O_2 would take about 7 months to reach 1/3 of the RDA. Thus, the cost of packaging could be balanced out by greater nutritional value. Otherwise, an overrun of six times more ascorbic acid would be needed to supply 1/3 RDA at the end of two months. The cost of packaging in this case could be less than the vitamin C costs on an industrial scale. The data also show that from a nutritional standpoint, ascorbic acid loss would be a major mode of deterioration if a nutritional claim were made for the product. These results further bear out the previous recommendations that ascorbic acid either not be added or be added in some type of protective coating. The data also show that using a high temperature shortens the required test period to under one week.

c. Non-enzymatic browning

In this storage study, as illustrated in Figure 4, non-enzymatic browning detected as pigment formation, increased with temperature but was slightly lower in the N_2/H_2 atmosphere. An increased rate and a greater amount of pigment formed in the air

Figure 4. Effect of Storage Temperature and Atmosphere on Non-Enzymatic Browning of Hennican (a_w 0.86).



system may be due to some lipid oxidation occurring in those systems even though peroxide values were low. Lipid oxidation supplies carbonyl groups which could interact with the amino groups of amino acids leading to the production of brown pigments. The increased rate of ascorbic acid degradation in the air system could also cause a larger amount of pigment formation since it can eventually lead to the formation of furfurals which will react with amino compounds to produce brown pigments.

The product is browning at a significant rate as was found in Hennican in the previous contract (NAS 9-12560, Phase I). In that study at a_w 0.83, 35°C, in a product to which antioxidants were added and which was held in a vacuum sealed pouch, after 50 days the A_{420} was 0.7 starting from a value of 0.2 showing an increase in browning of 0.5 units. In the present study, the ΔB is only 0.2 to 0.3 units, about half the deterioration. The lower initial value shows that a better product was made with a lower amount of either reducing sugars or browning precursors. The increase over the same time period for the 25°C is less than 0.1 browning units. As is well-known, the rate of deterioration is less if a better initial quality exists.

Table 8 shows the time for the product to reach unacceptability on a browning basis from both a chemical and a visual standpoint. An absorbancy of 0.35 at 420 nm was used as the chemical index of unacceptability since at this point the product in air at 35°C was rated visually unacceptable by the panel. The trend exists that the samples in air reach a chemical and visual cut-off before

TABLE 8

NON-ENZYMATIC BROWNING - RESULTS

Temp (°C)	System	Visual Shelf-Life, Days	Days to Reach B = 0.35	Q_{10} (from E_a plot)
25	AIR	NR	145*	
				3.13
35	AIR	30	30	
				2.91
45	AIR	7	10	
25	N_2/H_2	NR	245*	
				3.74
35	N_2/H_2	124	45	
				3.44
45	N_2/H_2	36	15	

NR = not reached
* = projected from data

E_a = 20.8 Kcal/mole in air

E_a = 24 Kcal/mole in N_2/H_2

E_a = 20-25 Kcal/mole (literature)

the samples in the N_2/H_2 systems, as would be expected. As seen from Table 8, browning causes the product to become rapidly unacceptable and it would thus become the major mode of chemical deterioration other than ascorbic acid loss. The use of an accelerated temperature is very useful in this case since browning is so slow. This should be verified with the visual test samples. In the O_2-free environment, the accelerating factor at 45°C is about 16 times as compared to room temperature. This means the study could be carried out in less than two weeks. The activation energy also is comparable to that found from the literature and is only slightly changed in the oxygen-free environment.

A further index of browning deterioration is the toughening of a product during storage. Figure 5 illustrates the increase in toughness of the product with an increase in storage time and temperature. Toughness or Kg of force was measured by compressing pieces of uniform size on an Instron Universal tester using an 8 wire grid. There appears to be little difference in toughness detected by this method for the product stored in air vs. the product stored in the N_2/H_2 atmosphere at a given temperature, although a difference occurred in the extent of browning. Therefore, toughening may be a different mode of deterioration.

Table 9 lists the days for Hennican to reach an Instron value of 8.3 Kg. An Instron value of 8.3 Kg was chosen as the mechanical index of unacceptability. This was the mechanical toughness measurement at the time the panel rated the 35°C product unacceptable on a toughness basis as recorded in Table 9. The

Figure 5. Increase in Toughness in Hennican Stored at Three Temperatures and in Two Atmospheres.

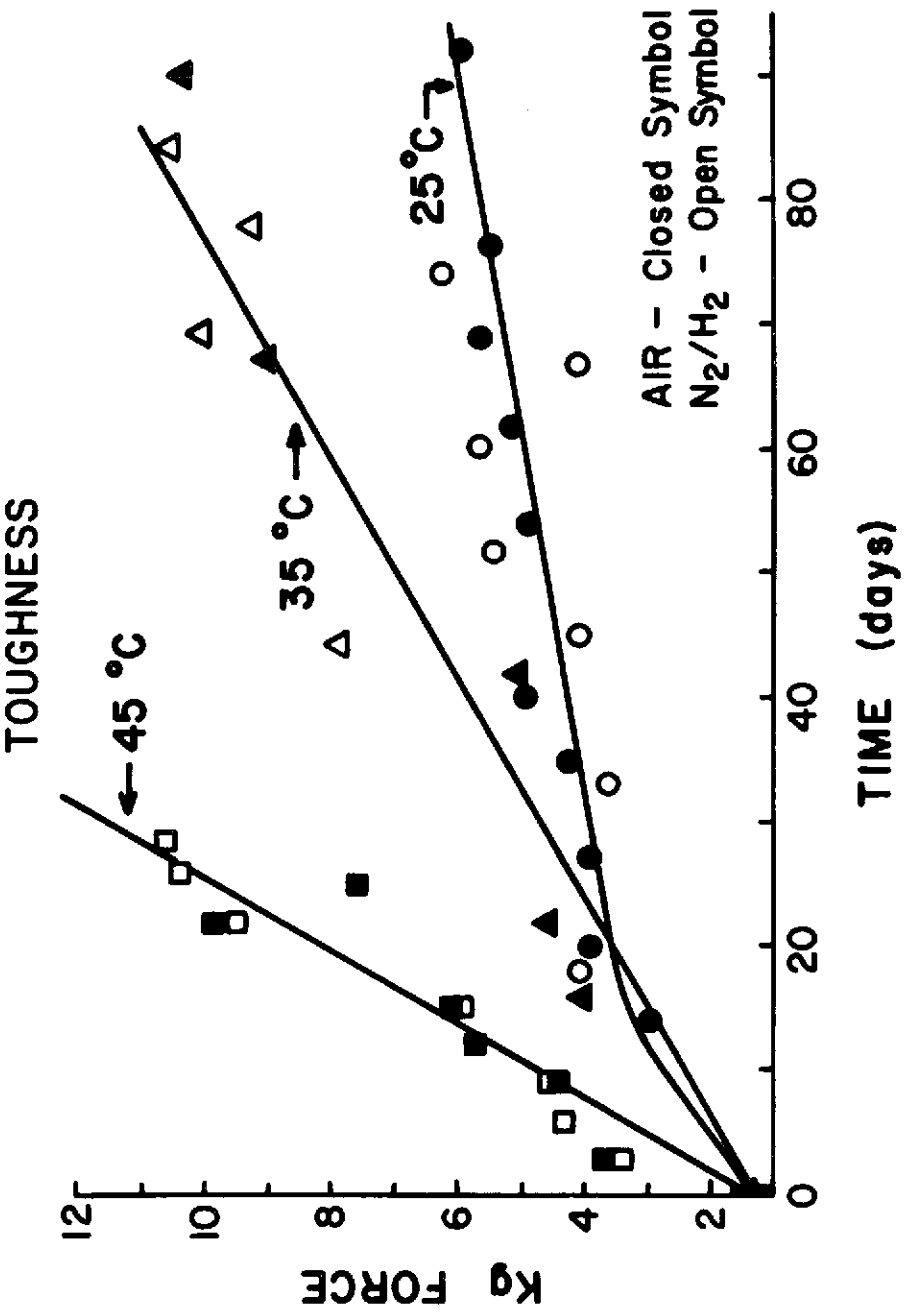


TABLE 9

HENNICAN STORAGE STUDY

TOUGHNESS RESULTS

Temp (°C)	System	Days to Reach Unacceptability		Q_{10}
		Organoleptic	Instron	
25	AIR	NR	162	
				3.22
35	AIR	63	63	
				3.00
45	AIR	17	21	
25	N_2/H_2	NR	162	
				3.22
35	N_2/H_2	84	63	
				3.00
45	N_2/H_2	36	21	

NR = not reached
E_a = 21.3 Kcal/mole

activation energies of toughening and non-enzymatic browning are similar (toughening having an E_a of 21.3 Kcal/mole and non-enzymatic browning having an E_a of 20.8--24 Kcal/mole) which could indicate that toughening is a result of non-enzymatic browning. However, when comparing the organoleptic evaluation to Table 8 it is obvious that there is not a direct correlation with visible browning. Except for the 35°C data in the low oxygen atmosphere, the visual browning occurs faster. Using the chemical indices would also indicate that browning occurs more rapidly than does toughening, thus it is the major mode of deterioration.

Table 10 contains the data for the change in available lysine content in Hennican at 25 and 35°C. No samples were available at 45°C. Lysine would be considered the most sensitive amino acid with respect to browning because of the ε-amino nitrogen group.

At 25°C, after an initial decrease of about 25% in 6-10 days, the values do not seem to be decreasing any more. At 35°C, however, in around 10 days the decrease is about 50%. After that the lysine is going down only slowly. The Q_{10} based on available lysine is only 2 as compared to the 4 or 8 for browning based on this preliminary data. This suggests that browning alone cannot be used for determination of nutritional losses of proteins since it involves a much more complex mechanism. What value the lysine loss has with respect to nutritional value from a biological standpoint remains a question.

4. Summary and Conclusion

The results of the major tests as summarized in Table 11,

TABLE 10

AVAILABLE LYSINE CONTENT OF HENNICAN IN STORAGE

mg Lysine/16 mg Protein Nitrogen

	25°C		35°C	
Day	can	pouch	can	pouch
0	4.88	4.88	4.88	4.88
4	3.75			
6			3.00	4.50
7	3.91			
10		3.25		
13	3.50			
15			2.25	3.25
17		3.63		
18			2.25	2.63
20	4.00			
21			2.50	2.00
24		3.38	2.25	2.38
27	3.50			
29			2.00	1.88
32		3.22		
34	2.88			
36			1.25	2.13
39	2.63			
43		3.25		
44			1.72	2.13
52	2.50			
55			1.58	1.75
58		3.38		
60	3.06			

TABLE 11

OVERALL EVALUATION: HENNICAN SHELF-LIFE STUDY

DAYS TO REACH UNACCEPTABILITY

Temp (°C)	System	Subjective Tests			Objective Tests		Vitamin C Half-life
		Visual Browning	Toughening	Overall	Non-Enzymatic Browning B = 0.35	Instron 8.2 Kg	
25	AIR	NR	NR	NR	145	162	10
35	AIR	30	63	30	30	63	5
45	AIR	7	17	9	10	21	2
25	N_2/H_2	NR	NR	NR	245	162	70
35	N_2/H_2	124	84	82	45	63	18
45	N_2/H_2	36	36	30	15	21	6

NR = not reached

indicate that browning is the major mode of deterioration in Hennican with respect to organoleptic qualities, since the results bear closely with the overall organoleptic shelf-life. Use of an accelerated temperature did not seem to change the mode of deterioration and decreased the time necessary to evaluate the rate by over 15 times. This verifies that high temperature can be used to accelerate shelf-life testing. In the present product, lipid oxidation was much slower and did not contribute to deterioration. From a nutritional standpoint, vitamin C degraded rapidly as was found in previous studies. From the standpoint of protection, these studies also suggest that intermediate moisture foods be packaged in a low oxygen environment as it significantly increases shelf-life. With respect to visual browning, the use of the American Can Co., Maraflex 7F Oxygen Scavenger Web increased the shelf-life by a factor of over 4 to 5 times. This would suggest that the higher cost could be borne out by the longer shelf-life achieved. The factor is only about 3 times on the overall organoleptic basis, but that is still significant. The overall data, however, do not indicate as good a correlation between the chemical and organoleptic data. Until the subjective data is complete at 25°C so that a verification of the Arrhenius plot can be made, it may still be premature to suggest 45°C as an acceleration temperature. The general recommendations for processors based on this study are:

(1) The use of high temperature to accelerate deteriorative reactions to shorten shelf-life testing

(2) The use of high quality ingredients and antioxidants retards rancidity

(3) Vitamin C stability is very poor in intermediate moisture foods

(4) A low oxygen environment significantly increases product shelf-life and would be worth the expense to create if shelf-life is needed.

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