Storage Stability and Improvement of Intermediate Moisture Foods

CONTRACT NAS 9-12560

Final Report
March 27, 1972 to March 17, 1973

National Aeronautics and Space Administration
Food and Nutrition Office
Houston, Texas 77058

Principal Investigator
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Summary

Studies were made of the rates of chemical reactions which deteriorate foods prepared to an intermediate moisture content and water activity (A_w 0.6 to 0.9). The phenomenon of sorption hysteresis was used to prepare model systems and foods to similar A_w's but different moisture levels so that the separate effects of water binding and water content could be elucidated.

It was found that water content is the controlling factor for lipid oxidation in model systems comprised of a solid support and an oxidizable lipid. Water exerts its influence in several ways: (1) Water dilutes the aqueous phase viscosity making diffusion of metal catalysts easier to the reaction site; this increases the rate of oxidation. (2) Water swells the solid support exposing new catalyst sites thereby increasing the oxidation rate. (3) Water causes a concentration dilution of trace metals present in the aqueous phase. At low metal content this has no effect and thus water exerts an accelerating effect by (1) and (2). At high metal content this dilution decreases oxidation rates. Based on these studies it was proposed that metal chelating agents like EDTA should give good protection to oxidation. EDTA exhibited the highest efficacy, about 10-15 times better than BHA which is a radical scavenger when studied in the model systems.

In studies in model systems for browning and decrease in available lysine, no correlation could be found for NEB, lysine and protein solubility. The protein solubility and the TNBS method for available

lysine were found to be unsatisfactory.

The stability of vitamin C in model systems was found to be directly related to the A_w of the system. At intermediate moisture content and 35°C the half life is less than 1 week showing vitamin C to be very unstable. The hysteresis phenomenon illustrated the strong influence of trace metals on the stability of vitamin C. Thiamine destruction also increased with increasing A_w.

Several methods were developed for producing intermediate moisture foods for use on the space shuttle. A modified soak-infusion technique produced an acceptable chicken cube if consumed in a sweet/sour type sauce. In a storage test below A_w 0.83 the product was acceptable for over 3 months, however, the sweet taste of glycerol was objectionable. Oxidative rancidity followed the same pattern as in the model systems and no free fatty acids were formed. The product was challenged with four microorganisms. It was found that each system has a different limiting A_w for growth so that general tables of limiting A_w may not be very meaningful. For the yeast and Pseudomonas species tested, a higher limiting A_w occurred for the adsorption prepared systems. This means that total water content is also important, the water binding factor (A_w) is not the only factor limiting growth. Similar results were found in model systems studies. Of great importance, it was also found that although death rate increases as A_w decreases a crossover point occurs at which A_w the organisms neither grow nor die. This could be due to the complete shut down of the metabolic processes.

A cold mixing technique was used to produce a highly acceptable IMF product called "Hennican." This was based on a mixture of dehydrated

cooked chicken, peanuts, peanut butter and raisins. With respect to rancidity, the rate of peroxide formation and oxygen consumption decreased as water content increased. This indicated that the product was similar to the model systems with high trace metal content. Nonenzymatic browning increased during storage and led to a toughening of the product both determine instrumentally and organoleptically. The product was acceptable for 2 months at 35°C indicating more than 6 months at room temperature. An additional product based on marshmallows and granola was made but was not tested for stability.

This study indicates the extreme usefulness of model system experiments for predicting stability in actual foods. Based on these studies, a very highly acceptable intermediate moisture food could be made to qualify for the space shuttle. Further testing is required, however, to improve the shelf life to more than six months.

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I. Introduction

In the past two years several basic physical chemical phenomena have been investigated under NASA contracts in the area of intermediate moisture foods. Basically, work was done in model systems and some liquid IMF (Intermediate Moisture Food) systems to determine the principal factors governing the rates of deterioration of these foods. Chemically, lipid oxidation and non-enzymatic browning were studied and, biologically, microbial growth was determined as a function of water activity and moisture content.

Two basic conclusions were drawn from these studies. In the first place, if microbiological growth was prevented chemical deterioration proceeded at a very rapid rate if not controlled by some means. Lipid oxidation caused rancidity to occur within two weeks if no antioxidants were added and if not protected against oxygen. Non-enzymatic browning, although slower than lipid oxidation, occurred with a corresponding decrease in the acceptability of the food item. These reactions should also decrease the nutritional value of the product. Of even more importance, it was found that the method of preparation of the food had a profound effect on the chemical and biological stability of the product. The phenomenon of sorption hysteresis was utilized in the method of preparation. In this method food items were produced at the same water activity but with different moisture contents. This was accomplished

by going down the desorption isotherm for the food from the wet state or by going up the adsorption isotherm from the dry state. It was found that lipid oxidation was slower if the food was on the adsorption branch of the isotherm and microbial growth was greatly inhibited as compared to the desorption prepared foods. Although this is a much more expensive way to prepare the food, the stability benefits could outweigh the costs. Results of non-enzymatic browning were not conclusive in terms of differences in rates.

The overall objective of this study is twofold.

(1) To continue work in IMF-model systems to further elucidate the mechanisms of the deteriorative reactions with respect to A_w (water activity) and moisture content and especially with respect to rancidity and vitamin stability.

(2) To utilize the results in model systems to prepare a shelf stable chicken IMF system and test the acceptability of this food.

In order to accomplish the objectives this study was divided into several areas of research important to both production and stability of IMF products. Areas of investigation included:

1. Production characteristics
    a. Infusion techniques
    b. Extrusion processing
    c. Cold mixing
2. Stability characteristics
    a. Model systems
        (1) Lipid oxidation
            (a) Effect of A_w - water content

(b) Antioxidant effectiveness

(2) Non-enzymatic browning

(a) Color

(b) Texture

(c) Protein solubility

(d) Nutritional loss of lysine

(3) Vitamin stability

(a) Thiamine

(b) Ascorbic acid

(4) Microbiological

b. Food systems

(1) Soak-infusion foods

(a) Rancidity

(b) Microbiological safety

(2) Cold mixed products

(a) Rancidity

(b) Flavor acceptance

(c) Nutrient stability

II. Literature Survey

A. General Characteristics of IMF

The introduction of intermediate moisture food (IMF) processing in the pet foods area has caused a great surge of interest in this type of food for human foods. IMF pet foods have captured over 25% of the multimillion dollar pet food market and the growth potential is expanding. Because of the growth in this area much interest has been stirred up among food companies to expand IMF technology into the human food market.

Several human food items made by past technology fit into the IMF category. These include various candies, like marshmallows, bakery goods, like fig newtons and various sausage products. These are listed in Table 1. In most of these products salt or sugar is added. These molecules bind the water in the food and thus give the product the desired stability against microorganisms without the need for refrigeration. This stability is due to the lowering of the water activity (A_w) of the product. In addition, since small molecules like salt or sugar hold more water per weight than larger molecules like starch, the product has a soft, moist texture. Using these principles, several large food companies have developed breakfast toaster tarts of IMF technology. These products rely on sugar and the acidity of the fruit for shelf stability without the need for refrigeration. As with the pet foods, these products are eaten directly. Rehydration with water is not needed and, thus, they are very convenient to use. Table 2 lists the general characteristics of intermediate moisture foods.

The pet food products that have been developed are basically a meat-sugar-cereal food which is extrusion processed at a reduced moisture content. Table 3 shows a typical composition for a dog food product. As seen, usually a mold inhibitor like sorbate is added since the water activity (A_w) is not reduced to below the limits for molds. Table 4 lists some of the IMF pet food products available in Europe and in this country.

Very little is known about the shelf stability of IMF pet foods except with reference to microorganisms. Based on the work of Labuza (1971a) as seen in Figure 1, these foods would be expected to deteriorate

TABLE 1

IMF Foods

Dried Fruits Soft Candies Marshmallows Jams & Jellies Honey & Syrups	Sugared
Fruit Cake	Baked
Pepperoni Dry Salami Beef Jerky	Salted & Dried
Country Ham Pemmican	Dried, Salted & Sugared
Cheese	Salt, Low Water

TABLE 2

Intermediate Moisture Food Characteristics

Moisture content - 10-40% (controllable)

A_w - 0.65 to 0.84

Directly edible - soft moist texture
- no rehydration
- no refrigeration

Completely manufactured - composition can be completely controlled

Texture - hard brick to very soft pliable

Nutrient levels - can be adjusted for specific requirements

TABLE 3

Typical IMF Dog Food Composition

Meat and Meat Byproducts	32 %
Cereal or Soy Flakes	33 %
Sugar	23 %
Nonfat Dry Milk	2 %
Calcium and Phosphorus	3 %
Propylene Glycol	2 %
Sorbitol	2 %
Fat and Emulsifiers	2 %
Salt	0.6%
K-sorbate	0.3%
Vitamins, Minerals, Colors	0.1%
	100.0%

TABLE 4

Semi-Moist Pet Foods - International and

Canada: Quaker Oats and Others

(*Denotes Semi-Dry Products)

CANADA

Product	Pet	Company (Mfg.)
Burger	Dog	Quaker Oats
Cheeseburger	Dog	Quaker Oats
"Flavour Morsels" (Liver, Tuna, Chicken & Beef)	Cat	Quaker Oats
Dr. Ballards (Liver, Tuna, Chicken & Beef)	Cat	Standard Brands, Ltd.
Top Breed (Chicken and Beef)	Cat	Martin Feed Mills, Ltd.
"Tender Vittles" (Liver, Tuna, Beef & Gourmet)	Cat	Purina
"Top Choice"	Dog	Gaines

INTERNATIONAL

Minced Morsels	Dog	Quaker Oats, Southall
Flora Tartaar (Dutch)	Dog	Quaker Oats, Nakskov
Doggy American (Belgium)	Dog	Quaker Oats, Nakskov
Fido Steaky (France)	Dog	Quaker Oats, Nakskov
Kennel Hund (Denmark)	Dog	Quaker Oats, Nakskov
Hunky Chunks*	Dog	Quaker Oats, Southall
Flora Maise Krachtbrokken*	Dog	Quaker Oats, Netherlands
Frolic*	Dog	Quaker Oats, Belgium

UNITED STATES

Ken-L Ration Burger	Dog	Quaker Oats
"Top Choice"	Dog	General Foods
Gaines Burger	Dog	General Foods
"Tender Vittles"	Cat	Purina
Tabby	Cat	Lipton
Special Cuts	Dog	Quaker Oats

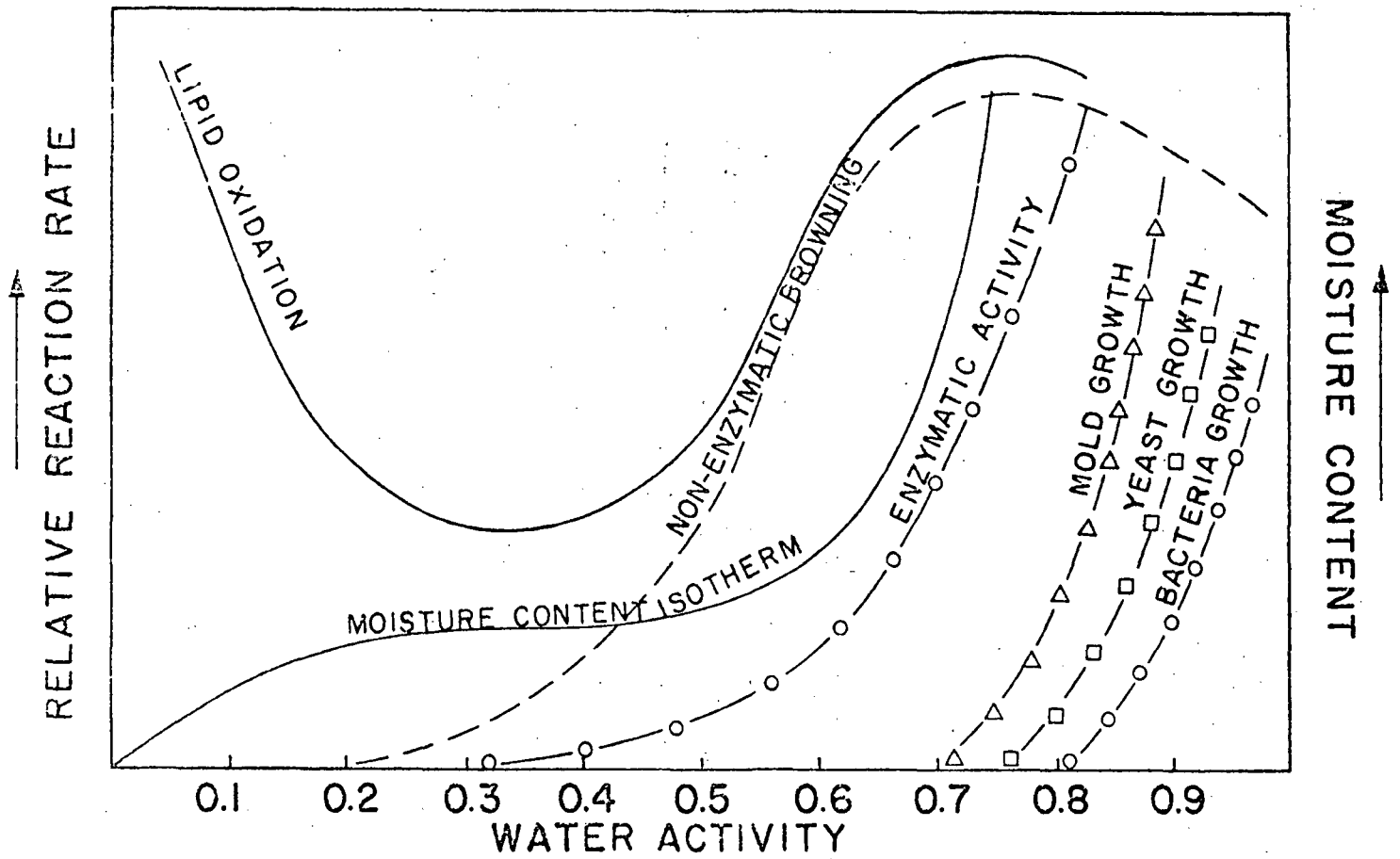


FIGURE 1. FOOD STABILITY AS A FUNCTION OF A_w

rapidly due to hydrolytic reactions, non-enzymatic browning, and especially due to lipid oxidations. These reactions lead to off-odors and off-flavors, color changes, toughening and decrease in nutritional value. Fortunately, since the pet food products are viewed as a commodity item, their turnover is very rapid and, thus, these deteriorative reactions do not progress very far. In addition, with respect to rancidity, little is known if the off-odors and off-flavors that are developed are objectionable to the animals. With respect to foods for humans, however, a much longer shelf life would be required since the product would be used most likely as a meal substitute. Several food companies that are now working on IMF foods and on IMF-complete meal substitutes are listed in Table 5. Some of these already have products out in test markets in spite of the fact that the shelf life of the products is not really known (Table 6). Table 7 lists some of the food products that can be adapted to IMF technology.

Some food companies are investigating intermediate moisture foods from the standpoint of special dietary foods. In view of the extrusion processing technology that the pet food companies use, it is possible to completely formulate a shelf stable food for any specific need such as for low sodium, low potassium and low protein. The combination of these latter ingredients are very important in chronic renal failure where, if not controlled in the diet, the patient usually dies. In addition, these people need a high caloric intake to prevent protein catabolism which the sugar (humectant) could provide. Even more interesting is that IMF foods have a low water content. Patients on renal dialysis usually have to be dialyzed two or three times a week at a substantial

TABLE 5

Companies Doing Research on IMF for Humans

Quaker Oats
Nabisco
Hunt-Wesson
General Foods
General Mills
Ralston Purina
Archer Daniels Midland
Internatinal Multifoods
American Potato
Swift & Co.
Hormel
Land O' Lakes
H. P. Hood and Sons
Peavy
Pillsbury
Mead-Johnson
Kraftco
Green Giant

TABLE 6

Human Type Recent IMF Foods on Market

National Biscuit Co.	Pastry Toastetts (10-12% H_2O) Fast Break Meal Bar (test market)
General Mills	Breakfast Squares (test market)
General Foods	Pastry - Toastems (15% H_2O)
Pillsbury	Space Sticks Candy (11% H_2O) Meal Bar (prototype)
Kelloggs	Toastem Pop Up Pastry (13% H_2O)
Kraft	Up and At Em (test market)

TABLE 7

Potential IMF Systems

Complete Meal Bar
Nutritious Candy Substitute for Children
Shelf Stable Salads
Shelf Stable Hash Browns
Shelf Stable French Toast or Pancakes
Campers and Hikers Trail Food
Breakfast Substitute
Special Dietary Food

cost which sometimes exceeds $20,000 per year. As shown by Nelson et al. (1972), if a diet is controlled in terms of water intake, dialysis would only have to be done every 7 to 10 days at a substantial cost savings to the patient. This would also allow dialysis units to treat more people. Thus, intermediate moisture foods of special dietary composition should be of extreme benefit for treatment in this disease and allow these people to have a food that they can eat away from home.

The space program has a very big interest in an IMF human food item. NASA has supported two previous contracts by the present investigator for initial development work (NAS 9-9426 and NAS 9-10658). An IMF item which could be highly nutritious and eaten without hydration would be extremely desirable for the space program. This is especially true for the space station and shuttle where much extra vehicular activity (EVA) could be taking place. A device to dispense the food in pellets or cubes through the face plate or within the helmet could be used, thus enabling the astronaut to continue working and be supplied with the nutrients he needs.

Table 8 lists the specific benefits of using an IMF system in the space program. These same benefits apply to military use. As with the pet foods, these products could be eaten directly without the need for rehydration. This is important since in the Gemini and Apollo programs a major complaint was the problem in rehydration of the freeze-dried products and the excessive time this took for many of the foods. The ease of handling afforded by IMF products eliminates this time consumption and problems with water rehydration.

TABLE 8

Contribution of IMF to NASA Program

1. High density - low volume
2. High caloric density
3. Can be made to specific nutrient needs of each astronaut
4. Low water content - minimizes urine volume
5. No refrigeration - shelf stable
6. Completely balanced emergency ration
7. Can be adapted to any configuration
8. Can use KCl to up K load in diet without adverse effects
9. Can be used in space helmet
10. No crumbs in weightlessness
11. Can be used under stress or diversion
12. Package damage not critical

Several factors make the IMF product very suitable for space flight. It is a shelf stable product, thus energy consumption for refrigeration is zero. It can be molded into any configuration thus making it suitable for in-suit consumption as was done in the moon walk in the Apollo 15 mission. The soft, moist texture eliminates any of the crumbing problems that could cause instrumentation problems. Lastly, it is a concentrated food energy source because of the lowered moisture content, and thus the thrust requirement is reduced especially in the shuttle where no meals are planned except for emergencies.

With respect to nutritional benefits, because the product is completely formulated it can be matched to the individual astronaut's needs. Potassium, which is a problem, can be added and it would help in turn to lower the water activity of the product. In fact, many of the same concerns for renal dialysis patient diets are similar to those for the astronaut diet as seen in Table 9.

Although development and manufacture of an IMF food for astronauts might be expensive, food systems for space are a small fraction of the cost of the total space program. The manufacturing cost, however, may be significantly high enough to preclude it from the commercial market. This present study is designed to look at several products from the stability standpoint to see if the shelf life obtained justifies the cost. The real question remains, however, as to why there have been no commercially produced meat type items for humans in the marketplace.

The most significant problem is the taste of the product. Present procedures rely on salt, glycerol and sugar to reduce the A_w of the

TABLE 9

Biomedical Problems

	Acute Renal Patient	Astronaut
Potassium	must be kept to minimum < 3 g/day	must maximize < 5 g/day
Sodium	minimize < 2 g day	adequate to prevent K loss and diuretic effect 3 to 6 g/day
Protein	keep to minimum to avoid renal damage 20-60 g/day	adequate for catabolism and regrowth 90-120 g/day
Water	minimize 300-1500 cc/day	minimize under adverse conditions otherwise normal
Calories	high to prevent catabolism	high because of stress

product. The A_w lowering occurs because of Raoult's law which describes the water binding of solutes. Unfortunately, to obtain the low A_w needed (less than 0.85 to prevent food poisoning from Staphylococcus organisms) high levels of salt and/or sugar are necessary. High levels are needed in spite of the fact that these compounds behave non-ideally, lowering A_w more than expected. The need in this area is to find a humectant (A_w lowering chemical) that:

(1) is low in molecular weight

(2) if high in molecular weight then is very non-ideal, i.e. lowers A_w more than expected

(3) has no flavor or odor

(4) is non-toxic to humans and possibly toxic to microbes

(5) is preferably liquid and completely miscible with water

(6) does not react in the food to cause deterioration

(7) has a large A_w stabilizing effect

Of course, this is not the scope of the present study and certainly the development of such a chemical would require years of animal tests. However, it is possible that this chemical can be found and used in the future for IMF. Table 10 shows why sucrose and glycerol have been used. They have a very stabilizing effect with respect to A_w, and thus large changes in moisture content do not cause a very large change in A_w.

Hollis et al. (1969) of General Foods reported several procedures developed on U. S. Army Natick contracts using glycerol as the humectant for meat and vegetable type food items. Similarly, Pavey (personal communication) of Swift & Co. has been working on several meat items

TABLE 10

A_w Stabilizing Effect

Increase in H_2O from A_w 0.85 ⟶ 0.90

100 g solids	water increases
food	3-8 g
sucrose	22 g
glycerol	71 g

on Natick contracts. In these studies they have used a cook-soak procedure to get the humectant into the food to lower A_w and give stability. Variable times and temperatures have been used without much consideration for the physical-chemical principles involved, however, some of the products were shelf tested for flavor acceptability at water activities expected for commercial development. As seen in Table 11, the products started out at a medium score (about 6) on a ten point hedonic scale and were not very good after even 3 months. This was most likely due to the use of glycerol as the humectant even though its flavor was partially masked by a sour or mustard flavor addition. Much more work is needed in this area besides work on stability of the product.

B. Shelf Life Stability

1. General Problem

One major problem of IMF items is their shelf stability. Stability is a problem even with the present dog foods on the market, however, the turnover rate is so rapid that usually they are not rejected at use time as mentioned previously. Shelf life is felt to be 3-4 months for the dog foods. Deterioration occurs by lipid oxidation and non-enzymatic browning both of which cause a toughening due to protein aggregation and, in addition, loss of biological value takes place. Dogs, however, are accustomed to eating dry foods directly, so the toughening is not a problem. Similarly, they cannot tell us if they have a nutritional deficiency until it becomes very manifest. IMF foods for humans presents an entirely different problem. These foods will be bought to replace a meal at a future time when preparation time is not available. It will most likely sit on the shelf at home or in

TABLE 11

General Foods/Natick Taste Panel*

	Initial	3 mo.	6 mo.	A_w
Pork w/BBQ	6.28	5.55	5.17	0.86
Pork w/sour sauce	6.38	4.93	5.14	0.84
Pork w/mustard	6.21	5.86	5.48	0.85
Ham w/mustard	5.86	5.52	5.48	0.81

*Johnson et al., 1972

the market for months. The product will need at least 6 months shelf life and possibly 1 year. Thus, deterioration, if occurring, will be much more evident if the product is not used immediately. Rancidity will occur and cause rejection of the food item and the product will get tough. These problems could also occur in the environment of the space craft where long-term storage is a necessity.

Even more serious is the fact that since an IMF food for humans is a convenience item which will supposedly be a meal replacement, the FDA will require nutritional labeling. Moreover, the label will have to be correct for the actual nutrient content over the entire shelf life of the product. The assurance of nutritional value is also a requirement for the NASA foods since the astronauts will have no choice as to the foods available. Thus, deterioration must be prevented or controlled and a knowledge of the nutrient losses must be obtained.

2. Chemical Deterioration

The rates of chemical deteriorative reactions in various model systems of intermediate moisture foods were investigated under NASA Contract NAS 9-10658 (Labuza, 1971). Among other findings this investigation elucidated the stability aspects of intermediate moisture systems that reach their final water activity by each of two specific processing techniques, namely: water desorption or dehydration as compared to water adsorption. The storage stability differences noted between systems prepared by these two methods offer great potential for development of stable and improved intermediate moisture food systems (IMF) especially for NASA items where as noted the cost factor in processing is not a problem.

In the major commercial process technique used in the manufacture of IMF pet foods the principal components "meat and meat by-products" are mixed with various binding agents and humectants and cook extruded to form the product. The meat components, therefore, undergo a desorption (i.e. removal of water and replacement by humectant) process which brings the system to an equilibrium somewhat lower on the sorption isotherm. This same process occurs in the cook-soak technique of intermediate moisture food preparation designed by Hollis et al. (Contract DAAG 17-67-C-0098, U. S. Army Natick Laboratories, 1969) in which the food was infused with a solution of low water activity in exchange for tissue water.

In comparison, the other basic process for preparation of IMF uses the technique of mixing a dry blend with water or humidifying so that the food approaches final equilibrium by ascending the adsorption isotherm. No current commercial processes utilize this method. It is more costly due to the fact that the ingredients have to be dehydrated first. Because of the phenomenon of sorption hysteresis, the adsorption technique achieves a lower water content at the identical water activity as a similar composition food prepared by the desorption technique. Figure 2 shows a typical food isotherm with the hysteresis loop.

In NASA Contract NAS 9-10658, the stability of various types of model food systems was tested under conditions which would be expected to maximize deterioration. With respect to lipid oxidation, those foods which are high in unsaturated fatty acids such as chicken or pork became rancid very rapidly when made into an IMF system. This occurred even though they were at a high water activity. In fact, both the chicken

FIGURE 2

TYPICAL HYSTERESIS LOOP AND ISOTHERM

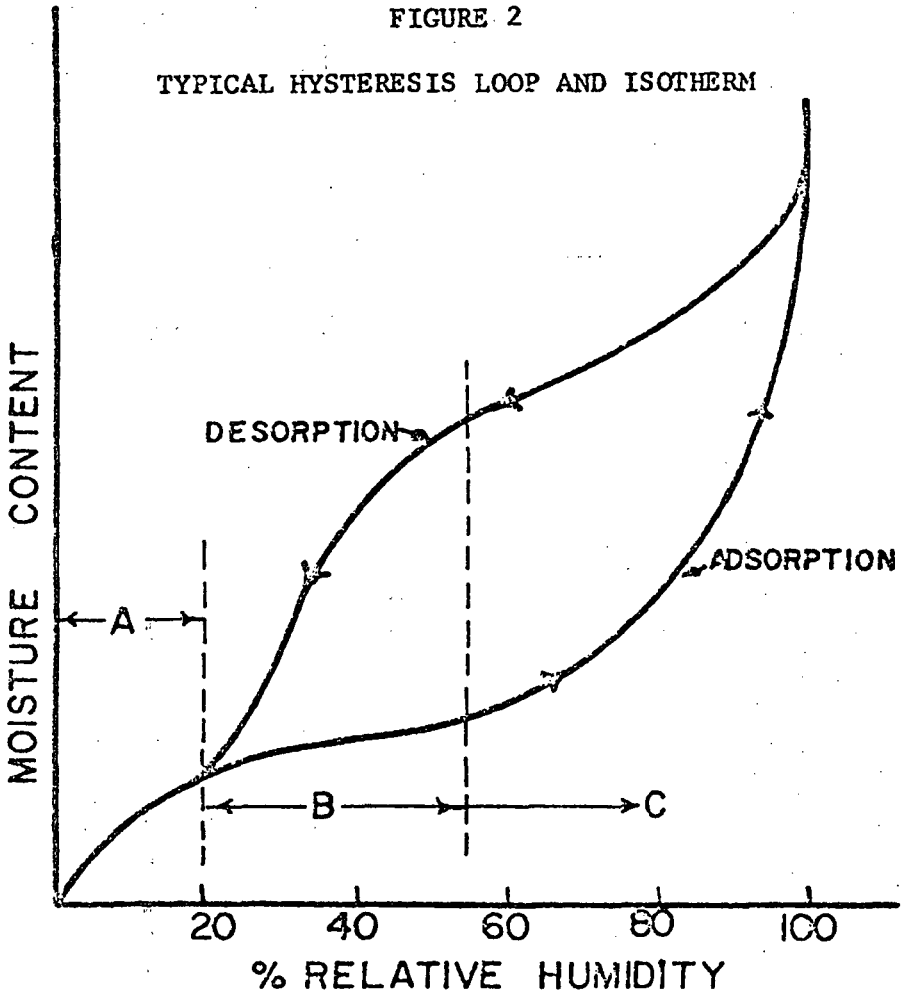


FIGURE 2. TYPICAL HYSTERESIS LOOP AND ISOTHERM

and pork prepared either by desorption through addition of a humectant or by the cook-soak process were found to have less than one month stability. Addition of antioxidants gave very little added storage life. However, when these same foods were prepared by dehydration followed by humidification to the same water activities, they had up to 2 months stability with addition of antioxidants. This suggests that adsorption prepared foods can have significantly increased storage life. This was further reported by Labuza et al. (1972a).

The mechanism for the slower oxidation rate for the adsorption system has not been thoroughly elucidated. At low water activity, lipid oxidation decreases as moisture increases. At higher water activities the rate increases as was shown by Labuza et al. (1971). The water present presumably mobilizes the catalysts and swells the solid matrix exposing new catalyst sites so that the rate of oxidation increases over that of foods at lower water activity. Such an hypothesis might explain the mechanism of the faster oxidation rate for the desorption foods which are also more swollen and contain more water than adsorption foods.

Non-enzymatic browning is probably the other significant chemical deteriorative reaction occurring in intermediate moisture foods. Work under Contract NAS 9-10658 has shown that browning proceeds at a rate which would make many fruit products unacceptable in 2 months. Meat products will undergo this reaction, but in addition, lipid oxidation occurs and could make the food unacceptable first because of the faster reaction rate under normal package conditions. Under high vacuum and with addition of antioxidants, however, browning could become a significant

deteriorative reaction in meat type IMF systems. The significance is that the browning would cause protein aggregation with subsequent toughening and loss of biological value. This area needs serious investigation.

Enzymatic deterioration is usually assumed to be unimportant in intermediate moisture foods because of the heat treatment used in processing. However, in the future, preparation of certain highly heat sensitive IMF foods may be desirable and enzymes may be active in the product. In addition, many high temperature/short-time processing techniques do not completely destroy enzymes. Thus, a knowledge of the extent of enzyme activity in food systems at intermediate moisture content is necessary. Preliminary work in NAS 0-10658 has pointed this out.

Some knowledge of nutrient loss rates in IMF systems must also be obtained if nutritional claims are to be made and the product is to supply the complete balanced meal. The areas of non-enzymatic browning, protein solubility and lysine loss are all intertwined. Labuza (1972) has recently reviewed this area with respect to dehydrated foods. As would be expected, the higher the temperature used during drying and the longer the drying time, the greater are the losses of biological value. One advantage to IMF processing is that any heat processing can be minimized since the water activity controls microbial growth and, thus, there would be less effect on the destruction of protein biological value.

In the heating of soybean meal, for example, the data of Taira et al. (1971) show a half life (time for 50% loss) for lysine of 4 hr at 126°C in the wet state (about 50% wet) and a projected half life of about 12 hr in the dry state at the same temperature. Presumably, the loss

at an intermediate A_w would be somewhere between this. However, Loncin et al. (1965) showed that the maximum lysine loss rate during storage at 40°C occurred at A_w = 0.68 and followed non-enzymatic browning closely in dry milk. Sharp (1962) showed that browning occurred at a maximum rate at intermediate A_w in freeze-dried pork, and Mizrahi et al. (1970) showed this for cabbage. These studies did not relate browning to either lysine loss or textural change. They indicate, however, that in extrusion processing it is possible that a high loss of biological value might occur since the IMF products are at the moisture content where the rate is a maximum. In addition, the activation energy of non-enzymatic browning is 40-50 Kcal/mole which means that the rate increases over 6-8 times for every 10°C rise in processing temperature. Thus, a process designed to minimize temperature may be necessary.

With respect to storage losses in the dry state both Dvorak (1965) and Cole (1962) showed losses of lysine and soluble protein for beef at less than 37°C. This could be due to interaction of oxidizing lipids or from the slower mechanism of browning. Toyomizu et al. (1963), for example, correlated protein denaturation with lipid oxidation in freeze-dried mackeral (< 1% water). If lipid oxidation is allowed to occur (i.e., no antioxidants added) in IMF systems, the protein and biological value losses could be high since oxidation is so fast. However, with proper controls only browning interactions should occur which are much slower so that the 3 to 6 month shelf life should be possible if storage temperature is low. Storage at high temperatures (>40°C) would favor browning reactions and might not be indicative of actual storage problems.

Very little data is available as to vitamin losses in dry or intermediate moisture foods. Losses of all vitamins can be minimized by maintaining low process and storage temperature and by control of lipid oxidation. The latter would be protective partially to the fat soluble vitamins (A and E) as well as to vitamin C. Vitamin C is the least stable of the vitamins but no data is available in the intermediate moisture range. From the analysis of Labuza (1972) it would be expected to be lost quite rapidly as A_w increases, however, this must be confirmed. For example, Figure 3 shows kinetic data from various sources on destruction of vitamin C. It can be seen that ascorbic acid is destroyed rapidly in the IMF range. The half life (time for 50% to be destroyed) varies from about 70 days in a flour product to 7 days in a sugar product. There is no data for this loss in IMF dog foods since dogs do not require vitamin C. Humans require ascorbic acid so this data will be needed especially if the IMF product is to be a balanced meal. Also, a study must be made to determine methods to protect vitamin C from destruction. Based on a half life of 1 month and a required shelf life of 6 months (at which point the product would still supply one-third of the RDA) if it is to be a complete meal, 1000% overage would be necessary (Figure 4). This amount of ascorbic acid would make the flavor of the product unacceptable

Thiamine (vitamin B_1) is the most studied of the B vitamins and is felt to be the most reactive. Data at high moisture content (as in canning) shows that it deteriorates at about 100 times slower than ascorbic acid (vitamin C), however, in the dry state it may react even faster than vitamin C (Labuza, 1972).

FIGURE 3. EFFECT OF A_W ON RATE OF LOSS OF ASCORBATE

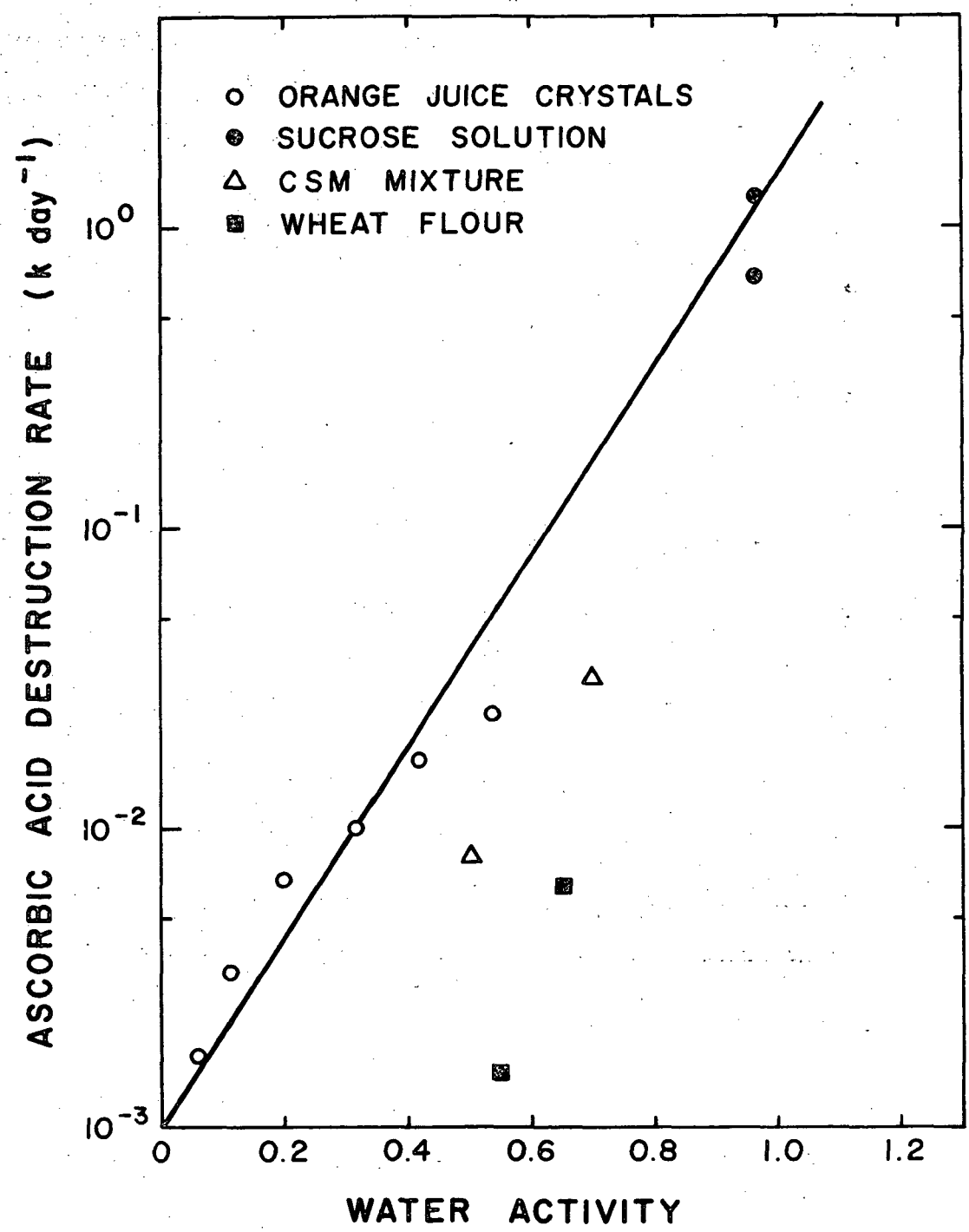
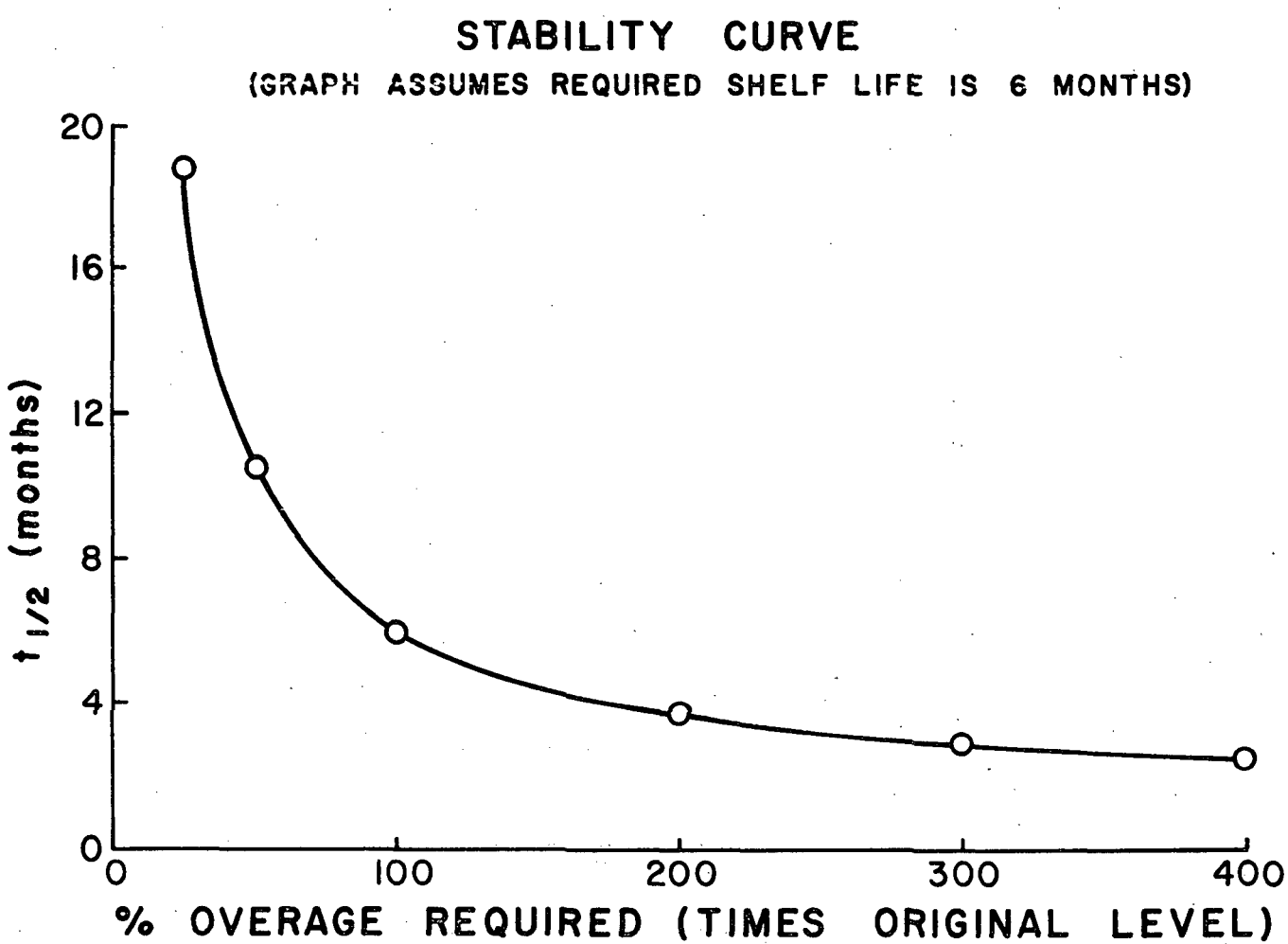


FIGURE 4. STABILITY CURVE FOR ASCORBIC ACID AS A FUNCTION OF HALF LIFE



According to Kandutsch and Baumann (1953) when a laboratory rat food was desiccated over CaO in vacuum, the thiamine was completely stable for two weeks at room temperature. Desiccation over CaO in the presence of air also markedly retarded the rate of thiamine destruction, and under these conditions thiamine was actually somewhat more stable than when stored under nitrogen.

While it is of great importance to produce dehydrated foods without marked loss of thiamine during dehydration, it is equally important to prevent considerable losses during the period between dehydration and consumption.

Rice et al. (1944) made a thorough study of the destruction of thiamine in dehydrated pork. (Pork is known as an excellent source of thiamine). To determine the extent of thiamine destruction, dehydrated pork was placed in a vacuum with samples at various moisture levels and stored at 49°C. Anhydrous pork retained its thiamine well, but as the moisture content increased, the rate of loss also increased until at six percent it had reached a maximum rate of loss. Since thiamine is quite readily inactivated by hydrolytic cleavage, it might be assumed that water is responsible for its destruction in foods.

The experimental data on the effects of temperature, container, incorporation of soya flakes, and moisture content on thiamine contents of dehydrated pork loaves during storage were presented by Nymon and Gortner (1947) determined by the thiochrome method. The data indicated the influence of moisture content of the sample upon thiamine retention. A two percent moisture content promoted better thiamine retention than at a level of four percent. The higher the storage temperature, the

more rapid was the destruction of thiamine. But the destructive effect at the higher temperature appeared to be balanced by the protective action of a lower moisture content (2.2 percent in the loaf stored at 100°F as compared with a moisture content of 3.8 percent in the loaf stored at 70°F). The container alone did not appear to exert any direct effect on thiamine reaction. The data are in agreement with Rice et al. (1944) and Tressler et al. (1943).

In spray dried whole egg, Klose et al. (1943) found virtually no loss of thiamine at -9.5°C in 9 months. Olsen and Weybrew (1948) reported that in dried whole egg the thiamine retention after 20 and 57 weeks' storage at 37°C varied inversely with the moisture content in the range 1 to 6%. At high temperature levels a temperature of 37°C (98.5°F) brought about marked changes in thiamine content. The data showed that the relationship between thiamine retention and moisture was linear within the experimental error of the determinations by a modified thiochrome method.

It is interesting to compare thiamine stability in dried eggs with that in dehydrated pork. According to Rice and Robinson (1944) dehydrated pork has a very high initial thiamine content (15μg/g). However, after 130 days of storage at 37°C only 0.62μg/g is retained. Eggs dried to the two percent moisture level retained 2.62μg per gram after a similar storage treatment. It should be pointed out that the dehydrated pork studied contained a comparatively large amount of moisture (probably about six percent). The data presented for eggs suggest that if it were practical to dehydrate pork to low moisture levels the stability of its thiamine might be very greatly increased.

Bayfield and O'Donnell (1945) observed the change in thiamine content in stored wheat. The data showed that there was a loss of thiamine in the wheat during storage particularly when the wheat contained high moisture content.

As pointed out above it is clear that moisture affects the stability of thiamine during storage. However, there is no data available in the IMF range. Although the work of Rice et al. (1944) suggests a maximum occurs at 6% water, the amount of data they collected does not support this clearly. A definitive study with respect to A_w must be done in order to predict the shelf life of the product especially since the data suggest a high destruction rate at high A_w.

C. Microbiological Stability

Besides chemical and biochemical deterioration the most significant route of destruction of IMF systems could be by microbial growth. Most systems are prepared to water activities at which only molds could grow, but Hollis et al. (1969) showed significant increases in total counts of bacteria in several food items.

The area of microbiological safety of these foods has been investigated in previous NASA contracts as well as by others. Basically, it is known that below certain A_w (water activity) limits no growth occurs for microorganisms. However, these limits vary with the microbe, the system composition, and the method of preparation. For example, as was shown by Labuza et al. (1972b) the limiting A_w is higher for adsorption prepared foods than for desorption prepared foods. The absolute limits, however, are not certain but can be classified as in Table 12 (Leistner, 1970). Based on this table, if the product is prepared below an

TABLE 12

A_w Growth Minima for Microorganisms*

A_w	Bacteria	Yeast	Molds
0.96	Pseudomonas		
0.95	Salmonella		
	Eschericia		
	Bacillus		
	Clostridium		
0.94	Lactobacillus		
	Pediococcus		
	Microbacterium		Rhizopus, Mucor
0.93			
0.92		Rhodotorula	
		Pichia	
0.90	Micrococcus	Saccharomyces	
		Hansenula	
0.88		Candida, Torulopsis	Cladosporium
0.87		Debaryomyces	
0.86	Staphylococcus		
0.85			Penicillium
0.75	Halophilic Bacteria		
0.65			Aspergillus
0.62		Zygosaccharomyces	
0.60			Xeromyces

*adapted from Leistner (1970)

A_w of 0.86, it should be safe with respect to food pathogenic organisms. If acid is added, higher limits of A_w can be used since below a pH of 5, Staphylococcus should be inhibited. The basic problem of IMF systems will thus be molds since the product would have to be made to an A_w of less than 0.6 to prevent growth. Thus, the activity of growth inhibitors must be tested in IMF systems prepared to an A_w of between 0.60 and 0.85.

Recently, Insalata (1972) has reviewed some of the microbiological problems in manufacture of IMF products. He has pointed out that although A_w is a slight function of pressure, under extrusion cooking where pressures may exceed 100 atmosphere, the A_w may increase significantly. This may induce microbial growth as well as chemical reaction. In addition, there may be microclimates within the food which are at higher A_w than measured for the whole food where growth may occur.

Chordash and Potter (1972) have studied the death of various microbes in intermediate moisture foods. They used strained baby foods (custard, pea, beef and ham) and inoculated the food as is, then dried it down in vacuum to the final moisture desired. The vacuum oven was operated at 40 mm Hg and 40°C and was felt not to be detrimental to the test organisms. Their results showed that Pseudomonas aeruginosa growth became inhibited below A_w 0.96 in all products whereas Staphylococcus aureus being more resistant grew down to A_w 0.94. For the Pseudomonas below A_w 0.96, they died out in 2 to 5 days, whereas the Staphylococcus died only slowly seeming to remain stable. In fact, there seems to be more stability at low water activity (0.68) than at intermediate values (0.76 to 0.87). This is similar to the work reported by Plitman (1973) for Staphylococcus. None of their products were acceptable from a

textural standpoint being too dry. This is why most of the products produced commercially use high sugar or glycerol contents to keep the texture soft.

Several recent studies have been made on the state of water and microbial activity. Koga et al. (1966) showed that water is bound to dried yeast in a manner similar to that in food. As moisture is decreased, the rate of growth decreases and can be quantitated by the drop in oxygen metabolism suggesting one of the effects of A_w on an enzyme system. Diener and Davis (1970) studied the production of aflatoxin by Aspergillus flavus as a function of A_w and temperature on peanuts. At A_w 0.98 the temperature limit for production is between 13°C to 40°C. Out of this range no aflatoxin is produced but this constitutes the normal range of storage to be expected for intermediate moisture foods. At 30°C no aflatoxin was produced below A_w 0.83; at 20°C the limit was slightly higher for some peanut products. This shows that to be absolutely safe for humans, IMF products should either contain a mold inhibitor or be below A_w 0.80.

Charlang and Horowitz (1971) showed that lowering of A_w has three effects on microbes (1) prolongation of the lag phase especially for conidia formation in molds; (2) reduction of growth rate and; (3) reduction of total growth. These responses are observed regardless of the type of substance used to lower A_w (electrolyte vs non-electrolyte). For the mold Neurospora crasa, the toxic effect of A_w lowering substances is in the order glycerol < NaCl < glucose < sucrose at the same A_w showing that there is a secondary effect of the added compound. They showed that some added salt ions at low concentration could overcome some of the

toxic effects, as did increasing the oxygen concentration. Their results indicate that the biochemical effect of reducing A_w is the loss of a germination-essential substance from the conidia as well as plasmolysis of the cell at low A_w. This work suggests a possible mechanism for growth inhibition of other microorganisms as well.

Marshall et al. (1971) showed that glycerol is not as inhibitory to the growth of salt-sensitive bacteria but was more inhibitory than NaCl to salt tolerant species. Cocci were more sensitive to glycerol whereas rods are more sensitive to salt. Thus, for example, the growth rate of Staphylococcus aureus was lowered 10% greater in glycerol than in NaCl, suggesting that it could be used to advantage in IMF systems. Christian's (1955) work confirms this in that salmonellae being rods are more sensitive to salt than to glycerol as is also the case with Clostridium botulinum (Baird-Parker et al.,1967) and Clostridium perfringens (Kang et al., 1969). These latter species, fortunately, are inhibited at A_w's higher than the level used for IMF systems.

Strong et al. (1970) has studied the effect of A_w on C. perfringens. The limiting A_w was 0.96 to 0.97 depending on the medium salts used and the response to A_w was as stated above based on the work of Charlang and Horowitz (1971). Interestingly enough, the stress of lowering pH was not as effective at lower A_w, i.e. A_w was more important to the growth. This work justifies the importance of studying growth in the actual food and not in synthetic media since the food contains various salts, sugars, acids, etc. which will have a different effect on growth even though it may be at the same A_w as the solution. These results also explain the results of Limsong and Frazier (1966). They felt that the Pseudomonas

fluorescens they studied was adapting to growth at lower A_w when certain salts or sugars were added. It is just that the organism has a different response to various salts. The limiting A_w for this organism was A_w 0.965.

Cotterill and Glauert (1972) reported on the destruction of Salmonella oranienburg in egg yold to which salt was added. They used salt concentrations of 10 to 35% and stored at -25 to 36°C. At 10% salt the A_w is already 0.92 so that death should be expected in all cases. At 36°C the organism disappeared in 1 to 4 weeks, the shorter time at the highest salt concentration. As temperature decreased, survival increased, but no growth was apparent. This is similar to the results of Labuza et al. (1972b) for staphylococci survival in food media at low A_w. At 16°C, A_w 0.85, the salmonellae survived for over 6 months. This could be important from a public health standpoint especially if the product picked up water so growth could start again. The organism also survived through 28 weeks at -25°C. This could be due to the lowering of A_w stress at low temperature or the fact that NaCl has a eutectic at -21°C and would have crystallized out of solution.

Patel and Miller (1972) have reported that glycerol stimulated sporulation of yeast at up to 16%. This could occur in IMF systems if glycerol is used.

These above studies all indicate that there is a limiting A_w for organism growth but that it varies with the medium used and especially with the food. Thus, for safety in preparation of IMF systems studies should be carried out with test organisms.

In NAS 9-10658 and Labuza et al. (1972b) an investigation was conducted on the effect of sorption hysteresis on microbial growth.

The preliminary work showed that the water activity minimum for growth of destructive microbes is much higher for adsorption prepared foods than for a desorption IMF. The mechanism for this was not elucidated. It was found that bacteria and yeast died out rapidly but molds could grow at low A_w. Potassium sorbate was found to be an excellent antimycotic at the maximum allowable level. Questions unanswered in that study which need answers are: (1) does the humectant inhibit growth over and above its A_w lowering effect and (2) is the water sorption hysteresis effect repeatable in solid food system.

Overall, many questions about the stability of intermediate moisture foods remain unanswered. No data are available to answer whether these foods can be relied upon for greater than 3-4 months stability. There is a need to investigate the possible processing techniques and variables for the production of a shelf stable IMF. A chicken cube product would be the most likely candidate for such a study because of the prior work done with chicken and the fact that lipid oxidation, browning, enzymatic action and mold growth can all occur in this product. The results collected could be applied to production and storage testing of a highly nutritious, high protein, high fat, intermediate moisture food bar with an acceptable flavor and long storage stability.

In summary, to this date very little data have been reported as to the shelf stability of intermediate moisture foods except for work conducted on prior NASA contracts and those reported in recent meetings (Table 13). The past data suggest that most of these foods have very limited stability, but that by adjustment of processing conditions, composition, use of additives and proper packaging of food for human consumption a long shelf life

TABLE 13

Conferences with IMF Emphasis	# of Papers
Food Industry Week, VPI, February 1972	2
University of Minnesota Short Course, February 1972	(1 day)
St. Louis Annual Small IFT, March 1972	5
American Society of Microbiology, April 1972	5
Institute of Food Technologists, June 1972	2
University of Massachusetts, Physical Properties of Foods, August 1972	(½ day)
MIT Summer Course, August 1972	(1 day)
Cereal Chemists Society, November 1972	3
6th Int. Short Course on Freeze-drying and Advanced Food Technology, June 1973	(1 day)
1st Annual National Renal Diet Symposium, Minneapolis, March 1973	1

is possible. In addition, on the basis of these results it would be possible to prepare a nutritionally complete food item.

III. Procedures

A. Chemical Studies

1. Fat analysis for model systems and foods

Extraction

a. Add 40 ml of chloroform : methanol (3:1) to sample and flush with N_2 until the outside of the flask was cool to the touch. Stopper tightly.

b. Shake on reciprocal shaker for ½ hr at speed #5.

c. While shaking, weigh empty 24/40 125 ml round bottom flask.

d. Filter mixture by suction through 2 layers of No. 50 Whatman filter paper into previously weighed round bottom flask. Wash filter paper and original sample flask with 20 ml of 3:1 $CHCl_3$: MeOH. Make certain final solution is crystal clear.

e. Place this clear solution on a rotary evaporator. Turn on condenser water, then turn on vacuum water. Hold flask in place until vacuum gauge reads 10. Then turn on rotator. Make certain that the liquid does not bump. When quiet, apply heat at setting 40°C. (Be careful if, 45-50°C, water bath is warm when putting on new sample. Liquid in the sample may bump; it may bump more vigorously when rotation is turned on).

f. Evaporate 30 to 40 min or until no solvent remains in the flask. Break the vacuum with N_2. Stopper, cool and weigh. Fat weight is this weight minus tare weight.

$$\% \text{ fat} = \frac{\text{fat weight} \times 100\%}{\text{sample weight}}$$

2. Fat Acidity (Free Fatty Acid Determination)

a. Extract fat as in the Fat Analysis. Determine sample weight on a dry solids basis.

b. Dissolve fat with 50 ml of benzene-alcohol-phenolphthalein solution.

c. Titrate solution with KOH solution to distinct pink, or in case of yellow solution, to orange-pink.

d. If emulsion forms during titration add a second 50 ml portion of benzene-alcohol-phenolphthalein solution.

e. End point should match color of solution made by adding 2.5 ml of 0.01% $KMnO_4$ solution to 50 ml $K_2Cr_2O_7$ solution of proper strength to match color of original solution (orange) being titrated. (Add 0.5% $K_2Cr_2O_7$ solution dropwise to 50 ml H_2O until color is matched. Then add 2.5 ml of the 0.01% $KMnO_4$ solution. This final solution may be used as a standard for titrating end point of the extract.)

f. Make a blank titration on 50 ml of bezene-alcohol-phenolphthalein solution and subtract this value from titration value of the sample. If the additional 50 ml of benzene-alcohol-phenolphthalein solution was added

to break up an emulsion, double the blank titration.

g. Report fat acidity at mg KOH required to neutralize free fatty acids from 100 g sample (dry basis). Fat acidity = Y* x (titration-blank).

Y* = whatever factor you have to multiply by the dry weight to get 100

h. Preparation of Solutions

(1) Benzene-alcohol-phenolphthalein solution: To 1 ℓ C_6H_6 add 1 ℓ alcohol and 0.4 g phenolphthalein to form 0.02% solution.

(2) Potassium hydroxide solution: 0.0178 N, CO_2-free. 1 ml = 1 mg KOH.

3. Moisture Content AOAC

Freeze sample by immersion in liquid nitrogen for 1 min. Grind immediately in a C.R.C. micromill for 15-20 sec. Dry in either:

a. Vacuum oven - 65°C for 18 hr

b. Air oven - 110°C for 24 hr

Report moisture as weight lost divided by grams left after drying (g H_2O/g solids).

4. Moisture Content - Measurement by GLC

Moisture content was measured by extraction of 2 g samples (weighed exactly) for 30 min at 21°C on a reciprocal shaker with 20 ml of anhydrous methanol and measuring the water content by a GLC technique. A 3' x ⅛" column was used packed with Poropack Q.

a. Column temperature - 110°C

b. TC - detector - 250°C

c. Sample size - 5 μl

An internal standard was used ($MeOH/H_2O$ Ratio Method) and a Perkin Elmer Computerized Electronic Integrator printed out the moisture content in terms of peak area. Figure 5 shows as standard curve for one of the representative runs.

5. Glycerol Measurement by GLC

The same extraction procedure as for moisture contents was used, however, a different amount of methanol was used and a different internal standard system was used for the glycerol determination. To each system after extraction with methanol, either 1 ml of octanol or 1 ml of amyl alcohol was added, and the ratio of the peak height of glycerol to that of the alcohol was determined in each sample. Figure 6 shows the standard curves found for a representative run for glycerol. A new standard curve was run each time an analysis was made. The conditions used for glycerol analysis are:

a. Column temperature - 245°C

b. Column length - 60 cm

c. Column diameter - 0.32 cm

d. Detector temperature - 280°C

e. Carrier gas flow rate - 42 cc/min

f. Sample size - 2 μl

6. A_w Measurement (VP device)

The vapor pressure apparatus described in NAS 9-10658 was

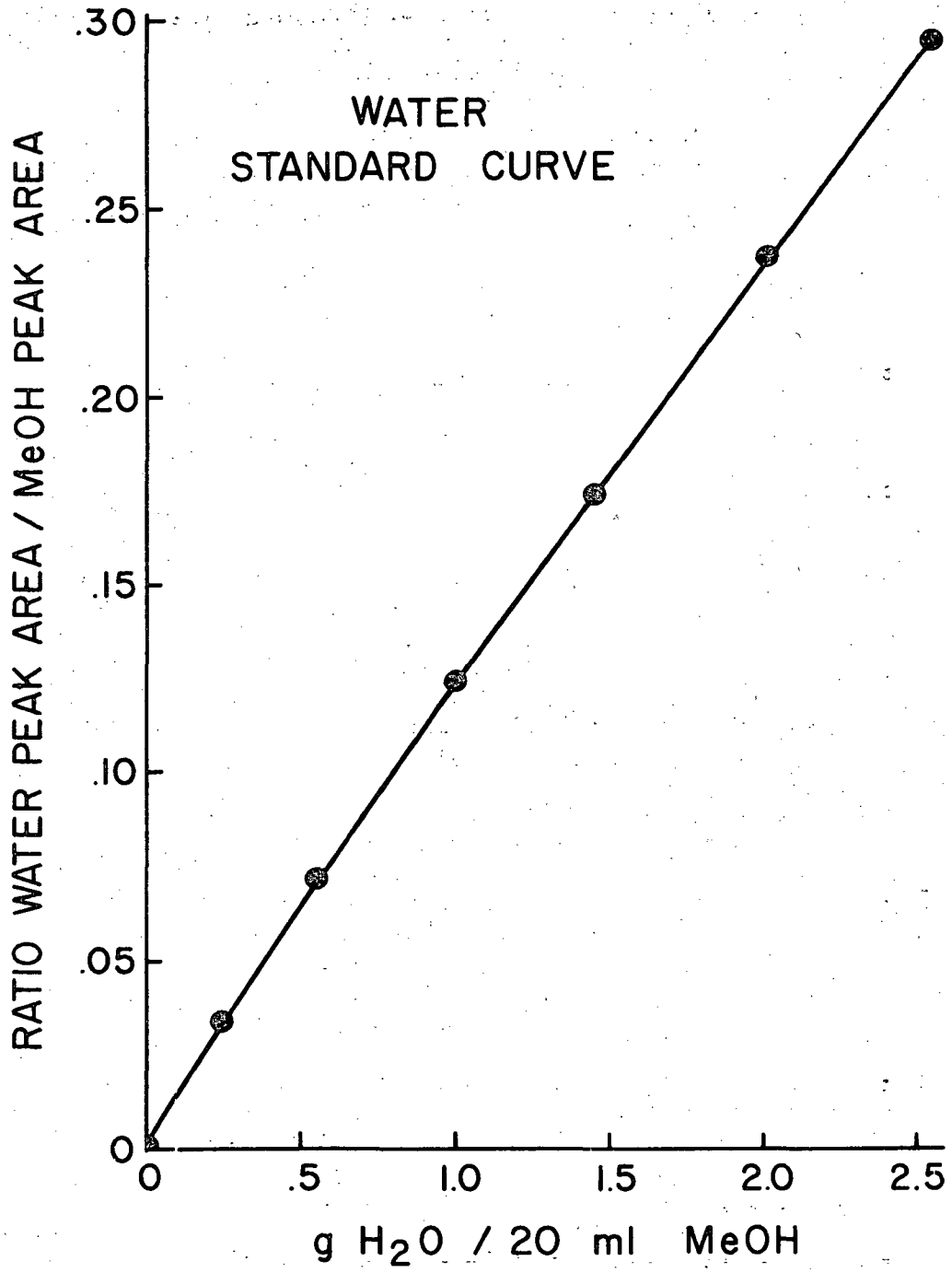


FIGURE 5. STANDARD CURVE FOR GLC ANALYSIS OF WATER

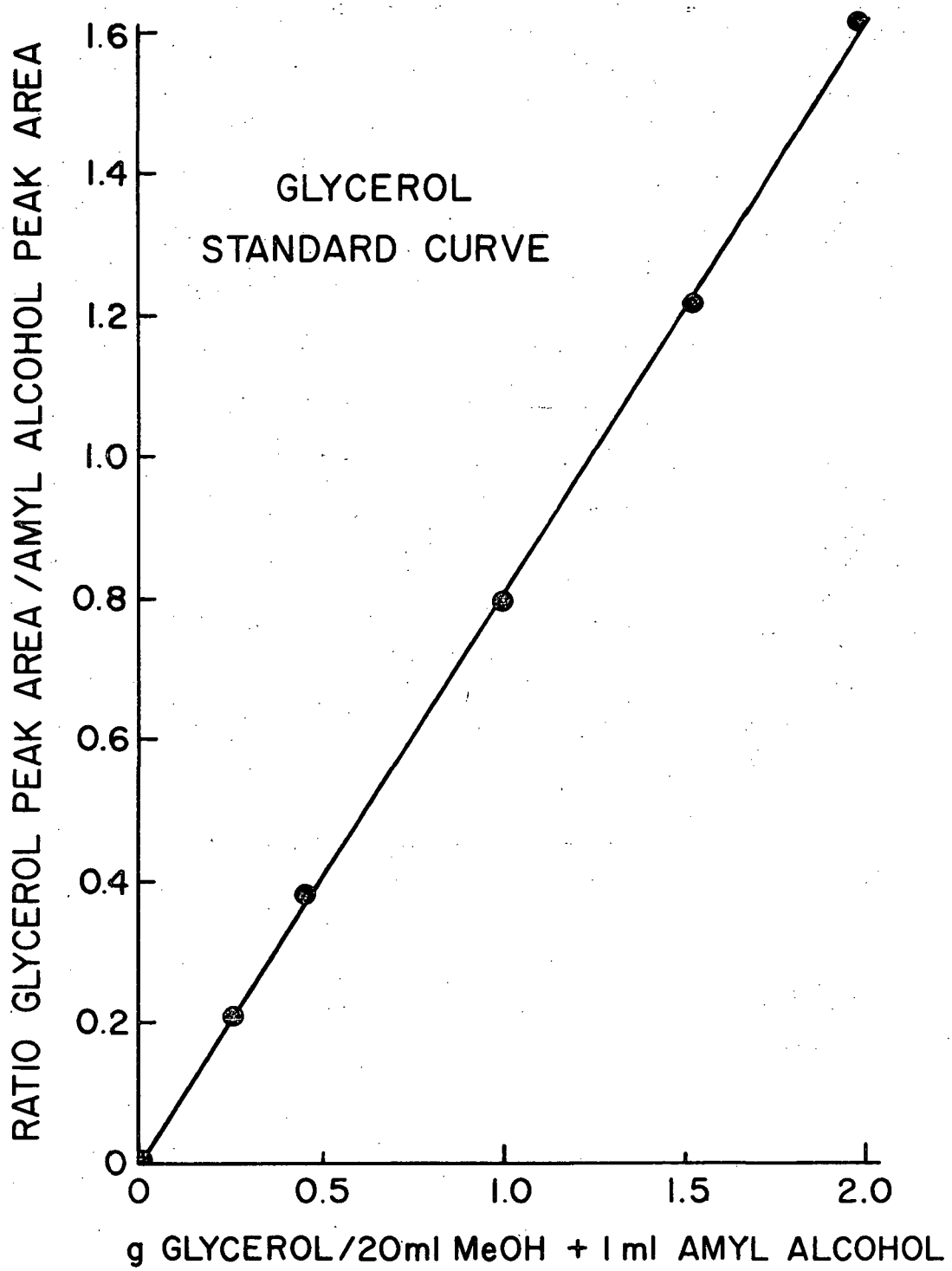


FIGURE 6. STANDARD CURVE FOR GLC ANALYSIS OF GLYCEROL

rebuilt and modified to reduce error to ± 0.5% RH. With standard salt solutions the error is actually ± 0.1% RH. Measurements were made at 20°C in a constant temperature room. The device is shown schematically in Figure 7.

7. Lipid Oxidation Measurement

a. Manometric Determination of Oxygen Absorption

Samples (in triplicate or duplicate) of each IMF system were prepared for oxidation studies by weighing directly into 30 ml Warburg manometer flasks. These were specially made without the standard side arms and center well. After preparation, the flasks were individually attached to manometers containing mercury as the manometer fluid. These were then placed in water baths at 35°C and closed. A thermal barometer, consisting of an empty flask attached to a manometer, was also placed in the bath to account for atmospheric pressure changes. The oxygen absorbed by each sample was then measured over a period of up to 8 weeks by measuring the change in pressure across the manometer. The method of Umbreit et al., 1964 was used to calculate oxygen uptake. Essentially, this consists of the following calculations:

$$\frac{\mu l\ O_2 \text{ absorbed (STP)}}{\text{mm manometer reading}} = k_f = \frac{T_o}{T_1} \quad \frac{1}{P_o} \quad \times V \times 10^3$$

k_f = flask constant in μl oxygen per mm of manometer change

T_o = 273°C

T_1 = run temperature, 310°K

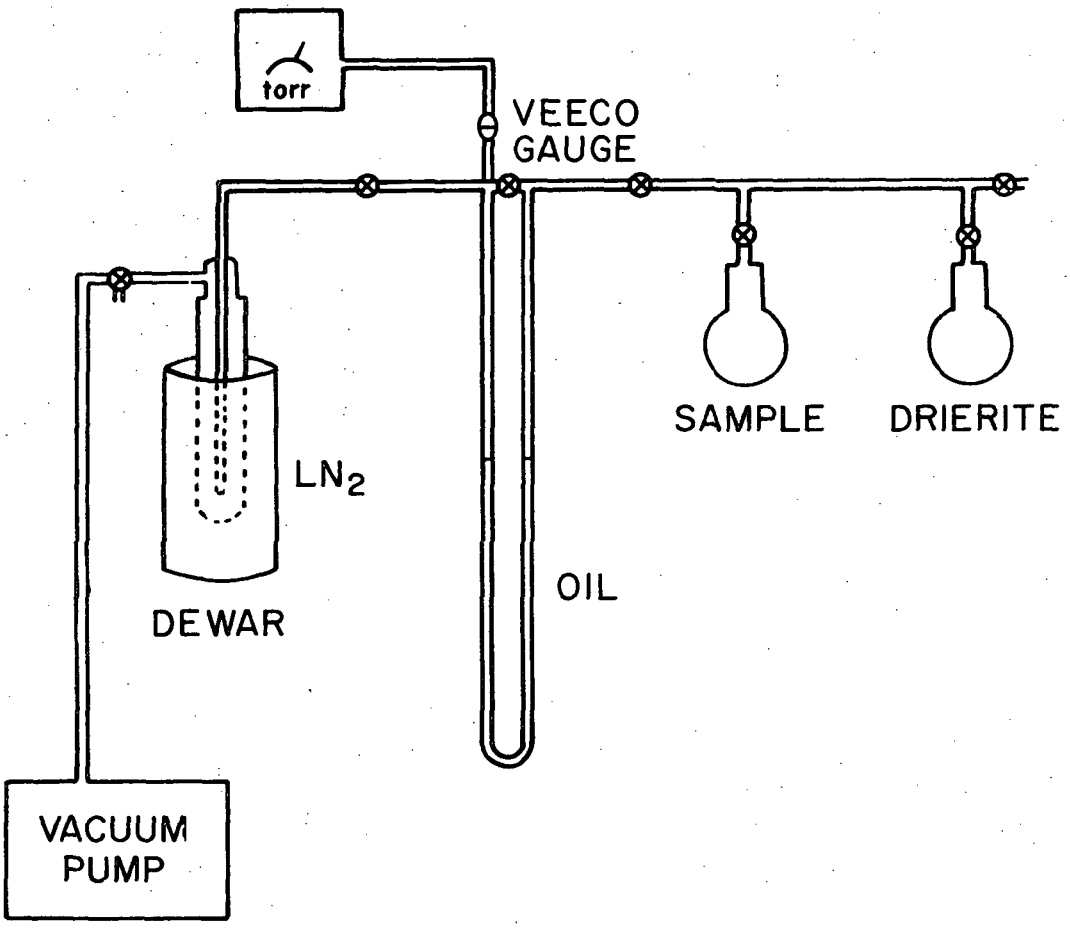


FIGURE 7. SCHEMATIC - VAPOR PRESSURE MEASUREMENT DEVICE

P_o = 1 atm of manometer fluid = 760 mm Mercury

V = flask and manometer volume less the sample volume

The model system sample volume was measured on representative samples using a Beckman Model 930 Air Comparison Pycnometer to give the average sample densities. The flask and manometer volumes were then measured by mercury displacement. The flask constant is then multiplied by the corrected manometer change (corrected for ambient pressure changes as measured by the thermal barometer) for each time period over which a measurement is made and these changes are summed up and divided by the lipid weight or the solids weight so that a comparison between samples can be made. This method measures only total oxygen absorbed, so it does not correct for oxidation of components other than the lipid or production of gases such as CO_2 during non-enzymatic browning (Cole, 1967). All data was calculated by the use of the IBM 366-30 computer system located on the St. Paul Campus of the University of Minnesota. From the oxygen uptake curves various kinetic constants were calculated according to the method of Labuza (1971b). These include; Θ_i, the induction time; K_M, the rate constant in the initial rate period; and K_B, the rate constant in the accelerated rate period.

b. Peroxide Value (A.O.C.S. Method Cd 8:53)

Representative samples of the IMF systems containing between 0.1 to 0.25 g lipid were weighed into a 125 ml Erlenmeyer flask and 40 cc of chloroform : methanol (3:1 v/v), were

added; the flasks shaken for ½ hour after flushing with nitrogen, and their contents filtered under vacuum, on a 55 mm Buchner funnel using 40 ml of solvent to wash the residue. The filtrate was collected into a tared 125 ml flask (with a 24/40 ground glass joint) and the solvent was evaporated for 30 min on a rotary vacuum evaporator, and the vacuum was broken with nitrogen. For initial samples, the system was evaporated to dryness. The weight of lipid was then calculated by weighing the flask. This value was then used for all other samples so that less evaporation time was needed. The residue remaining on the Buchner funnel was saved for determination of the extent of lysine and nonenzymatic browning in cases where necessary.

The lipid in the flask was dissolved with 10 ml of a mixture of glacial acetic acid : chloroform (3:2). Then 0.5 ml of freshly prepared potassium iodide (saturated solution) was added and after exactly 2 min, 15 ml distilled water were added to stop the reaction. The mixture was then titrated with 0.01 N $Na_2S_2O_3$ using 1 ml 5% starch solution as an indicator. The thiosulfate was standardized periodically against potassium dichromate. The peroxide value was calculated as:

$$\frac{\text{meq } O_2}{\text{Kg fat}} = \frac{(\text{ml } Na_2S_2O_3)\ (\text{Normality } Na_2S_2O_3) \times 1000}{\text{grams fat}}$$

8. Protein Solubility

a. Food Samples

A pulverized sample, 1 g, was suspended in 10 ml 3% NaCl solution and mixed via Vortex mixer for 10 min. The system was then centrifuged at 1000 G for 10 min and decant was filtered through Watman No. 3 filter paper, filtrate being the soluble protein. Precipitate remaining in centrifuge tube was resuspended with 10 ml NaCl and treated as the original sample. Titrates of both solubilization steps are combined for % Protein Analysis. Biuret assay (J. Biol. Chem. 1949. 177:751) was used to determine the amount soluble protein, i.e. amount of protein in combined filtrate. Total protein was done by Kjeldahl-N determination on a pulverized 2 g chicken sample.

Percent soluble protein is (Biuret protein - Kjeldahl protein), quantity multiplied by 100%.

b. Casein Model Systems

(1) 0.25 g casein system suspended in 10 ml 3% NaCl solution (pH 6.7; I.S. 1.034).

(2) Extract soluble protein by agitating saline-casein system for 1 hr, 120 cycles per min, room temperature.

(3) Following extraction process, centrifuge the system at 1000 x G for 10 min to separate soluble from insoluble protein fractions.

(4) Filter supernatant through Watman #3 filter paper.

(5) Determine protein content of filtrate with modified Biuret protein determination procedure (J. Biol. Chem. 1949. 177:751).

(a) To 4.0 Biuret reagent, add 1.0 ml sample aliquot containing 1-10 mg protein. Mix well.

(b) Allow 30 min for color development at room temperature.

(c) Read absorbance against 0.00 O.D. of blank. (Blank has 1.0 ml sample aliquot replaced with 1.0 ml 3% NaCl solution).

(d) Determine amount protein in sample from a standard curve.

9. Total Protein - A.O.A.C.

a. Macro-kjeldahl total nitrogen (6.25 x %N = % Protein). 2 g sample size

10. Available Lysine

Modified Wartheson (M.S. Thesis, University of Minnesota, 1971).

a. To 0.05 g pulverized sample in a 50 ml screw-cap test tube, add 5 ml 4.0% $NaHCO_3$ buffer, pH 8.5. Mix sample well and incubate it in 40°C water bath shaker (120 cpm) for 10 min to solubilize the protein.

or

(In high protein system) 250 mg of sample added to 100 ml 4% (wt/vol) $NaHCO_3$, pH 8.5 buffer. This was heated to 70-85°C with stirring until dissolved. A 5 ml aliquot is then used as the sample.

b. Add 5 ml 1% TNBS (trinitrobenzene sulfonic acid, wt/vol),

mix sample well, and incubate in 40°C water bath shaker for 2 hr at 120 cpm.

c. Carefully add 15 ml conc. HCl, mix sample well, and autoclave sample at 122°C for 1 hr.

d. Quantitatively transfer contents of test tube to 50 ml vol flask; bring to volume with H_2O.

e. Filter (Whatman #3 filter paper) about 10 ml sample solution into separating funnel, and extract 3 times with diethyl ether. Drive off excess ether by placing collected aqueous phase in 40°C water bath shaker for 15 min at 120 cpm.

f. Dilute sample with 30% HCl (vol conc. HCl/vol conc HCl plus H_2O) to bring O.D. into readable range (usually 1:10 dilution).

g. Read O.D. at 346 nm.

h. Calculate g available lysine/g protein from a standard curve.

i. Blank (0.00 O.D.) has 15 ml conc. HCl added before TNBS added to sample with HCl addition omitted prior to autoclaving; other steps are same as for regular samples.

11. Non-Enzymatic Browning

Modified Choi et al. (1949) by Labuza (Contract NAS 9-10658, 1971)

a. 0.5 to 2 g pulverized sample suspended in 22.5 ml of a ~1% trypsin solution (0.25 g trypsin/22.5 ml H_2O); system incubated in 45°C water bath shaker for 2 hr at 120 cpm (cycles per min).

b. Following 2 hr incubation, trypsin is denatured with 2 ml 50% (wt/vol) trichloroacetic acid, and 0.1 g Celite (filter aid) is added.

c. System filtered with Watman #3 filter paper and O.D. of filtrate read at 420 nm with Beckman Jr. spectrophotometer.

d. Blank (0.00 O.D.) has no sample added to trypsin solution, but treated otherwise as regular samples.

12. Trace Metal Level

Trace metal analysis was performed on various samples by the University of Minnesota Agricultural Experiment Station Soil Laboratory. Tests were made for Fe, Ca, Cd, Ni, Mn, Zn, Cu, Mg, and Cr by atomic absorption spectroscopy using a Perkin Elmer 303 A.A. Spectrophotometer. Table 14 shows the conditions used for the heavy metal determination in both solid supports used(about 5 g samples in duplicate). Table 15 contains the results.

13. Vitamin Analyses

a. Thiamine

(1) Reagents

(a) Sodium hydroxide solution - 15%: Dissolve 15 g of NaOH in H_2O to make 100 ml.

(b) Potassium ferricyanide solution - 1%: Dissolve 1 g of $K_3Fe(CN)_6$ in H_2O to make 100 ml. Make solution on day it is used.

(c) Oxidizing reagent: Mix 4.0 ml of the 1% $K_3Fe(CN)_6$ solution with the 15% NaOH solution to make 100 ml.

TABLE 14

Conditions for Trace Metal Analysis

Metal	Flame	Slit	Wavelength (nm)
Fe	Oxidizing	4	249.8
Ca	Reducing	4	211.5
Cd	Oxidizing	4	229
Ni	Oxidizing	3	232
Mn	Oxidizing	5	280.5
Zn	Oxidizing	4	214.7
Cu	Oxidizing	4	325
Mg	Oxidizing	4	285.7
Cr	Reducing	4	368.5

Burner Head: 3-slot
Fuel: C_2H_2 (acetylene) at 8 psig
Oxidant: Air at 30 psig
Temperature: 2300°C

TABLE 15

Heavy Metal Content (ppm)

	Fe	Ca	Cd	Ni	Mn	Zn	Cu	Mg	Cr
Microcrystalline Cellulose									
Sample 1	0.68	--	Trace	Trace	0.11	0.16	0.35	--	Trace
Sample 2	0.48	0.40	--	--	0.08	0.04	0.20	0.33	--
Amylopectin									
Sample 1	115.00	250.00	Trace	Trace	3.64	1.36	5.25	570.00	Trace
Sample 2	76.00	287.00	--	--	3.55	1.00	5.25	627.00	--

Use the solution within 4 hr.

(d) Isobutyl alcohol: Redistilled in all-glass apparatus. Use redistilled product as anhydrous or H_2O saturated.

(e) Quinine sulfate stock solution: Use quinine sulfate solution to govern reproducibility of fluorometer. Prepare stock solution of this reagent by dissolving 10 g of quinine sulfate in 0.1 N H_2SO_4 to make 1 ℓ. Store in light-resistant containers.

(f) Quinine sulfate standard solution: Dilute 1 vol of the quinine sulfate stock solution with 39 volumes 0.1 N H_2SO_4. (The solution fluoresces to approximately the same degree as does isobutyl alcohol extract of thiochrome obtained from 1 μg of thiamine HCl). Store the solution in light-resistant containers.

(g) Thiamine hydrochloride stock solution I: Weight accurately 50-60 mg of USP thiamine hydrochloride reference standard that has been dried to constant weight over P_2O_5 in a desiccator. Since the reference standard is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH 3.5-4.3 with HCl, and dilute to 500 ml with the acidified alcohol. Add enough additional acidified alcohol to make

the concentration exactly 100 ug/ml. Store at approximately 10°C in a glass-stoppered, light-resistant bottle.

(h) Thiamine hydrochloride stock solution II: Dilute 100 ml of thiamine HCl stock solution I to 1 ℓ with 20% alcohol adjusted to pH 3.5-4.3 with HCl. Store at approximately 10°C in a glass-stoppered, light-resistant bottle. 1 ml = 10 µg thiamine HCl.

(i) Thiamine hydrochloride standard solution: To 10 ml of thiamine HCl stock solution II, add approximately 50 ml of approximately 0.1 N HCl, digest or autoclave as in extraction, cool, and dilute to 100 ml with the 0.1 N acid (1 ml equivalent to 1 µg thiamine HCl). Prepare a fresh solution for each assay. For materials containing free thiamine, dilute 20 ml of this solution of 100 ml with 0.1 N HCl (1 ml = 0.2 µg thiamine HCl).

(2) Extraction

Place a measured quantity of sample in a flask of suitable size. Add a volume of 0.1 N HCl equivalent in milliliters to not less than 10 times the dry weight of the sample in grams. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid; then wash down the sides of the flask with 0.1 N HCl. Digest 30 min at 95°-100°C. Cool, and if lumping occurs, agitate the mixture until the particles are evenly dispersed. Dilute with 0.1 N HCl to a

measured volume containing approximately 0.2-5.0 µg of thiamine per milliliter.

(3) Oxidation of Thiamine to Thiochrome

To each of four or more approximately 40 ml tubes (or reaction vessels) add about 1.5 g of NaCl or KCl and 5 ml of the standard solution. (Precision and accuracy of results depend upon uniform technique in conducting the following oxidation procedure. Protect the solution from light, which destroyes thiochrome. Use a pipette that delivers 3 ml in 1-2 sec for addition of the oxidizing reagent). Place the tip of the pipette containing the oxidizing reagent in the neck of the tube and hold it so that the stream of solution does not hit the side of the tube. Swirl the tube gently to produce a rotary motion in the liquid and immediately add 3 ml of the oxidizing reagent. Remove the pipette and swirl the tube again to insure adequate mixing. Immediately thereafter, add 13 ml of isobutyl alcohol, stopper, and shake the tube vigorously at least 15 sec. Treat one or more of the tubes similarly, and treat each of two or more of the remaining tubes (standard blanks) in the same manner, except to replace the oxidizing reagent with the 15% NaOH solution.

To each of four or more similar tubes add 5 ml of the assay solution. Treat these tubes in the same manner as directed for tubes containing the standard solution.

After isobutyl alcohol has been added to all the tubes, shake again approximately 2 min. (The tubes may be placed in a shaker box for this additional shaking). Centrifuge the tubes at low speed until clear supernatant extract can be obtained from each. Pipette or decant approximately 10 ml of the isobutyl alcohol extract (the upper layer)

from each tube into the cell for measurement of thiochrome fluorescence.

(4) Thiochrome Fluorescence Measurement

Thiamine content of the oxidized assay solution is determined by comparing the intensity of fluorescence of the extract of this solution with that from the oxidized standard solution. The intensity of fluorescence is proportional to the quantity of thiamine present and may be measured with a suitable electronic fluorometer. An input filter of narrow transmittance range with a maximum of approximately 365 nm and an output filter of narrow transmittance range with a maximum of approximately 453 nm have been found satisfactory. Use quinine sulfate standard solution to govern the reproducibility of the fluorometer.

Measure the fluorescence of isobutyl alcohol extract from the oxidized assay solution, and call this reading A. Next, measure the fluorescence of the extract from the assay solution which has been treated with 3 ml of the 15% NaOH solution, and call this reading (assay blank) b. Then measure the fluorescence of the extract from the oxidized standard solution, and call this reading S. Finally, measure the fluorescence of the extract from the standard solution which has been treated with 3 ml of the 15% NaOH solution, and call this reading (standard blank) d.

(5) Calculation

Calculate as follows: µg thiamine hydrochloride in 5 ml assay solution = (A-b)/(S-d).

b. Ascorbic Acid

(1) Reagents

(a) Metaphosphoric acid-acetic acid stabilizing extracting soln. Dissolve, with shaking, 15 g

glacial HPO_3 pellets or freshly pulverized stick HPO_3 in 40 ml HOAc and 200 ml H_2O; dil. to ca 500 ml, and filter rapidly through fluted paper into glass stoppered bottle. (HPO_3 slowly changes to H_3PO_4, but if stored in refrigerator, soln remains satisfactory 7-10 days). Prepare fresh weekly.

(b) Ascorbic acid std.

USP Reference Ascorbic Acid; keep cool, dry , and out of direct sunlight in desiccator.

(c) Indophenol std. soln.

Dissolve 50 mg 2,6-dichloroindophenol Na salt (Eastman No. 3463), that has been stored in desiccator over soda lime, in 50 ml H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye dissolves, dil. to 200 ml with H_2O. Filter thru fluted paper into amber glass stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make end point indistinct occur in some batches of dry indophenol and also develop with time in stock soln. Add 5.0 ml extracting soln. containing excess ascorbic acid to 15 ml dye reagent. If reduced solution is not practically colorless, discard, and prepare new stock soln. If dry

dye is at fault, obtain new speciman).

(2) Standardization

Weigh accurately (± 0.1 mg) ca 100 mg of the Reference Standard ascorbic acid, transfer to 100 ml glass stoppered vol. flask, and dil. to mark (room temp.) with the HPO_3-HOAc reagent. Standardize indophenol soln at once as follows: Transfer three 2.0 ml aliquots of the ascorbic acid soln to each of three 50 ml Erlenmeyers containing 5.0 ml of the HPO_3-HOAc reagent. Titrate rapidly with the indophenol soln from 50 ml buret until light but distinct rose-pink persists at least 5 sec. (Each titration should require ca 15 ml of the indophenol soln, and titrations should check within 0.1 ml). Similarly, titrate 3 blanks composed of 7.0 ml of the PHO_3-HOAc reagent plus vol H_2O ca equivalent to vol indophenol soln used in direct titrations. After subtracting average blanks (usually ca 0.1 ml) from standardization titrations, calculate and express concentration of indophenol soln as mg ascorbic acid equivalent to 1.0 ml reagent. Standardize indophenol soln daily with freshly prepared standard ascorbic acid soln.

(3) Sample Procedure

(a) Transfer the entire content of one jar into 100 ml volumetric flask - weigh accurately.

(b) Add about 80 ml HPO_3-HOAc reagent and shake on shaker for 20 min.

(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 HPO_3-HOAc reagent.

(f) Titrate with the indophenol solution

$$E = \frac{(mgAA)}{100} \times \frac{2}{(ml\ dye)}$$

$$mgAA/100g = \frac{ml\ dye \times E}{gram\ sample} \times D \times 100$$

D: Dilution Factor

E: Equivalent Factor (mgAA/ml dye)

(g) Report as mg ascorbic acid/gram solids

14. Viscosity Measurement

The viscosity of glycerol water solutions of calculated concentrations were measured by using a Brookfield Synchro-Lectric Viscometer model LVF (for lower viscosity systems) to predict the theoretical viscosity in the aqueous phase of the systems.

The Brookfield Viscometer, fitted with a special bar-type spindle and supported by a Helipath Stand, was used to try to measure the viscosities of the systems directly. However, great variations resulted possibly due to the different solid support systems and stickiness. Therefore, the viscosities of systems with higher moisture contents were measured with the HBT viscometer which was found to be suitable. These viscosities should be useful for estimating the true system viscosities, at least to some degree, at lower moisture contents.

B. Model System Preparation

1. Lipid Oxidation Systems

To prepare the systems for oxidation studies glycerol and methyl linoleate were first mixed in a beaker using a glass stirring rod. To this the solid support (either microcrystalline cellulose (Avicel, FMC Corp. Pennsylvania) or amylopectin was added and mixed thoroughly. For the direct mix system (DM) the amount of water necessary to achieve the desired water activity (as previously determined) was added. This gives the desorption system. Although the water was added to the dry support, previous work showed this method to be no different than adding excess water and desorbing it in a desiccator. For the humidified systems (DH), after mixing the support with the lipid and glycerol, the system was transferred into desiccators containing saturated salt solutions and held for 48 hr at 37°C. Usually, the system was weighed directly into the Warburg manometer flasks before humidification to facilitate ease of handling. The direct mixed system was also held simultaneously in the desiccator so that the extent of oxidation was the same. Vacuum desiccators were used to prevent or to slow oxidation during the humidification procedure. After equilibration the flasks are connected to the manometers and oxidation was measured. Table 16 shows the composition of the systems used and Figures 8 and 9 show the isotherms for the two systems.

2. Vitamin Stability Systems

Table 17 contains the composition for the systems used for the thiamine stability tests. These systems were prepared according to the direct mix procedure (DM) used for the lipid oxidation studies for the adsorption systems. The same samples were prepared by one of two ways:

TABLE 16

System Compositions of Model Systems

Cellulose Systems	
Methyl linoleate	10
Glycerol	40
Microcrystalline cellulose	50
Water	Per isotherm
Amylopectin Systems	
Linoleate	10
Glycerol	40
Amylopectin starch	50
Water	Per isotherm

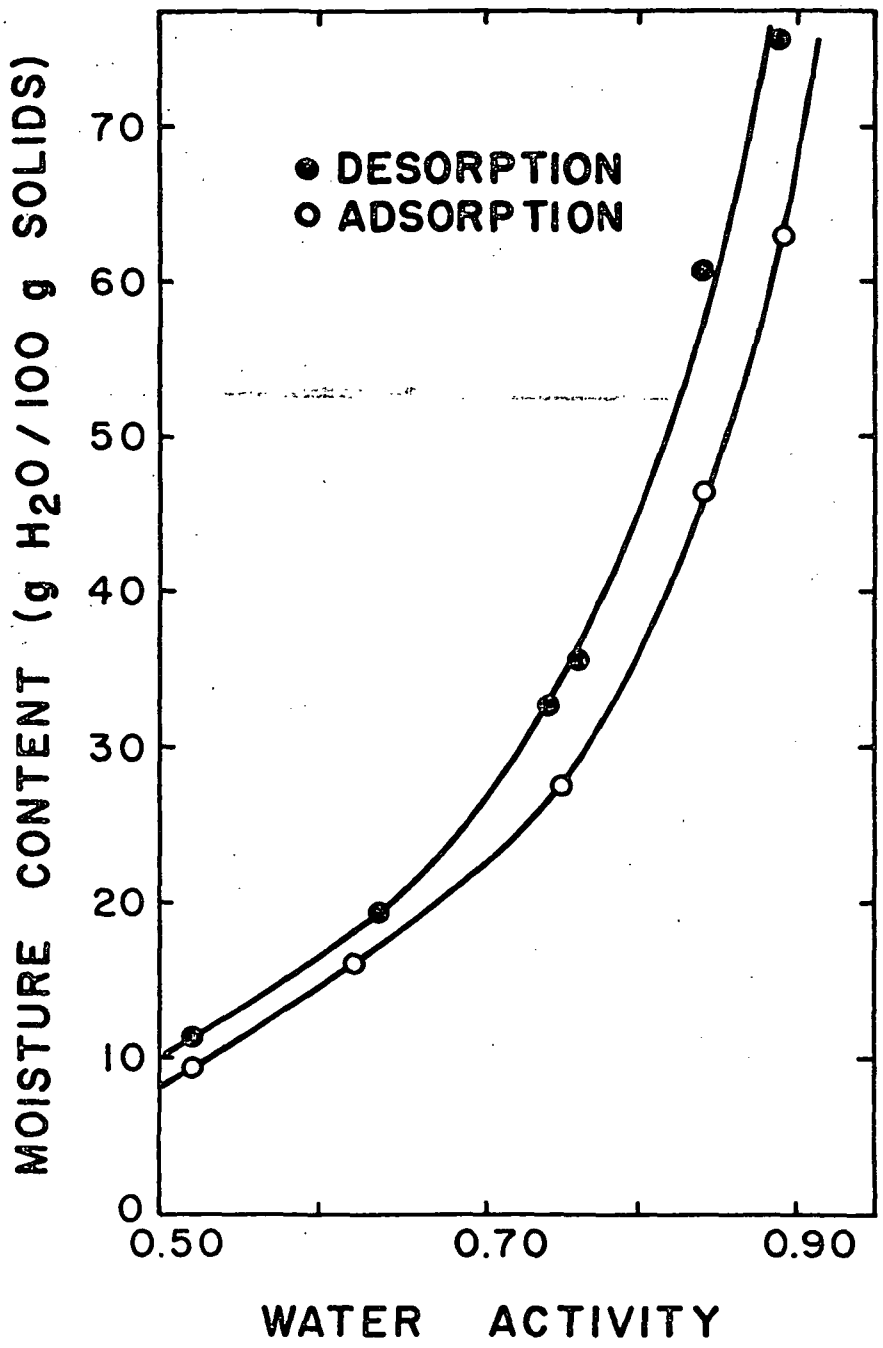


FIGURE 8. MOISTURE SORPTION ISOTHERM FOR MICROCRYSTALLINE CELLULOSE SYSTEM

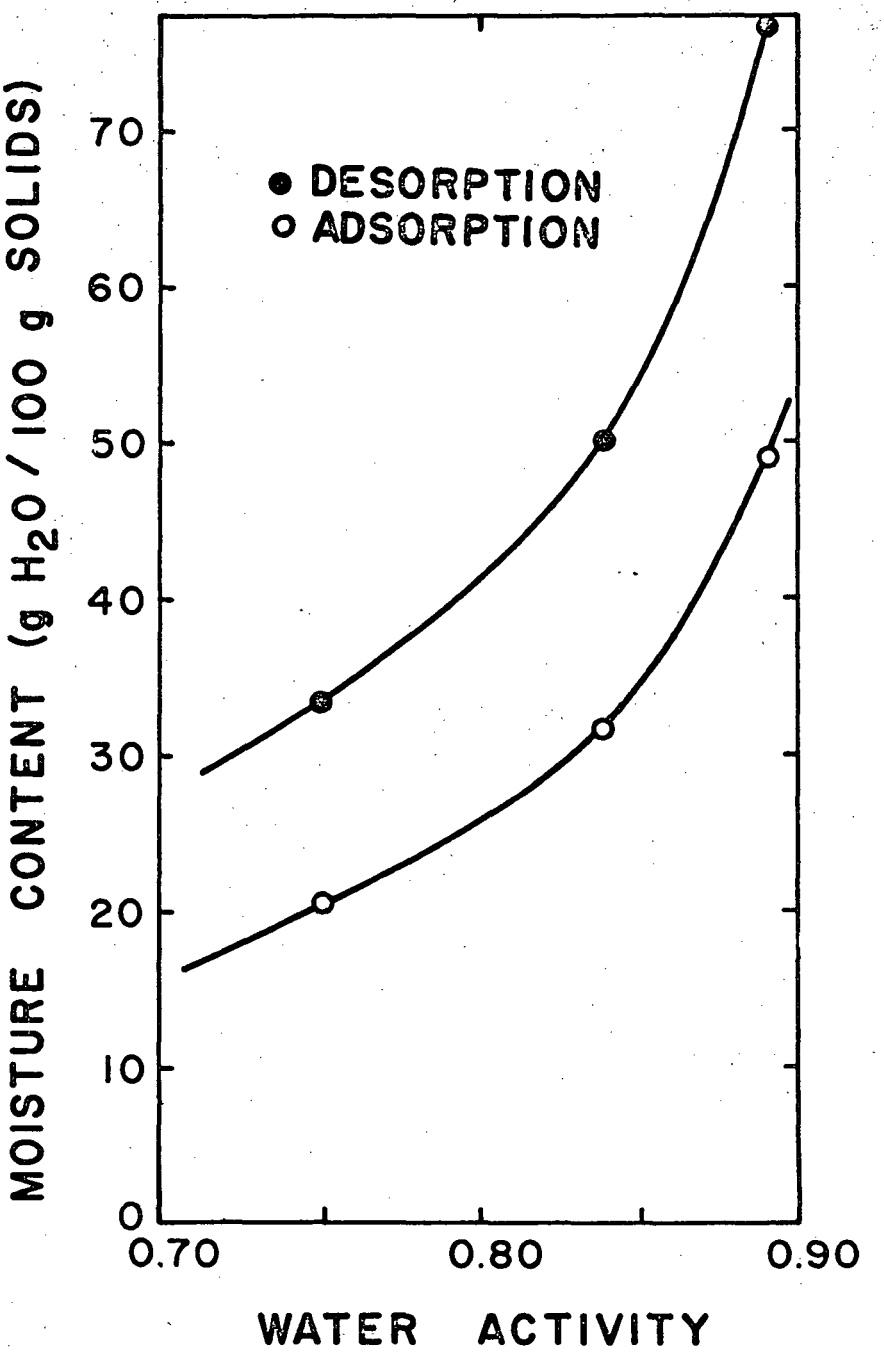


FIGURE 9. MOISTURE SORPTION ISOTHERM FOR AMYLOPECTIN MODEL SYSTEM

TABLE 17

Vitamin Stability Test System Composition

	A_w				
	0.84	0.75	0.61	0.51	0.2
Corn oil	5 g	5 g	5 g	5 g	5 g
Cellulose	50 g	50 g	50 g	50 g	50 g
Glycerol	40 g	57.5 g	75 g	80 g	94 g
Buffer solution	59 g	41.5 g	24 g	19 g	5 g
Vitamin solution	1 ml	1 ml	1 ml	1 ml	1 ml

a. Freeze-dried Rehumidified (FDR)

Direct mix system was frozen at -20°C followed by drying for 18 hr at 75°F and 100 μ Hg. The samples were then humidified to the desired humidity for 3 days in vacuum desiccators over the necessary salt solutions. No change in ascorbic acid occurred as shown in Table 18 from the freeze-drying

b. Dry Mix Humidified (DH)

The dry ingredients are mixed together with the lipid and then humidified as above.

C. Microbiological Studies in Model Systems

1. Test Organisms Used

a. Pseudomonas fragi - Grown in TSY broth in a shake flask @ 22°C for 18 hours. Population was estimated at 450 nm with a Turner nephelometer measuring the turbidity.

b. Candida cypolytica - Same as "a."

c. Staphylococcus aureus (F265) - Same as "a."

d. Aspergillus niger - Streaked onto TSY plates and incubated for 72 hours at 35°C for spore growth. Spores washed from plates with sterile PO_4 buffer (0.125%) and the turbidity was measured on this suspension.

2. Sampling Procedure

One gram of the sample was asceptically transferred into 9 ml of (0.125%) phosphate buffer. Surface plates on TSY agar were made from .1 ml of this solution in duplicate. All organisms were incubated at 35°C for 24 hours except for the Pseudomonas which were incubated at 22°C for 48

TABLE 18

Changes in Ascorbic Acid during Freeze-drying

System A_w	Before FD (mg)	After FD (mg)
0.51	100.4	99.6
0.67	97.73	99.6
0.75	99.73	99.14
0.84	95.0	97.9

hours. The population curve vs O.D. is shown in Figure 10.

3. System Preparation

Overall the method used was to take a semi-solid food (Heinz pork commercial baby food was used) and add to it enough glycerol to lower the water activity to the desired level. To weighed portions of this the microorganisms were added and then the portions were divided into 2 oz screw cap storage jars. About 5-6 grams were placed in each jar. The samples up to this point comprise the direct mix procedure, i.e. the desorption process. To obtain the adsorption samples, one-half the jars from each treatment before additon of the microbe were frozen, freeze-dried, and then rehumidified back to the same water activity and inoculated. These were then placed in storage (about 3 days later) with the direct mixed systems usually at 22°C. Samples were then taken periodically for testing.

a. Direct Mixing (Desorption Process) (DM)

The food was removed from the jars as purchased, and combined in a Waring Blender jar. To this water was added. Enough glycerol was added to give an A_w of 0.68 as measured on the VP apparatus. To this system water was then added to increase the A_w to give higher A_w systems. Systems at 0.68, 0.75, 0.84, 0.89 and 0.94 water activity were made. The composition used is shown in Table 19. In each system the glycerol to pork solids ratio is kept constant.

For the actual storage test the procedure was done using aseptic techniques using a sterile blender jar and mixing for 3 minutes. After dividing in half, half of each system was then divided into four sterile blender jars and a different test organism added to each. Inoculation was

FIGURE 10

STANDARD CURVE

MICROBIAL POPULATION - 24hr at 25°C

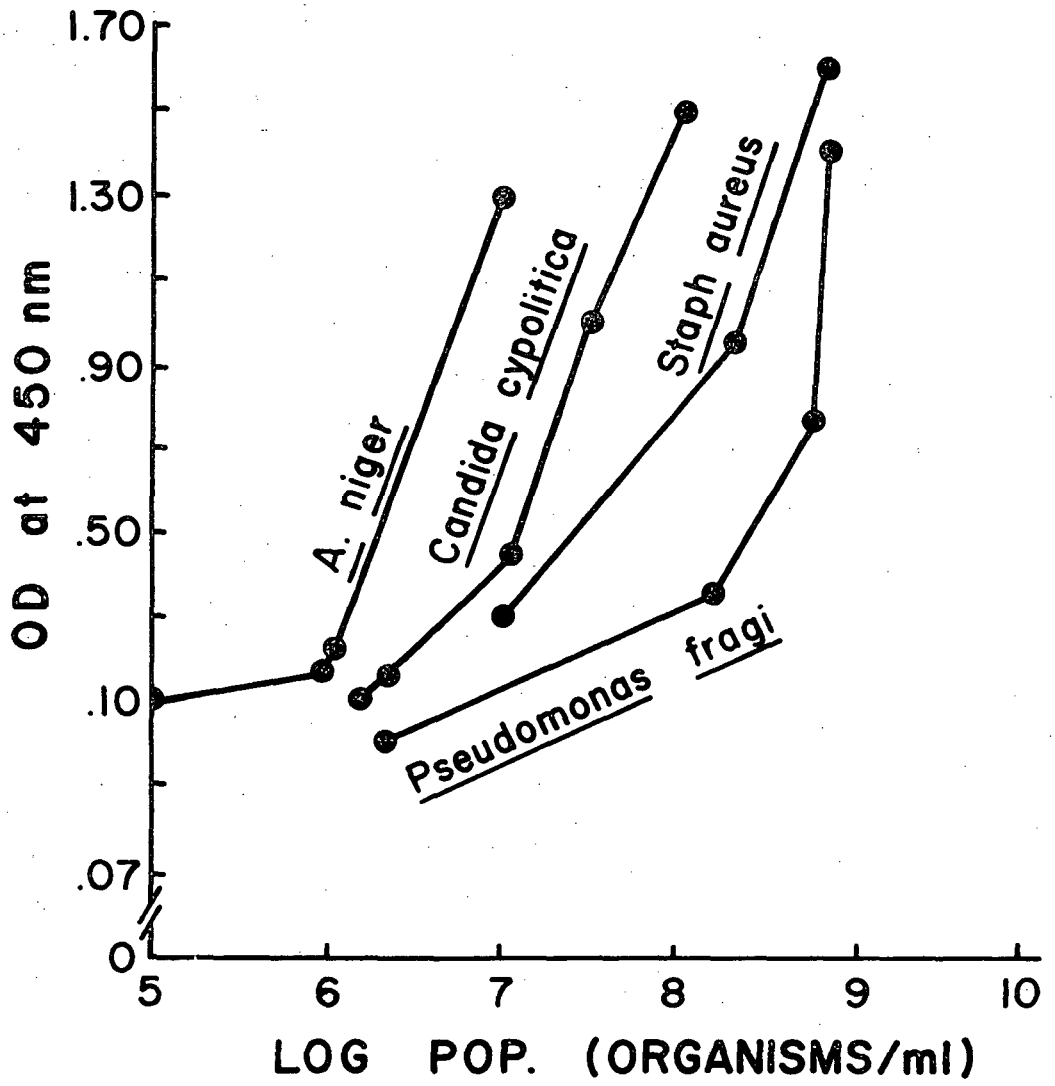


FIGURE 10. POPULATION DENSITY CURVE FOR TEST ORGANISMS

TABLE 19

Pork System Composition

Run M1

A_w System*	Pork	Glycerol	g H_2O Added
0.67	5 g	7 g	0.0 g
0.71	5 g	7 g	0.4 g
0.81	5 g	7 g	1.4 g
0.86	5 g	7 g	3.0 g
0.92	5 g	7 g	7.0 g

*As measured after 4 weeks storage. Storage in 2 oz. screw cap jars, taped with electrician's tape.

Heinz Pork Baby Food

M content g H_2O/g solids 3.88

% H_2O 79.4%

done at an approximate level of 10^5 cells/gram solids of the particular system. This was blended for 3 minutes and 2.5-3 g were transferred into sterile 2 oz screw cap storage jars and sealed with electrician's tape. The jars were stored at 21 $\pm$ 1°C either capped or in desiccators at the appropriate humidity to insure no loss of water and equilibirum.

b. Freeze-dried Rehumidified System (FDR)

The other half of the direct mix system prepared above was divided into four parts but not inoculated with microbes. Known weights were transferred into 2 oz sterile storage jars, frozen at -20°C and freeze-dried at 75°F, 200 μHg for 18 hr so that no glycerol was lost. The vacuum was broken with nitrogen through a bacteria filter and the jars were capped immediately. The samples were then partially rehumidified with a suspension of the test microorganism to give an initial count of 10^4 to 10^5 cells/gram solids. The amount of water used was 5% less than that needed for equilibrium as found by previously humidifying test samples. The jars were then held open in the various desiccators at 22°C for final rehumidification and then placed in storage.

The water activities were checked as described above. The isotherms obtained are shown in Figure 11 and the moisture contents obtained in the actual run in which all the samples were held for 3 days to 3 weeks in the desiccator are shown in Table 20. Some differences can be seen from the initially prepared isotherm for the moisture contents so that all samples were returned to the deisccators and held for 2-4 weeks with the caps open. At that time the A_w was again measured with the results shown in Table 21. The slow equilibration is probably due to the high glycerol content of the systems. Although pulling a vacuum on the desiccators

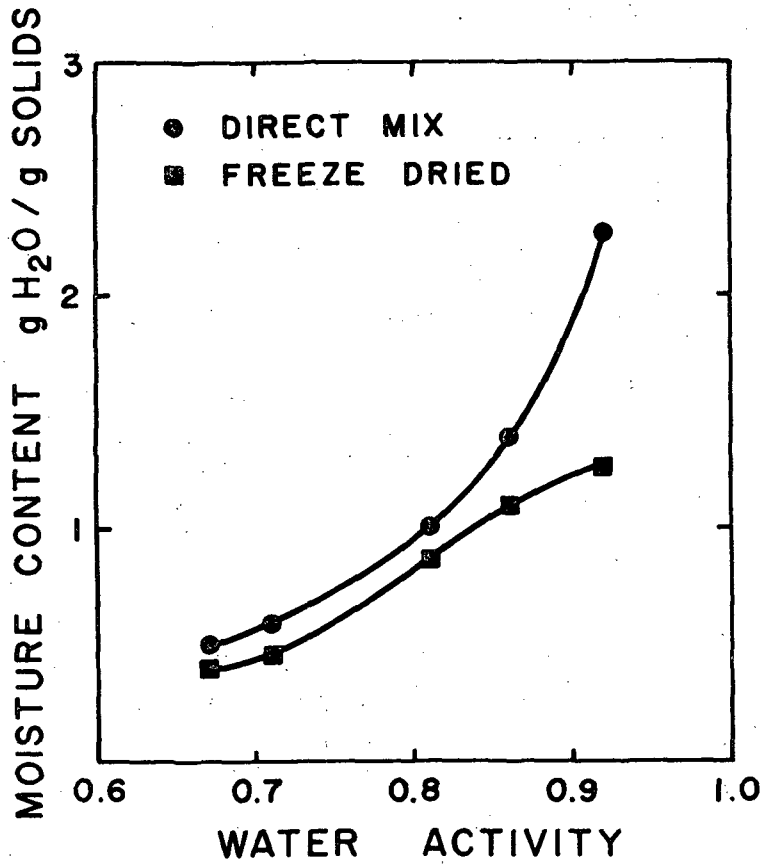


FIGURE 11. MOISTURE SORPTION ISOTHERM FOR PORK-SLURRY MODEL SYSTEM

TABLE 20

Pork IMF

Run M1

Moisture Content

A_w System (Measured)	DM* g H_2O/g solids		FDR** g H_2O/g solids	
	(GLC)	(From isotherm)	(GLC)	(From isotherm)
0.67	0.50	0.50	0.42	0.42
0.71	0.58	0.62	0.46	0.58
0.81	1.01	0.92	0.87	0.90
0.86	1.39	1.39	1.11	1.09
0.92	2.28	2.57	1.27	1.25

*DM - direct mix

**FDR - freezedried humidified

Table 21

Pork IMF

Run M1

Expected A_w	FDR A_w (3-6 days)	FDR A_w (4 weeks)	Measured A_w (Average)
0.68	0.65	0.68	0.67
0.75	0.70	0.72	0.71
0.84	0.80	0.81	0.81
0.89	0.86	0.86	0.86
0.94	0.90	0.93	0.92

might have increased the rate of equilibration, it was not done to prevent undue stress on the microorganisms.

IV. Results and Discussion

A. Production Characteristics

1. Soak Infusion Techniques

Hollis et al. (1968, 1969) developed procedures for soaking diced food materials in various low water activity solutions to obtain foods of intermediate moisture characteristics. These techniques were refined and studied in the present investigation in order to perfect a reproducible method which gives an acceptable product. The chicken used was a canned, sterile product (Aslesan's Deluxe Banquet Chicken Stock No. 3020, Minneapolis, Minnesota). The fat and moisture analyses are shown, respectively in Tables 22 and 23. As can be seen, there is quite a bit of variability, especially with the fat content. This will lead to variability in production of an IMF system as will be seen.

(a) Cook-Soak Method

Fresh chicken (from local supermarket) was cut into 1-1.5 cm pieces and was cooked for 15 minutes in the desired solution using 1 pt chicken to 2 pts (w/w) solution. The solution containing the chicken was then refrigerated for 16-18 hr at 4°C. After soaking, the pieces were drained, patted dry and then the A_w was measured on a 10 g sample.

In Run C1 the infusion systems used were based on the compositions developed in NASA Contract NAS 9-9426. The composition used is shown in Table 24 with the results obtained. Duplicate systems were tested. As seen in Table 24, the 18 hour soaking was not enough to lower the A_w to the desired levels.

TABLE 22

Aslesans Deluxe Banquet Chicken

Stock No. 3020

(2 lb cans - sterilized)

Chicken Sample Size (g)	Fat Analysis	% Fat (wet basis)
1.5	Freeze-dried Whole:Extracted Whole Batch A	16.67
1.5	"	20.53
1.5	"	21.33
3.0	"	16.50
3.0	"	15.18
3.0	"	14.19
1.5	Freeze-dried:Broken Up:Extracted Batch B	21.71
3.0	"	19.33
3.0	"	22.11
3.0	"	26.49
5.0	"	20.16
5.0	"	22.02
5.0	"	18.40
10.0	Ground in Omni Mixer:Freeze-dried Batch C	15.81
10.0	"	17.41
10.0	"	19.08
10.0	"	19.62
10.0	"	20.62
10.0	"	18.09
10.0	"	19.40
10.0	Extracted Fresh	22.00

AVG = 19.21% Fat

TABLE 23

Fresh Chicken

Moisture Content (gH_2O/g solids)

Sample	Method		% Fat in Batch
Batch A (Run 9C)	A*	B**	
I	1.761	1.772	23.6
II	1.666	1.754	17.0
III	1.670	1.788	16.9
IV	1.705	1.652	19.6
AVG	1.70	1.74	19.3
Batch B (Run 9C)			
I	1.815		23.1
II	1.849		26.3
III	1.786		23.9
IV	1.887		25.6
AVG	1.83		24.7
Batch C (Run 6C)			
I	1.94	1.94	
II	1.89	1.91	
III	1.91	1.87	
IV	1.95	1.87	
AVG	1.92	1.90	

Overall Average - 1.82 gH_2O/g solids

*A - Freeze-dried: 24 hr at 80°F, 100 μHg
**B - Vacuum Oven: 18 hr at 29" vac, 70°C

TABLE 24

Chicken Infusion

Systems Run C1

A_w (extrapolated)	0.68	0.75	0.84
Composition (grams)			
Glycerol	345	303	212
H_2O (deionized)	205	247	338
NaCl	15	15	15
Chicken Bouillon	27	27	27
K-sorbate	3.6	3.6	3.6
g H_2O/g solids	0.52	0.71	1.31
Measured A_w - Solution A	0.67	0.74	0.86
Measured A_w - Solution B	0.68	0.75	0.84
Measured A_w - Chicken Dice			
18 hours - 4°C	0.81	0.84	0.84
38 hours - 4°C	0.73	--	--
% Glycerol in solution	57.9	50.9	35.6

In Run C2 it was decided to increase the glycerol levels in each soak solution and keep the soak time constant. The composition used and results obtained are in Table 25. The same cook-soak method was used. As in the previous test, although the A_w could be lowered it could not be controlled to give the range of desired values. This could be due to the tremendous shrinkage occurring during the cooking with possible case hardening at the chicken surface. Hollis et al., (1968) used this method but did not report on different A_w levels. This procedure was thus abandoned as being unreliable as well as producing a very tough chicken texture.

(b) Soak Infusion Procedure (Preliminary)

In order to insure a consistent chicken source 100 lb of Aslesans Diced Chicken was obtained. This is a canned sterile product consisting of light and dark meat. The light meat was separated out and cut into 1-1.5 cm^2 pieces asceptically. To 50 g chicken pieces in a beaker, 500 ml of an infusion solution was added and the system was allowed to soak for various times and temperatures. The pieces were then removed, drained, patted dry and the A_w was measured. The solutions used were made based on a different principle. The amount of glycerol needed to give an A_w of 0.68 in the solution was determined and then this was kept constant in all the higher A_w systems in relationship to the solids level. Thus, only water content was changed. The systems used are shown in Table 26 along with the results. It can be seen that at 4°C and 35°C equilibrium is nearly reached in 24 hours, further soaking at 4°C did not improve the results. It should be noted that the pieces were not tough although the taste was somewhat sweet.

TABLE 25

Chicken Infusion System

Run C2

	System (% Glycerol)			
Composition (grams)	51%	64%	68%	72%
Glycerol	30.4	38.1	40.5	42.9
H_2O (deionized)	24.6	16.9	14.5	12.1
NaCl	1.5	1.5	1.5	1.5
Chicken Bouillon	2.7	2.7	2.7	2.7
K-sorbate	.36	.36	.36	.36
Measured A_w - Chicken				
18 hours @ 4°C	0.82	0.70	0.72	0.72

TABLE 26

Chicken Infusion System

Run C3

	A_w Theoretical				
% Composition	0.68	0.75	0.77	0.84	0.89
H_2O (deionized)	35.3	40.1	42.8	54.8	63.0
NaCl	2.5	2.4	2.3	1.8	1.5
Chicken bouillon	4.5	4.3	4.1	3.2	2.6
K-sorbate	0.6	0.6	0.5	.4	0.3
Glycerol	57.0	52.7	50.4	39.8	32.5
Measured A_w of solutions	0.69	0.75	0.77	0.84	0.89
A_w of Chicken after Treatment					
4°C - 24 hours	0.71	0.73	--	0.83	0.87
4°C - 48 hours	0.71	0.74	--	0.81	--
35°C - 24 hours	0.68	--	0.74	0.82	0.87

(c) Soak Infusion Studies

(1) System Preparation

The composition of the soak solutions used in these studies are reported in Table 27. The A_w's for the solutions were measured at 21 ± 1°C. In each case a ratio of 10:1, weight of infusion system to weight of fresh chicken, was used and the chicken was equilibrated under the conditions stated in Table 28. These systems constitute the direct mix (DM) preparation.

The weight change after soaking was measured after filtering the mixture in a suction flask using 8 layers of cheesecloth. The data is somewhat variable as seen in Table 28 because the pieces tend to break up slightly during soaking and some of this material passes through the filter system. This was especially true in the early runs (Run C4 and C5) where filter paper was used instead of cheesecloth.

To prepare the adsorption systems, after filtering, the chicken was frozen at -20°C and then freeze-dried at room temperature and 100 μHg pressure for 15 hr. This was found by Heidelbaugh and Karel (1970) to be mild enough to prevent glycerol loss. The systems after drying were then humidified over the appropriate salt solution to give as close as possible the same A_w as was found for the respective direct mix system. The salts used are listed in Table 29.

In order to check glycerol loss an infusion soak system was prepared according to Table 30. Chicken was soaked for 18 hr at 21°C and then after draining soaked again in a fresh infusion for the same time to insure adequate equilibration. A portion of the chicken was then freeze-dried for 15 hr at 25-27°C.

TABLE 27

Chicken Infusion Systems

% Composition	A_w Theoretical 0.68	0.69	0.73	0.75	0.77	0.84	0.88-0.89	0.94	0.95	0.96	0.99
H_2O (deionized)	34.4	35.3	40.1	41.8	42.8	54.8	63.0	75.1	78.8	78.8	88.6
NaCl	2.5	2.5	2.4	2.3	2.3	1.8	1.5	1.0	0.8	0.8	0.45
Chicken Bouillon	4.5	4.5	4.3	4.1	4.1	3.2	2.6	1.7	1.5	1.9	0.81
K-sorbate	0.6	0.6	0.6	0.5	0.5	0.4	0.3	0.3	0.2	0.2	0.2
Glycerol	57.9	57.0	52.7	51.4	50.4	39.8	32.5	21.9	18.7	18.3	10.9
Measured A_w	0.68	0.69	--	0.75	0.77	0.84	0.89	0.94	0.95	0.96	0.99

TABLE 28

Soak Infusion Methods

Run #	Method	°C	Chicken (g) To Soak Solution (g)	% Weight Change at Given A_w 0.68	0.75	0.84	0.89	0.94
C4	Rotary Shaker*- 24 hr	4	50/500	+12.7	+14.3	+9.7	+6.3	--
C5	Rotary Shaker - 24 hr	35	50/500	+10.1	+ 8.9	+3.0	-7.0	--
C6	Rotary Shaker - 18 hr	35	45/450	+ 7.5	+ 6.4	-4.2	-1.6	--
C7	Rotary Shaker - 18 hr	35	25/250	+13.4	+ 7.0	-0.5	-6.4	--
C8	Rotary Shaker - 18 hr	35	25/250	+10.0	+ 9.0	+4.0	+1.0	--
C9	Rotary Shaker - 18 hr	21	50/500	+11.7	+10.3	+4.3	+4.9	--
C10	Rotary Shaker - 18 hr	21	55/500	+13.5	+10.1	+6.7	+3.8	+0.7

*100 cycles/min

TABLE 29

Saturated Salt Solutions

Salt Used	Theoretical A_w at 20°C	Measured A_w at 21°C
$CuCl_2$	0.68	0.69
NaCl	0.75	0.76
$CdCl_2$	0.82	0.83
K_2CrO_4	0.88	0.87
KNO_3	0.95	0.92

TABLE 30

Glycerol Test - Soak System

Composition (grams)	System A	System B
Water	34.4	78.8
NaCl	2.5	0.8
Bouillon	4.5	1.5
K-sorbate	0.6	0.2
Glycerol	58.0	18.7
Ratio: infusion/chicken	10/1	10/1
A_w infusion	0.68	0.96
A_w chicken	0.65	0.93
% Δ wt after soak	+9.6	-4.1
Infusion		
g glycerol/100 g infusion	57.95	18.66
g H_2O/100 g infusion	34.43	78.79
g solids*/100 g infusion	7.6	2.5
Soaked Chicken (calculated)**		
g glycerol/100 g	38.63	12.28
g H_2O/100 g	22.95	51.93
g solids/100 g	38.91	35.77

* Not glycerol

**Original moisture 1.94 gH_2O/g solids

Table 30 shows the theoretical composition of the soaked chicken based on complete replacement of the internal fluid with infusion based on the composition given in Table 27. Table 31 shows the results of glycerol analysis compared to the theoretical calculations. For the direct mix it can be seen that extraction was complete when 80 ml of methanol were used, the measured value being very close to the theoretical predicted value. The results of glycerol analysis after freeze-drying are also very close to the direct mix system showing that very little glycerol has been lost. Any differences could be due to the increase in solids content due to the salts in the infusion system and any inherent error in glycerol analysis ($\sim$ 2-3%).

(2) Results of Soak Infusion

The results of the A_w achieved in Runs C4 through C10 for the direct mix soak infusion systems are shown in Table 32. The moisture contents achieved are presented in Table 33 along with the results for the humidified systems.

In Run C4 at 4°C it can be seen that the systems had not totally equilibrated even after 48 hr as was found in Run C3. This is due to the slow diffusion rate. By using a higher temperature as in Runs C5 through C8 the diffusion rate is speeded up and near equilibrium occurred in 18 hours.

In Run C6 after an 18 hr equilibration some pices were held for 24 hr at 4°C. As seen in Table 32, very little change occurred showing that the internal distribution of glycerol was adequate. The unusual aspect is that in many cases the measured A_w of the piece is less then that of the soak solution. This is possible if glycerol concentrates

TABLE 31

Effect of Preparation Method on Glycerol Content

	A_w 0.65		0.93	
	Theoretical	GLC	Theoretical	GLC
% Glycerol (Direct Mix) (g glycerol/g direct mix)	0.38	0.39	0.12	0.13
Glycerol Content (g glycerol/g solids)				
Direct mix	0.99	1.01	0.35	0.36
Freeze-dried		1.02		0.39

TABLE 32

Measured A_w (at 21°C) after Soaking

Direct Mix Systems

Run #	Measured A_w Soak Solution (21°C)	Soak Temperature	Hours of Soaking 18 hr	24 hr	48 hr	Equilibration 24 hr @ 4°C
C4	0.69	4°C		0.71	0.71	
	0.75			0.73	0.74	
	0.84			0.83	0.81	
	0.89			0.87	--	
C5	0.68	35°C		0.68		
	0.75			0.74		
	0.84			0.82		
	0.89			0.87		
C6	0.68	35°C	0.67			0.68
	0.73		0.72			0.72
	0.84		0.83			0.83
	0.89		0.87			0.86
C8	0.68	35°C	0.69			
	0.74		0.73			
	0.84		0.83			
	0.89		0.88			
C9	0.68	21°C	0.68			
	0.73		0.72			
	0.84		0.82			
	0.89		0.87			
C10	0.68	21°C	0.69			
	0.75		0.75			
	0.84		0.82			
	0.89		0.88			
	0.92		0.92			

TABLE 33

Moisture Content (g H_2O/g solids)

Run #	System	Method	A_w of Direct Mix System 0.68	0.72-0.74	0.82-0.84	0.87	0.92-0.94
C5	Direct Mix	(a)	0.31	0.42	0.70	0.91	
C6	Direct Mix	(a)	0.32	0.39	0.71	0.89	
	Humidified						
	(3 days)	(b)	0.30	0.31	0.45	0.55	
	(5 days)	(b)	0.29	0.31	0.50	0.67	
C9	Direct Mix	(a)	0.360	0.438	0.752	0.916	
	Direct Mix	(c)	0.500	0.543	0.788	0.981	
	Direct Mix	(d)	0.450	0.509	0.781	0.945	
	Freeze-dried - dry	(c)	0.066	0.039	0.016	0.015	
	Freeze-dried - humidified (48 hr)	(b)	0.456	0.570	0.670	0.968	
C10	Direct Mix	(a)	0.406	0.487	0.747	0.960	1.247
	Direct Mix	(c)	0.542	0.641	0.750	0.925	1.184
	Direct Mix	(d)	0.514	0.577	0.770	0.984	1.260
	Freeze-dried - dry	(c)	0.066	0.057	0.019	0.019	0.0
	Freeze-dried - humidified (48 hr)	(b)	0.435	0.485	0.572	0.608	0.916
	Freeze-dried - humidified (72 hr)	(b)	0.460	0.506	0.651	0.671	1.021
	Freeze-dried - humidified (122 hr)	(b)	0.465	0.558	0.717	0.785	1.267

(a) moisture based on grams lost in freeze-drying divided by grams left
(b) moisture based on weight gained in desiccator divided by dry solids weight
(c) by GLC method - grams water divided by weight not H_2O in product
(d) weight loss in freeze-drying plus moisture left in dry product on grams solids basis

at the surface or the measurement techniques causes a significant loss of water. Since neither occur and the difference is only 0.01 units , the problem may only be experimental error. The same conditions were used in Runs C7 and C8 and although the measured weight change after soaking varied, the A_w achieved was the same (Table 32).

In the previous runs 35°C was used. This could cause a problem if it stimulated growth of organisms that could cause food poisoning. Therefore, in the remaining tests, a lower temperature, 21°C, was used. This is also the temperature used for measurement of the A_w so that any influence of temperature on A_w would be negligible.

In Run C9 the water activities are close to the desired values but again are lower than the solution. These were measured after 24 hr equilibration at 4°C after soaking at 21°C. The soak systems were also used to make the freeze-dried humidified systems. As seen in Table 34, the desired humidities come close to, but did not exactly match, the A_w of the soak system. The moisture values for both the direct mix and freeze-dried systems are given in Table 33. Using the weight loss of drying gives a lower moisture by GLC since some water is retained. When this is accounted for the GLC values compare fairly well. As expected, the moisture for the FDR system is less due to hysteresis. The isotherm is shown in Figure 12 and is similar to that found by Wolf et al. (1972).

Run C10 was a repeat of some of the conditions of Run C9 to test reproducibility. As seen, in Tables 32 and 33 the results show fairly good reproducibility. The humidified samples gave closer agreement to the expected A_w of the desiccator after holding for 5 days, however,

TABLE 34

Measured A_w after Humidification

Run #	A_w of Soak System	A_w of Desiccator	A_w of Humidified Sample after 2 days	3 days	5 days
C9	0.68	0.69	0.72		
	0.72	0.75	0.77		
	0.82	0.83	0.80		
	0.87	0.87	0.85		
C10	0.69	0.68-0.69	0.66	0.66	0.67
	0.75	0.75-0.76	0.73	0.74	0.74
	0.82	0.82-0.83	0.77	0.80	0.82
	0.88	0.87-0.88	0.81	0.83	0.84
	0.92	0.94-0.95	0.88	0.91	0.93

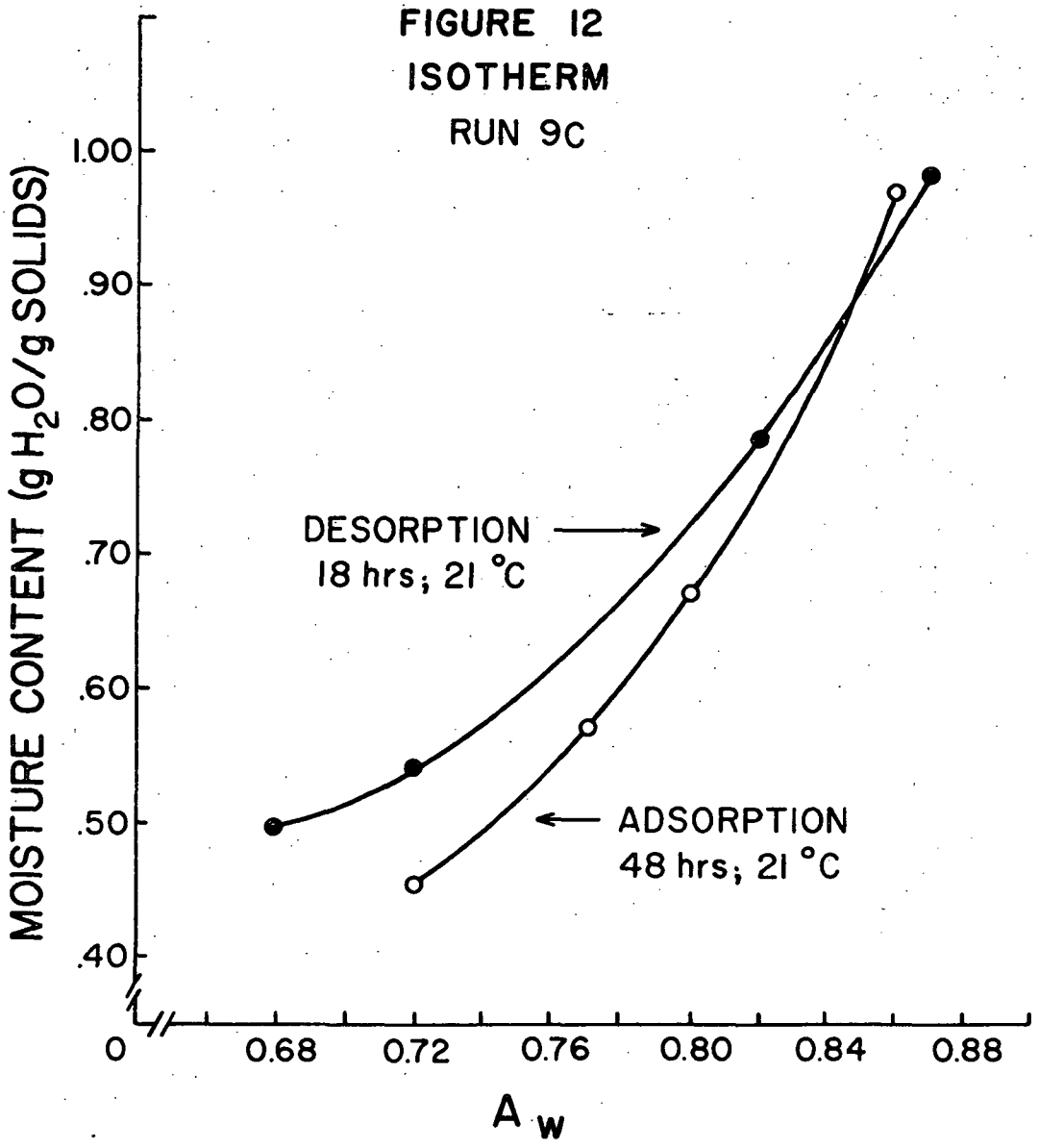


FIGURE 12. MOISTURE SORPTION ISOTHERM FOR SOAK-INFUSION CHICKEN SYSTEM - RUN 9C

the amount of hysteresis is reduced. The isotherm for Run C10 is shown in Figure 13. It has less hysteresis than Run C9 due to the longer equilibration time.

The system developed here shows good reproducibility in spite of the variation in the chicken itself. This system was used for testing of stability.

2. Extrusion System Studies

Chicken IMF systems were prepared using the industrial technique for IMF pet foods. A Wenger X5 extrusion cooker was set up with a pressure feed device and a steam jacketed system. A system of up to 8 head lengths is possible. Each head has a 44 mm length with an inside diameter of 25 mm yielding a maximum length/inside diameter ratio of 14/1 Each of these is individually controlled for temperature and pressure and can either be steam heated up to an internal temperature of 300°F or cooled with tap water. These are all connected to the control unit of the DEC PDP8 computer for control and collection of the data. Feed rate and, therefore, residence time are two other variable parameters. Residence time can be adjusted from 3 to 5 seconds. Each section inner tube wall is either spirally wound or parallel fluted for variation in heat transfer and mixing. However, with the type of product used, no differences occur with either type of wall. At the exit head is a die which can be varied in size and a cutter which automatically cuts off lengths of the extruded system.

Several extrusion systems were prepared to test the operation of the device and the suitability of the product.

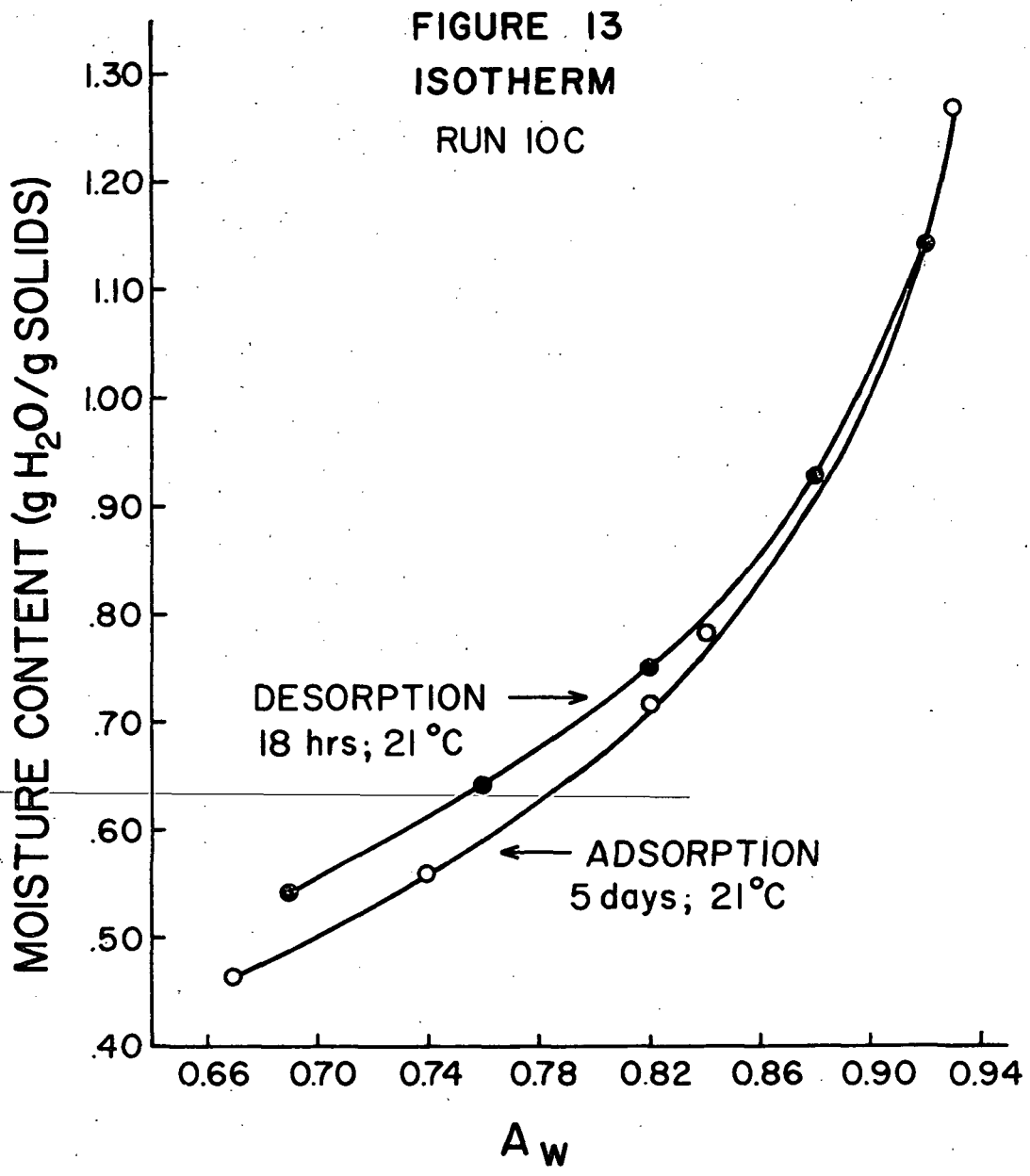


FIGURE 13. MOISTURE SORPTION ISOTHERM FOR SOAK-INFUSION CHICKEN SYSTEM - RUN 10C

Run E1

Two systems were studied as shown in Table 35 using both sterile chicken and chicken which had been freeze-dried at 80°F for 17 hr and broken into 1 cm pieces. The freeze-dried chicken was ground to a powder in a Hobart commutator (Model 8418ID). Each system was mixed in the commutator and then was cooked extruded according to the conditions shown in Table 36. The consistencies of both products was similar to a pasty puree which made them unacceptable.

As seen in Table 35, the infusion was based on the composition for A_w = 0.75 used in the soak infusion systems. Less water is used, however, in the direct mix since water is present in the chicken itself. The results show that, unfortunately, not enough water was removed in the formulation. The freeze-dried chicken itself lowered the A_w of the infusion by 0.07 A_w units due to its own internal soluble solids to give a final satisfactory value, however, the consistency was unacceptable.

Run E2

In Run E2 two systems were prepared using the freeze-dried chicken method (dried 17 hr at 32°C 100 μHg). The compositions used and A_w's obtained are shown in Table 37; the extrusion data are in Table 36. Although an A_w of 0.75 and 0.84 were theoretically prepared the results show similar water activities for the extruded product. This could be possible due to the variation in the chicken composition itself or the degree of hydrolysis of the chicken during the cook extrusion. Both systems were too pasty to be acceptable and had a very sweet flavor.

Run E3

In order to get systems closer to the desired A_w, the same systems

TABLE 35

System Composition for Extrusion

Run E1

Composition (grams)	Direct Mix System	Freeze-dry System
Water	387.21	457.14
NaCl	17.83	17.82
Bouillon	31.93	31.90
K-sorbate	4.15	4.14
Glycerol	414.63	414.28
Chicken	940.0	320.0*
Ratio infusion/chicken	91/100	240/100
A_w		
Infusion	0.76	0.80
Post-extrusion		
3 hr at 21°C	0.88	0.78
24 hr at 21°C	0.88	0.77
48 hr at 21°C	--	0.73
Moisture Content gH_2O/g solids	0.36	0.58

*freeze-dried

[illegible] Experiment Record and Data Sheet

Operator ____________________ Date ________

Run No. ____________________ Project ________

	Run 1E	Run 2E	Run 3E	Run 4E	Run
Head 1 (Inlet) Type / Heat or Cool	**Fluted Cool**	**Fluted Cool**	**Fluted Cool**	**Fluted (st) Cool**	
Head 2 Type / Heat or Cool	**Fluted Heat**	**Fluted Cool**	**Fluted Cool**	**Fluted Cool**	
Head 3 Type / Heat or Cool	**Spiral Heat**	**Fluted Heat**	**Fluted Heat**	**Fluted Heat**	
Head 4 Type / Heat or Cool	**Fluted Heat**	**Spiral Heat**	**Spiral Heat**	**Spiral Heat**	
Head 5 Type / Heat or Cool	**Spiral Heat**	**Fluted Heat**	**Fluted Heat**	**Fluted Heat**	
Head 6 Type / Heat or Cool	**--**	**Spiral Heat**	**Spiral Heat**	**Spiral Heat**	
Head 7 Type / Heat or Cool	**--**	**Fluted Heat**	**Fluted Heat**	**Fluted Heat**	
Head 8 [illegible] Type / Heat or Cool	**--**	**Spiral Heat**	**Spiral Heat**	**Spiral Cool**	
[illegible] pressure [illegible]	**Short 4 relief**	**Long 1 relief**	**Long 1 relief**	**Long 1 relief**	
[illegible] standard, [illegible]	**Std.**	**Std.**	**Std.**	**Std.**	
[illegible]	**No**	**Yes**	**Yes**	**Yes (not used)**	
[illegible]	**None, 1/8**	**1/8, 11/64**	**11/64**	**5/52**	
[illegible] RPM's	**400**	**400**	**350**	**350**	
Torque	**--**	**--**	**--**	**--**	
Temperatures	**140°F (outlet)**	**135-140°F (outlet)**	**155°F (outlet)**	**See Comments**	
[illegible]	**--**	**--**	**--**	**--**	
[illegible]	**70 psig (300°F)**	**70 psig (300°F)**	**70 psig (300°F)**	**70 psig (300°F)**	
Water Pressure	**70 psig (55°F)**	**70 psig (55°F)**	**70 psig (55°F)**	**70 psig (55°F)**	

No.	Run 1 E	Run 2 E	Run 3 E	Run 4 E	Run 5
- Solid Composition	Chicken	Chicken	Chicken	Chicken	
(Setting) Solid Feed Rate	20-25	15-25	15-20	5-10 (works better)	
Liquid	--	--	--	--	
Liquid Feed Rate	--	--	--	--	
w Speed	--	--	--	--	
Reading	12	12	12	12	
shed Product Processing?					
l Product Characteristics					
Moisture					
Density					
gth of Run	10 min	20 min	15 min	15 min	

ENTS:

hermocouple in second plate.

anitation by heating the barrel to 200°F for .5 to 1.0 hr and running chlorine solution approximately 200 ppm) prior to product.

ifficulties with smaller dies due to clogging.

4
.84 product outlet 110°F
.89 product outlet 120°F
.94 product outlet 122°F

e: Fluted or spiral refer to barrel configuration: Parallel ribbing on the inside face is referred to as fluting and spiral or screw-type ribbing is called spiral. The shape d to affect the types of products run by mixing differently; the effect is marginal, ever.

TABLE 37

System Composition and A_w Extruder Runs

Run E2

Composition (grams)	System A	System B
Infusion		
Water	91.85	109.6
NaCl	4.99	3.6
Bouillon	8.98	6.4
K-sorbate	1.19	0.8
Glycerol	112.99	79.6
Chicken	100	100
Measured A_w	0.76	0.82
Theoretical A_w	0.75	0.84
Ratio: infusion/chicken	220/100	200/100
A_w - Extrusion Product		
Post Extrusion		
0 hours	0.69	0.73
3 days	0.71	0.72
Moisture Content gH_2O/g solids	0.40	0.58

as in Run E2 were used but the ratios were changed slightly as shown in Table 38. Another system was also added at A_w 0.87. In addition, 2 g garlic powder were added to each system to mask the sweet taste. The extruder conditions are shown in Table 36.

As seen in Table 38, all products gave a lower A_w when mixed and extruded as would be expected, however this makes it difficult to predict the compositions needed for a direct mix vs freeze-dried humidified system. It is possible to make the latter by drying the product after extrusion and humidifying to see if a hysteresis phenomenon exists. The A_w's produced are in an acceptable range.

In terms of consistency, all products were fairly acceptable with A_w = 0.89 having the best firmness and 0.75 being the driest and crumbliest. However, the products were still not as firm as desired. The other problem was the extreme garlic odor and flavor showing that the amount used must be reduced.

Run E4

In order to obtain a more cohesive product it was decided to change the system by adding 20 g of whey protein concentrate in place of 20 g of glycerol. The compositions used are in Table 39 and the systems were cook-extruded under the conditions in Table 36. All products were firm enough to hold their shape which can be attributed to using both the whey concentrate and using cooling water on section 8 in the extruder. The A_w's are shown in Table 39. Except for the high A_w system which is out of the intermediate moisture range, the other products are acceptable.

These studies show that an extruded product can be made under laboratory conditions. No further tests were made, however, due to

TABLE 38

Extrusion Chicken System Composition

Run E3

Composition (grams)	System A	System B	System C
Water	41.8	54.8	63.0
NaCl	2.3	1.8	1.5
Bouillon	4.1	3.2	2.6
K-sorbate	0.5	0.4	0.3
Glycerol	51.4	39.8	32.5
Garlic powder*	2.0	2.0	2.0
Ratio: infusion/chicken	210/100	220/100	250/100
	A_w		
Infusion	0.71	0.83	0.87
Extrusion Product			
1 day	0.67	0.73	0.86
6 day	0.68	0.75	0.82
Moisture Content gH_2O/g solids	0.38	0.59	0.79

*Schilling

TABLE 39

Extrusion Chicken System

RUN 4E

Composition (grams)	System A	System B	System C
Water	172.7	120.6	157.5
NaCl	2.3	4.0	3.8
Bouillon	3.9	7.0	6.5
K-sorbate	0.7	0.9	0.8
Glycerol	30.4	67.6	61.3
Garlic powder*	0.3	0.3	0.3
Whey protein concentrate**	20.0	20.0	20.0
Freeze-dried chicken	100.3	100.1	100.1
A_w - Extrusion Product			
1 day	0.94	0.90	0.82
4 day	0.91	0.89	0.82
Moisture Content gH_2O/g solids	1.09	0.60	0.82

*Schilling
**ENR-PRO 50

problems with the control unit of the extruder. Plans for Phase II include a study of processing effects on acceptability, nutrient losses and microbial death.

3. Cold Mixing IMF Technology

(a) Hennican

Many intermediate moisture foods are prepared solely by mixing the various components together usually at or near room temperature. In fact, an ancient American Indian food, "Pemmican," as described in Figure 14 from the Good Earth Almanac is such a food. Nuts, dried beef and fat, as well as berries which supplied the sugar for the humectant, were ground together to give a paste which was allowed to equilibrate. This food was shelf stable and was used both to keep the Indians through the harsh winters as well as for a hunting and travel food (Brown, 1971). Since in preliminary tests, the soak infusion chicken systems had an objectionable taste by itself, it was decided to make a new food using chicken as the base, but formulated in a manner like "Pemmican." This food was named "Hennican."

In an initial trial the formula, as shown in Figure 14 was used except for substituting freeze-dried chicken for beef. The unroasted nuts and chicken were ground separately to a fine powder in a blender jar. The rest of the components were then placed in a plastic bag and kneaded for 20 min. To this the ground material was added during kneading. The system was held at room temperature. It was very sticky and in 3-4 days developed an undesirable soapy taste. This soapiness was probably due to the enzymes released from the unroasted nuts.

THE FIRST LIGHTWEIGHT CAMPING OR "TRAVELING" FOOD WAS THE INDIAN PEMMICAN, AND IS AS VALUABLE and EASY TO MAKE TODAY AS IT WAS THEN. THE ORIGINAL PEMMICAN WAS MADE FROM BUFFALO, ELK OR VENISON JERKY and WHATEVER ELSE HAPPENED TO BE HANDY. THIS INCLUDED ALMOST ALL KINDS OF BERRIES AND NUTS AND HONEY IF IT WAS AVAILABLE.

HAVE NATURAL LIVING IDEAS OF YOUR OWN? SEND THEM TO: "GOOD EARTH ALMANAC" C/O THIS NEWSPAPER

RECIPE FOR MODERN PEMMICAN

- DRIED BEEF ..... 8 oz.
- RAISINS ..... 8 oz.
- UNROASTED PEANUTS or PECANS ..... 8 oz.
- HONEY ..... 2 tsp.
- PEANUT BUTTER ..... 4 tblsp.
- CAYENNE PEPPER ..... 3/4 tsp.

1. CUT ALL FAT FROM BEEF, THEN CUT INTO THIN SLICES. DRY IN OVEN WITH OVEN AT LOWEST TEMPERATURE and DOOR OPEN SLIGHTLY UNTIL MEAT WILL BREAK and CRUMBLE.

2. POUND MEAT INTO POWDER or GRIND USING AN ELECTRIC BLENDER.

3. ADD RAISINS, DRIED BLUEBERRIES, CHOPPED DRIED APRICOTS, PEACHES, PECANS, PEANUTS.

4. HEAT HONEY and PEANUT BUTTER TO SOFTEN IT, THEN BLEND INTO THE MIXTURE. ADD THE CAYENNE PEPPER and MAKE SURE IT IS WORKED THOROUGHLY THROUGH THE MIXTURE.

5. IF YOU WANT TO GO COMPLETELY NATURAL, PACK THE MIXTURE INTO SAUSAGE CASINGS (AVAILABLE at MEAT COUNTERS) OR YOU CAN PUT THE MIXTURE INTO PLASTIC "TIE BAGS."

6. KEEP IN A COOL, DRY PLACE. PEMMICAN WILL KEEP INDEFINITELY AND CAN'T BE BEAT AS A SNACK or LUNCHEON ON THE TRAIL.

Mark Gregory

Run H1

In order to produce a more desirable product, the composition of Hennican was changed to that of Table 40. As seen, dry roasted nuts were used to alleviate the enzyme problem. In addition, the amount of raisins was doubled in order to increase the amount of sugar and thus bind more water. The same mixing procedure was used. The texture developed was very firm without being sticky, but as seen in Table 40, the A_w was on the low side.

Run H2

In order to improve the flavor and texture other formulations were tried. Table 41 lists the composition of the basic new system. The individual components were ground separately in a blender. The sorbate was added to the honey and the peanut butter to that mixture. All ingredients were then mixed together in the blender, kneaded for 20 min and then thoroughly mixed in a Brabender 300 g Farinograph mixing bowl. To 30 g samples of the basic system in Table 41, increasing amounts of water were blended in and the A_w was measured. The results are shown in Figure 15. As can be seen, only a little water is needed to increase the A_w rapidly. The problem with the systems above A_w 0.60 were that they were very sticky and they weeped fat on standing. To alleviate this, systems were tried with added non-fat dry milk solids and the extra water at various levels. As seen in Figure 15, the A_w is slightly increased. Moreover, the samples did not weep fat and had a fairly firm texture. The system at A_w 0.76 was studied for storage stability.

TABLE 40

"Hennican" Formulation

Basic

Run H1

Component	Source	Amount (grams)
Peanuts	Holiday House Dry Roasted	222.1
Dry Chicken	Aslesans*	222.1
Raisins	Sun Maid	444.2
Peanut Butter	Skippy Creamy	60.1
Honey	Adolph's Natural Honey	24.68
Cayenne	Schilling	0.64
K-Sorbate	(~0.3%)	2.92

System A - as above

System B - 0.5% tocopherol added

Moisture Content (6 samples) = 7 g H_2O/100 g solids

Protein Content = 27.09%

Fat Content** = 12.6 to 19.8%, Avg. = 16.4

Water Activity = 0.55

*Freeze-dried before use
**Avg. of 12 samples

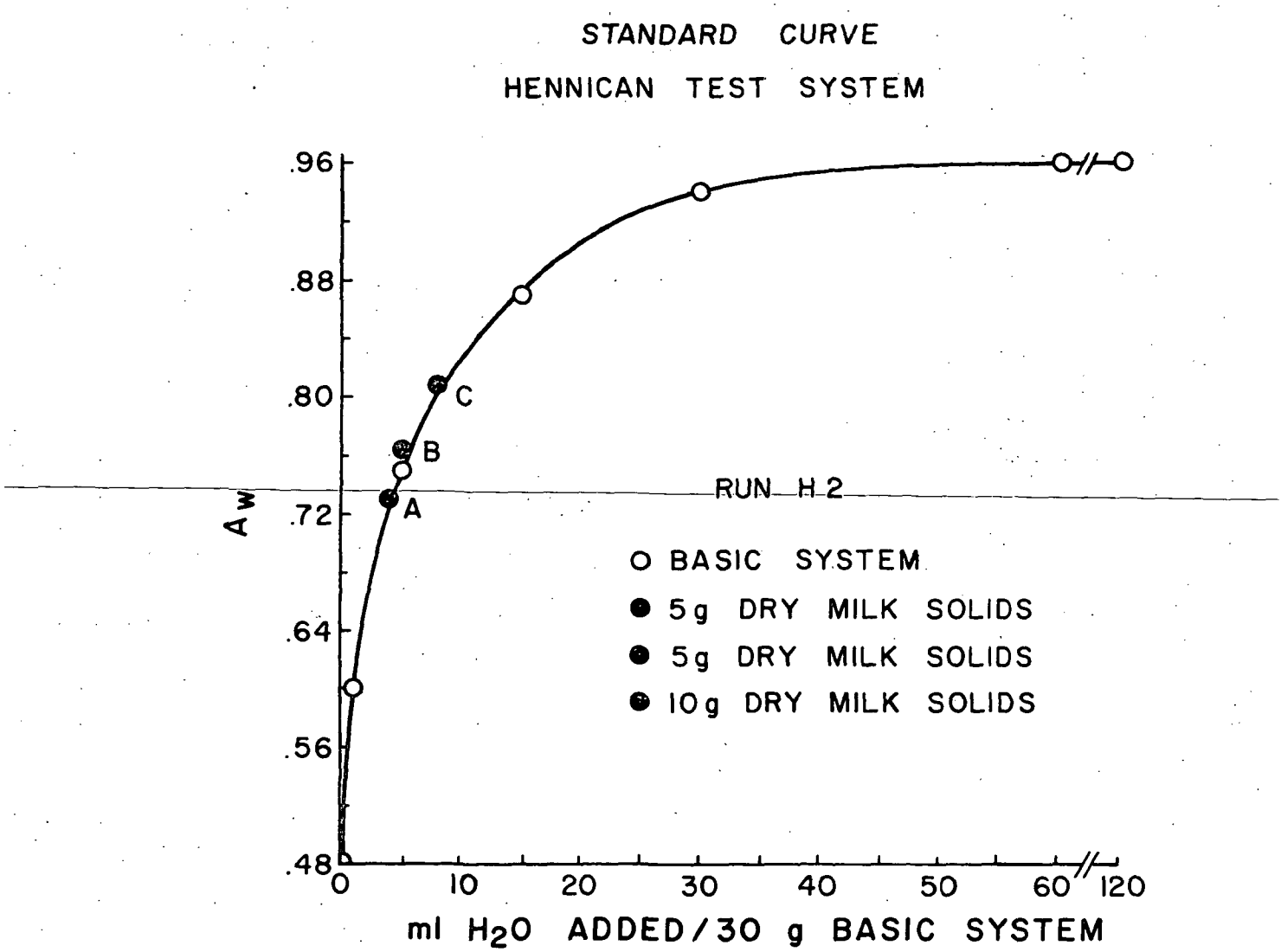
TABLE 41

Hennican Test System H2

Component	Source	Amount (g)	%
Peanuts	Planter's Dry Roast	100	22.8
Freeze-dried chicken	Aslesan's	100	22.8
Raisins	Del Monte Seedless	200	45.6
Peanut Butter	Skippy Creamy	26.4	6.0
Honey	Robb Ross Grade A	10.9	2.5
Cayenne Pepper	Durkee	0.14	0.03
K-Sorbate			0.3

A_w = 0.48

FIGURE 15. WATER CONTENT - MOISTURE ADDITION CURVE - RUN 2H



Run H3

From the results in Run H2 (Figure 15) both desorption and adsorption systems were prepared based on the composition to achieve an A_w of 0.68. The same principle as in the soak infusion systems was used. The glycerol content based on solids was kept constant and water was added to achieve higher A_w's. The mixing procedure was changed to eliminate the kneading. The preparation procedure was as follows:

a. Grind freeze-dried chicken in blender (Osterizer Classic VIII) speed-blend, using 8 oz blender jar.

b. Grind raisins using the same method. Raisins may be frozen with liquid nitrogen to facilitate grinding.

c. Grind peanuts using the above method.

d. Weigh freeze-dried chicken in a beaker, weigh in non-fat dry milk and ground peanuts (freeze-dried chicken mixture), mix with metal spatula.

e. Weigh K-sorbate and cayenne pepper in a beaker, crush using a glass rod.

f. Weigh honey and peanut butter into the beaker containing K-sorbate and cayenne pepper (peanut butter mixture), mix using a glass rod.

g. Using a large blender jar (5 cups) on the blender, add small amounts of freeze-dried chicken mixture, raisins, and peanut butter mixture. Blend (the resulting mixture will be dry). Transfer the mixture to a large beaker. Repeat this step until all components are mixed.

h. Add dry mixture to Brabender Farinograph Bowl. Add water using a volumetric pipette. Dissolved antioxidants are added at this time.

i. Knead on fast speed for 5 min. If the amount prepared is large (400-500 g) 10 min will be required.

The Hennican was prepared according to the composition shown in Table 42. The A_w's were measured on the vapor pressure device and the isotherm is shown in Figure 16. It can be seen that the curve is very steep with respect to moisture in the IMF range showing the A_w stabilizing effect of the added humectants. Table 42 also reports the moisture contents of the system. From Table 42 the system at A_w 0.68 is shown to be somewhat dry and the 0.91 system has too high a water content. This one was also sticky to the touch.

To produce the rehumidified system, the systems after mixing were formed into a sheet 0.5-0.75 cm thick, cut into bite-sized squares and were then freeze-dried 71 hr at 26°C. Samples in duplicate were then transferred into vacuum desiccators to determine the adsorption isotherm. All three systems were done in this manner to determine if initial structure would have any affect on the isotherm. The samples were weighed over a five day period to determine when equilibrium was attained. Figure 17 shows the results for the system initially prepared to 0.68 A_w. As seen, in 40 hr the system has attained equilibrium in the IMF range. The samples outside that range are still changing weight but would not be important in this study. The other systems showed similar results. The final equilibrium values are plotted in Figure 16. As seen, the direct mix system showed a higher moisture content at each A_w, but the amount of hysteresis, i.e. the difference with respect to the freeze-dried rehumidified system is very small. This is probably due to the grinding of the sample

TABLE 42

Hennican Run H3

Composition (g)	System A	B	C
Dry roasted peanuts	68.50	68.50	68.50
Freeze-dried chicken	68.50	68.50	68.50
Raisins	137.00	137.00	137.00
Peanut butter	18.11	18.11	18.11
Honey	7.44	7.44	7.44
Cayenne pepper	0.10	0.10	0.10
K-sorbate	0.90	0.90	0.90
Non-fat dry milk (5 g/30 g basic Hennican)	50.00	50.00	50.00
g H_2O added	25.00	50.00	155.00
Measured			
Moisture content (g H_2O/100 g solids)	14.35	21.48	53.53
A_w	0.68	0.78	0.91

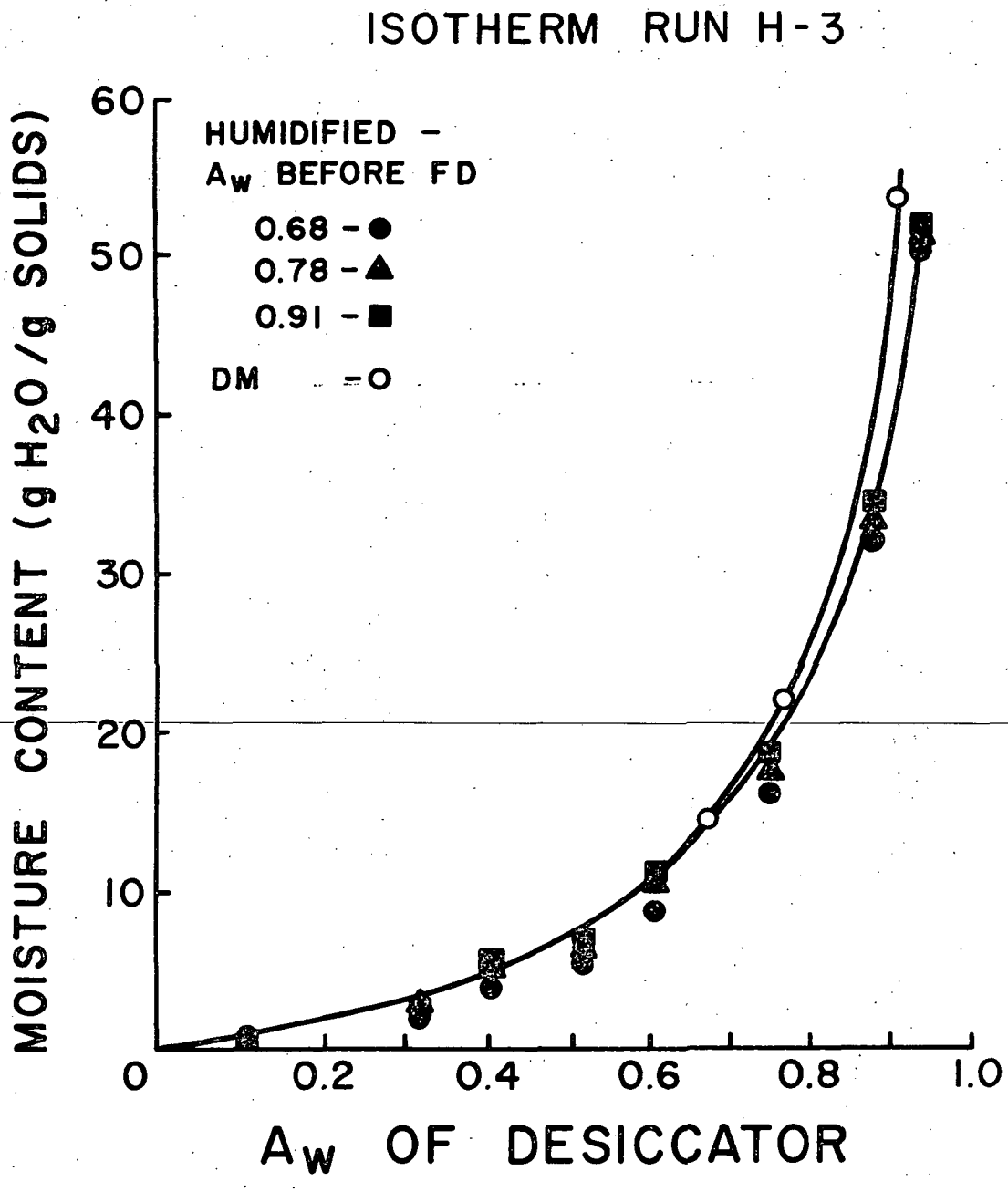
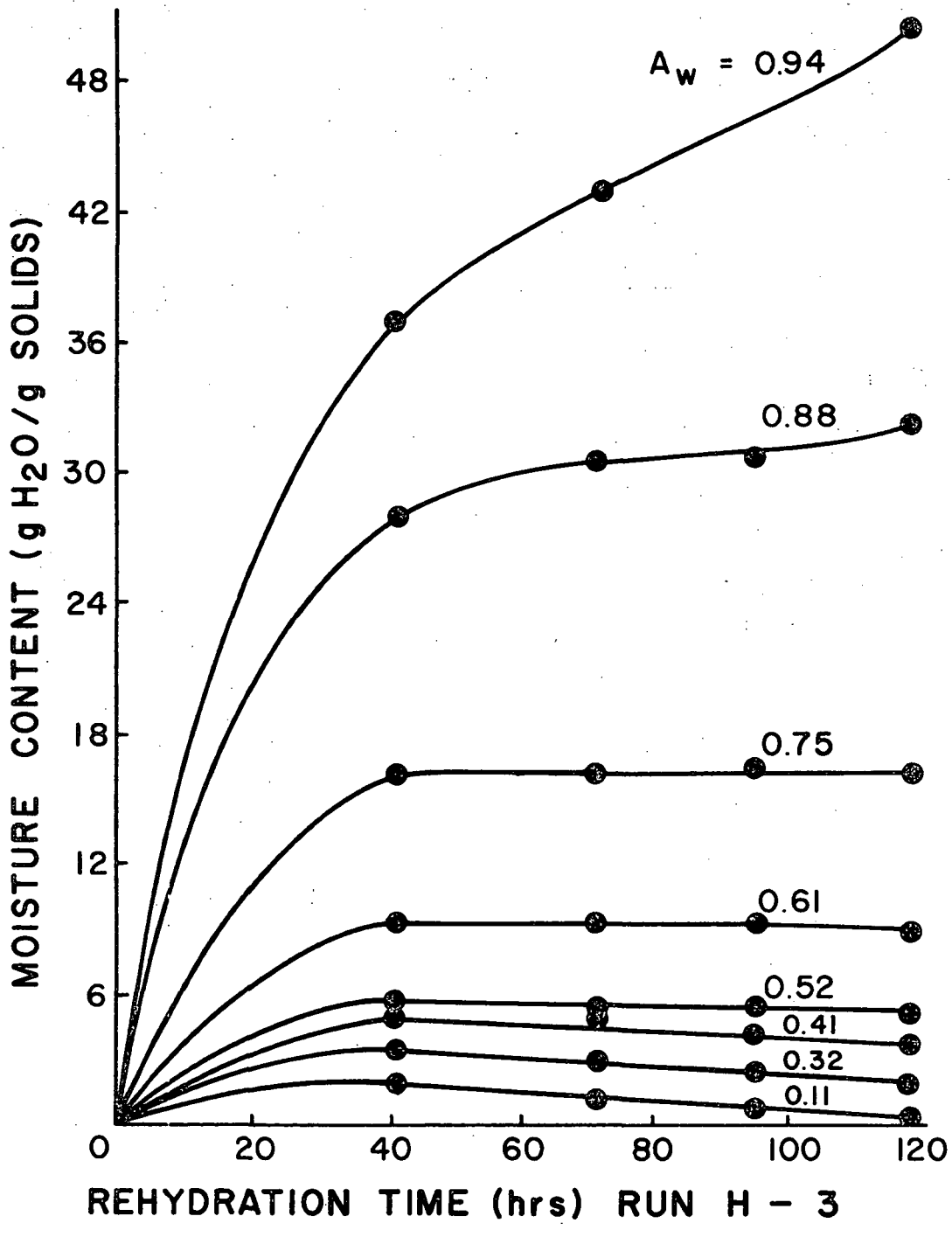


FIGURE 16. SORPTION ISOTHERM FOR HENNICAN - RUN 3H

FIGURE 17. REHYDRATION TIME FOR FREEZE-DRIED HENNICAN - RUN 3H



initially into a powder which eliminates much of the original pore structure.

The initial taste and palatability of the freeze-dried rehumidified samples were also excellent. Table 43 shows a complete analysis of the system at A_w 0.75. As can be seen, it has a high caloric density which makes it useful as an energy food. Over 3/4 of the calories come from the carbohydrate and fat so that the high protein content should not be catabolized for calories. About 300 g per day, taken partially as a meal and as between meal snacks would supply over 50% of some of the micronutrients. This system was designated as the major test system to fulfill the objectives of this contract.

(b) Granola Bars

A second IMF food product was developed that had a very high initial acceptance based on cold mixing technology. The formulation is shown in Table 44. The procedure for production is as follows:

1. Weigh components into separate beakers.
2. Add marshmallows into the bowl of the Brabender, knead on fast speed until the marshmallows become taffy-like.
3. Add non-fat dry milk
4. Add H_2O 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 throught the base (do not overmix).

This system will be tested for stroage stability in Phase II of this contract.

TABLE 43

Hennican Composition

$A_w = 0.75$

Component		%
Dry roasted peanuts		17.38
Freeze-dried chicken		17.38
Raisins		34.76
Peanut butter		4.60
Honey		1.89
Cayenne pepper		0.02
K-sorbate		0.23
Non-fat dry milk		12.71
H_2O		11.04
Antioxidant	BHA/BHT	200 ppm fat basis
	EDTA	500 ppm solids basis

	% (wet basis)
Protein	28.00
Fat	7.40
Carbohydrates	45.90
H_2O	18.70
Calories/g	3.62

Minor Nutrients

Vitamin A (IU/100 g)	14.0
Vitamin C (mg/100 g)	3.6
Thiamine (mg/100 g)	0.18
Riboflavin (mg/100 g)	0.17
Niacin (mg/100 g)	6.92
Iron (mg/100 g)	2.84

TABLE 44

Granola IMF Bars

Composition

Component	Brand	Grams	%
Granola	Chas. A. Pillsbury Basic	15.0	27.3
Marshmallows	Kraft Minature	15.0	27.3
Peanut butter	Skippy Creamy	16.0	29.1
Nonfat dry milk		5.0	9.1
H_2O		4.0	7.3

% by Weight
(based on wet weight)

Protein	14.13%
Fat	26.50%
Carbohydrates	50.38%
H_2O	8.99%

$A_w = 0.74$

B. Model System Studies

1. Lipid Oxidation in Model Systems

According to the stability map (Figure 1) lipid oxidation should be one of the primary modes of deterioration of intermediate moisture foods. The rate of deterioration is a function of many factors, the most important of which is the water content-water activity relationships.

In studying intermediate moisture foods, Labuza (1971) and Labuza et al. (1972a) found that at the same water activity, reaction rates for deterioration can be very different depending on the direction of reaching the final water activity, either adsorption by going up the isotherm or desorption by going down the isotherm from the natural moisture content. They reported that the lipids in the system on the adsorption branch of the hysteresis loop oxidized about 4-5 times slower than the desorption systems at the same water activity.

Labuza et al. (1969) have reviewed the kinetics of lipid oxidation as a function of water activity. It was shown that water was protective for dehydrated foods and as water activity increased the oxidation rate decreased. However, it was also shown that in the capillary region of the isotherm (intermediate moisture foods range) the oxidation rate increased. Labuza (1971a) discussed the chemical stability of foods as a function of both moisture content and water activity. The protective effect of water at low water activity and low moisture content was attributed to hydration of metal catalysts decreasing their effectiveness and hydrogen bonding of peroxides thus slowing the chain reaction. The accelerative effect of water at higher water activity and moisture content was postulated to be due to the soluble solids content, viscosity of the liquid phase and

swelling of the polymeric matrix. Normally, at higher water activities the water present presumably mobilizes the catalysts and swells the solid matrix exposing new catalyst sites so that the rate of oxidation increases over that of foods at lower water activity. Such a hypothesis might explain the mechanism of the faster oxidation rate for the desorption foods which are more swollen and contain more water than adsorption foods.

The purpose of this part of the study was to confirm the results found for the rate of lipid oxidation as a function of A_w in actual food systems, to elucidate the exact mechanisms and kinetics which control the rate of oxidation as affected by sorption hysteresis, and to determine the effectiveness of antioxidants. The model system approach was used in order to provide conditions more amenable to control.

Table 45 lists the parameters for the sixteen experiments performed to satisfy these purposes. The kinetics of oxidation as defined by Labuza (1971b) were used to elucidate the effects of A_w on oxidations. The parameters tested include:

1. Oxygen uptake curve
2. Induction time - time to reach either 1% (moles oxygen/mole linoleate) or 3% oxidation
3. K_M - the rate constant in the monomolecular rate period from plot of $(\text{extent})^{\frac{1}{2}}$ vs time
4. K_B - rate constant in accelerated oxidation period from plot of ln (extent) vs time

(a) Effect of Water Content - A_w on Oxidation

In the first run a repeat of previous studies done by Labuza (1971), Heidelbaugh and Karel (1970) and Heidelbaugh et al. (1971) was performed

TABLE 45

Parameters Tested in Model Systems

Run No.	Parameters Tested
1 A,B,C	Microcrystalline cellulose system: A_w = 0.75, 0.84, 0.89, 0.52 No additives (natural metals, less than 10 ppm)
2	Amylopectin system: A_w = 0.75, 0.84, 0.89 No additives (natural metals, 1000 ppm)
3	Microcrystalline cellulose system vs Amylopectin system: A_w = 0.84 No additives (see run 1 and run 2)
4	Microcrystalline cellulose vs Amylopectin system: A_w = 0.75, 0.84 Trace metals of same type in amylopectin added to cellulose to bring to 1000 ppm according to levels shown in Table 4.
5	Same as run 1 A_w = 0.75, 0.84, 0.89
6	Same as run 5 but with 20 ppm metal* A_w = 0.75, 0.84, 0.89
7	Same as run 5 but with 50 ppm metal* A_w = 0.75, 0.80, 0.84, 0.87, 0.89 (DM only)
8	Same as run 5 but with 100 ppm metal* A_w = 0.85, 0.84, 0.89 (DM only)
9	Same as run 5 but with 500 ppm metal* A_w = 0.75, 0.84, 0.89 (DM only)
10	Same as run 5 but with 1000 ppm metal* A_w = 0.75, 0.84

Table 45 (continued)

Run No.	Parameters Tested
11	Same as run 4 1000 ppm EDTA (solids basis) added to both systems (~0.08 moles/mole metal) vs controls
12	Same as run 4 0.5 moles sodium citrate/mole metal vs control
13	Same as run 4 Two antioxidant systems and control A - Isopropyl citrate (60% mono ester) 0.5 m/mole B - Sodium citrate - 0.5 m/mole
14	Microcrystalline cellulose system: A_w = 0.75, 0.84, 0.89 Additives: 100 ppm cobalt (lipid basis) in all systems Antioxidants (lipid basis): 100 ppm BHA vs 100 ppm BHT vs 50 ppm BHA plus 50 ppm BHT
15	Microcrystalline cellulose system vs Amylopectin system A_w = 0.75, 0.84, 0.89 Additives: 100 ppm cobalt (lipid basis) added to cellulose system Antioxidants: 50 ppm BHA
16	Same as run 4 A - Tocopherol 200 ppm fat basis B - BHA 200 ppm fat basis (only A_w 0.84 for cellulose) tested against control

in order to validate the test methods. In addition, studies were made at higher humidities then previously done.

Figure 18 shows the typical results obtained for oxygen absorption in Warburg manometers for duplicate flasks from Run 1A . The other runs were similar. Oxidation follows the typical autocatalytic pattern with a slow rate period followed by an increasingly faster rate. As expected, the oxidation rate increases with increased A_w. In addition, the direct mixed (DM) systems oxidized faster than the humidified systems (DH) at similar water activity as had been reported by Labuza et al. (1972 a). These phenomena are explained on the basis that water exerts an antioxidant effect at low A_w due to hydrogen bonding of peroxides and hydration of metal catalysts, but at higher A_w the water becomes a pro-oxidant. The pro-oxidant effect was presumed to be due to the increased mobility of the metal catalysts in the aqueous phase since the water increase lowers the liquid phase viscosity. In addition, possible exposing of new catalytic sites during swelling of the solid matrix as A_w increases could also occur. Of course, in the microcrystalline cellulose system swelling should not occur to any large degree since the cellulose particles are completely crystalline (Bluestein and Labuza, 1972). Thus, the mobility effect should be predominant for oxidation in this type of system.

It can be seen explicitly in Figure 18 that the rate of oxidation is faster at A_w 0.84 than 0.75 for both desorption and adsorption systems. The difference is more easily illustrated in Table 46 where the kinetic constants for oxidation are reported. These constants, θ_i (the time to reach 1% oxidation on a molar basis which is called the induction time) and K_M (the monomolecular rate constant) which is the rate during the initial

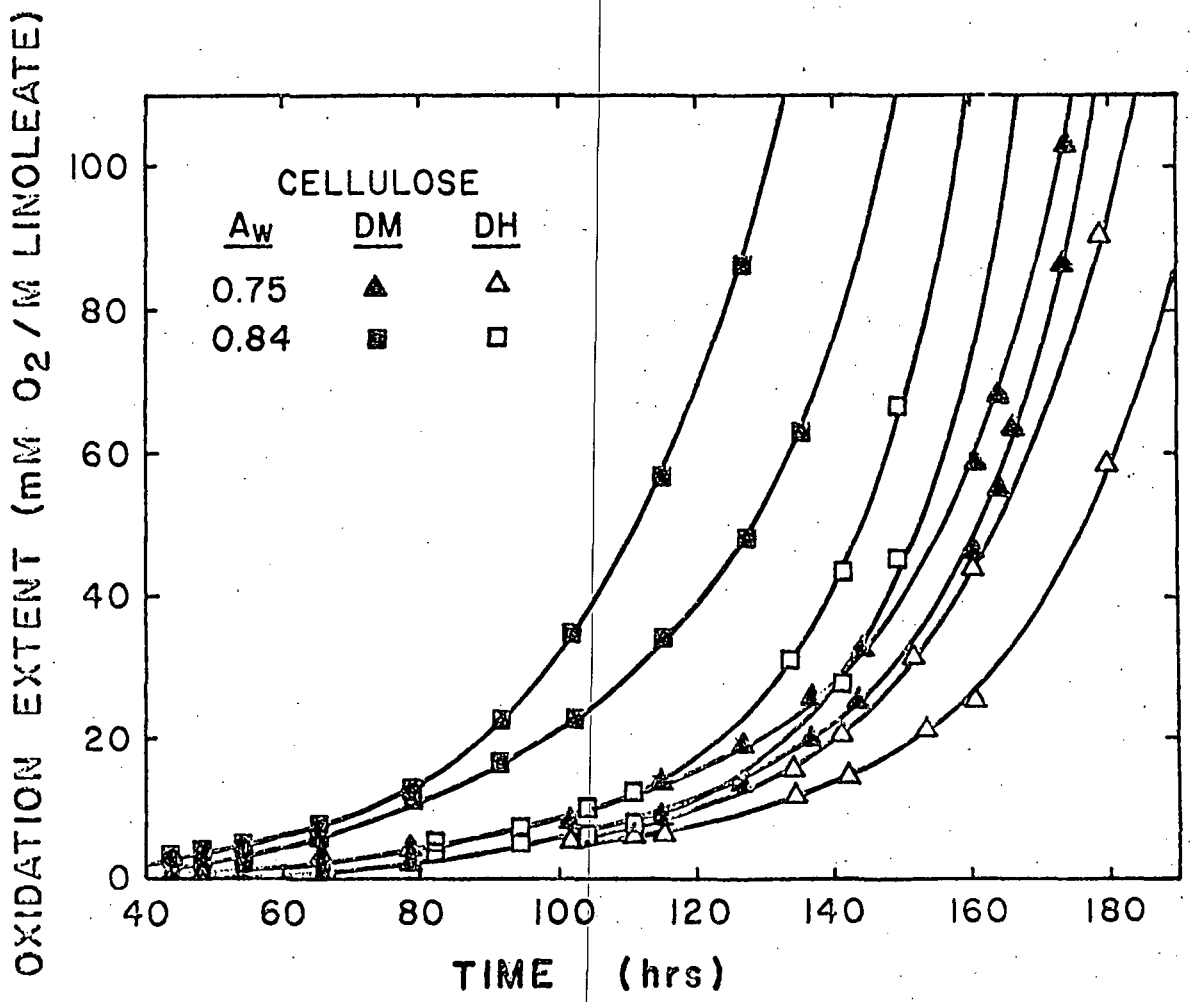


FIGURE 18. OXYGEN ABSORPTION CURVE FOR CELLULOSE MODEL SYSTEMS AS A FUNCTION OF A_w - RUN 1A

TABLE 46

Microcrystalline Cellulose Systems

Oxidation Constants

Run #	A_w	θ_i Induction Time (hr)		K_M $(M/M)^{\frac{1}{2}} \times 10^3$		K_B $hr^{-1} \times 10^2$	
		DM*	DH**	DM*	DH**	DM*	DH**
1A	0.75	109	137	1.17	0.82	4.82	4.36
	0.84	73	109	1.81	0.88	4.51	4.77
2B	0.52	183	197	0.82	0.49	4.09	4.13
	0.75	93	109	1.33	0.82	4.28	4.97
	0.84	72	110	1.51	0.92	3.07	4.69
	0.89	76	110	1.58	1.15	2.39	4.52
2C	0.52	158	179	0.88	0.70	4.12	--
	0.68	95	111	1.10	0.93	4.74	3.74
	0.84	67	110	1.47	1.04	3.21	3.88

*DM - direct mix
**DH - dry mix - humidified

stages of oxidation up to the time of possible rancidity are useful in assessing the effect of various additives and conditions on lipid oxidation.

K_M is directly dependent on catalyst activity as shown by Labuza et al. (1969), and one would expect the rate during the initiation period to increase if catalyst activity increased. Thus, as water content increases, the aqueous phase becomes less viscous, the catalyst is more mobile and thus more effective, the rate of oxidation increases and this is all reflected in an increased K_M with increase in A_w. The value of K_B, which is the rate in the fast period, shows both increases and decreases. This value is much more difficult to measure. Since, however, a food would be rancid before reaching the fast oxidation period, its value in terms of processing and storage predictions is of no interest.

It can also be observed in Table 46 that in each test the dry mix humidified systems show much slower rates of oxidation as compared to the direct mix systems by about a factor of 0.75 to 0.50. This is somewhat less than that reported by Labuza et al. (1972a) for actual liquid food IMF systems but confirms that oxidation is slower on the lower branch of the hysteresis loop. In Table 47 are shown the ratio of the theoretical viscosities of two of the systems in comparison to the ratio of the rates of oxidation. It can be seen that the viscosity effect can account for most of the decrease in oxidation in the humidified system, but not all. This confirms that catalyst mobility is probably a major factor in the non-swelling cellulose system for control of oxidation rate.

TABLE 47

Run 1A

	Theoretical Viscosity (centipoise)	K_M (a) (10^3 $(M/M^{\frac{1}{2}}/HR)$	Moisture Content $gH_2O/100$ g solids
A_W = 0.84			
Direct Mix	2.25	1.81	61
Humidified	3.00	0.88	47
RATIO	0.75	0.48	
A_W = 0.75			
Direct Mix	4.16	1.17	32
Humidified	5.37	0.82	27
RATIO	0.78	0.70	

(a) viscosities of aqueous phase due to glycerol

In Figure 20 are presented the peroxide values obtained for the systems of Run 1A. They follow the typical pattern of a maximum which shows that multiple time tests are needed if perioxide determinations are to be used to follow oxidation kinetics. The data confirm the results of direct oxygen uptake showing a faster rate of oxidation as moisture increases and a faster rate in the direct mixed systems. Table 48 compares the induction times (time to reach 1% oxidized) for both methods. It can be seen that the peroxide measurement gives higher times. This is due to the fact that during oxidation peroxides are decomposed during the initiation step and thus a lower value would be expected. The results, however, show the same pattern as above for oxygen uptake.

In Run 2 amylopectin was used as the support. This polymer contains the same basic glucose unit as in cellulose but has a different linkage thus giving it swelling properties. It also shows a larger hysteresis loop (Figures 8 and 9) than microcrystalline cellulose. Amylopectin also contains a very high concentration of trace metals, about 1000 ppm, as compared to the microcrystalline cellulose which has only about 3 ppm (Table 15). As seen in Figure 20, oxygen absorption was very rapid with very little induction time. This can be attributed to the high trace metal content. The oxidation constants are reported in Table 49. It should be noted that although the induction time is reduced by about 4 to 5 times as compared to the microcrystalline cellulose runs, the rate constant, K_M, is only about 2 to 3 times larger. This indicates that considerable oxidation has occurred during the preparation which is not recorded in the oxidation extent. This is difficult to prevent. The interesting factor, however, is that the oxidation pattern still seems

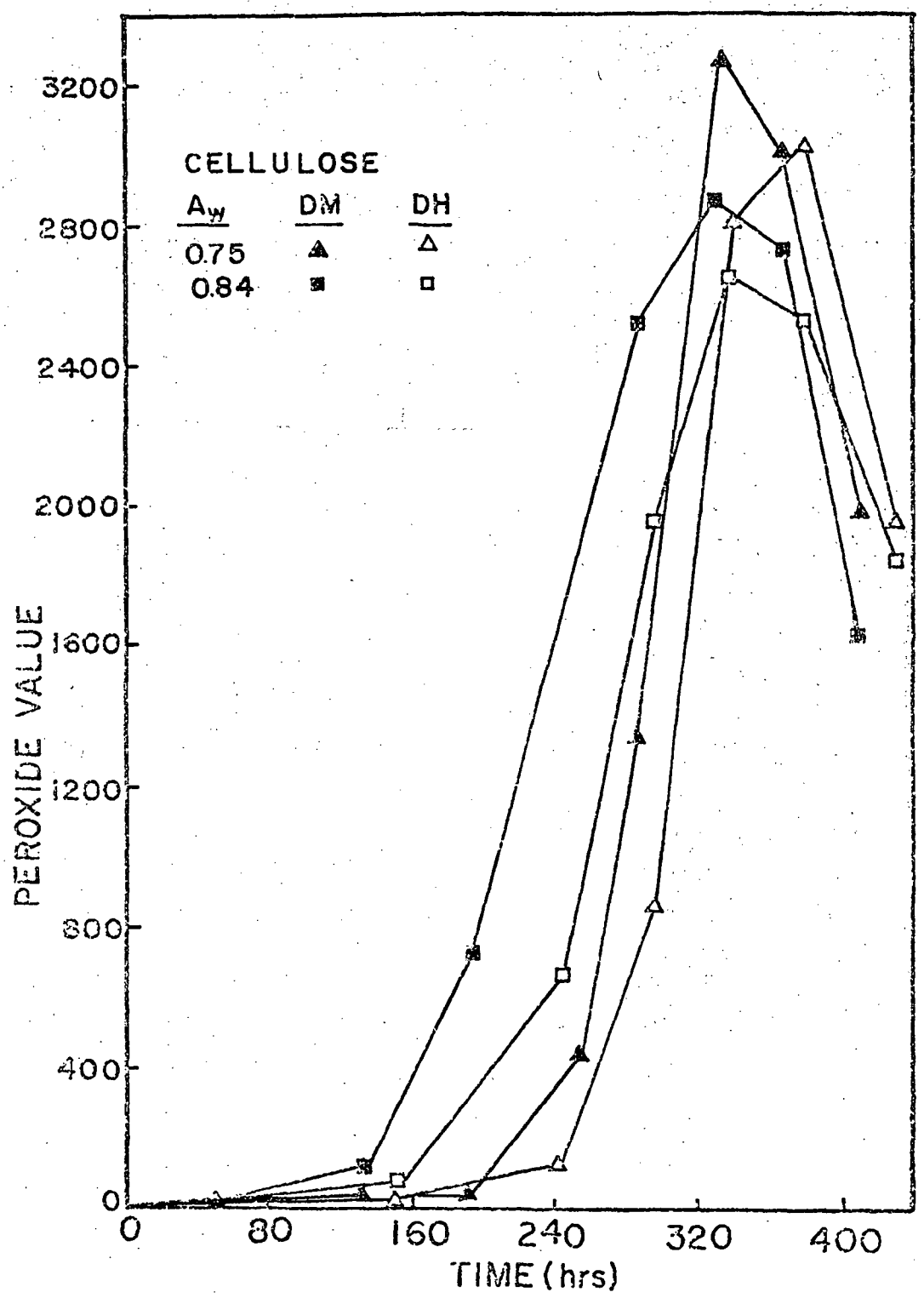


FIGURE 19. PEROXIDE VALUES - RUN 1A

TABLE 48

Time to Reach 1% Oxidation (hours)

Run 1

A_w		PV	Warburg
0.84	DM*	135	73
	DH**	168	109

*DM - direct mix
**DH - dry mix - humidified

FIGURE 20. RUN 2 - AMYLOPECTIN SYSTEM: OXIDATION EXTENT AS A FUNCTION OF A_w

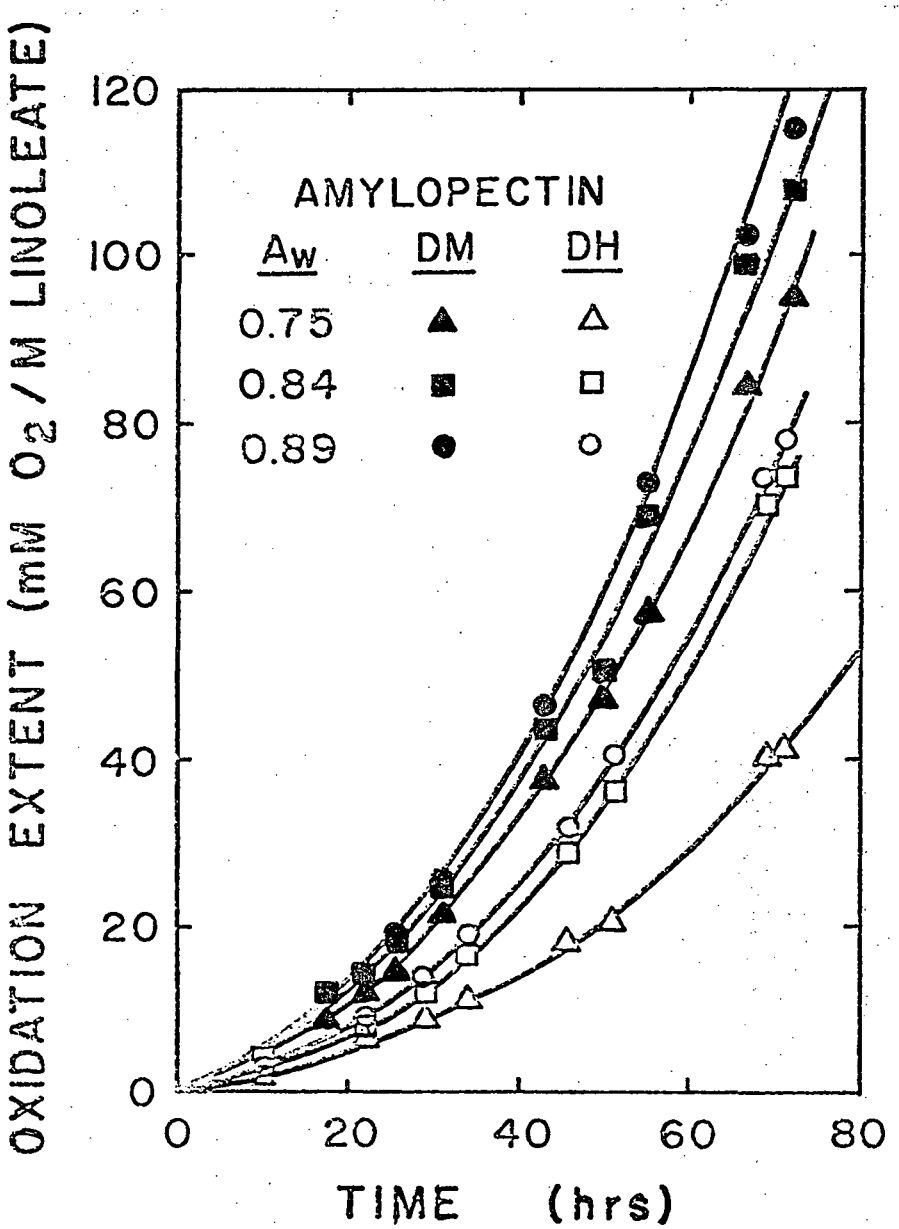


TABLE 49

Lipid IMF Model Systems

Oxidation Constants

Run #	Support	A_w	(a) θ_i hours DM(1)	(a) θ_i hours DH(2)	(b) K_M $(M/M)^{\frac{1}{2}}$ $hr^{-1}x10^3$ DM(1)	(b) K_M $(M/M)^{\frac{1}{2}}$ $hr^{-1}x10^3$ DH(2)
2	A*	0.75	22	32	3.81	2.32
		0.84	14	24	3.98	3.66
		0.89	16	23	5.30	3.49
3	C**	0.84	78	103	2.02	1.53
	A*	0.84	18	21	5.10	2.68

(a) time to reach 1% oxidized
(b) monomolecular rate constant

(1) direct mix
(2) dry mix - humidified

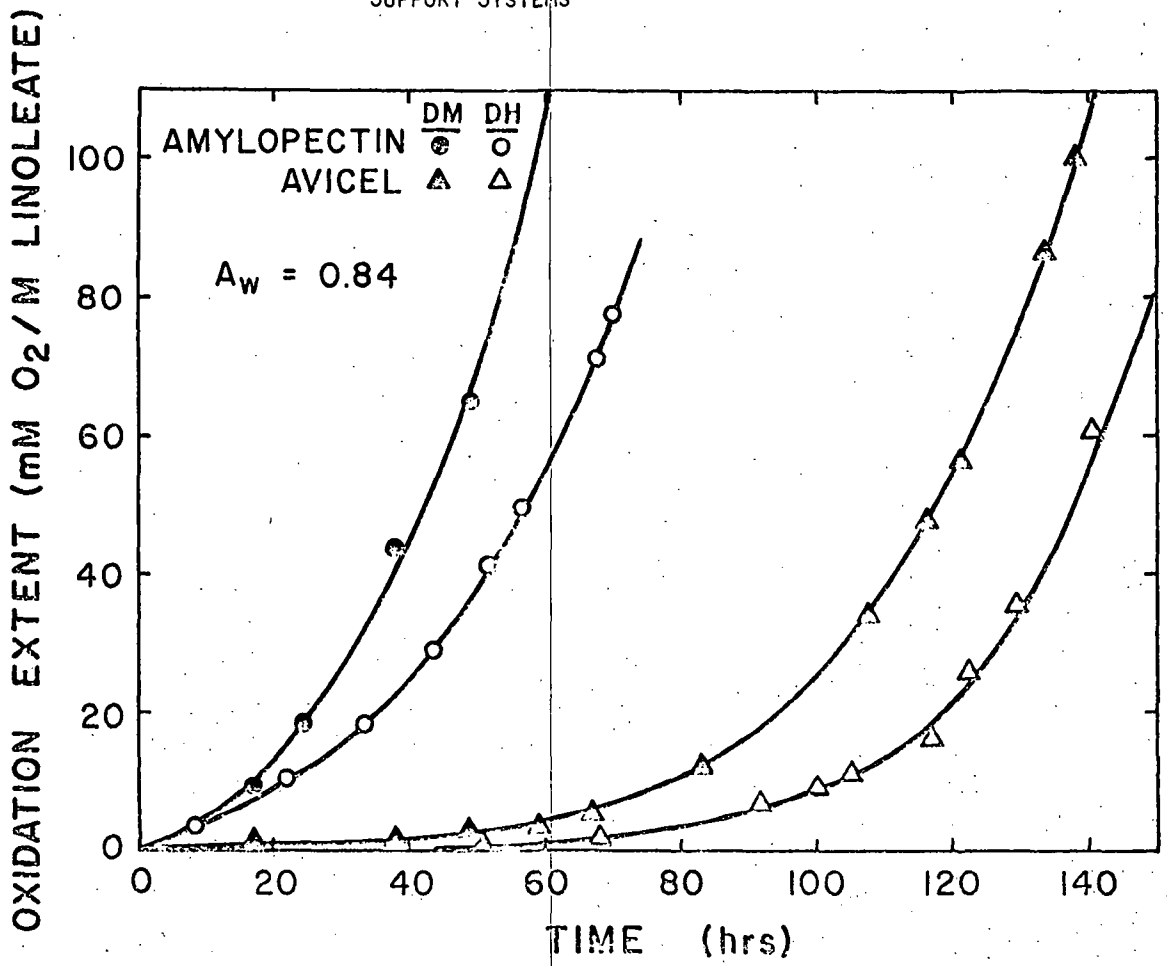
*A amylopectin
**C microcrystalline cellulose

to follow the normal autocatalytic pathway for the amylopectin system. Labuza (1971b) reviewed a number of studies in which high metal concentrations (500 to 1000 ppm) were used. In those studies oxidation extent followed a straight line with time which is attributed to the mechanism of maximum rate kinetics. Antioxidants had very little effect on the oxidation rate in those studies.

The most significant finding of Run 2 is that the system follows the same pattern as microcrystalline cellulose with respect to water activity and method of preparation. It is noted, however, that the desorption systems are closer together and do not cross over the adsorption systems as was found previously for cellulose. The adsorption systems show a larger spread possibly due to the increased swelling which may be exposing new catalysts or it may be due to the reduced viscosity as water increases. In Run 3 both amylopectin and microcrystalline cellulose were compared simultaneously using the same batch of lipid and humidifying at A_w 0.84. The oxidation constants derived from Figure 21 is reported in Table 49. The most significant finding of Runs 2 and 3 is that the amylopectin system follows the same pattern for lipid oxidation as microcrystalline cellulose with respect to water activity and method of preparation, however, due to its higher metal content the oxidation rates are faster.

In order to establish whether swelling or catalyst mobility and concentration is the predominating mechanism for the increase in oxidation rate with A_w, the two support systems were studied in Run 4 in a simultaneous experiment. In this case, in order to normalize the effects of catalysts, metal salts were added to the microcrystalline cellulose

FIGURE 21. RUN 3 - COMPARISON OF OXIDATION EXTENT FOR TWO SOLID SUPPORT SYSTEMS



system in the amounts equal to that measured in the amylopectin system. Soluble salts were added for each metal to give a total concentration of about 1000 ppm with the same distribution of metals.

Oxygen uptake was measured by a Warburg manometric system. The rates were so fast that no measurement of K_M or O_i was possible. In order to make comparisons the time to reach 3% oxidized was measured as shown in Table 50. This time should be of value since an examination of most curves shows a very rapid rise in extent after 1% oxidation is reached.

In Table 49, it is noted that, as in previous runs, for the amylopectin system an increase in A_w decreases the time to reach a given extent of oxidation. In addition, the humidified samples oxidized slower than the direct mix system. However, for the microcrystalline cellulose exactly the opposite is found to that which was reported earlier. The rate decreased with an increase in A_w and the rate increased for the humidified system. Although at first this might be attributed to using the 3% oxidation level, an examination of all previous data at 3% oxidized always showed the same previous trend as reported at 1% oxidized.

These results can be used to explain the separate effects of swelling vs catalyst mobility. In the cellulose system all the added trace metals are relatively free and are dissolved in the aqueous phase. At some point it should be expected that increasing the moisture content would tend to dilute the concentration of metals in solution and increase their diffusion path. Simultaneously, the phase viscosity is reduced as more water is added which should increase the diffusion rate constant. These two mechanisms work in opposite direction. If swelling is not significant, i.e. no new catalyst sites exposed as the matrix absorbs

TABLE 50

Time to Reach 3% Oxidation (hours)

(Total Metal Content - 1000 ppm)

Run 4

System	A_w	DM	DH	$\frac{DH}{DM}$
Microcrystalline cellulose	0.75	11.0	9.7	0.882
	0.84	18.5	12.5	0.676
Amylopectin	0.75	46.0	55.0	1.20
	0.84	38.0	43.5	1.14

more water and swells, then the two solution mechanisms presented above would predominate. The higher A_w system would have a lower catalyst concentration than the lower A_w system since it has more water present. At the high trace metal concentration the diffusion rate is no longer as strongly controlling as in the low trace metal systems previously studied so that the concentration effect predominates. Thus, an increase in A_w should reduce the rate because of a dilution of metals. As to the reason, the humidified systems now oxidize faster, the same explanation holds true. The free metal concentration is higher than at the corresponding A_w for the direct mix system because less water is present. The increased viscosity, although possibly slowing diffusion, is masked by the concentration effect.

In the amylopectin system exactly the opposite happens. Physically, the gel structure of amylopectin system seems to have a higher viscosity than that of the cellulose system, so that any increase in moisture, although diluting the metal level, also reduces viscosity and increases the reaction rate. Although direct measurement of the system viscosity is very difficult (due to different solid support and system texture) in higher moisture content systems where the Brookfield BHT viscometer can be used the differences in viscosity between the two systems are obvious. Some of the measurements are shown in Table 51.

A swelling effect should also occur in amylopectin in addition to the viscosity effect. One could expect that the metals in amylopectin are more strongly bound to the solid matrix due to the structural characteristic of the amylopectin and the fact that the oxidation rate is much slower than for cellulose at the same metal content. In order to

TABLE 51

System Viscosities [a]

% H_2O [b]	Spindle # [c]	rpm	Viscosity (cp x 10^3) Amylopectin System	Viscosity (cp x 10^3) Cellulose System
79.2	6	5	97	54
71.6	6	2.5	256	160
65.5	7	5	307	179

(a) at 20°C
(b) g H_2O/100 g solids
(c) Brookfield viscometer Model BHT

test this hypothesis the degree of binding of metals to the solids was tested. At first, free metal salts in water solution were added to cellulose to the same level as that of amylopectin as shown in Table 52 and equilibrated for 4 hours. Then the water was removed by freeze-drying.

About 10 grams each of amylopectin and cellulose with impregnated metal were mixed with distilled water at the ratio of about 1 to 5. They were then dialyzed in dialyzer tubing (Fisher Scientific Co.) against deionized water for 8 hours. After the dialysis, the contents in the tubing were transferred to beakers and freeze-dried. The dry weight of each sample was recorded and the trace metal contents were measured with an atomic absorption spectrophotometer. The results are shown in Table 52. As can be seen, on an overall basis, amylopectin has 70.8% metals left after dialysis, while cellulose has only 48.3% left although two metals Fe and Mn were retained better in the cellulose. This indicates that most of the metals in amylopectin are more strongly bound to the solid matrix than to the cellulose. In addition, the metals in amylopectin are thus probably more hidden in unswollen pores because of the branching structure and swelling properties. Therefore, water would swell the solid matrix and open up new catalytic sites and thus increase the effective catalyst concentration for lipid oxidation. Water should at the same time lower the viscosity in the aqueous phase. Consequently, as water content increases, the oxidation rate increases. The higher viscosity and the more strongly bound, hidden metals in the amylopectin should explain the fact that at the same metal level, water activity and sorption isotherm branch, this system oxidized much slower

TABLE 52

Heavy Metal Contents (ppm)

Systems	Ca	Fe	Mg	Cu	Mn	Total
Pure microcrystalline cellulose	0.4	0.58	0.33	0.27	0.08	1.7
Metal content before dialysis of cellulose and amylopectin	285	90	570	5.86	4.13	955.5
% Left after Dialysis[(a)]						
Amylopectin	83.5	77.1	63.3	88.3	70.0	70.8
Microcrystalline cellulose	51.9	91.2	39.0	60.1	87.5	48.3

(a) 8 hour dialysis in dialyzer tubing (Fisher Scientific Co.) with distilled water

than the cellulose system to which metal was added.

A comparison of Runs 1 and 4 shows that for the cellulose system a reversal occurs for lipid oxidation kinetics with respect to water content-A_w. At low trace metal concentration, the higher the moisture content and A_w, the faster the rate; at high metal concentration the opposite occurs, i.e. the rate is slower. For the high metal system when they are added, the metals should be expected to be readily free in the aqueous environment. At the low metal content, most of these metals are bound tightly. As seen in Table 53, as moisture is increased the metal concentration, if all is available, decreases. The theoretical aqueous phase viscosity also decreases. It is postulated, therefore, that at low trace metal concentration, the dilution effect of water is unimportant since the amount of trace metals present is small. However, the lower viscosity as caused by the addition of water causes the rate increase for oxidation due to easier diffusion.

At high added metal concentrations the system is abundant in free metals which should all be in solution. Increasing the A_w or water content will dilute the free metal concentration. If a great excess of trace metals are present, any increase in metal content due to swelling of the matrix would be insignificant, but the decrease in metal concentration is very significant (Table 52). This should then cause a decrease in the oxidation rate. At some metal concentration inbetween the effect of dilution of the metals and decrease in phase viscosity shculd work in opposite directions and cancel each other out.

Experiments were carried out to test this above hypothesis. Microcrystalline cellulose with metal contents ranging from $<$ 3 ppm to 1000 ppm

TABLE 53

Moisture Content, Theoretical Viscosity and Theoretical Metal Concentration

A_w system	0.75		0.84		0.89	
	DM	DH	DM	DH	DM	DH
Moisture content	34	28	61	47	78	63
Theoretical viscosity (cp)	4.16	5.37	2.25	3.00	1.80	2.10

Metal content (ppm/solid basis)	Theoretical metal concentration in aqueous phase (ppm/H_2O basis)						ppm**	
	DM	DH	DM	DH	DM	DH	DM	DH
5	14.7	17.8	8.2	10.7	6.3	7.9	8.4	9.9
10	29.4	35.7	16.5	21.5	12.6	15.8	16.8	19.9
20	58.8	71.4	33.0	43.0	25.2	31.6	23.6	39.8
50	147	--	82.5	--	63.0	--	84	--
100	294	--	165	--	126	--	168	--
500	1470	--	825	--	630	--	840	--
1000	2940	3570	1650	2150	1260	1580	1680	1990

** ppm decrease of metal due to increase A_w from 0.75 to 0.89

was used. The use of cellulose also minimizes the swelling phenomena. The test systems are summarized in Table 45 for Runs 5 through 10. Oxygen uptake was measured as shown in Figure 22 (a-f) and the induction times are reported in Table 54. A complete reversal of the kinetics occurs as the metal content increases. At low metal content the higher the A_w the faster the oxidation rate and the DM oxidizes faster than the DH. As the metal concentration is increased to 50 ppm, all systems oxidize at about the same rate as predicted. At 500 ppm the system oxidation rate pattern has completely reversed with the rate of oxidation being faster at the lower water content.

These results are explained on the basis of the dilution effect as described above. At high metal concentration increasing the A_w, and therefore the amount of water, significantly decreases the free metal concentration even though the phase viscosity is reduced. This dilution effect which decreases the effective metal catalyst concentration for oxidation predominates in the system where the metals are fairly free as opposed to amylopectin where they are not. It would be expected, therefore, that metal chelators would be very effective in these systems as compared to where the metals are tightly bound.

In order to further elucidate the effect of A_w-water content on oxidation, the results of Runs 1 through 10 were examined in light of the sorption hysteresis effect. As discussed previously, at low moisture content water exerts an antioxidant effect through hydration of metal catalysts lowering their activity and hydrogen bonding peroxides thereby retarding chain initiation. As water content increases, the phase viscosity decreases increasing catalyst mobility, however, the effective

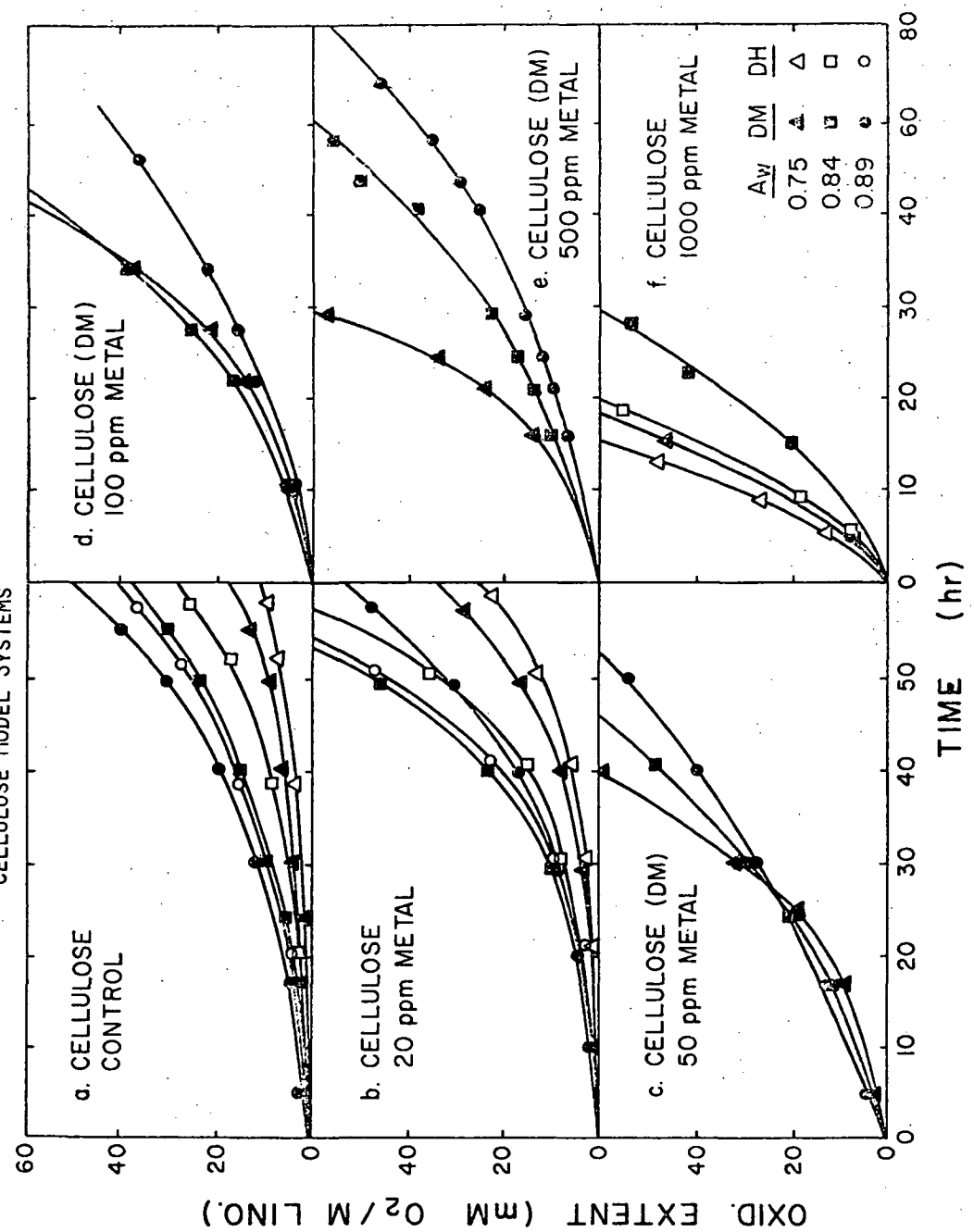


FIGURE 22. EFFECT OF METAL CONCENTRATION ON EXTENT OF OXIDATION IN CELLULOSE MODEL SYSTEMS

TABLE 54

Microcrystalline Cellulose Systems

Oxidation Constants

Run No.	Metal content (ppm)	A_w	θ_i [a] hours DM	DH
5	Trace*	0.75	72	80
		0.84	55	62
		0.89	49	53
6	20	0.75	58	63
		0.84	44	49
		0.89	50	46
	Trace*	0.84	73	91
7	50	0.75	29	--
		0.80	31	--
		0.84	30	--
		0.87	31	--
		0.89	32	--
8	100	0.75	31	--
		0.84	29	--
		0.89	40	--
9	500	0.75	23	--
		0.84	34	--
		0.89	44	--
10	1000	0.75	11	9.7
		0.84	18.5	12.5

[a] Time to reach 3% oxidation

*Less than 10 ppm (natural metal).

metal concentration decreases.

The water activity of the system is also an index of reactivity since it measures the availability of the water. The lower the A_w, the more strongly bound is the water to the system components. Thus, when water is an antioxidant, the rate of oxidation should decrease as A_w increases because the water becomes more available. On the other hand, when water acts as a pro-oxidant the rate of oxidation should increase as A_w increases as more water becomes available.

These effects are illustrated in Figures 23 through 26 in which the induction time was plotted vs A_w or moisture content. What is obvious from these figures is that in the intermediate moisture range it is the moisture content that is controlling the rate of oxidation. As seen in Figure 25 and 26, there is very little difference in the oxidation rate between the two hysteresis branches if the systems are compared at similar water content and metal content. Thus, at high A_w, where the water is not bound tightly, it is the moisture content which controls oxidation rate. In a system where metals are fairly free, greatest stability is obtained at the lowest moisture, or by going to very high moisture contents. In a system like meat, however, where the metals are bound tightly as in amylopectin (Figure 26), above a certain moisture any increases do not affect the oxidation rate. Thus, in the manufacture of an intermediate moisture food one could go to as high a moisture as possible to attain the desired textural characteristics without much change in oxidation rate; the only limitation being microbial growth. These studies also suggest that metal chelating agents would be very effective in systems where the metals are fairly free.

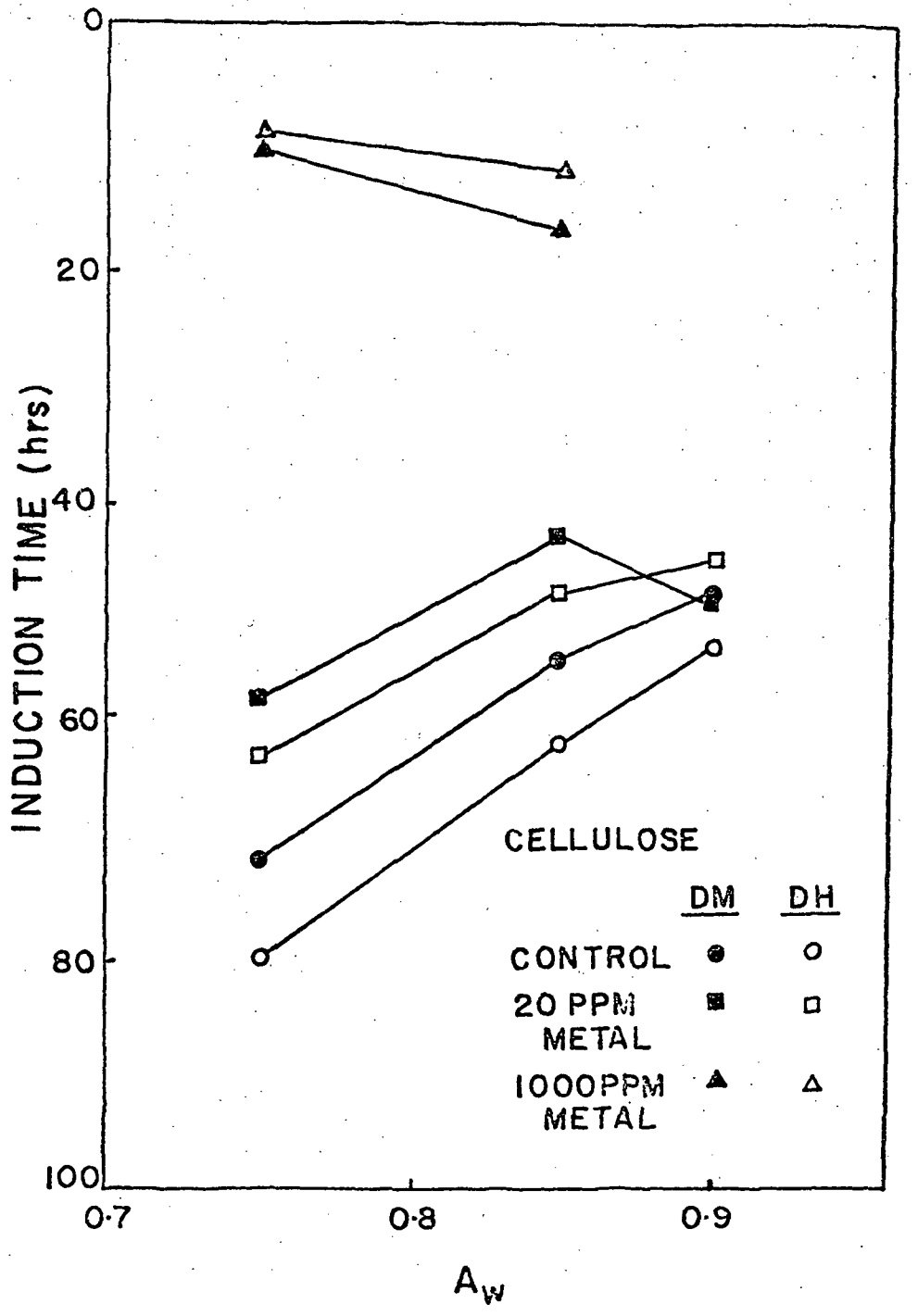


FIGURE 23. INDUCTION TIME VS WATER ACTIVITY - CELLULOSE SYSTEM

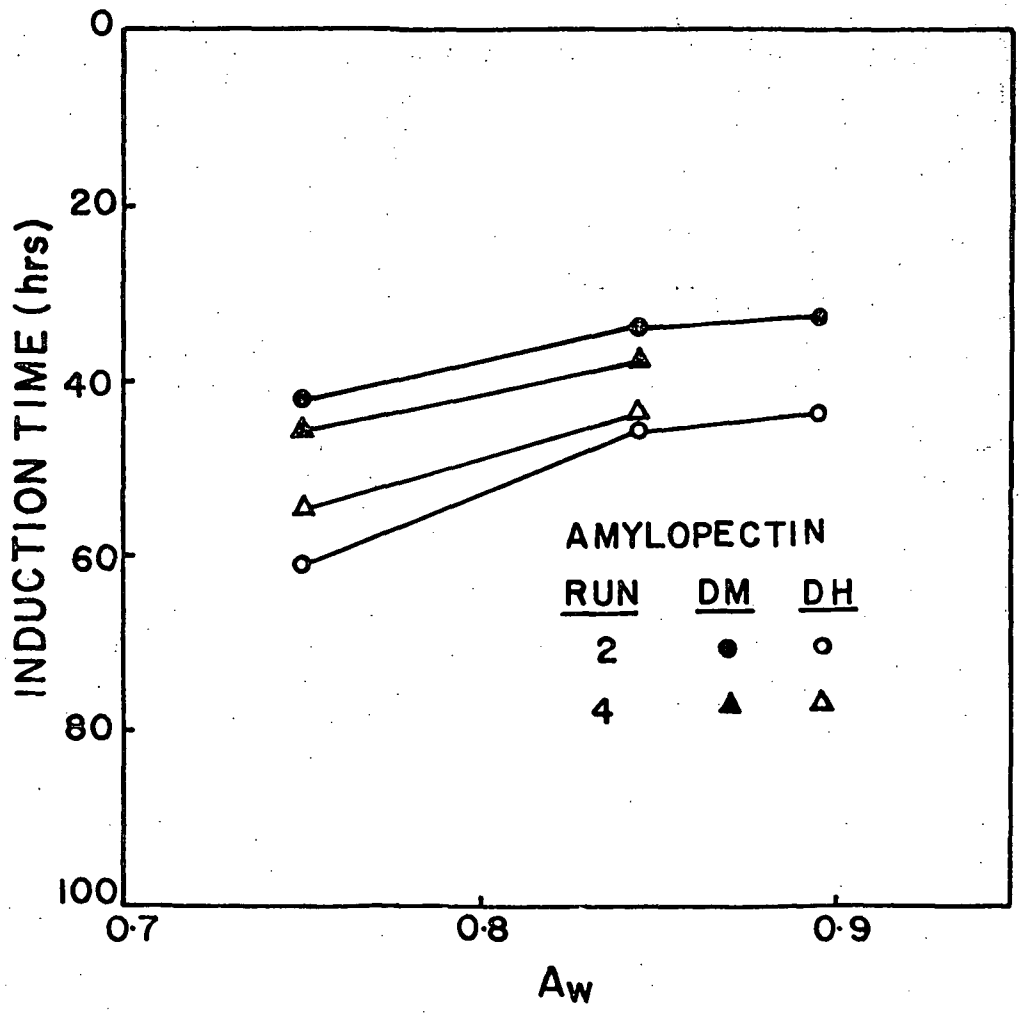


FIGURE 24. INDUCTION TIME VS WATER ACTIVITY - AMYLOPECTIN SYSTEM

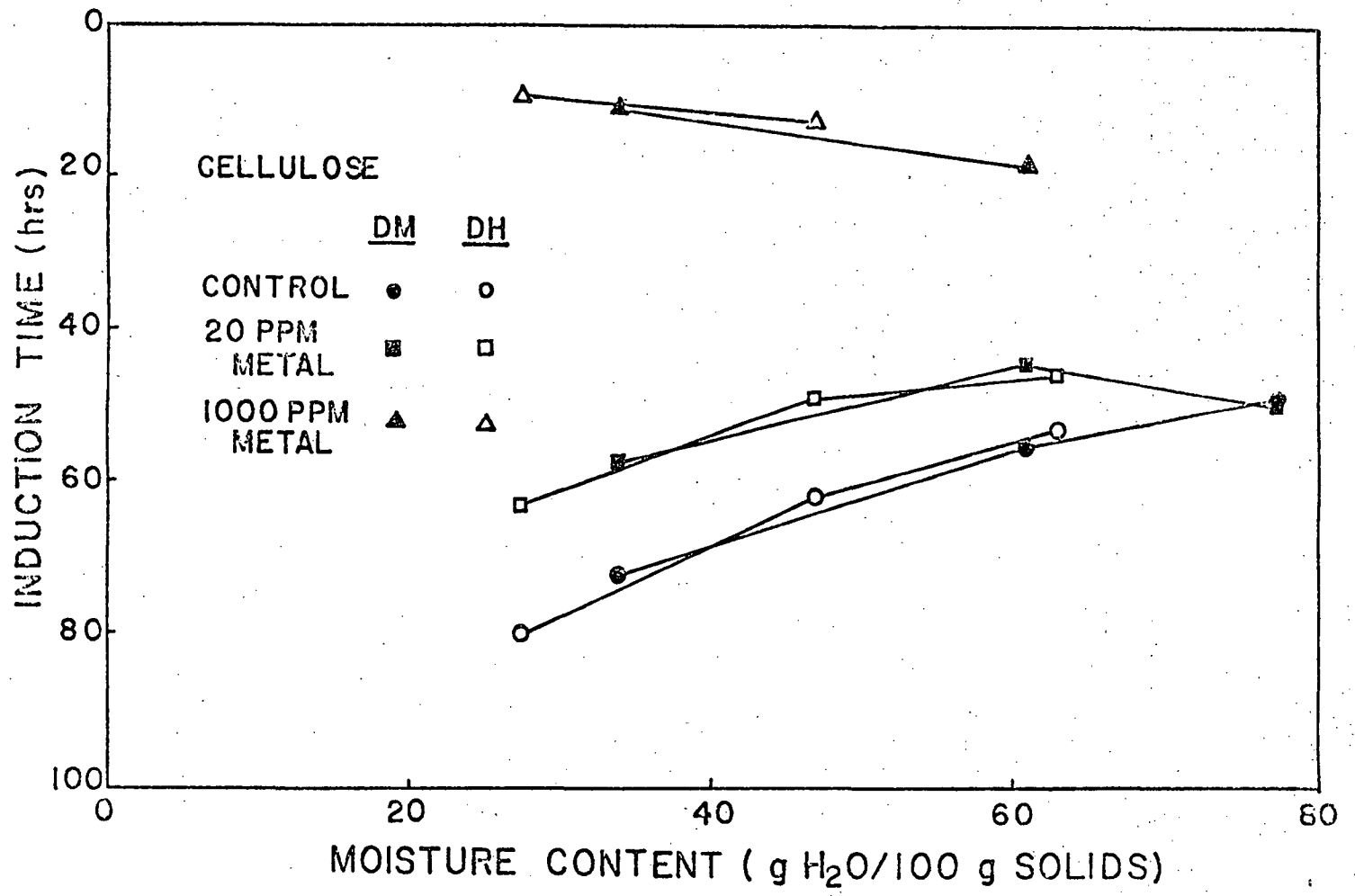


FIGURE 25. INDUCTION TIME VS MOISTURE CONTENT - CELLULOSE SYSTEM

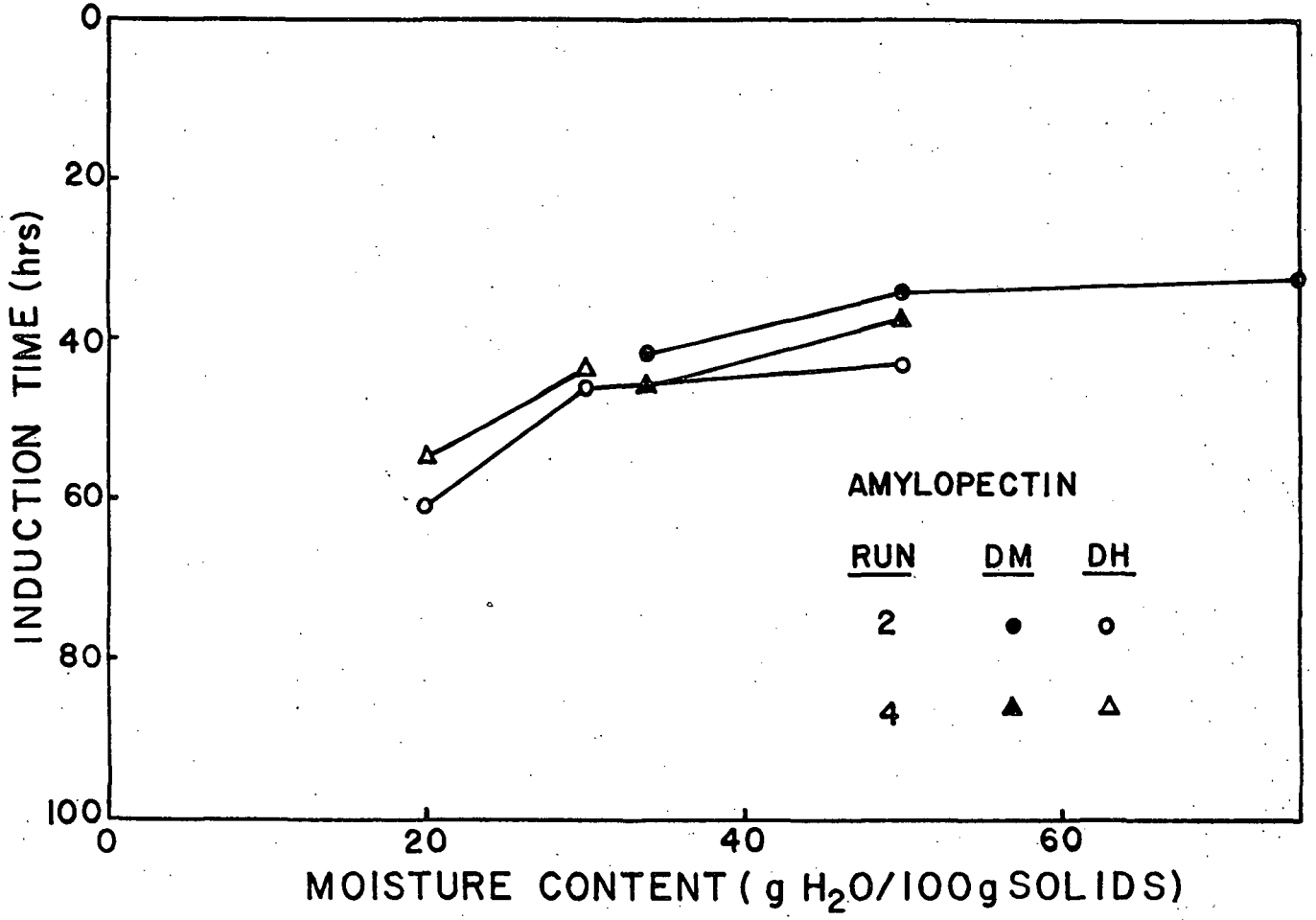


FIGURE 26. INDUCTION TIME VS MOISTURE CONTENT - AMYLOPECTIN SYSTEM

(b) Antioxidant Effectiveness

It has been shown that metal catalyst solubility and mobility were the most important reasons for the catalytic effect of water at high humidity. The top two sections of Figure 27 summarizes these results. In pure microcrystalline cellulose and amylopectin the direct mixed desorption systems oxidize faster than the humidified systems. In addition, the rate increases as A_w increases. These increases can be attributed to the higher water content which swells the system and exposes new catalysts. When free metals were added to the cellulose (Cellulose II in Figure 27) to match the level in the amylopectin, however, the reversal occurs, i.e. the higher moisture system oxidizes slower with respect both to method of preparation and A_w.

Further studies were made to test antioxidant effectiveness in model systems in the IMF range. Various water soluble and lipid soluble antioxidants were tested. Antioxidants which chelate metals (Type II) like citric acid or EDTA were tested against those which terminate free radical reactions like BHA or tocopherol (Type I). The test systems are summarized in Table 45 for Runs 11, 12, 13, and 16. Oxygen uptake was measured in each run and both induction time and K_M were calculated. Because of the high rates of oxidation, induction time was measured at 3 mole % oxygen uptake. The kinetic values are summarized in Table 55. Figure 27 is a summary of all the data normalized by using the control data for each run and setting induction times equal for the controls compared to the results for Run 4 (top of Figure 27) in which only the controls were tested. Thus, Figure 27 gives a good visual picture of antioxidant effectiveness. Each run will be discussed individually.

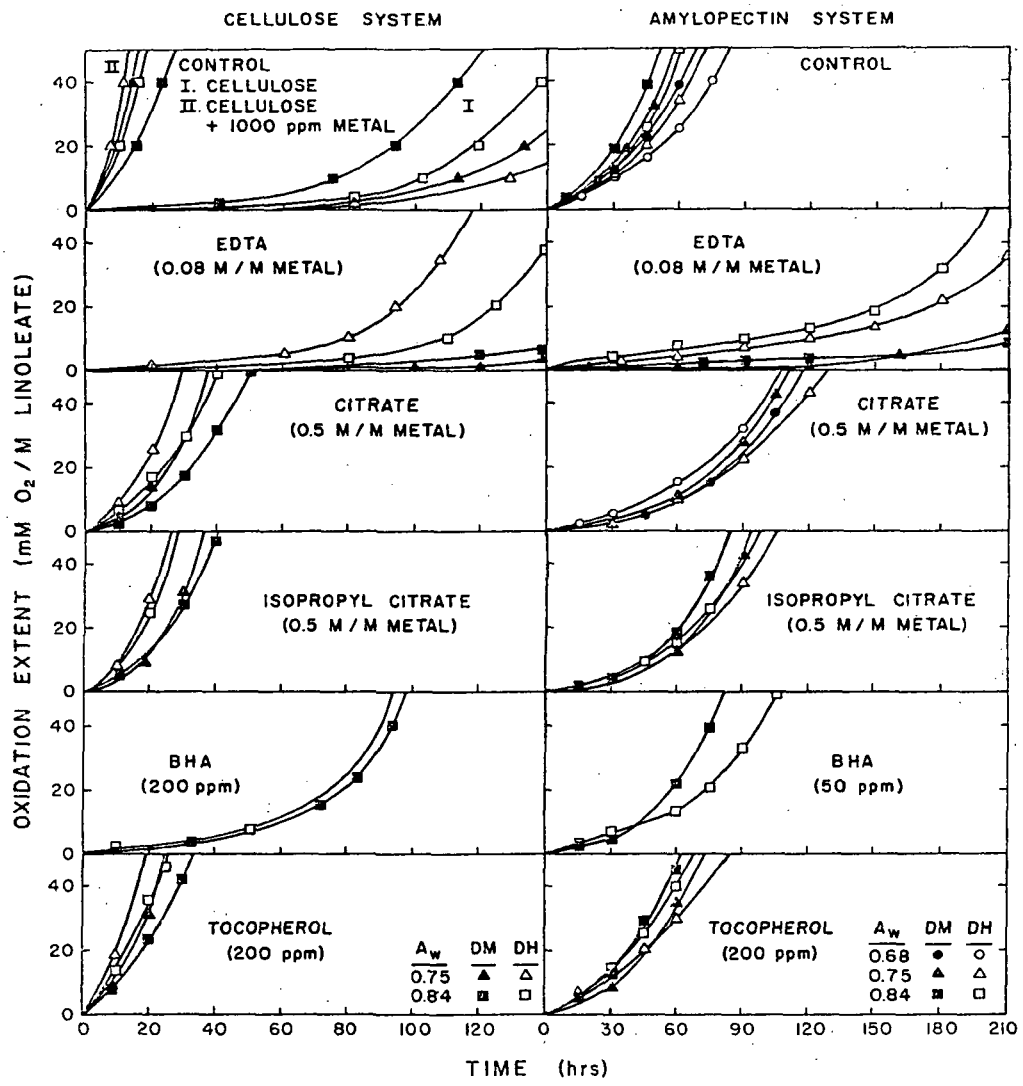


FIGURE 27. EFFECT OF ANTIOXIDANTS ON EXTENT OF OXIDATION IN CELLULOSE AND AMYLOPECTIN MODEL SYSTEMS

TABLE 55

Lipid IMF Model Systems[(a)]

Oxidation Constants

Run #	Support	Antioxidants	A_w	θ_i DM*	θ_i DH**	K_M (M/M) $hr^{-\frac{1}{2}}$ $\times 10^3$ DM*	K_M (M/M) $hr^{-\frac{1}{2}}$ $\times 10^3$ DH**
11	C[(1)]	EDTA	0.75	247	104	0.845	2.64
		EDTA	0.84	230	135	1.16	1.90
		None	0.75	11.0	9.7	nd[(c)]	nd
		None	0.84	18.5	12.5	nd	nd
	A[(2)]	EDTA	0.75	250	198	0.490	0.684
		EDTA	0.84	261	177	0.460	0.718
		None	0.75	46.0	55.0	2.81	2.71
		None	0.84	38.0	43.5	3.66	3.13
12	C	Sodium citrate	0.75	30.0	23.0	5.14	6.40
		Sodium citrate	0.84	40.0	31.0	3.27	4.96
		None	0.75	10.8	9.5	nd	nd
	A	Sodium citrate	0.68	97.0	89.0	0.880	1.44
		Sodium citrate	0.75	92.0	102.0	1.41	1.09
		Sodium citrate	0.84	86.0	95.0	1.53	1.36
		None	0.68	53.0	67.0	2.71	1.88
		None	0.75	50.0	57.0	2.85	2.39

Run #	Support	Antioxidants	A_w	θ_i DM*	θ_i DH**	K_M $(M/M_\infty^{\frac{1}{2}})$ $hr^{-1} \times 10^3$ DM*	K_M $(M/M_\infty^{\frac{1}{2}})$ $hr^{-1} \times 10^3$ DH**
13	C	Isopropyl citrate	0.75	29.0	20.0	4.80	9.65
		Isopropyl citrate	0.84	32.0	22.0	4.85	7.92
		Sodium citrate	0.84	42.0	34.0	3.61	4.73
		None	0.84	20.0	11.0	8.56	11.96
	A	Isopropyl citrate	0.75	84.0	90.0	1.79	1.58
		Isopropyl citrate	0.84	76.0	85.0	2.19	1.89
		Sodium citrate	0.84	98.0	103	1.33	1.30
		None	0.84	43.0	54.0	3.28	2.96
16	C	BHA	0.84	88.0	84.0	1.02	1.09
		Tocopherol	0.75	19.0	13.0	7.47	12.3
		Tocopherol	0.84	24.0	18.0	5.8	10.4
		None	0.75	12.0	9.0	nd	nd
		None	0.84	19.0	15.0	8.65	10.0
	A	Tocopherol	0.75	56.0	59.0	2.27	1.71
		Tocopherol	0.84	50.0	47.0	3.46	2.36

(a) Average Value of duplicate samples
(b) Time to reach 3% oxidized
(c) nd = not determined. (Too fast to be measured)
(1) Microcrystalline cellulose
(2) Amylopectin
* Direct mix
** Dry mix rehumidified

It should be noted that in this Figure (27) all the antioxidant cellulose systems have 1000 ppm of added trace metals. In Run 11 the metal chelating agent, ethylenediaminetetraacetic acid (EDTA) was tested since metal catalysis has been proposed as the major relationship to oxidation rate increase with moisture content. The EDTA was added by dissolving in a minimum amount of water then was mixed with glycerol.

In the cellulose system the pattern followed by the control was repeated for the EDTA. The humidified system oxidized faster than the direct mix system and oxidation was faster at the lower A_w. Even though the EDTA is very effective, especially in the direct mix system, the pattern of oxidation does not change to that of a low metal system. Based on the induction time, EDTA reduces the rate by 10 to 20 times in the cellulose system (Table 56). The amylopectin systems show an effect similar to the cellulose system but opposite to that of the amylopectin system without EDTA with respect to method of preparation. The effectiveness is also less as seen in Table 56, most likely because the metal is more tightly bound. There is, however, very little difference in oxidation rate with respect to A_w. These changes in rate with respect to water content can be explained by the solubility of EDTA as seen in Figure 28. The isotherm for EDTA shows that it goes into solution at about A_w 0.94. Thus, when the direct mix system is made the EDTA can chelate the metals in the aqueous solution. The metals are strongly chelated in both direct mix systems giving an extremely slow oxidation rate. However, in the humidified systems the higher the moisture content, the greater is the antioxidant effect. This means either more metal was chelated in the initial preparation or the EDTA

TABLE 56

Antioxidant Effectiveness

System	Antioxidant	A_w	Effectiveness Ratio[a] DM(b)	DH(c)
Cellulose II*	EDTA	0.75	22.5	10.7
		0.84	12.4	10.8
	Sodium citrate	0.75	2.8	2.4
		0.84	2.2	2.2
	Isopropyl citrate	0.75	2.6	2.1
		0.84	1.6	2.0
	α-tocopherol	0.75	1.6	1.4
		0.84	1.3	1.2
	BHA (200 ppm)	0.84	4.6	5.6
Cellulose I (100 ppm cobalt)	BHA (50 ppm)	0.75	3.2	3.1
		0.84	3.1	2.8
		0.89	2.2	2.7
	BHA (100 ppm)	0.75	4.0	4.6
		0.84	4.8	4.0
		0.89	2.6	3.8
	BHT (100 ppm)	0.75	3.7	3.9
		0.84	2.8	2.1
		0.89	2.3	1.9
	BHA/BHT (50 ppm/50 ppm)	0.84	4.8	4.3
Amylopectin	EDTA	0.75	5.3	3.6
		0.84	6.9	4.1
	Sodium citrate	0.68	1.8	1.3
		0.75	1.8	1.8
		0.84	2.3	2.0
	Isopropyl citrate	0.75	1.7	1.6
		0.84	1.8	1.6
	α-tocopherol	0.75	1.2	1.1
		0.84	1.3	1.1
	BHA (50 ppm)	0.75	2.1	2.1
		0.84	3.3	1.9
		0.89	2.5	2.0

*1000 ppm metals
[a]ratio of induction time divided by induction time of respective control
(b)direct mix
(c)dry mix humidified

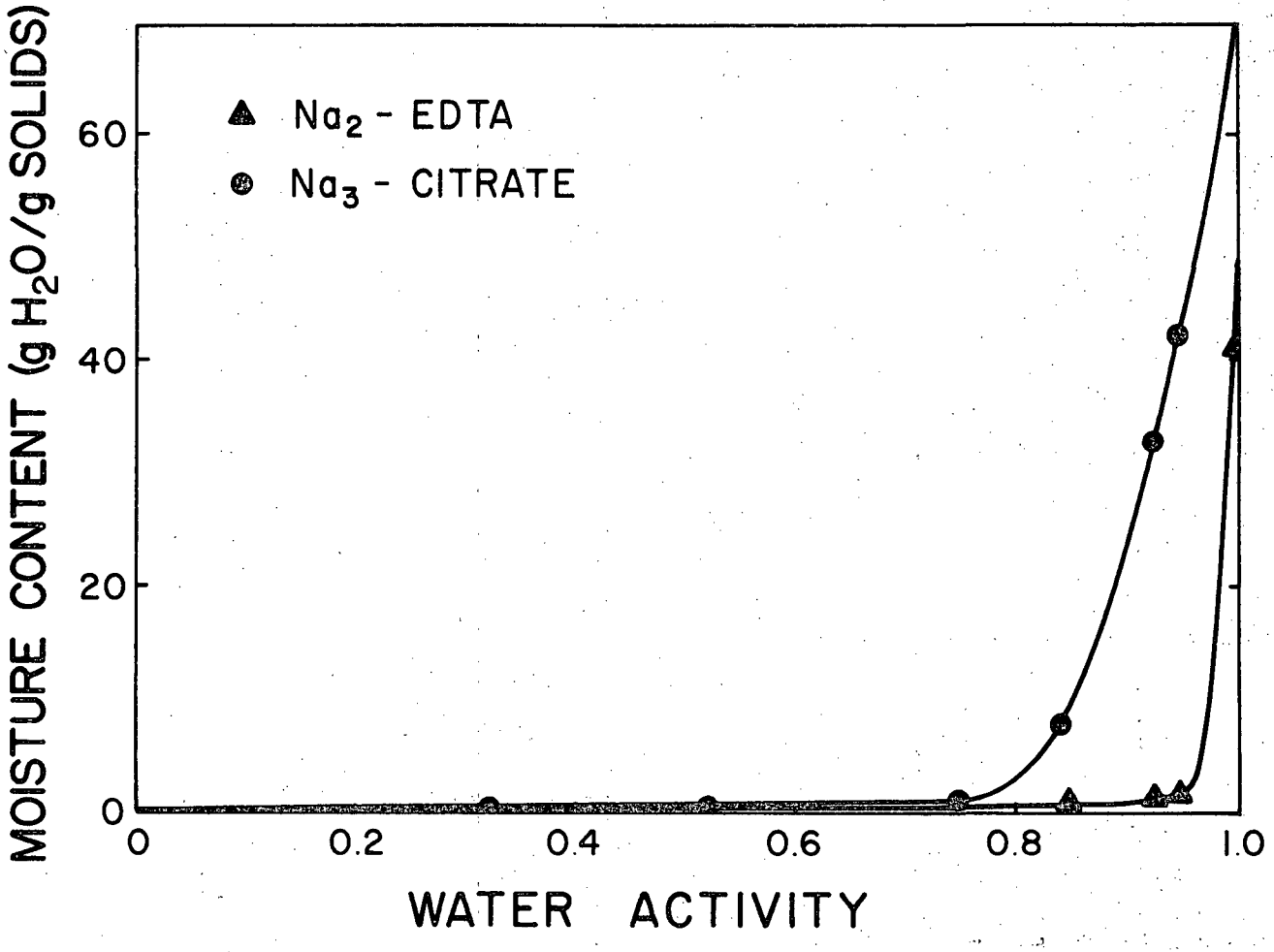


FIGURE 28. SORPTION ISOTHERM FOR EDTA AND SODIUM CITRATE

has greater solubility and mobility as the A_w increases. Also, the higher viscosity at the lower A_w should slow the EDTA which is a fairly large molecule. These results support the original ideas about the effect of A_w and moisture content on oxidation.

In Run 12 sodium citrate, another chelating agent, was tested. Its solubility point as shown in Figure 29 is at about A_w 0.75. As seen in Table 56 and Figure 27, the citrate is a poorer antioxidant than EDTA reducing the rate by a factor of only about 2 times.

In the cellulose direct mix system the citrate is more effective at the higher A_w probably due to solubility as in the case of EDTA. The difference is less since the solubility point is about A_w 0.75 indicating that most of the citrate may have gone into solution. This also explains the slightly greater effectiveness in the direct mixed system. These differences are less obvious in the amylopectin system. The rate decreases as A_w increases in the direct mix, but there is a rate minimum at A_w 0.75 for the humidified system. The citrate, as was the EDTA, is less effective in the swelling system (amylopectin) than in the cellulose as seen in Table 56. It should also be noted that much more citrate was used than was added for EDTA (about 6 times as much). The maximum effectiveness occurred at the highest A_w.

Isopropyl citrate was studied in Run 13. It is partially water soluble and would tend to operate at the water-lipid interface but should be less affected by A_w. It was dissolved directly in the linoleate during mixing. The pattern follows the effects of A_w on the control in exactly the same pattern for both solid supports. It must tie up some free metals initally and is not transported in the aqueous phase. Sodium citrate was

compared in the same run (Table 55). The sodium citrate was about 50% more effective than the isopropyl ester on an overall basis as seen in Table 56.

In Run 15 and 16 both BHA and tocopherol were tested as antioxidants, both of which are free radical terminators having no effect on the metals. It can be seen that BHA is extremely effective in the cellulose system at 200 ppm (Table 56) but is less effective at 50 ppm. In the amylopectin system, BHA is about the same as citrate. The oxidation rate followed the same pattern as in the controls since BHA does not effect metal catalysis. The kinetic data for Run 15 is in Table 57. Since less metal was added to the cellulose the time to 1% oxidation could be used as the induction period. The results indicate that in cellulose BHA is more effective at the lowest moisture content. With amylopectin, however, there is very little difference with respect to A_w.

The tocopherol, which has vitamin E activity and is therefore non-toxic, is fairly poor as an antioxidant as seen in Figure 27 and Table 56. In amylopectin its effectiveness is very small. Due to its expense and poor ability as compared to EDTA or BHA, it is not useful as an antioxidant.

With respect to antioxidants the results of these tests indicate that BHA and EDTA either alone or possibly in combination would be very effective in controlling lipid oxidation at intermediate A_w. Further additional gain could be attained in vacuum packaging since the ratio of oxygen to lipid would be lowered to effectively slow the rate (Labuza, 1971). The results suggest that EDTA would be best in a food made by direct mixing if it is a swelling system, which most are, whereas BHA

TABLE 57

Lipid IMF Model Systems

Oxidation Constants

Run #	Support	Antioxidant	A_w	(a) θ_i hours DM(1)	(a) θ_i hours DH(2)	(b) K_M $(M/M)^{\frac{1}{2}}$ $hr^{-1} \times 10^3$ DM(1)	(b) K_M $(M/M)^{\frac{1}{2}}$ $hr^{-1} \times 10^3$ DH(2)	(c) K_B DM(1)	(c) K_B DH(2)
14	C**	100 ppm BHA	0.75	372	498	0.245	0.228	4.24	3.06
			0.84	349	435	0.253	0.199	2.86	3.92
			0.89	197	415	0.427	0.205	1.62	4.17
		100 ppm BHT	0.75	341	428	0.492	0.370	3.73	3.46
			0.84	199	227	0.652	0.602	2.34	2.74
			0.89	173	207	0.754	0.688	1.90	2.57
		50 ppm BHA + 50 ppm BHT	0.84	344	473	0.270	0.204	2.21	3.99
15	A*	50 ppm BHA	0.75	47	68	1.44	0.74	3.12	2.70
			0.84	46	46	1.62	1.22	3.43	2.73
			0.89	40	45	1.70	1.26	3.47	2.95
	C**	50 ppm BHA	0.75	293	340	0.269	0.257	0.816	0.936
			0.84	226	311	0.340	0.283	0.715	0.962
			0.89	168	299	0.444	0.321	0.590	0.997

(a) time to reach 1% oxidized
(b) monomolecular rate constant
(c) biomolecular rate constant

(1) direct mix
(2) dry mix - humidified

*A amylopectin
**C microcrystalline cellulose

is about equal in both systems. Thus, the combination should be very effective.

In Run 14 a study was made of the typical antioxidants added to intermediate moisture dog foods, namely butylated hydroxy toulene (BHT) and butylated hydroxy anisole (BHA). The values of θ_i, K_M and K_B as taken from the results of oxidation in the cellulose system are presented in Table 57 and the effectiveness ratios are in Table 56. It should be noted that the addition of antioxidants did not change the shape of the oxidation curve as was shown in the previous runs except to increase the induction periods. This is because these types of antioxidants are Type II antioxidants which help to terminate free radicals. They lower free radical concentration which lowers the rate and, thus, K_M is lowered and θ_i increases correspondingly.

As seen in Table 56 and 57 both these effects occur. The induction time is about 2 to 5 times longer than without antioxidant and K_M is about 4 to 5 times lower. Very little effect occurs with K_B because prior to the latter oxidation period, the antioxidant should be used up and cannot affect the rate of oxidation.

The same pattern of the rate being faster at higher A_w and the direct mix being faster than the humidified systems occurs in the presence of antioxidants at 100 ppm added metals. These antioxidants do not affect the metal catalyst concentration or catalyst mobility as do the chelating agents but terminate free radical production. Thus, the changeover in kinetics does not occur as was seen at 100 ppm metals in Figure 22 since the antioxidant effect predominates.

In comparing the antioxidants in Table 56, BHA seems to be more effective especially in the humidified system. In combination, a 50/50 mixture of these two were as effective as 100% BHA. Since BHT is less

expensive this combination would be more economical to use in conjunction with a chelating agent.

In terms of actual IMF food systems, the real problem will be incorporating the lipid antioxidants into the food. This is possible if a cooker-extruder system or a cold mix system is used but will be difficult in a soak system since the amount going into the food may be variable during infusion.

2. Non-Enzymatic Browning in Model Systems

As discussed in the literature survey, non-enzymatic browning is a second major mode of deterioration of intermediate moisture foods. Besides causing a brown color with the development of a bitter off-flavor, browning leads to a loss of protein solubility and the destruction of lysine. These latter reactions decrease the nutritional value of the protein.

Several studies were designed to test the suitability of the methods proposed for studying non-enzymatic browning. Table 58 shows the composition of the system used. Only one A_w was studied. The system was stored in jars at 44°C.

The results of Run P1 are summarized in Figure 29 with the actual data shown in Tables 59, 60 and 61. With respect to browning, the expected trend occurred. After an initial induction period, the rate of browning increases linearly (Labuza, 1972). The sample variability is very small between the three replicates for browning optical density. The lysine losses and decrease in protein solubility do not show any significant changes in relationship to the increase in browning. As seen, the replicates show a large variability for lysine. The fact that no loss

TABLE 58

Browning Model System

Composition

Component	grams
Casein	50.00
D-glucose	2.50
K-sorbate	0.75
Water	12.50

Moisture g H_2O/g solids = 0.21

A_w = 0.87

Protein content = 72.38%

FIGURE 29. RUN P1 - PROTEIN-SUGAR INTERACTIONS IN A CASEIN MODEL SYSTEM

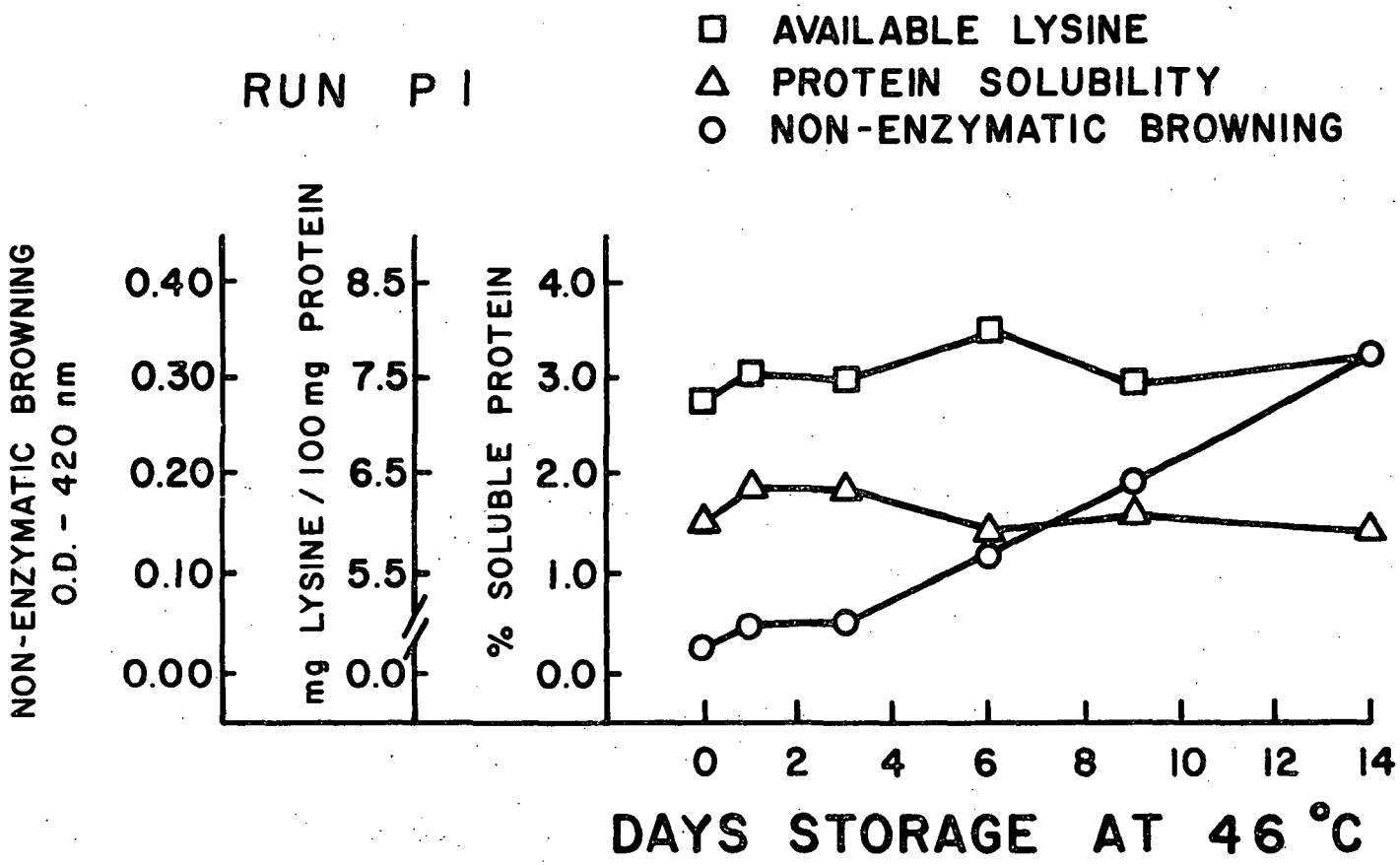


TABLE 59

Run P1 Results

Non-Enzymatic Browning at 44°C

	O.D. @ 420 nm Sample #			
Day	1	2	3	Avg. O.D.
0	.034	.037	.038	.036
1	.051	.048	.054	.051
3	.049	.051	.063	.054
6	.124	.125	.125	.125
9	.202	.192	.203	.199
14	.337	.327	.348	.337

TABLE 60

Run P1

Results - Available Lysine at 44°C

(mg lysine/100 mg protein)

Day	Replicates 1	2	3	Avg.
0	--	6.9	7.6	7.3
1	7.6	8.3	6.8	7.6
3	7.9	7.1	7.6	7.5
6	7.8	8.2	8.2	8.1
9	7.5	7.8	7.3	7.5
14	7.9	7.5	7.9	7.8

TABLE 61

Results - Protein Solubility at 44°C

Run P1

(mg soluble protein/100 mg total protein)

Day	Replicates 1	2	3	Avg.
0	1.6	1.6	--	1.6
1	1.7	2.0	1.9	1.9
3	2.1	1.7	1.8	1.9
6	1.4	1.6	1.4	1.5
9	1.6	1.6	1.6	1.6
14	1.6	1.4	1.3	1.5

Avg sample variability $\pm$ 0.1

in lysine occurred may be due to the recent evidence (Carpenter, K., AMA Conference on Proteins in Foods. Nov. 17, 1972. Chicago, Illinois) that suggests that the TNBS reagent may be so powerful as to be able to bind to lysine groups that have reacted in the NEB scheme with the reducing sugars. With respect to protein, the amount of salt soluble protein is so small that the test does not really indicate the total effect on protein. This test did not seem to be adequate so that a different solubility test was tried.

In Run P2 the suitability of an enzymatic digestion procedure was tested for protein solubility. The same system as used for Run P1 was used. Three samples ($\sim$ 1 g) were held for 3 hr at 95°C to induce browning and then cooled at 25°C. To these 25 ml of 10^{-4}ml trypsin solution (pH 7.8) buffer were added and digestion was allowed to occur for 1 hr at 45°C. If lysine becomes unavailable then less of the protein would be chopped up by the enzyme. Following digestion, the samples were centrifuged for 10 min at 1000 g and were then filtered through Whatman #3 filter paper. The filtrate was then analyzed for total protein by the biuret procedure. A control was made by holding triplicate samples for 3 hr at 25°C. The results indicated in Table 62 that reading the sample at 555 nm for measurement of protein was inadequate since the brown pigment also absorbed in this region and gave a higher reading than the controls.

In Run P3 the same test as Run P2 was repeated except that soluble protein was determined by the micro-Kjeldahl procedure. The results shown in Table 62 verify that the NEB sample had less soluble protein (significant at 95% level), however, this is in a model system. Since

TABLE 62

NEB - Protein Results

Run P2

Sample	$OD_{555\ nm}$ Protein	$OD_{420\ nm}$ Browning
Control	0.392	0.121
NEB (a)	0.463	0.513

Run P3

Control	% Soluble Protein
A	10.01
B	9.83
C	9.88
NEB(a)	
A	9.07
B	9.02
C	9.07

(a) held 3 hr at 95°C

the chicken used in both the soak-infusion procedure and Hennican is already cooked, its protein solubility should be low and may not be easy to measure. In addition, the sample protein content variability may be quite large. No further model systems were studied as it was decided to test the methodology on the actual food systems.

3. Vitamin Stability: Model Systems

(a) Thiamine

In Run V1 the stability of thiamine as a function of water activity was studied at 55°C using the direct mix model system as described in Table 17. The results are plotted as a first order plot in Figure 30. As can be seen, the thiamine is rapidly lost as A_w increases. The calculated rate constants and half lives are presented in Table 63 with the projected half life for storage at room temperature based on an activation energy of 30 Kcal/mole (Labuza, 1972). As seen, in the IMF range of A_w from 0.75 to 0.84 the thiamine half life is 5 to 7 months. Due to the high activation energy, the half life would be only 1 to 2 months at 35°C for the same range. Thus, thiamine is very unstable and considerable overrun may be necessary if the food product is to be a major supply of this vitamin in the diet.

(b) Ascorbic Acid

In Run V2 the destruction of ascorbic acid was studied in a model system similar to that used for the lipid oxidation studies. The systems used are shown in Table 64. These systems were weighed into 2 oz flasks after preparation by the direct mix methods previously reported. Samples were taken periodically and tested for ascorbic acid.

The results are presented in Table 65 for ascorbic acid retention.

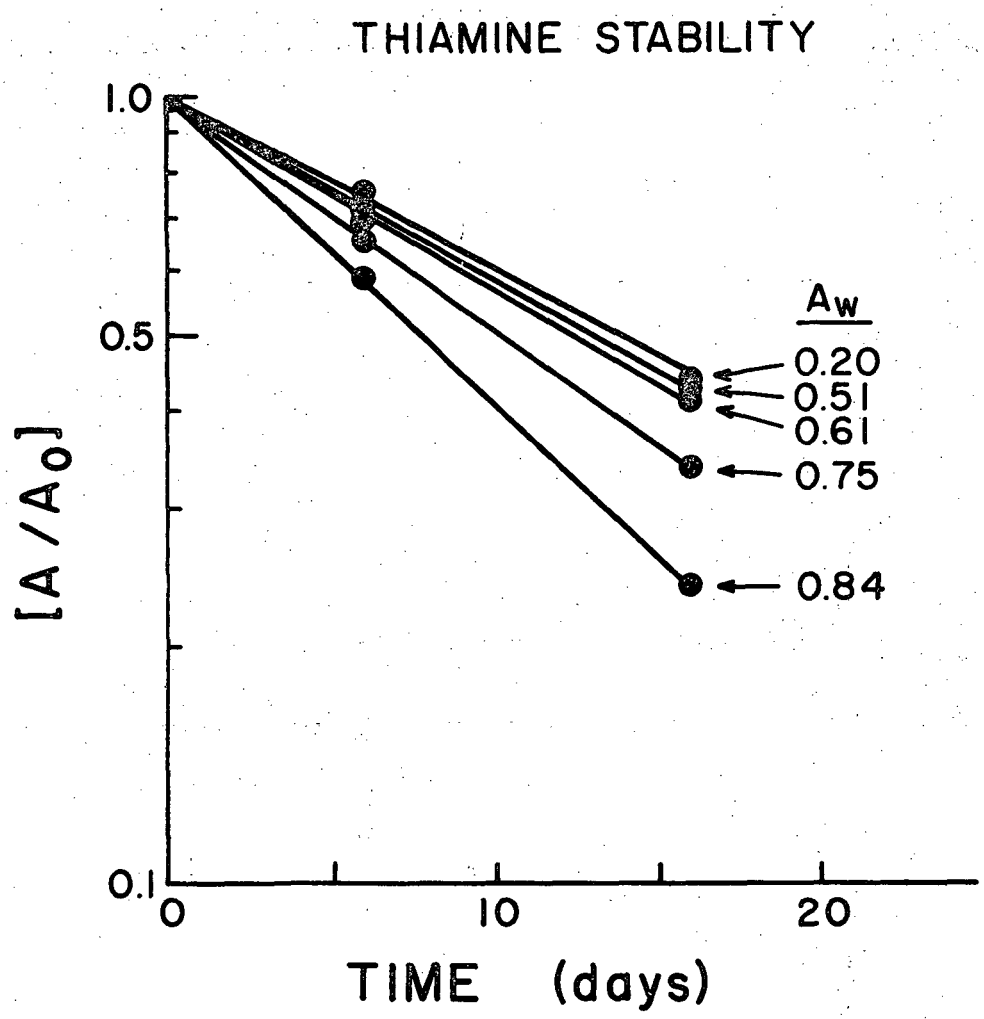


FIGURE 30. THIAMINE STABILITY AT 55°C FOR VARIOUS A_W'S IN A MODEL SYSTEM

TABLE 63

Thiamine Destruction Rate Constants

A_w	k day^{-1} (55°C)	Predicted half life (days) $\theta_{\frac{1}{2}}$ (55°C)	$\theta_{\frac{1}{2}}$ (25°C)
0.20	5.13×10^{-2}	13.5	297
0.51	5.44×10^{-2}	12.7	279
0.61	5.78×10^{-2}	12.0	264
0.75	7.03×10^{-2}	9.9	217
0.84	9.24×10^{-2}	7.5	165

TABLE 64

Vitamin C Stability Tests

Composition of Model System (grams)

System	A_w:	0.51	0.67	0.75	0.84
Corn oil		10	10	10	10
Glycerol		40	40	40	40
Cellulose		50	50	50	50
Moisture content (g*/100g solids)		9.0 g	19.7 g	31 g	57.3 g
Ascorbic acid		100 mg	100 mg	100 mg	100 mg

*per isotherm

TABLE 65

Ascorbic Acid Retention RUN V2

(mg/100 g solids)

Time*	A_w 0.51	0.67	0.75	0.84
19				85.0
21			71.4	
25		92.2		
44	87.9			
47				40.1
48			52.3	
54		68.5		
64	83.8			
67				12.2
68			39.2	
74		43.3		
87	77.7			
90				4.1
91			27.3	
97		40.9		
110	62.4			
114			14.9	2.7
119		24.4		
132	64.8			
135				0
136			6.4	
141		14.1		
153	42.7			
158				0
159			1.71	
162		17.0		
182	48.0			

*hours

The data were plotted on the basis of a first order reaction as ln (% retention) vs time and the half lives as determined from the slope of the line are shown in Table 66. As compared to Run V1, the rate of ascorbic acid destruction is much faster than for thiamine. At 35°C thiamine should be lost at about 10 to 20 times slower. Ascorbate is very unstable, for example at A_w 0.75 the half life of vitamin C is about 2 days. In this kind of system, as shown in Figure 4, vitamin C could not be added if 6 months shelf life is desired. These data indicate that nutrient destruction is significant in the intermediate moisture range and may limit the applicability of these foods to human feeding unless vitamins are protected or they are given as supplements.

The composition used in Run V3 was the same as in Run V2 except that pH 6 buffer was used instead of distilled water. This buffer was prepared by adding 12.3 ml of 0.2 M $Na_2HPO_4 \cdot 7\ H_2O$ solution to 87.7 ml of 0.2 M $Na_2HPO_4 \cdot H_2O$ and diluting to 200 ml.

The direct mix (DM) was prepared as described in the procedure section. Ten gram samples were placed in 2 oz jars, sealed and stored in a desiccator at 35°C at the respective A_w to prevent water loss. The freeze-dried rehumidified (FDR) samples were prepared by freezing at -20°C followed by drying for 18 hr at 75°F and 100 μHg. The samples were held open in respective desiccators for 3 days and then were sealed in jars. Zero time for both the DM and FDR systems was taken at the time at which the jars were sealed. Samples did lose some ascorbate during humidification as moisture increased so zero time was adjusted to the time at which they were sealed. The moisture contents attained and A_w's achieved are shown in Table 67. As seen, due to the short humidification time,

TABLE 66

Half Life: Ascorbic Acid Destruction

(days @ 35°C)

A_w	Direct Mix			Humidified		Thiamine*
	Run V2	Run V3	Run V4	Run V3	Run V4	Run V1
0.44	--	6.9	4.6	4.5	--	--
0.45	--	--	--	--	11.6	--
0.51	8.1	--	--	--	--	54
0.62	--	--	--	2.7	8.4	--
0.66	--	4.2	2.4	--	--	--
0.67	3.0	--	--	--	--	--
0.69	--	--	--	1.8	--	--
0.72	--	3.0	1.4	--	--	--
0.75	2.0	--	--	--	7.4	42
0.77	--	--	--	1.0	--	--
0.82	--	--	--	--	5.7	--
0.84	1.0	1.8	0.8	--	--	32

*estimated

TABLE 67

Moisture Characteristics

Vitamin Stability Tests

Run V3

Theoretical A_w	0.51	0.67	0.75	0.84
Measured A_w (3 day)				
FDR	0.44	0.61	0.69	--
DM	0.45	0.66	0.72	0.84
Moisture (g H_2O/100 g solids)				
DM	9.0	19.7	31.0	57.3
FDR (3 day)	8	17	17	27

Run V4

Theoretical A_w	0.51	0.67	0.75	0.84
DM				
0 day	0.45	0.66	0.72	0.84
7 days	0.43	0.65	--	0.86
DH				
4 days	0.41	0.61	0.72	0.80
7 days	0.43	0.63	0.75	0.82
Moisture content (g H_2O/100 g solids)				
DM	9.0	19.7	31.0	57.3
DH				
5 days	8.4	17.4	26.4	50.8
7 days	8.2	17.2	26.3	50.3

the FDR systems are at a lower humidity then the desiccators used.

The results of ascorbic acid loss plotted as (A/Ao) is shown in Figure 31. As found in Run V2, a rapid destruction rate was found which increased with increased A_w. Also, the humidified systems oxidized faster than the direct mix system. This could be due to metal catalysis since the salts in the buffer added before freeze-drying could be concentrated in the lower moisture system, similar to that found for lipid oxidation. In Run V4 the same composition as in Run V3 was used except that the direct mix samples were kept exposed in the desiccators and the humidified samples were prepared as in the oxidation studies; instead of freeze-drying the dry ingredients were mixed together and held in a vacuum desiccator over the desired relative humidity for 3 days. Zero time was then taken at the end of this period when the vacuum was broken, however, all samples were kept exposed in the desiccators.

The moisture-A_w data is shown in Table 67. As seen, equilibrium was established in all the systems except at 0.51 where a lower equilibrium value was found.

The data of Run V4 are shown in Figure 32. The calculated rate constants are plotted vs A_w in Figures 33 and 34 for Runs V3 and V4. In Run V4 the ascorbic acid in the humidified systems oxidized slower than the direct mix. This is opposite to the results of Run V3. This reverse can be explained on the basis of system preparation. In Run V3, the humidified system was prepared by freeze-drying the direct mix system which had been buffered to pH 6. The system with the higher moisture content (i.e. DM) should thus oxidize slower due to dilution of the ascorbate in the available water. In Run V4, however, the

FIGURE 31. FIRST ORDER RATE PLOT FOR ASCORBIC ACID DESTRUCTION IN A CELLULOSE MODEL SYSTEM - RUN V3

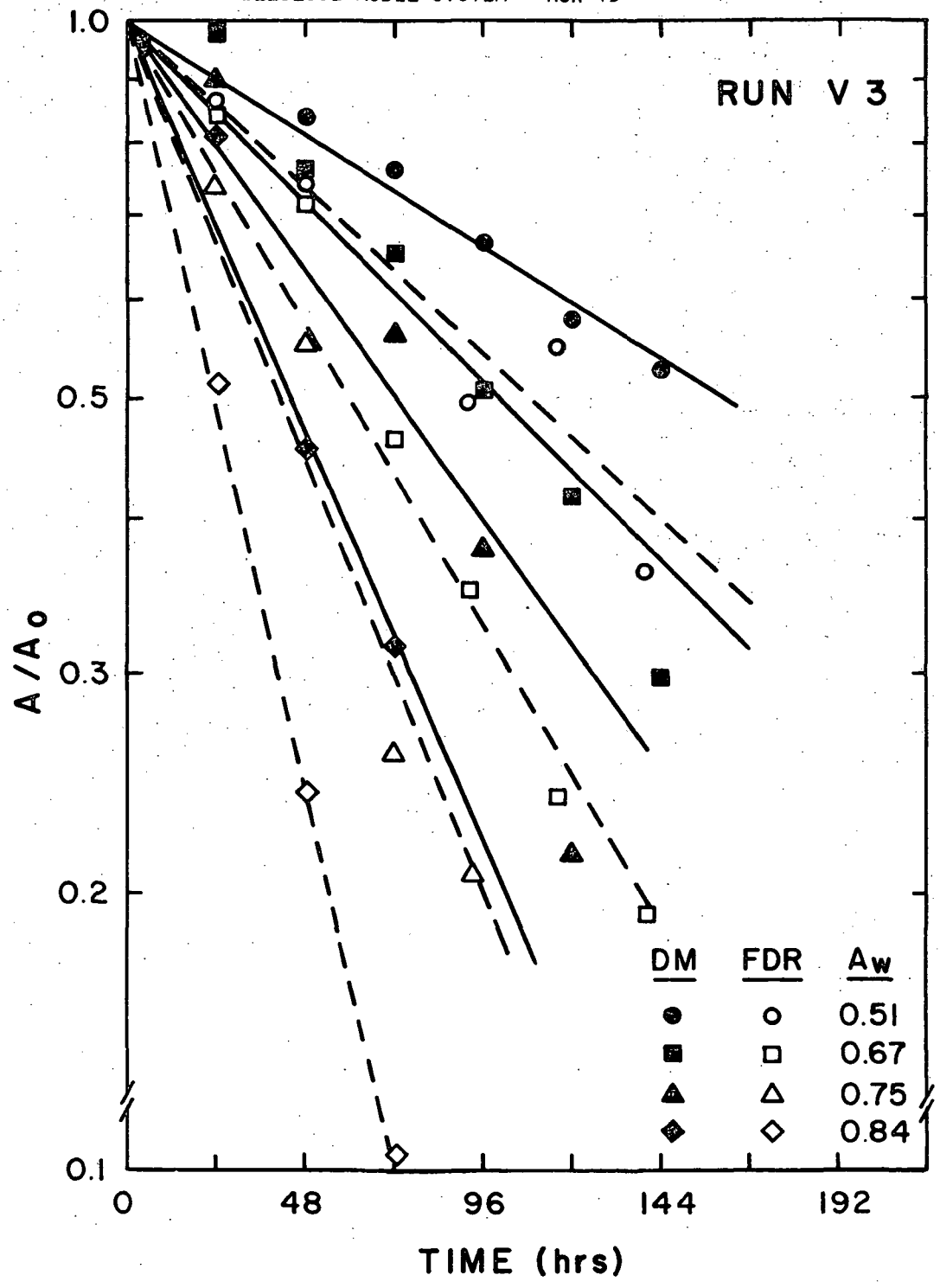
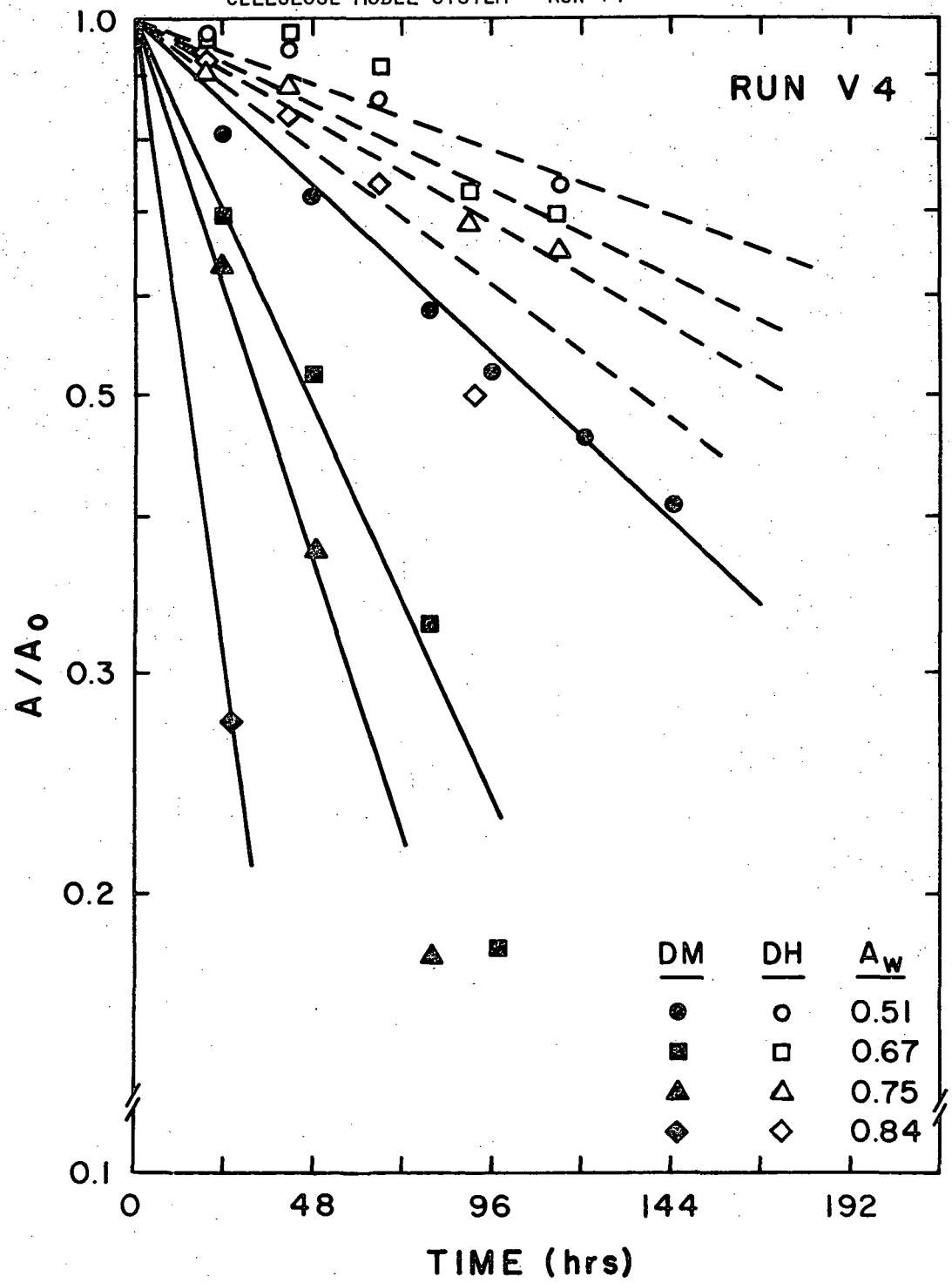


FIGURE 32. FIRST ORDER RATE PLOT FOR ASCORBIC ACID DESTRUCTION IN A CELLULOSE MODEL SYSTEM - RUN V4



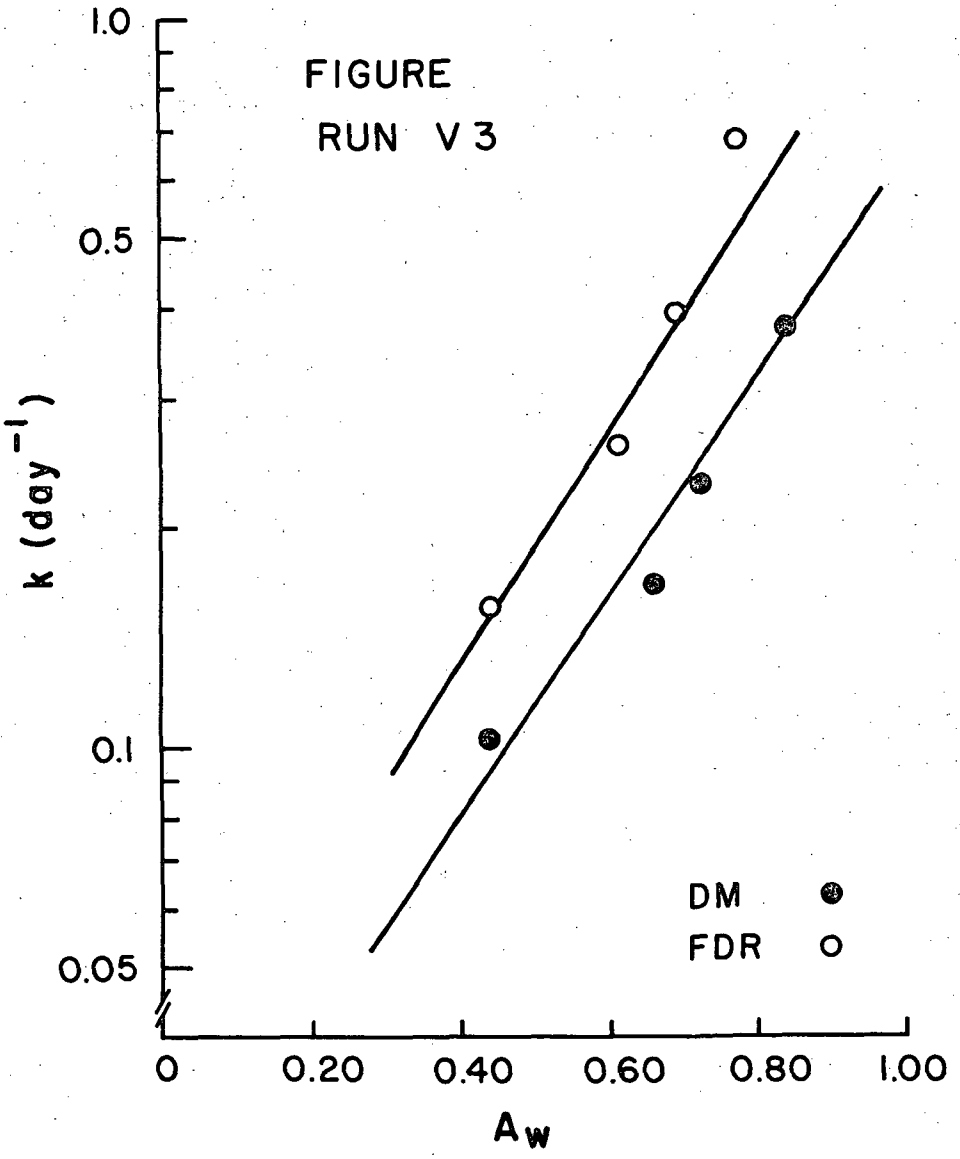


FIGURE 33. DESTRUCTION RATE CONSTANT (DAY^{-1}) FOR ASCORBIC ACID AS A FUNCTION OF A_W AND SORPTION HYSTERESIS - RUN V3

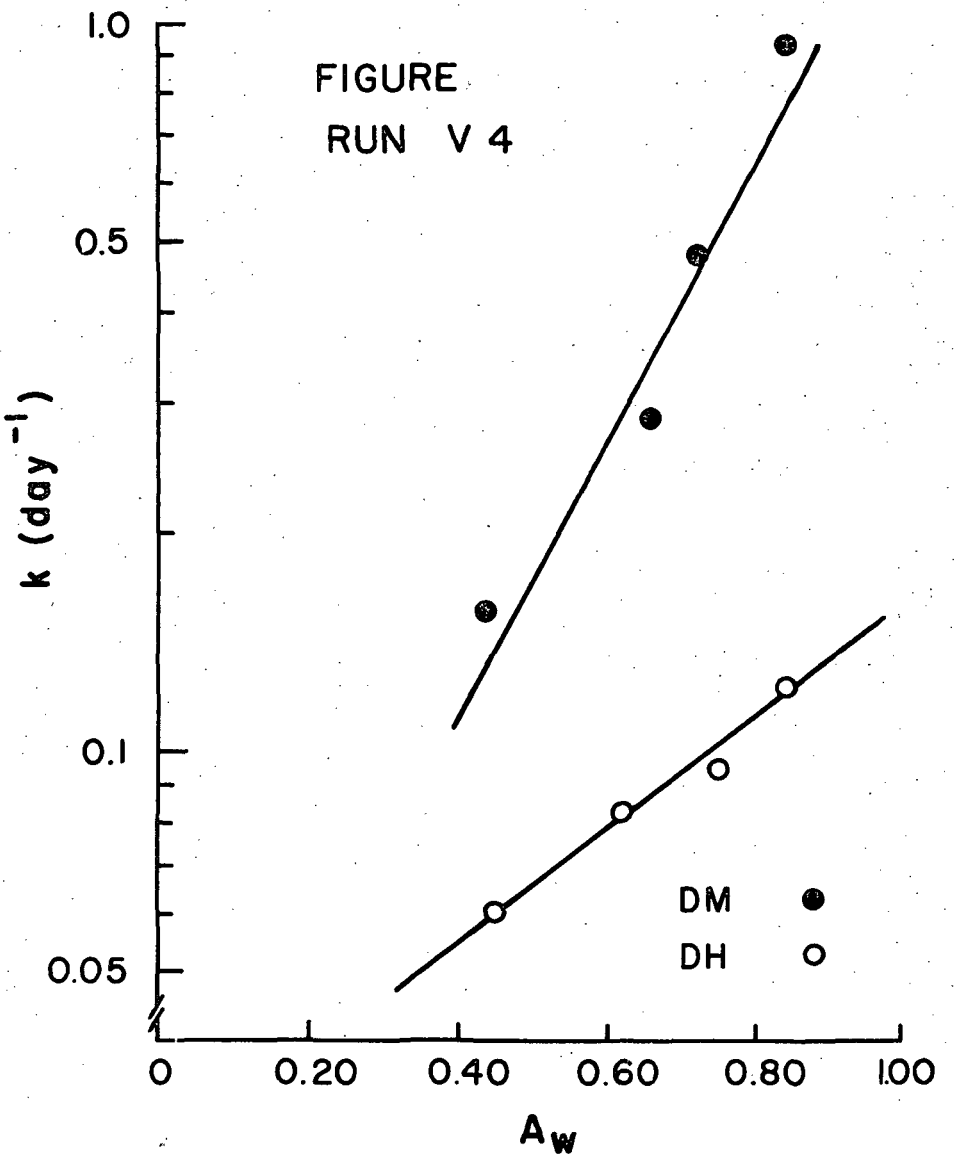


FIGURE 34. DESTRUCTION RATE CONSTANT (DAY^{-1}) FOR ASCORBIC ACID AS A FUNCTION OF A_w AND SORPTION HYSTERESIS - RUN V4

humidified system was made by direct humidification with no buffer added and it oxidized slower than the DM which was buffered to pH 6. In this case the higher moisture system unexpectedly showed the higher rate for ascorbate oxidation. These results show that the trace metal content of the buffer is very important. Most likely the trace metal content of the buffer acts as a significant catalyst for ascorbate oxidation.

The half lives for all three ascorbate runs vs thiamine are compared in Table 66. For ascorbate the values are all about the same order of magnitude but the change in A_w varies between each run. This could be due to small differences in the trace metals present. It is obvious, however, that in the IMF range of A_w 0.8 to 0.83 the half life of ascorbate is less than 1 week. Thus, either the food cannot be used as a source of vitamin C or the C must be protected from moisture. Thiamine although having a longer half life shows only a 1 month half life in this A_w range which again is not very good if the food is to be a thiamine source.

4. Microbiological Study: Model System

The methods for preparing a liquid pork model system was described in the procedures. Systems containing pureed pork and glycerol were made to five water activities (0.68, 0.71, 0.82, 0.86 and 0.92) by both adsorption and desorption methods. These were prepared and held at room temperature (24 $\pm$ 1°C) for up to a period of eight weeks after inoculation with the four different species of microbes at a level of about 10^5 organisms/gram. The results obtained are reported in Table 68 and Figures 35, 36 and 37.

Pseudomonas species are quite common contaminants of meat products

especially when held under refrigerated conditions. They produce slime and cause off-odors. As can be seen in Table 68, they are fairly sensitive organisms to the stress of low A_w and die out at all humidities below 0.92 in 4 days. This is expected as they are gram negative bacteria. The species used in this study was even less resistant than that reported by Labuza et al. (1972b). In that study a different organism was used and the liquid pork system contained salt and MSG both which might have allowed growth to a lower A_w since less glycerol was needed. In any case Pseudomonas should be no problem in IMF systems as also was found by Marshall et al., 1971.

Figure 35 presents the data for the yeast, Candida cypolytica, on the basis of the change in viable count. For the direct mix, growth occurs at A_w 0.92 but the organism dies out below that within a few weeks. No significant growth occurs for the rehumidified systems with rapid death in all cases. In addition, death occurs at a faster rate in the FDR systems as was found by Plitman et al. (1973). This could be due to an inhibition of the repair mechanisms.

Staphylococcus organisms being gram positive are more resistant to stress and can tolerate low A_w. This is a serious problem for intermediate moisture foods as the foods are usually made to as high an A_w as possible to insure enough water for palatability. The results of this test for Staphylococcus aureus (F265) are shown in Figure 36. For the direct mix system the organisms are able to grow at A_w 0.92, but at 0.86 and below they die out rapidly. For the rehumidified system no growth occurs at any A_w as was previously reported by Labuza et al. (1972b).

It is interesting to note that from a production standpoint if

TABLE 68

Micro-Model System

Viable Organisms/Gram Pork

Pseudomonas fragi

A_w → / STORAGE TIME ↓	0.67		0.71		0.81		0.86		0.92	
	DM	FDR	DM	FDR	DM	FDR	DM	FDR	DM	FDR
0 DAYS	$<10^2$	0	$<10^2$	<10	$<10^2$	<20	$<10^2$	<40	$<10^2$	<60
4 DAYS	0	0	0	0	0	0	0	0	<10	0
1 WEEK	0	-	0	-	0	-	0	-	0	-

FIGURE 35. STABILITY OF CANDIDA CYPOLYTICA IN A PORK SLURRY MODEL SYSTEM AS A FUNCTION OF A_W AND SORPTION HYSTERESIS

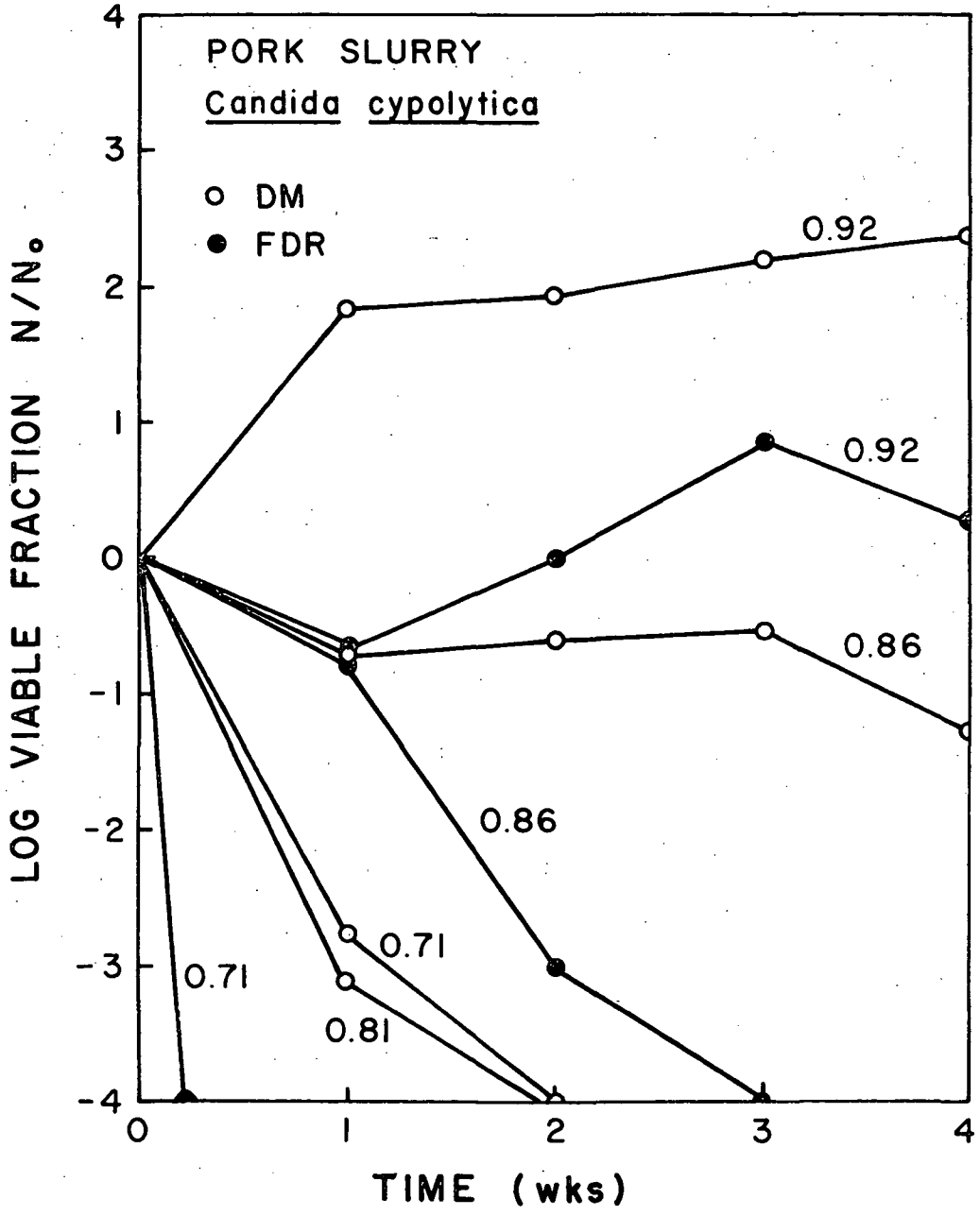


FIGURE 36. STABILITY OF STAPHYLOCOCCUS AUREUS IN A PORK SLURRY MODEL SYSTEM AS A FUNCTION OF A_w AND SORPTION HYSTERESIS

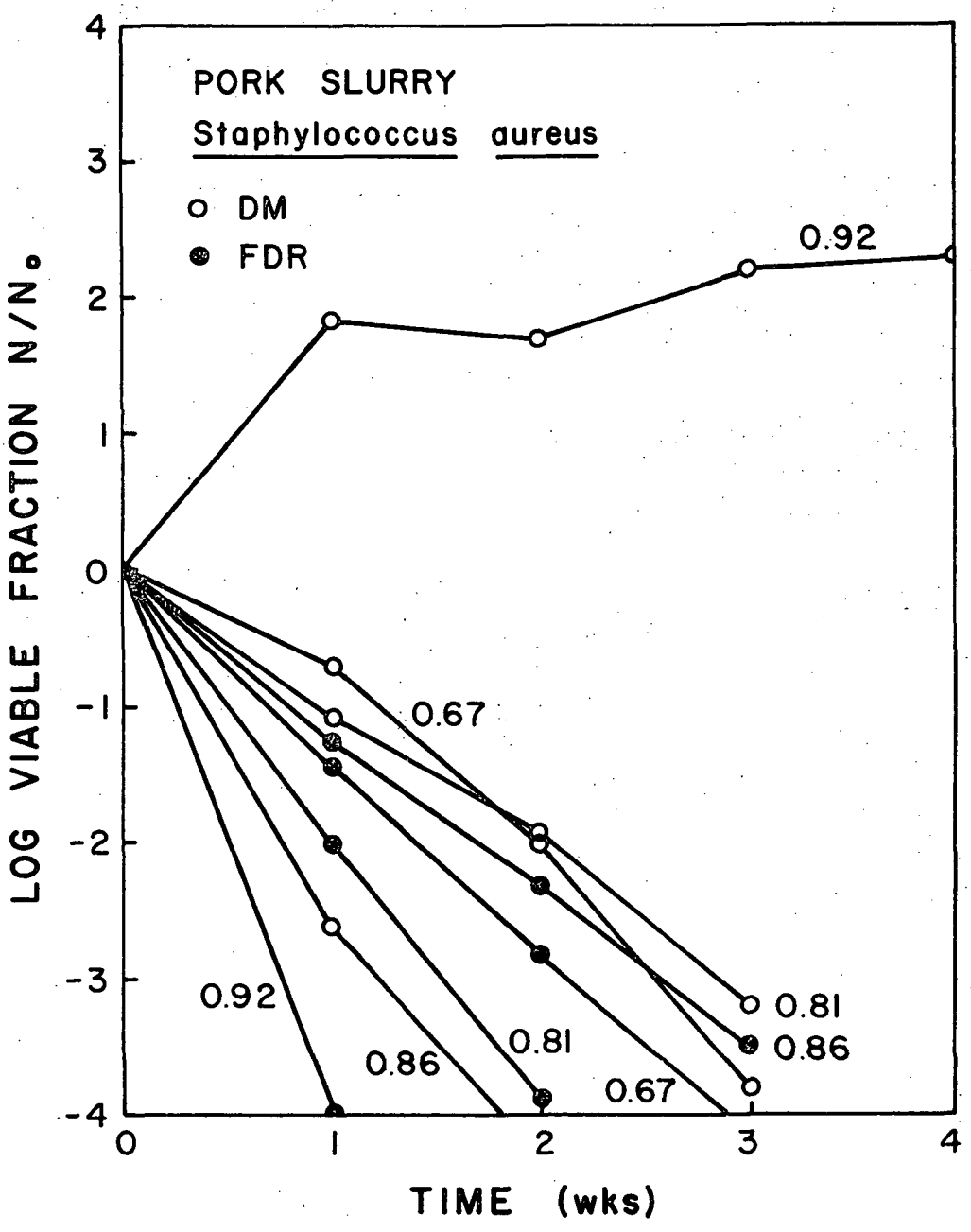
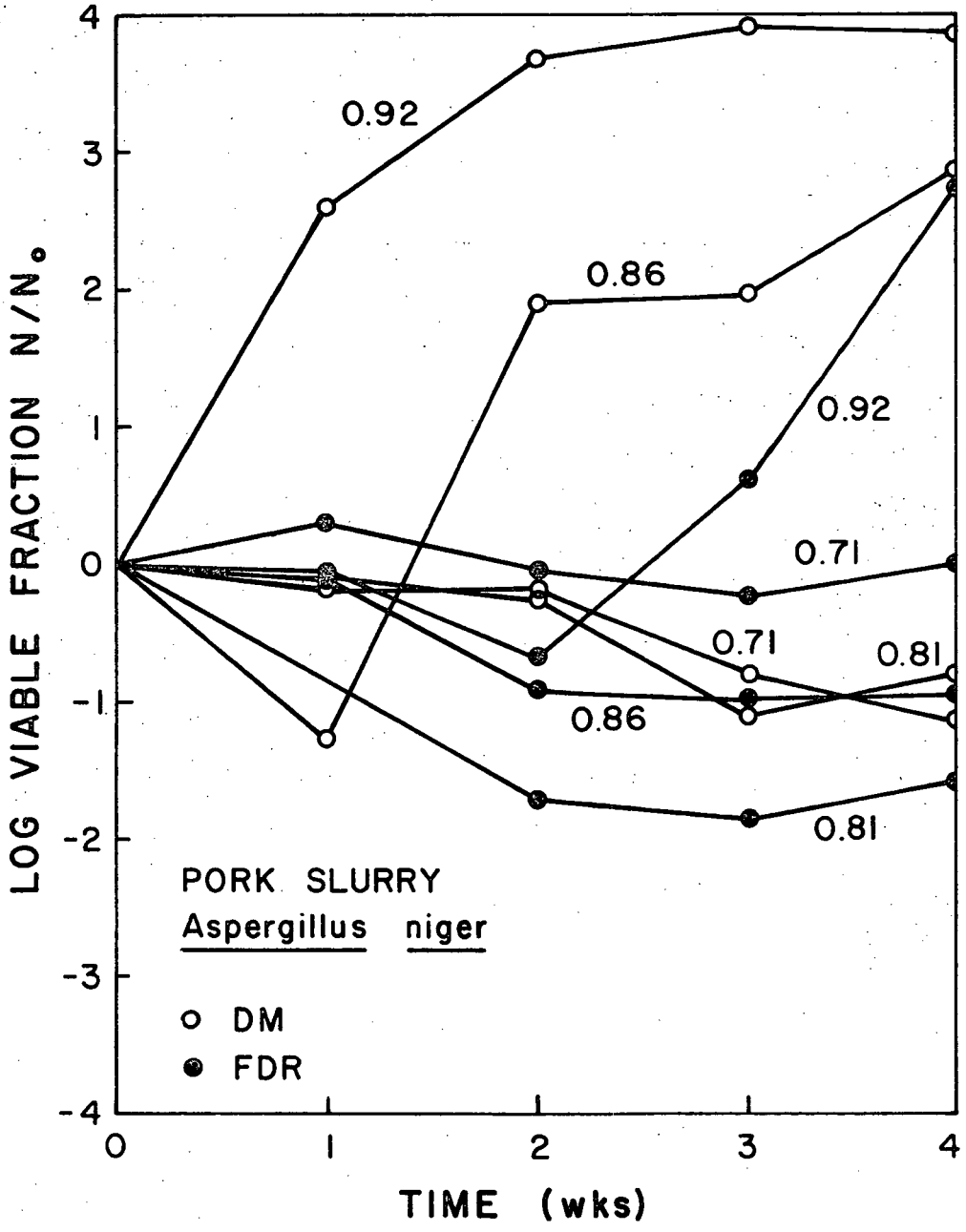


FIGURE 37. STABILITY OF ASPERGILLUS NIGER IN A PORK SLURRY MODEL SYSTEM AS A FUNCTION OF A_w AND SORPTION HYSTERESIS



quality control checks were made in 2-3 weeks after production it is possible that the product would be condemned due to the presence of staph organisms which are pathogenic organisms. However, these are not growing and certainly are not producing toxin. They should not be a problem with IMF systems. In addition, the same phenomenon as found by Plitman is observed in the FDR systems. As A_w decreases there is a reversal and the rate of death actually decreases. This psuedo-protective mechanism could be due to the fact that as A_w decreases, different enzyme systems are shut off. It is possible that at higher A_w, intermediates are produced which kill the cell. As the A_w goes lower, the whole enzyme system shuts down so that the organism does not die but is stabilized. Again, with respect to storage these pathogens should not be a problem as the product would lose moisture most likely in storage and, thus, they would not regrow.

The most resistant organisms to low A_w stress are molds. In Figure 37 are presented the results for Aspergillus niger in the liquid pork model system. For the direct mix the organism grows down to A_w 0.86; below that it is dying out slowly but is still present. This could be a problem if the food is not protected from moisture pickup in humid environments since the limit of growth is right on the IMF A_w limit. For the humidified systems growth occurs at A_w 0.92 but below that it dies out. Again, a reversal in death rate occurs in the FDR systems at A_w 0.71 as for the Staphylococcus.

Overall, these results as summarized in Table 69 show that the range of A_w for limitation of growth is higher for the rehumidified systems than for the direct mix systems. This is similar to that reported

TABLE 69

Microbiological Model System

Limiting A_w Range for Growth

	DM	FDR
Staphylococcus aureus	>0.86 <0.92	>0.92
Candida cypolytica	>0.81 <0.86	>0.86 <0.92
Pseudomonas fragi	>0.92	>0.92
Aspergillus niger	>0.81 <0.86	>0.86 <0.92

TABLE 70

Chicken Infusion System

Storage Study

A_w infusion	0.68	0.75	0.83	0.88
Weight change % (Avg. of two flasks)	16.7	15.9	10.9	9.8
Moisture content (g H_2O/g solids)				
direct mix (a)	0.477	0.581	0.785	1.021
direct mix (b)	0.423	0.513	0.786	1.05
rehumidified (c)	0.404	0.548	0.671	0.694
rehumidified (b)	0.395	0.563	0.754	0.786
A_w				
direct mix	0.68	0.74	0.81	0.86
rehumidified	0.66	0.73	0.80	0.84

(a) Weight lost in freeze-drier corrected for water left by GLC
(b) by GLC
(c) Avg. of 6 samples based on weight gain

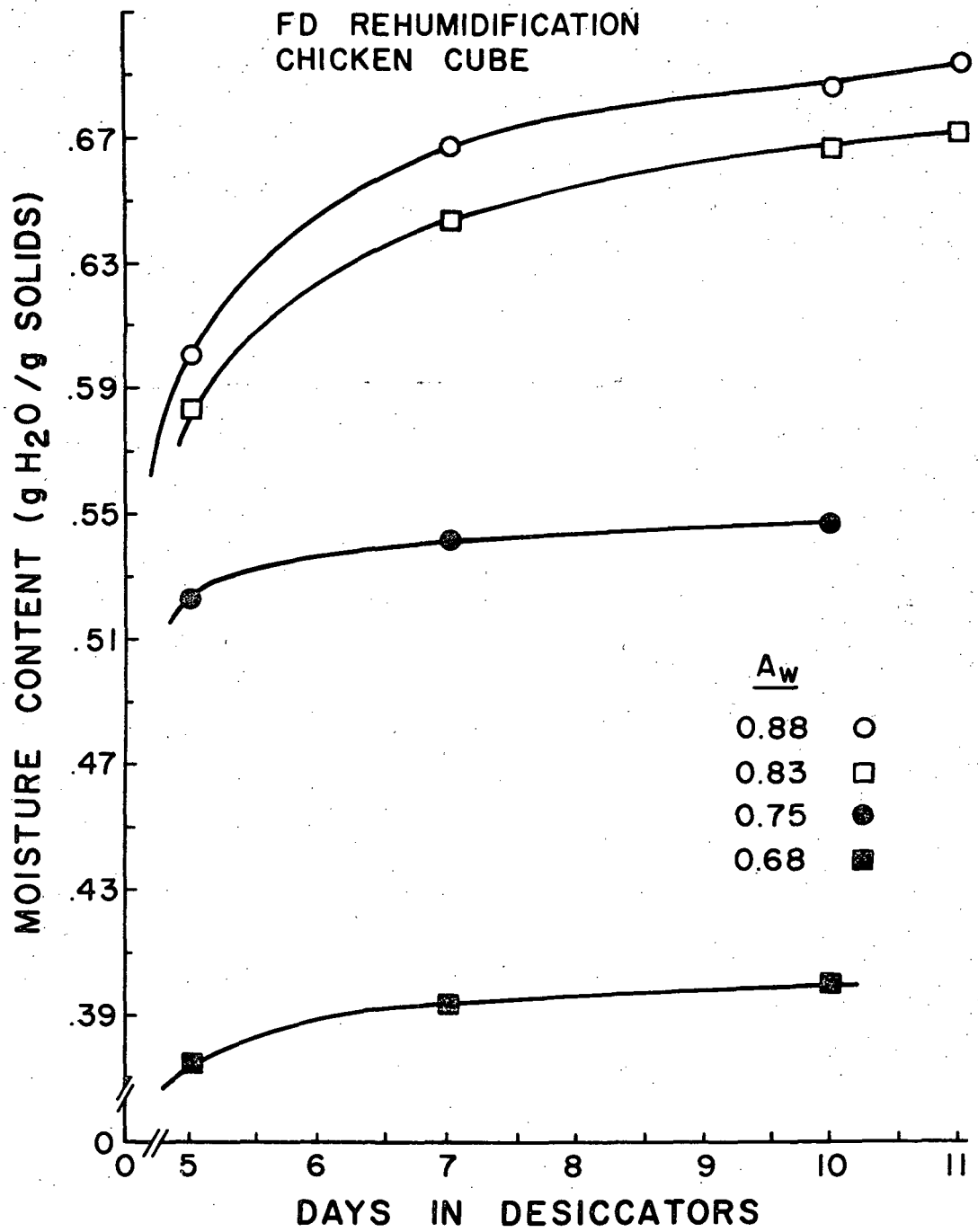


FIGURE 38. REHUMIDIFICATION TIME FOR FREEZE-DRIED SOAK-INFUSION CHICKEN CUBES AT VARIOUS A_w'S

TABLE 71

Chicken Infusion System

Peroxide Value 37°C Storage

A_w	0.68		0.75		0.83		0.88	
Days	DM[(a)]	FDR[(b)]	DM[(a)]	FDR[(b)]	DM[(a)]	FDR[(b)]	DM[(a)]	FDR[(b)]
0	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
5	N.C.		N.C.					
7		N.C.		N.C.				
8					N.C.	0.95	N.C.	1.24
15	N.C.	0.64	N.C.	0.94	N.C.		1.53	
16						2.98		3.48
21	N.C.	N.C.	0.60	1.51	1.13		8.05	
22				0.80		3.12		3.70
28						3.94		
29	N.C.		2.01		2.87		4.51	2.78
34	N.C.	2.16	0.87	1.22	4.21		7.86	
35						4.95		6.25
42	N.C.		0.42					
43		N.C.		1.75	5.43		33.37	
45						6.56		5.37
47	N.C.		1.75					
49	N.C.				3.37		21.21	
51		0.87		2.45		5.08		5.32
56		0.62		2.79		6.71		7.81
57	N.C.		1.77		7.92			
60							23.64	
64	--		1.42		8.57		25.05	
69	0.85		3.96		7.29		10.19	
92	--	2.03	--	1.44		15.96		20.88
102	1.08		1.91		7.14		21.23	

(a) Direct mix
(b) Freeze-dried rehumidified

TABLE 72

Free Fatty Acid Value

Storage at 37°C

A_w	0.68		0.75		0.83		0.88	
Days	DM[a]	FDR[b]	DM[a]	FDR[b]	DM[a]	FDR[b]	DM[a]	FDR[b]
0	176	205	169	238	182	228	165	184
5	175		201		224		248	
7		215		221				
8						233		226
14	191		192		207		299	
15		190		199				
16						229		211
20	198	194	176	200	189	196	314	
21								
22								
28	226	228	213	219	212	214	425	
29								250
33	204		162		205		415	
34		204		229		221		225
41	169		163					
42						208	406	
43		194		172				
46	221		121		193		355	
47						239		
48								190
51		177		183				
52						197		263
56	160	217	177	229				
57						269		219
59					210		401	
63	169		236		187		372	
68	188		231		209		504	

(a) Direct Mix
(b) Freeze-dried rehumidified

by Labuza et al. (1972b), however, the A_w limits for desorption are higher in this present study. This could be due to the change in composition of the system from the previous study due to the manufacturers elimination of salt and MSG from the product. These results were used to study the microbiological stability of the IMF systems.

C. Intermediate Moisture Food Storage Studies

1. Chicken Infusion System Storage Study

500 g chicken were infused at A_w's 0.68, 0.75, 0.83 and 0.88 according to Table 27. These were done in 250 g batches added to 2.5 liters of infusion for 18 hr at 80 RPM and 75°F. The freeze-dried samples were humidified for 10 days. All samples were stored ($\sim$ 10 g) in 2 oz jars which were covered first with 2 layers of Reynolon PVC film, then the cover was screwed on and taped shut with 3M black electrician's tape. These were stored at 37°C.

The pertinent moisture data are shown in Table 70. The moisture data compares well with Run C10 as does the hysteresis loop. Figure 38 shows the approach to equilibrium for the humidified samples. At 10 days the samples were very close to equilibrium and were then sealed in jars and stored as with the direct mix system.

The results for peroxide value and free fatty acids are shown in Tables 71 and 72, respectively. Organoleptic testing was attempted but was not recorded as the product had a very sweet taste. This would not be objectionable, however, if the product were presented in a sweet/sour sauce, but this was not done because the sauce might have complicated the stability of the product. It can be seen that the peroxide data of Table 71 show an increase in oxidation rate as A_w

increases for both the direct mix and humidified systems. This is as would be expected based on the model system data. At A_w's below 0.88 no large difference between the two preparation methods exists. At A_w 0.88 the direct mix system is oxidizing faster than the FDR system. In the direct mix system at A_w 0.88 after sixty days a definite off-odor developed, as was also evident in both the A_w 0.83 systems after 90 days. The odor was hay-like indicating rancidity. This is also evidenced by the higher peroxide values in these products at the same time. The systems at lower A_w show only a very small increase in peroxide value and did not produce a rancid off-odor. However, their taste and odor were not acceptable when eaten directly. This might be due to non-enzymatic browning as all the samples darkened with age.

With respect to free fatty acid production there is no apparent pattern below A_w 0.88. In the direct mix system at 0.88 the acid value increases with time. Most likely this is due to acid compounds produced from lipid oxidation and is not due to enzymatic processes since no change occurred at the lower A_w's. It can be concluded that below A_w 0.88 where microbial growth should not occur the product is stable for at least 2 months. Samples of these systems which had been kept at 4°C were plated at 75 days to check their sterility. The system had sorbate added as a microbial preservative. Table 73 shows the results. At A_w 0.83 and below the samples were not sterile, but contained less than 300 organisms per gram in all but one case. The freeze-dried rehumidified systems had only rod shaped colonies as might be expected in the higher glycerol content per gram from the data of Marshall et al. (1971). The direct mix systems had both cocci and rods. At A_w 0.88 there was significant

TABLE 73

Microbial Contamination

Storage at 4°C for 2.5 months

	A_w 0.68	0.75	0.83	0.88
Direct Mix	$< 3 \times 10^2$	$< 3 \times 10^2$	$< 2 \times 10^2$	1.7×10^4
FDR	$< 3 \times 10^2$	$< 7 \times 10^2$	$< 3 \times 10^2$	$< 1 \times 10^2$

growth in the direct mix system. However, in the rehumidified system there was less than 100 organisms per gram. This supports the previous data of growth with respect to A_w and method of preparation. It is possible, however, that more growth might have occurred at higher temperature.

In order to study nonenzymatic browning and its related reactions chicken cubes were prepared at A_w 0.70, 0.76 and 0.89. These were sealed in 2 oz jars (~ 10 g each) and stored at 46°C and tested periodically. The results for browning are shown in Tables 74. The data for browning show increases at both A_w 0.70 and 0.89 but not at 0.76. It is possible that there may be interference with other substances or that the method is not very precise.

To test the precision of the NEB method six samples prepared at A_w 0.76 were tested. The results are seen in Table 75. As seen, the method has a large variation so that sampling only one sample at a given time could give a large error. Based on this more samples should be tested at each time.

The results for available lysine are shown in Table 76. Although there is a decrease in available lysine, it does not occur with respect to any pattern. Again, this can be due to sample variability.

Three, one gram portions of chicken meat were randomly selected and tested in triplicate for lysine. The TNBS procedure was used except that the sample was prepared by putting in a Sorvall mixer to which 100 ml of 4% $NaHCO_3$ buffer at pH 8.5 was added and blended at maximum speed for one minute. The results are shown in Table 77. From a statistical analysis of the results it can be seen that there is an inherent variability

TABLE 74

Non-Enzymatic Browning of Chicken Infusion Systems

Storage at 46°C

(O.D. 420 nm)

	Day				
A_w Sample	0	6	12	22	29
.70	.128	.152	.172	.172	.178
.76	.147	.160	.137	.142	.142
.89	.124	.112	.240	.242	.228

TABLE 75

NEB Precision Testing

Sample #	O.D. 420 nm
1	0.282
2	0.207
3	0.178
4	0.207
5	0.197
6	0.187

Average	=	0.210
Range	=	0.187-0.282
Standard Deviation	=	0.037
95% Confidence	=	$\pm$0.074

TABLE 76

Available Lysine: Chicken Infusion System @ 46°C

A_w of Chicken	mg lysine/100 mg protein Days				
	6	14	22	29	65
.70	2.1	2.3	2.7	2.7	2.1
.76	3.0	4.1	3.2	1.7	2.0
.89	3.0	2.0	2.8	1.9	1.9

*initial value = 3.1 $\pm$ 0.2 lysine/100 mg protein

TABLE 77

Results of Variability of Available Lysine

Sample #	O.D. 346 nm	% Protein	mg Lysine / 100 mg Protein	Mean	Variance	Standard deviation
10	0.00	31.08				
11	.293		2.81			
12	.291		2.78	2.84	.01	.07
13	.305		2.92			
20	0.00	32.47				
21	.347		3.19			
22	.354		3.55	3.26	.07	.26
23	.331		3.04			
30	0.00	31.50				
31	.318		3.01			
32	.320		3.03	3.09	.016	.13
33	.342		3.23			

(90% confidence) in sample selection due to the variability in the chicken itself. This means that multiple samples must be tested for meaningful results as with the non-enzymatic browning study.

As was reported in Table 73 very little growth occurred in the chicken infusion dice stored for 2½ months at 4°C making these foods safe. This was because of the low temperature and possibly the sorbate which was added.

One of the important principles of IMF technology is that the foods are stable towards growth of microorganisms which are pathogenic. For each microbe species there is an A_w level below which it will not grow as was shown in Table 12. As seen, below 0.86 the food should not cause poisoning from pathogenic organisms, however, yeasts may grow in it and spoil the product. In addition, molds could grow causing spoilage as well as produce potential toxins. Examining Table 12 shows, for example, that one would have to be below A_w 0.62 to prevent growth of A. flavus, a potential source of aflatoxin which may be carcinogenic. Because of this most IMF systems made for commerical development contain a growth inhibitor such as sorbate, propionate and propylene glycol. Sorbate which was present in the cubes is most widely used in conjunction with glycol but its effectiveness is limited since most of meat products are in the pH range of 5.5 to 6.5 and sorbate is most effective at pH 4.8. Acid could be added but this causes hydrolysis of sucrose with attendant browning and loss of nutritional value. Figure 39 shows that if a shelf life of only 6 months is needed one could prepare the food at pH 3.5 to 4.0 which would make them safer microbiologically if 50% hydrolysis could be tolerated. If one year shelf life is desired then the minimum pH should be about 40 which is still better for sorbate control.

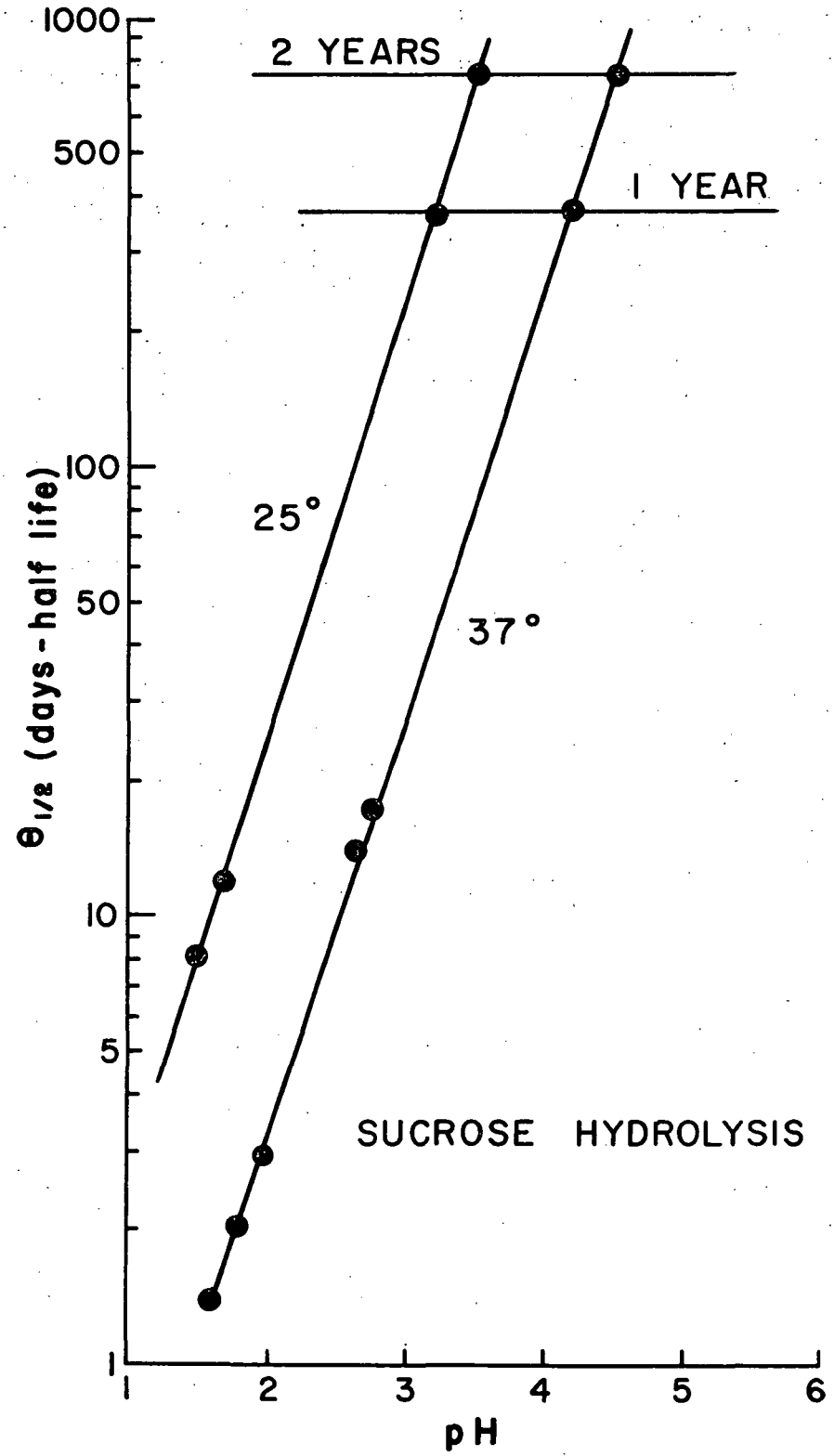


FIGURE 39. HALF LIFE FOR SUCROSE HYDROLYSIS AS A FUNCTION OF pH AND TEMPERATURE

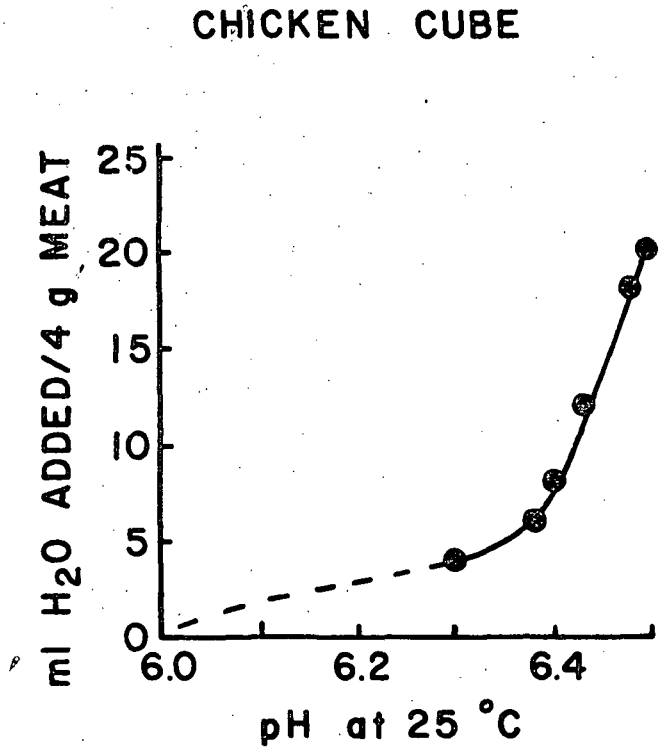


FIGURE 40. pH OF SOAK-INFUSION CHICKEN SYSTEM

Unfortunately, as seen in Figure 40 the predicted pH of the food is probably around 6.0 so sorbate should have very little effect. Further studies were made with inoculated systems to determine the growth characteristics of the four test organisms at 21 to 25°C storage without the addition of sorbate.

In order to prevent contamination of the chicken cubes during the soak infusion from other than the test organism, the infusion solution was pre-sterilized. Since glycerol itself is sterile and is highly volatile, the infusion solutions (Table 27) were made without glycerol, sterilized and to this the amount of water lost was added back. Then the sterile glycerol was added. No sorbate was added.

The soak technique used then was as follows:

Canned white chicken was cut into ½" cubes under sterile conditions. Known amounts were infused at the desired A_w. The chicken was soaked for 18 hr at 21°C at 100 RPM on a rotary shaker using three liter flasks. The chicken was drained and then the infusion at each A_w was divided into four, 600 g quantities and the chicken was divided into four, 60 g quantities. The test organisms were added, respectively, into flasks containing sterile infusion at each desired A_w. The cultures were grown at room temperature in TSY broth except for the mold which was cultured on TSY agar for five days. The inoculum was determined from the optical density of a solution containing the microbes, as shown in Figure 10. A dilution was made of each organism to give one ml inoculum that would give a population in the infusion at about 10^5 organisms per ml. With a dilution of 60 g chicken per 600 ml this would yield an inoculation of about 10^4 organisms/g of chicken. Since some growth or death could

occur when the organisms were put into the infusion solution, after their addition the chicken was immediately added and the system was shaken for 15-20 min at room temperature. The chicken was again immediately drained and then divided into 2 oz jars (~ 5 g each) and put into storage. Half of the original chicken, after the soak infusion but before inoculation with the test organisms, was frozen at -20°C for subsequent freeze-drying. Freeze-drying was done at 60-100 μHg starting with the platens at -18°C. These were shut off when the drier was started and warmed up to room temperature (~ 25°C). Drying time was 18 hr. The samples were dried in 2 oz flasks containing 4-5 g and were covered with gauze to prevent contamination.

In order to rehumidify the FDR samples and inoculate with the test organisms, a known amount (~ 4 g) of freeze-dried chicken was weighed into 60 x 5 mm petri dishes and the amount of water expected at equilibrium as shown in Table 33 was calculated using the 5 day values. To the pieces this amount of water less by 5% was added but containing the test organism to give an initial population of about 10^4 to 10^5/gram. These were then transfered into the desiccator at the proper humidity for fine tuning to the equilibrium humidity. Weight changes of representative samples were followed. Table 78 contains the summary preparation data for the microbiological test. The FDR systems were held in the desiccators for storage at 21°C whereas the DM systems were held in sealed jars.

The isotherms for these systems are shown in Figure 41. As seen, a very large hysteresis loop occurs. In the first phase, the direct mix samples lost considerable weight during storage especially after six weeks as can be seen on the isotherm. Fortunately, the effect

TABLE 78

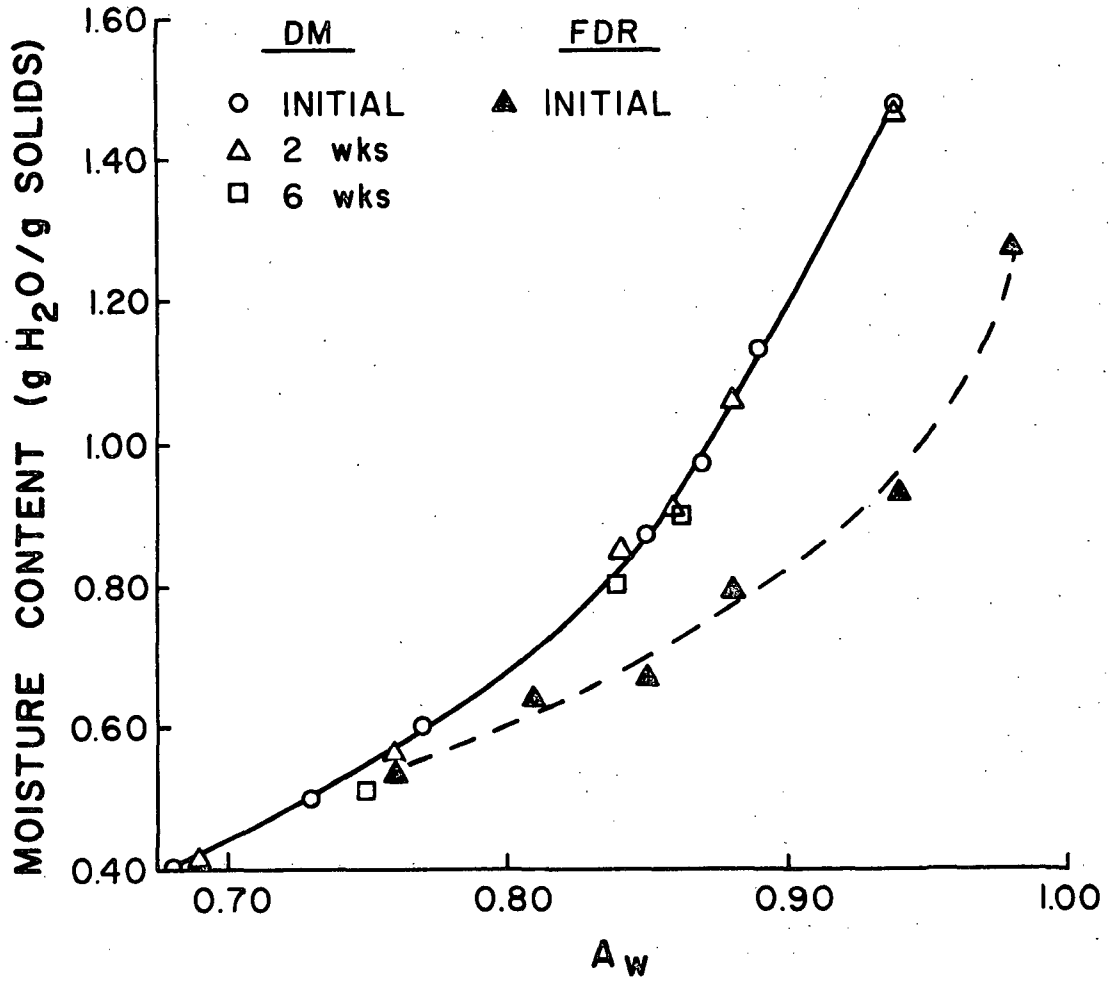
Summary Preparation Data

Chicken Infusion Microbiological Test

Infusion A_w**	0.69	0.75	0.83	0.88	0.90	0.96	0.99
A_w soaked chicken	0.73	0.75	0.82	0.86	0.89	0.93	0.97
Measured moisture content (g H_2O/g solids)	0.42	0.53	0.75	0.91	1.16	1.40	1.72
Weight change after soaking (%)	+5.9	+5.5	+0.02	-0.35	-0.3	-0.57	-3.0
Desiccator salt	$CuCl_2$	NaCl	$CdCl_2$	$LiSO_4$	K_2CrO_4	KNO_3	Na_2HPO_4
Desiccator A_w	0.68	0.75	0.82	0.85	0.88	0.94	0.99
A_w humidified pieces	--	0.75	0.81	0.86	0.88	0.94	0.98
Moisture humidified* (g H_2O/g solids)	--	0.52	0.64	0.67	0.78	0.93	1.27

*Average of 15 samples after 6 and 12 days of storage in desiccator
**pH of all infusion 6.4

FIGURE 41. SORPTION ISOTHERM - CHICKEN CUBE - SOAK-INFUSION MICROBIOLOGICAL STUDY



of A_w on growth can be seen readily in the first month so that this did not effect the results significantly, however, some of the direct mix systems were reprepared and held open in the appropriate desiccators to prevent water loss.

Figures 42 through 45 and Table 79 summarize the results of the microbiological tests for the chicken cube infusion system at 21°C. A pattern very similar to that found for the pork slurry model system was observed. The Pseudomonas fragi were the least resistant organisms especially in the rehumidified systems where they died out even at A_w 0.93. Growth took place at 0.93 for the direct mix system but not for the rehumidified system. As seen in Table 79 where no growth took place, the IMF product had very few live organisms left after one month.

The yeast Candida showed a pattern in Figure 43 also similar to that found in the model system studies. The rates of death were faster in the FDR systems and below a certain level a crossover occurred where there was a lower death rate at the lower A_w. This crossover has also been observed by Plitman et al. (1973). No organisms were present below A_w 0.85 after 2 months.

With respect to the pathogen Staphylococcus, a similar pattern to the yeast exists as seen in Figure 44. The limiting water activity may be the same in both FDR and DH since growth only takes place at the surface as opposed to the slurry system for the model system studies. With respect to toxin production, no toxin is formed if growth does not occur. Thus, at or below A_w 0.88 the product should be safe since the organisms are dying out. In liquid systems Troller (Appl. Micro., 1971, 1972) showed that toxin production ceases at an A_w above the

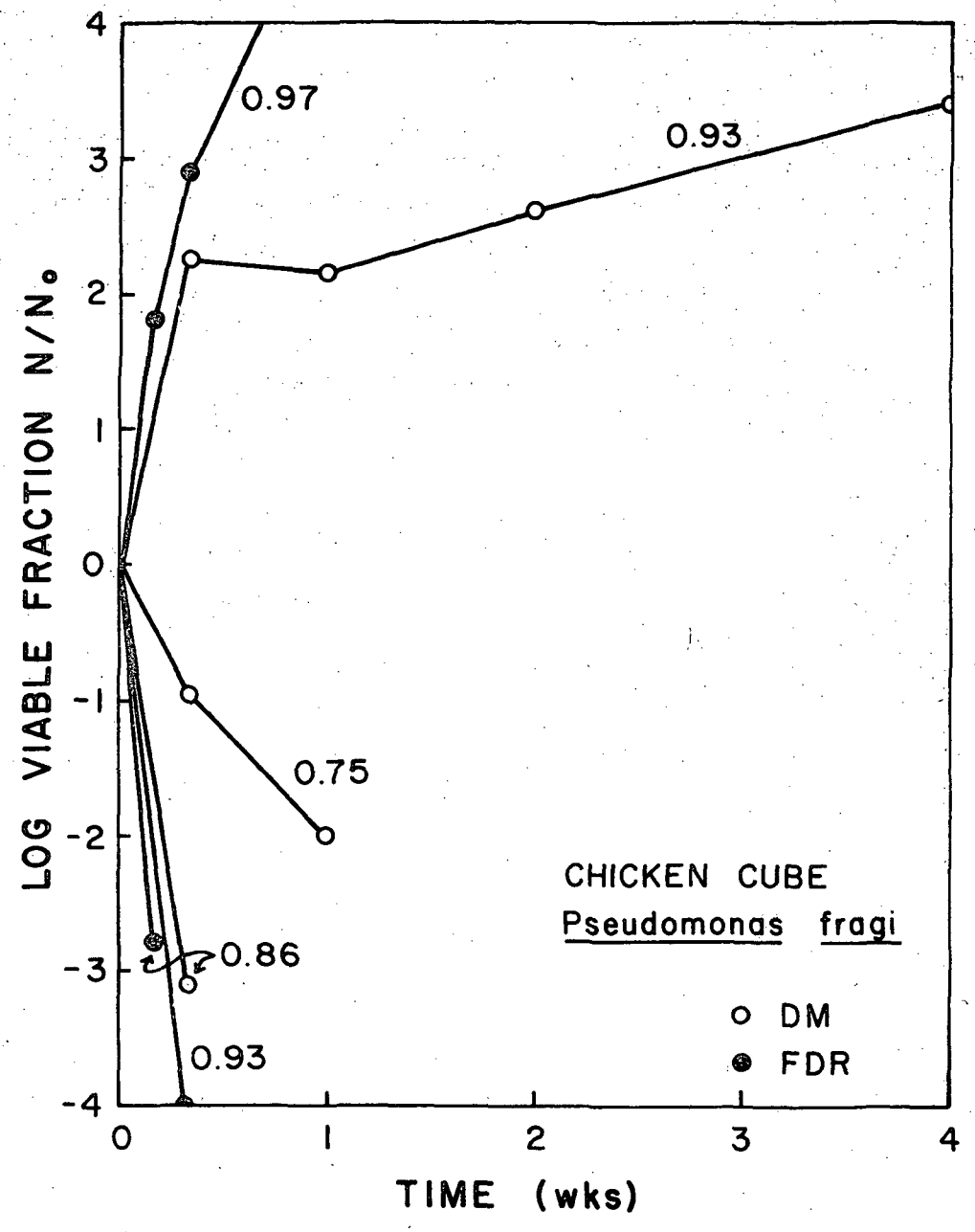


FIGURE 42. STABILITY OF PSEUDOMONAS FRAGI ON A SOAK-INFUSION CHICKEN CUBE SYSTEM AS A FUNCTION OF A_w AND SORPTION HYSTERESIS @ 21°C

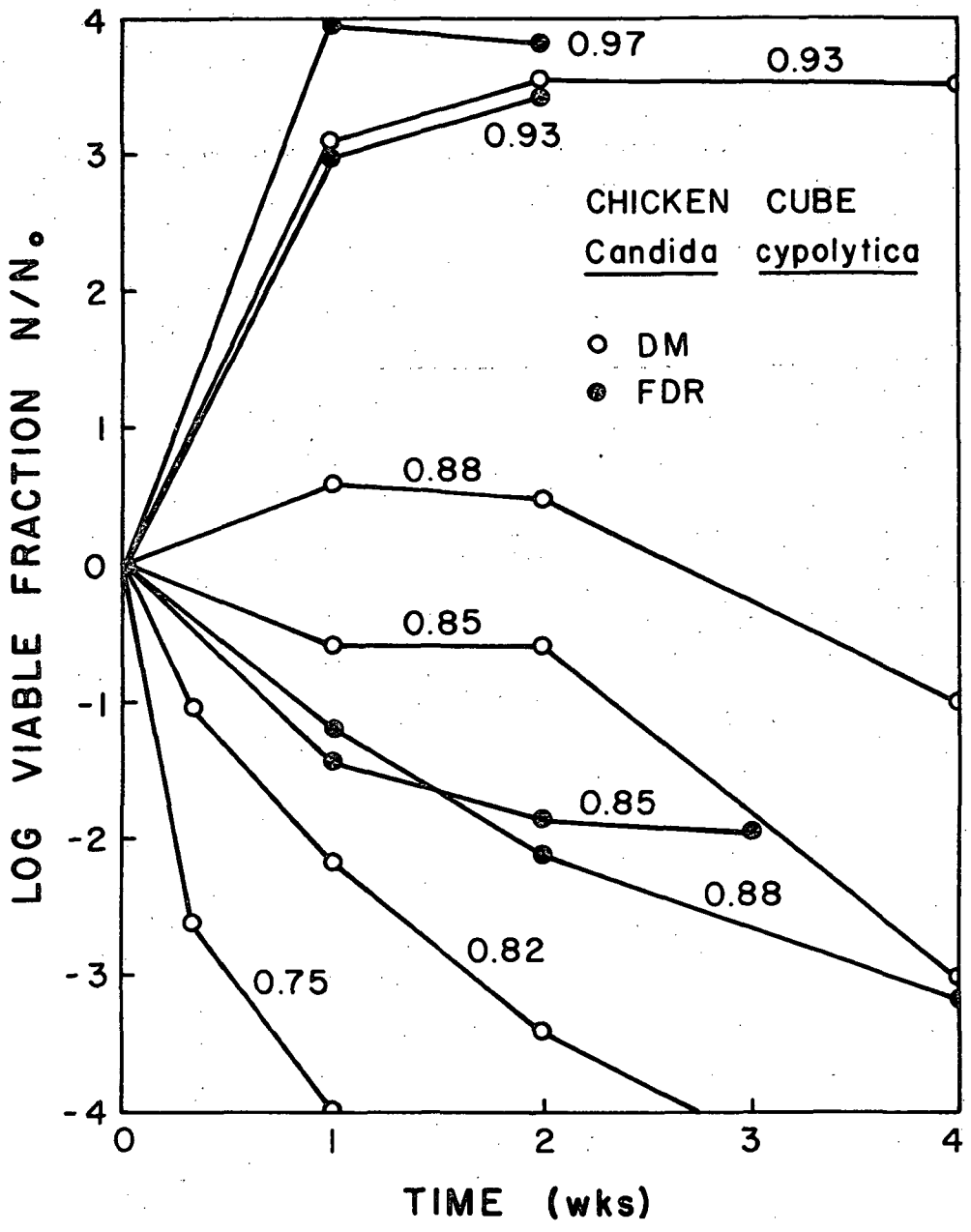


FIGURE 43. STABILITY OF CANDIDA CYPOLYTICA ON A SOAK-INFUSION CHICKEN CUBE SYSTEM AS A FUNCTION OF A_w AND SORPTION HYSTERESIS

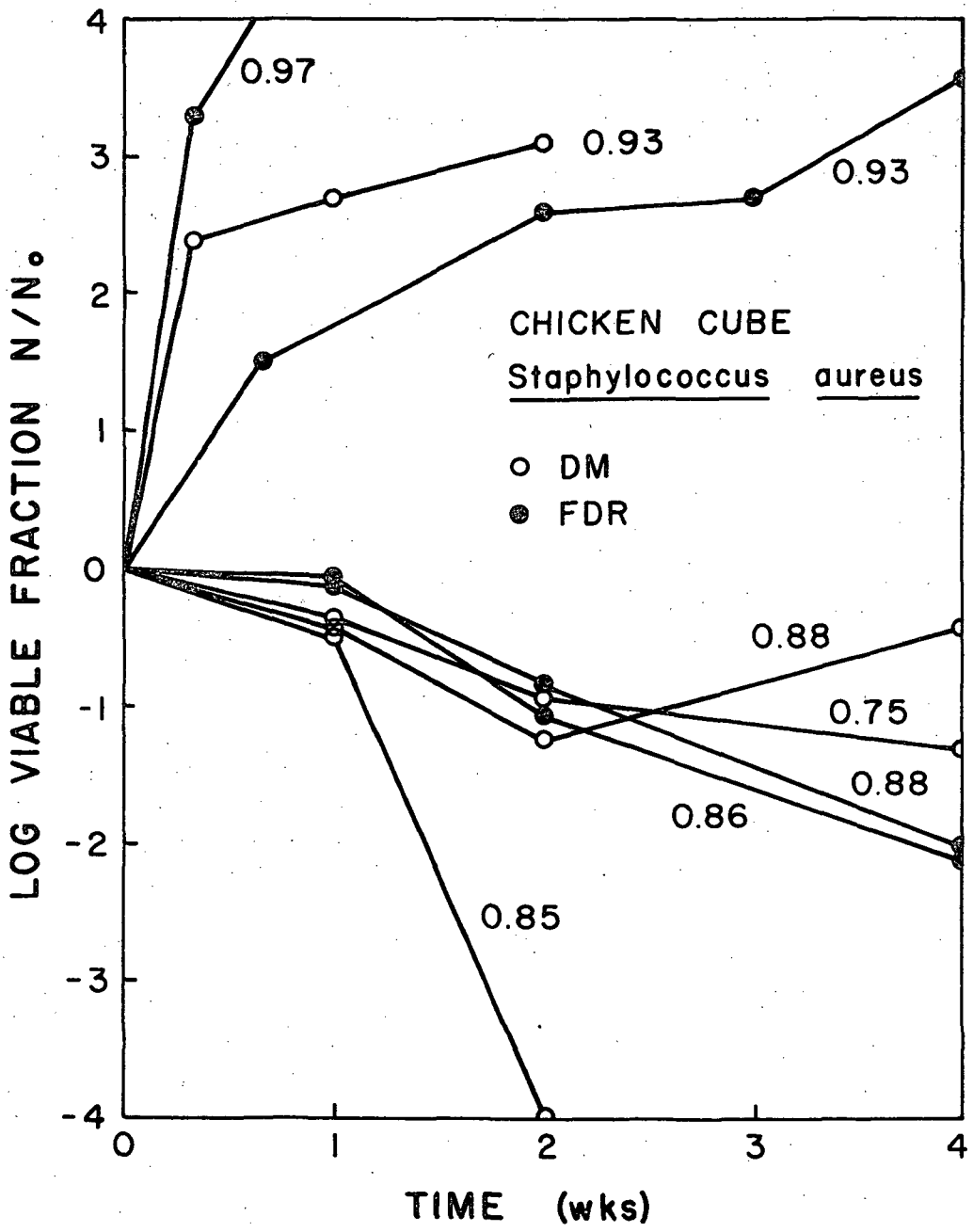


FIGURE 44. STABILITY OF STAPHYLOCOCCUS AUREUS ON A SOAK-INFUSION CHICKEN CUBE SYSTEM AS A FUNCTION OF A_w AND SORPTION HYSTERESIS @ 21°C

TABLE 79

Viable Count after 1 Month (per gram)

Chicken Infusion Systems*

Organism	Time	A_w 0.75 DM	0.75 FDR	0.82 DM	0.82 FDR	0.85 DM	0.85 FDR	0.93 DM	0.93 FDR
Pseudomonas fragi	4 weeks	0	0	0	0	0	0	10^9	0
Candida cypolytica	6 weeks	0	0	0	0	10^4	10^3	10^9	10^9
	8 weeks	-	-	-	-	0	0	-	-
Staphylococcus aureus	6 weeks	10^3	-	10^1	-	10^4	10^2	$>10^9$	$>10^9$
	8 weeks	10^3	-	0	-	10^2	0	$>10^9$	$>10^9$
Aspergillus niger	6 weeks	10^3	-	10^6	10^7	-	-	-	-
	8 weeks	10^3	-	10^6	10^6	$>10^9$	$>10^9$	$>10^9$	$>10^9$

*initial inoculum about 10^5 organisms/gram
0 denotes no organisms found in 4 gram sample

growth limit. For example, enterotoxin A production stops at A_w 0.86 in protein media, at 0.88 in NaCl media and 0.92 in KCl media. Thus, since no growth occurs at A_w 0.88 the product should be safe.

The mold was studied at both 20°C and 25°C storage temperatures as seen in Figures 45 and 46. The isotherm for the 25°C study is shown in Figure 47. Similar patterns exist at both temperatures in that the growth rate is less for the FDR systems. The mold is inhibited below an A_w of 0.75. Table 80 shows that the morphology of the mold is both affected by the A_w and the method of system preparation. These differences reflect changes in the biochemistry of the growth processes as was shown by Charlang and Horowitz (1971).

Table 81 compares the growth limits for both the model system and the soak infusion chicken. In all cases except for the FDR model study the growth limits overlap showing good correlation . For both the Pseudomonas and the yeast the limiting A_w for growth is higher for the adsorption prepared systems (FDR) than the desorption systems (DM). This is similar to what was found by Labuza et al. (1972b), however, no explanation can be made. Most likely the difference is tied to the sensitivity of enzyme systems to water content-water activity. For the mold and Staphylococcus not enough data points were taken to show if there is a difference in limiting A_w as caused by the preparation method.

2. Hennican Storage Stability

As indicated in the procedure a IMF system based on chicken, nuts and peanut butter was prepared by cold mixing. This product "Hennican" was studied under various storage conditions and compositions.

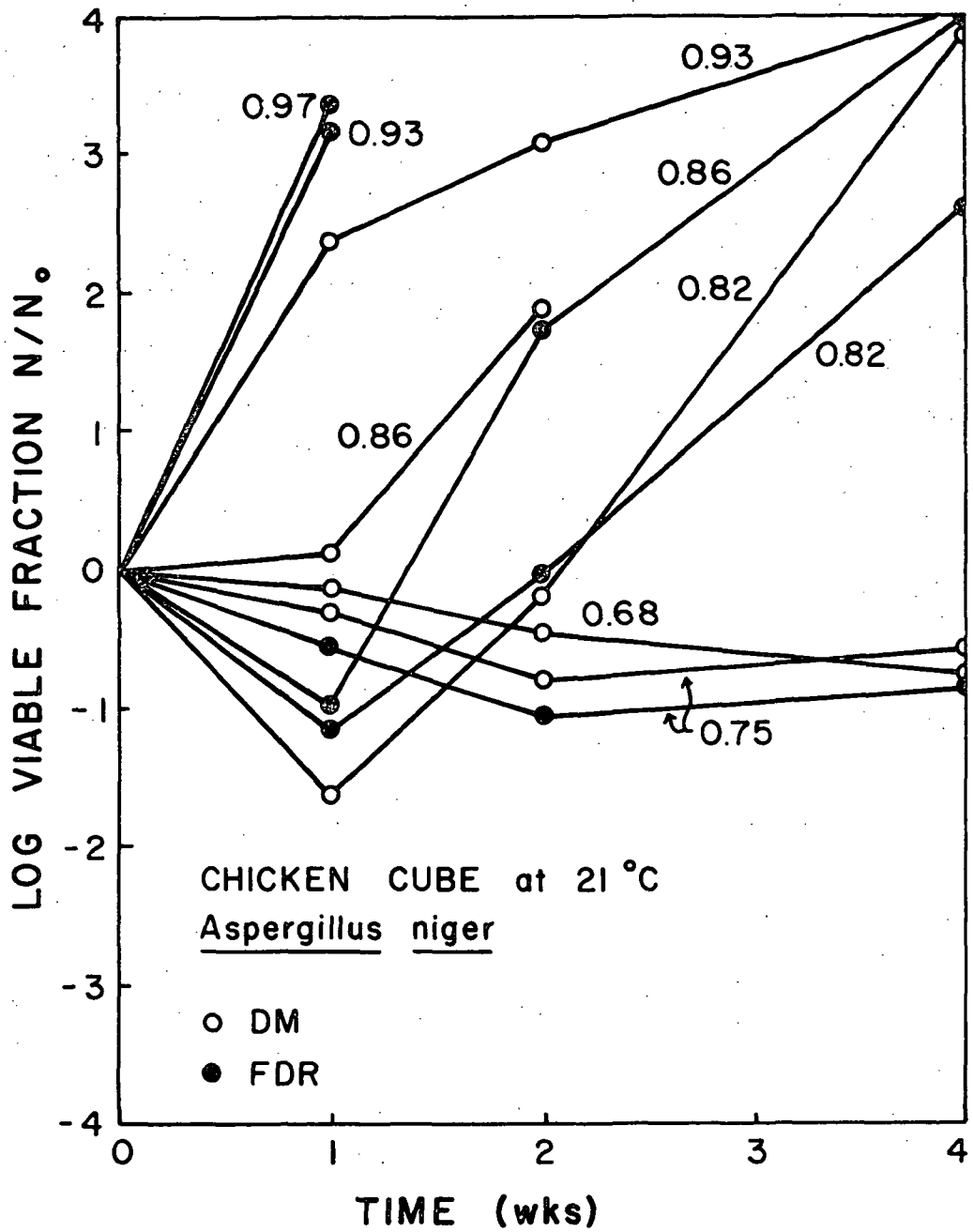


FIGURE 45. STABILITY OF ASPERGILLUS NIGER ON A SOAK-INFUSION CHICKEN CUBE SYSTEM AS A FUNCTION OF A_W AND SORPTION HYSTERESIS @ 21°C

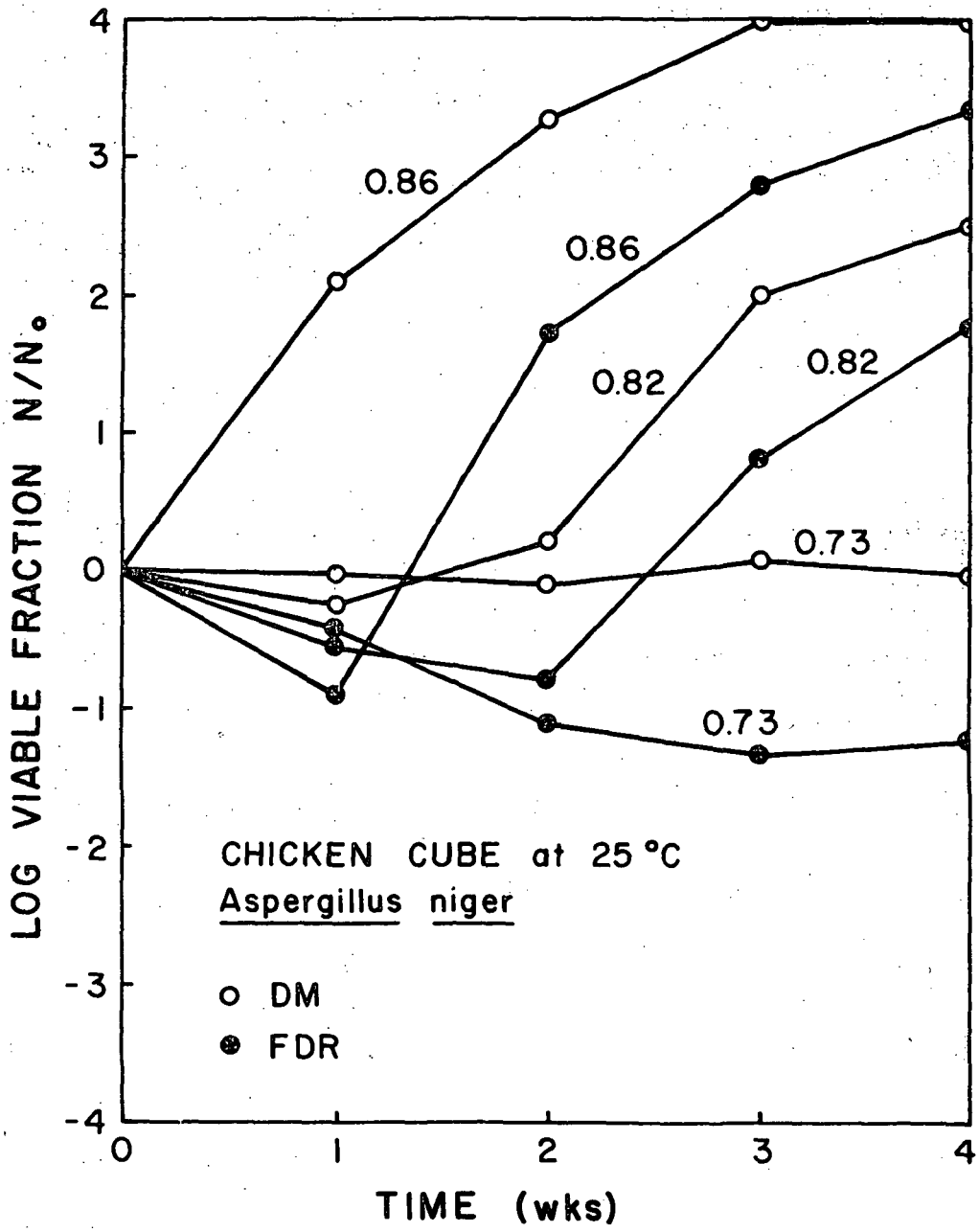


FIGURE 46. STABILITY OF ASPERGILLUS NIGER ON A SOAK-INFUSION CHICKEN CUBE SYSTEM AS A FUNCTION OF A_W AND SORPTION HYSTERESIS @ 25°C

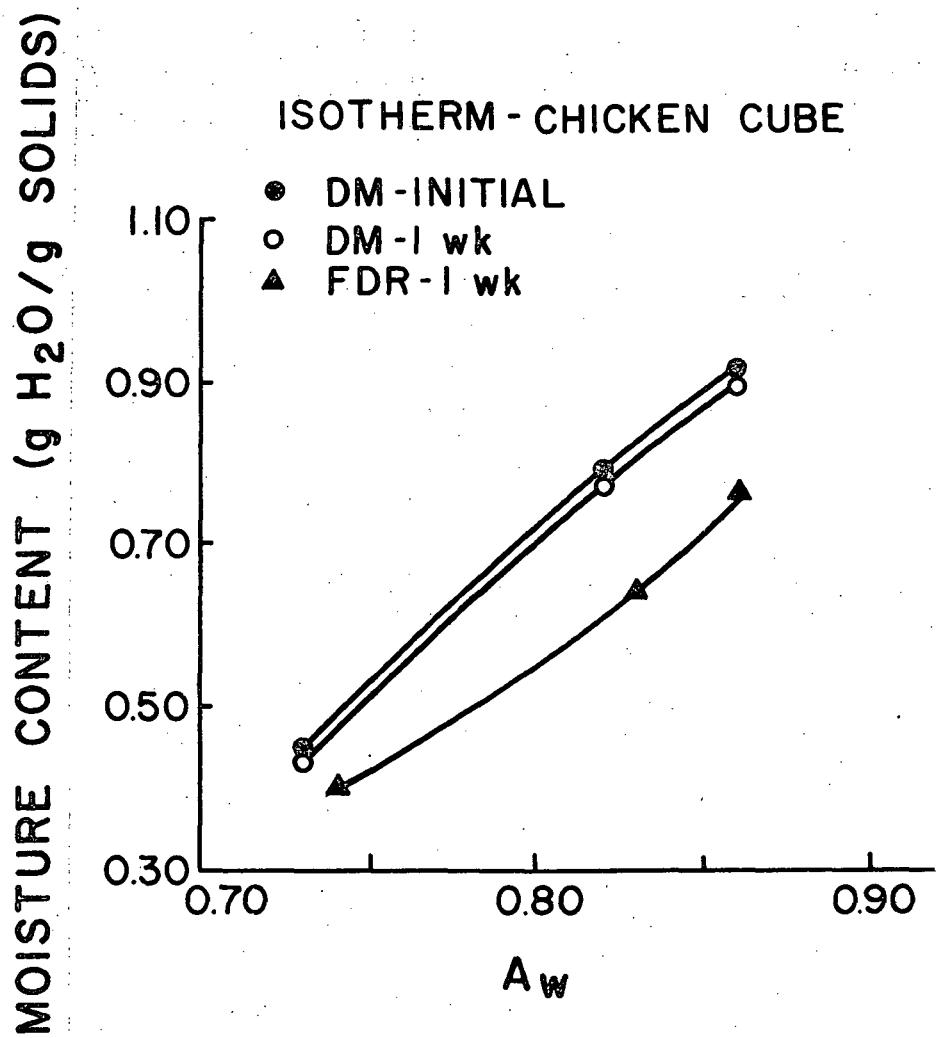


FIGURE 47. ISOTHERM - CHICKEN CUBE SOAK-INFUSION SYSTEM @ 25°C

TABLE 80

Morphology Differences of
Aspergillus niger ("smut")

(@21°-23°C on chicken cubes)

Run M2

Aw	DM
0.75	No visible mold.
0.82	Many small conidia and short conidiophore. General appearance is Black.
0.86	Conidia are few and small. Mycelium is dense. General appearance is White.
0.93	Mycelium are very long. Conidia are large and irregular. General appearance is White.
	FDR
0.75	No system.
0.82	Few small conidia on short conidiaphores. Mycelium is dense. General appearance is White.
0.86	Not all the conidia are stunted. 25% are typical size but symmetry of conidia is irregular. General appearance is White but darker than at 0.82.
0.93	Heavy production of black conidia. Conidiophores longer than 0.97, FDR. Mycelium only slightly visible. General appearance Black.
0.97	Very heavy production of conidia. Conidophores are shorter than FDR 0.93. Mycelium is not visible. General appearance is Black.

TABLE 81

Limiting A_w Range for Growth

Organism	Pork Slurry System		Chicken Infusion Systems		Limiting A_w
	DM	FDR	DM	FDR	
Pseudomonas fragi	>0.86 - <0.92	>0.92*	>0.88 - <0.93	>0.93 - <0.97	0.96
Candida cypolytica	>0.81 - <0.86	>0.86 - <0.92*	>0.85 - <0.88	>0.88 - <0.93	0.88
Staphylococcus aureus	>0.92	>0.92	>0.88 - <0.93	>0.88 - <0.93	0.86
Aspergillus niger	>0.81 - <0.86	>0.86 - <0.92*	>0.75 - <0.82	>0.75 - <0.82	0.64

*from Table 12

In Run H1, ten gram samples of system A and B (Table 40) were put into 2 oz jars, sealed and stored at 46°C and 37°C. In addition, several were vacuum sealed in a low oxygen permeability film and stored at both 46°C and 4°C. Peroxide values were measured at appropriate intervals and the samples were subjectively tasted. The vacuum sealed systems were held at 4°C for seven days before sealing and storage.

The results of this storage are shown in Table 82. At 46°C and 37°C storage in air, the system with the added antioxidant, tocopherol, did not oxidize slower as was found in the model system studies. The greater peroxide value for the antioxidant system is possible since tocopherol is a Type I antioxidant which terminates free radicals and could cause an accumulation of peroxides. This is fairly obvious at 37°C where the untreated system is rancid at 33 days, whereas the system with tocopherol having a much higher peroxide value was still acceptable in flavor and odor.

The data for samples sealed in vacuum are also in Table 82. At 46°C the samples were unacceptable at 4 months being almost black, very hard and dried out. The refrigerated samples, however, still retained a good flavor and odor although they were slightly dry. This should be expected if oxidation is the limiting reaction since it has an activation energy of about 20 Kcal/mole. A reduction in temperature of 40°C should increase the shelf life from about 15 to 80 X. However, the product is not acceptable in its present form at room temperature due to the off-odors and flavors developed in less than 6 weeks and its sticky texture.

Based on the flavor and texture problems new systems were designed

TABLE 82

Peroxide Values

Hennican

Run H1

Day	46°C System A	46°C System B*	37°C System A	37°C System B
0	13.3	13.3	13.3	13.3
4	12.0	18.2	--	--
8	15.4	59.8	--	--
12	19.7(R)	38.6(R)	--	--
14	13.0	24.7	13.5	16.0
18	8.5	17.3	--	--
20	36.5	73.9	--	--
29	20.0	129.5	--	--
33			40.5(R)	133.5
39	25.6	119.9	--	--
47	18.0	115.1		
62	--	--	20.8	146.8(R)
90	--	--	8.2	177.7

(R) Rancid

Vacuum Sealed

Day	46°C System A	46°C System B	4°C System A	4°C System B
0	17.6	23.9	17.6	23.9
13	43.6	34.5	43.7	30.7
120	9.0(R)	63.2(R)	16.8	56.7

(R) Rancid

*antioxidant added

in Run H3 (Table 42) and tested in storage in Run H4. Systems were prepared at A_w's 0.68, 0.76 and 0.85 according to the composition of Table 83 Both direct mix and freeze-dried rehumidified systems were made. A system also containing EDTA (500 ppm - solids basis) and BHA (200 ppm - fat basis) was made at A_w 0.76 since the model systems studies showed these to be very effective. In addition to peroxides, the rate of oxygen uptake was measured on all systems by Warburg techniques as used for model systems. Organoleptic testing was also done by 4 to 5 researchers at periodic intervals. The Hennican was rated on a nine point hedonic scale according to Table 84. A frozen control was thawed out and tasted each time as a comparison to an initial value for each sample. Samples were stored in 2 oz jars taped shut with black electricians' tape. They were stored in a chamber at 75% RH and 35°C. The initial A_w's and moisture contents are shown in Table 85 and Figure 48. As seen, with this product no hysteresis occurs probably because of the communation of the product, the high protein content and the low dissolved solids. This is similar to that found by Wolf et al. (1972). Further studies did not incorporate the hysteresis effect for Hennican as no gain would be obtained, however, all products were tested in Run 4H.

The storage study data are shown in Tables 86 and 87, respectively for peroxide values and organoleptic scores. As seen in Table 86, all the systems started out at a high peroxide value and reached a maximum in five to eleven days. This indicates an extremely fast rate of oxidation leading to rancidity. The high rate was verified by the fact that the organoleptic scores indicated that none of the samples were acceptable after 11 to 13 days. There is no difference with respect

TABLE 83

Composition (grams)

Run H4

Components	Basic Composition	Theoretical A_w:	Direct Mix Systems A 0.68	B 0.76	C 0.85	D EDTA & BHA 0.76
Dry roasted peanuts	50.00		66.50	105.00	66.50	105.00
Freeze-dried chicken	50.00		66.50	105.00	66.50	105.00
Raisins	100.00		133.00	210.00	133.00	210.00
Peanut butter	13.22		17.58	27.76	17.58	27.76
Honey	5.43		7.22	11.40	7.22	11.40
Cayenne pepper	0.07		0.09	0.15	0.09	0.15
K-sorbistat	0.66		0.88	1.39	0.88	1.39
H_2O	--		24.32	64.54	86.14	64.54
g H_2O added/100 g basic Hennican	--		8.33	14.00	29.50	14.00
Non-fat dry milk	5 g/30 g		48.65	76.80	48.65	76.80
EDTA (500 ppm)						0.2535
BHA (200 ppm)						0.0151
% Fat						
Direct mix			8.67	8.42	7.68	7.12
Freeze-dried rehumidified			9.53	9.38	8.63	9.56

Protein content (avg)

Direct mix - 26.34
FDR - 31.06

TABLE 84

Hennican Sample Score Sheet

SPACE FOOD QUALITY EVALUATION

Taster _____ Date _____

Direct Mix A_w				Freeze-dried Rehumidified A_w				Evaluation
0.68	0.75	0.85	BHA	0.68	0.75	0.85	BHA	
								9 Like strongly
								8 Very good
								7 Good quality
								6 Fair quality
								5 Acceptable
								4 Barely acceptable
								3 Unacceptable
								2 Dislike moderately
								1 Dislike highly

TABLE 85

Run H4

Moisture Changes Before and After Storage

Measured A_w — System: Theoretical A_w:	A 0.68	B 0.75	C 0.85	D 0.76
Direct Mix				
Initial	0.67	0.73	0.85	0.77
33 days storage	0.64	0.68	0.76	0.68
Freeze-dried rehumidified(3)				
Initial	0.64	0.71	0.78	0.69
19 days storage	0.61	0.71	0.73	0.75
26 days storage	0.64	0.71	0.70	0.70
Moisture (g H_2O/100 g solids)				
Direct mix(1)				
Initial	12	18	34	20
38 days @ 35°C(2)	11	15	21	11
Freeze-dried rehumidified(3)				
Initial	11	17	24	17
32 days at 35°C(2)	14	19	23	17

(1) by isotherm
(2) by vacuum oven average of 4 to 6 samples
(3) rehumidified 48 hr at 35°C

FIGURE 48

WATER SORPTION ISOTHERM - HENNICAN

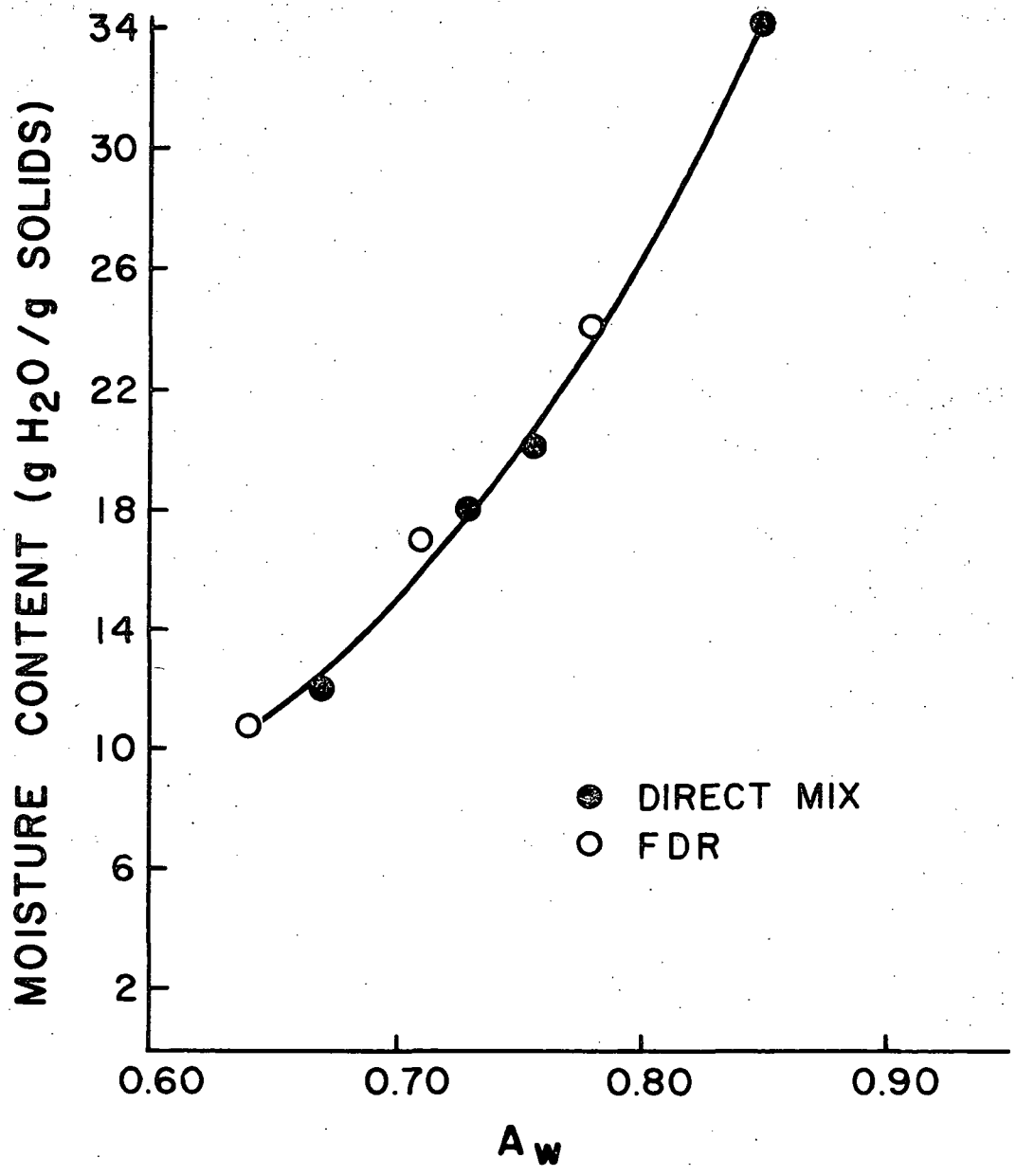


FIGURE 48. WATER SORPTION ISOTHERM - 21°C - HENNICAN

TABLE 86

Run H4

Peroxide Value at 35°C

System:	A		B		C		D	
Theoretical A_w:	0.68		0.76		0.85		0.76	
Days	DM	FDR	DM	FDR	DM	FDR	DM	FDR
0	29.00	41.94	32.97	25.46	15.69	18.75	54.84	16.67
4	61.43		38.07		27.33		48.49	
5		46.97		78.75[M]		66.55[M]		33.70[M]
8	82.81	56.67[M]	70.50[M]	55.77	33.33	43.71	60.94[M]	33.33
11	126.50[M]	8.05	57.71	6.48	65.39[M]	24.00	61.43	21.54
13		N.C.C.		N.C.C.		21.61		21.43
14	21.05		7.84		1.41		15.79	
15		12.03		9.47		7.81		10.22
16					15.79		20.21	
18	11.39		13.22		14.15		11.00	

M = approximate maximum in peroxide value

TABLE 87

Run H4

Organoleptic Score Summary*

Days	0.68		0.76		0.85		EDTA & BHA 0.75	
	DM	FDR	DM	FDR	DM	FDR	DM	FDR
0	8	5	8	7	9	5	7	7
4	7		6		6		6	
5		4		5		6		6
8	5	5	5	4	5	4	4	3
11	5	3	4	5	6	5	3	6
13		4		5		5		4
14	4		5		5		3	
16	4		4		4		4	

*Average of 5 scores each

to hysteresis as expected from the isotherm.

The oxygen uptake data are shown in Figures 49 and 50. As seen, the rates are very fast. The average slopes of the lines are tabulated in Table 88. These data indicate a very high oxidation rate with maximum rate kinetics applying as described by Labuza (1971b). The rates fall in the same range as for oxidation of poultry products. Labuza (1971b) reported for turkey a rate of 260 to 480 μl O_2/g day in the wet state which reduces to 60-150 μl O_2/g day in the dry state. The intermediate rates in this present study fit the turkey rates very well. The unusual aspect is the very rapid rate of oxidation for the antioxidant treated system, however, Hill et al. (1969) have shown that in systems with an initial high peroxide value, the antioxidant BHA tends to induce peroxide breakdown rather than being a free radical terminator. Thus, the rate of oxidation could increase. The EDTA could possibly also complex some metals naturally present and make them more effective catalysts as had been shown by Chalk and Smith (1957). The results indicate only a very slight oxidation rate increase with A_w and a faster rate for the FDR system. This would be similar to the cellulose system with high trace metal content which is free. However, the values are not very different.

The results of Run H4 were disappointing since the system had less than 2 weeks stability. This was attributed to several factors:

(a) The initial peroxide values of the peanuts and peanut butter were very high being 18.6 and 26.6, respectively. A new source of these materials was found (Skippy Peanut Butter Factory, Minneapolis) which had initial peroxide

FIGURE 49. RUN 4H - OXIDATION EXTENT AS A FUNCTION OF A_w FOR HENNICAN - DIRECT MIX SYSTEM @ 35°C

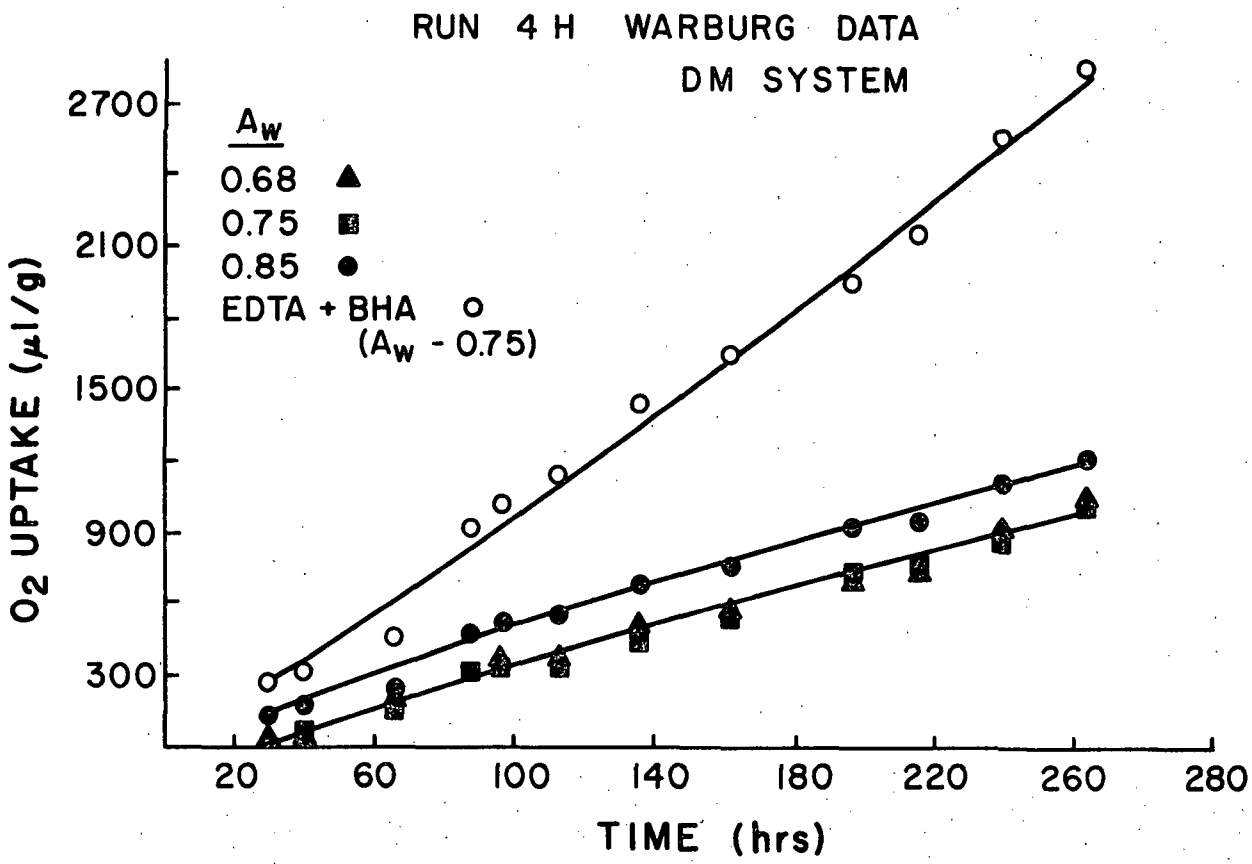


FIGURE 50. RUN 4H - OXIDATION EXTENT AS A FUNCTION OF A_w FOR HENNICAN - FREEZE-DRIED REHUMIDIFIED SYSTEM @ 35°C

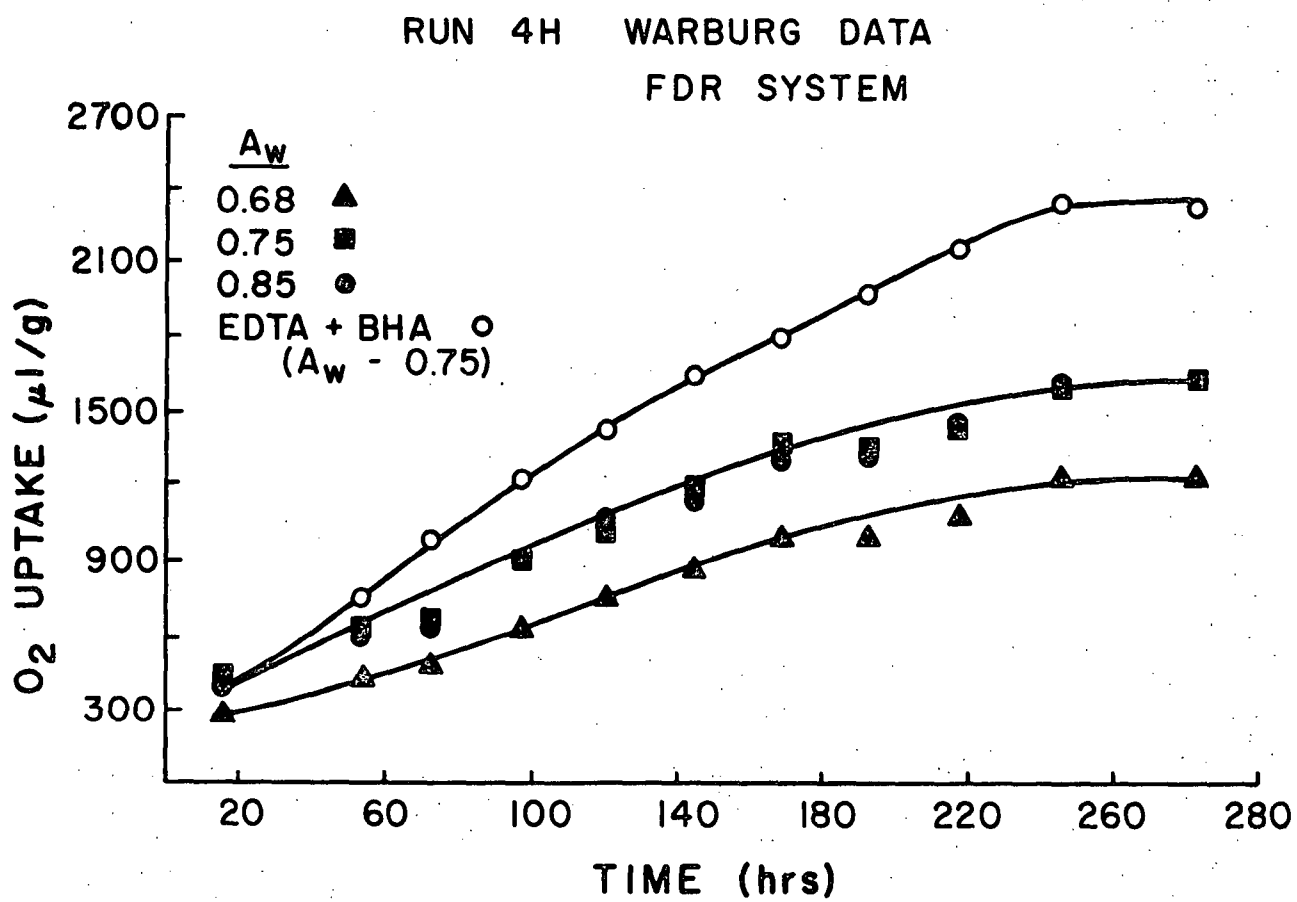


TABLE 88

Warburg Oxidation Constants

ul/ $_2$/gram/day

	Run H4		Run H5			Run H6
A_w	FDR	DM	DM^a	DM^b	θ_i**	DM
0.64	110					
0.68		96	90	127	4	
0.71	123					
0.73		96	84	97	5	85
0.78	123					
0.85		115	88	92	5.5	
0.75*	211	281	156	192	2	
0.83						82

(a) first 10 days
(b) 10-30 days
* BHA and EDTA
** induction time (days)

values of 3.5 and 1.5, respectively. This would insure an initially low value for further tests.

(b) The chicken was not freeze-dried just prior to mixing and, thus, also had peroxide value of 12 which is high.

(c) Most of the samples lost water in storage even though stored in supposedly sealed jars. The data in Table 85 indicate a moderate leak rate, the higher concentrations of metals resulting from this may have created the high oxidation rates. To insure against moisture loss the Hennican should be vacuum packed in foil bags.

(d) The antioxidant combination predicted to be most effective by the model systems did not work in the food but in fact accelerated oxidation.

(e) The BHA imparted an undesirable flavor and its level should be reduced.

In Run H5 Hennican was prepared to three humidities by direct mixing. A system containing antioxidants was also employed. Care was taken to insure a high quality initial product so as to obtain as long a shelf life as possible. The peanuts were cooked in an attempt to inactivate any possible enzymes but this gave the nuts a very bitter flavor, so the nuts were used as is, i.e. dry roasted. The peanuts and peanut butter were obtained fresh from the Skippy Peanut Butter Co., and the product (10-14g) was packed in foil laminated Scotchpak #20 bags (Minnesota Mining and Manufacturing Co.-3M) under vacuum and stored at 35°C. The BHA was reduced to 100 ppm to eliminate the off-flavor. Also, since hysteresis was minimal only the direct mix system was prepared. This eliminates

any variables due to drying and humidification and shortens the initial preparation time. The direct mix systems also had a better organoletpic rating in Run H4.

Several preparation technique changes were made to insure a more homogeneous product. The raisins were initially frozen in liquid nitrogen to facilitate grinding. The non-fat dry milk was first mixed with the freeze-dried chicken and ground peanuts to which the remaining ingredients were then added while on the mixer.

Table 89 contains the results of the microbiological examination of the Hennican components and of the actual product after 21 days storage at 35°C. As seen, the product microbial counts are very low except for the system at A_w 0.85. These results show that the combination of the reduced A_w and sorbate help to maintain the product safety, however, at the highest A_w the sorbate is not effective since the pH is high (Table 89).

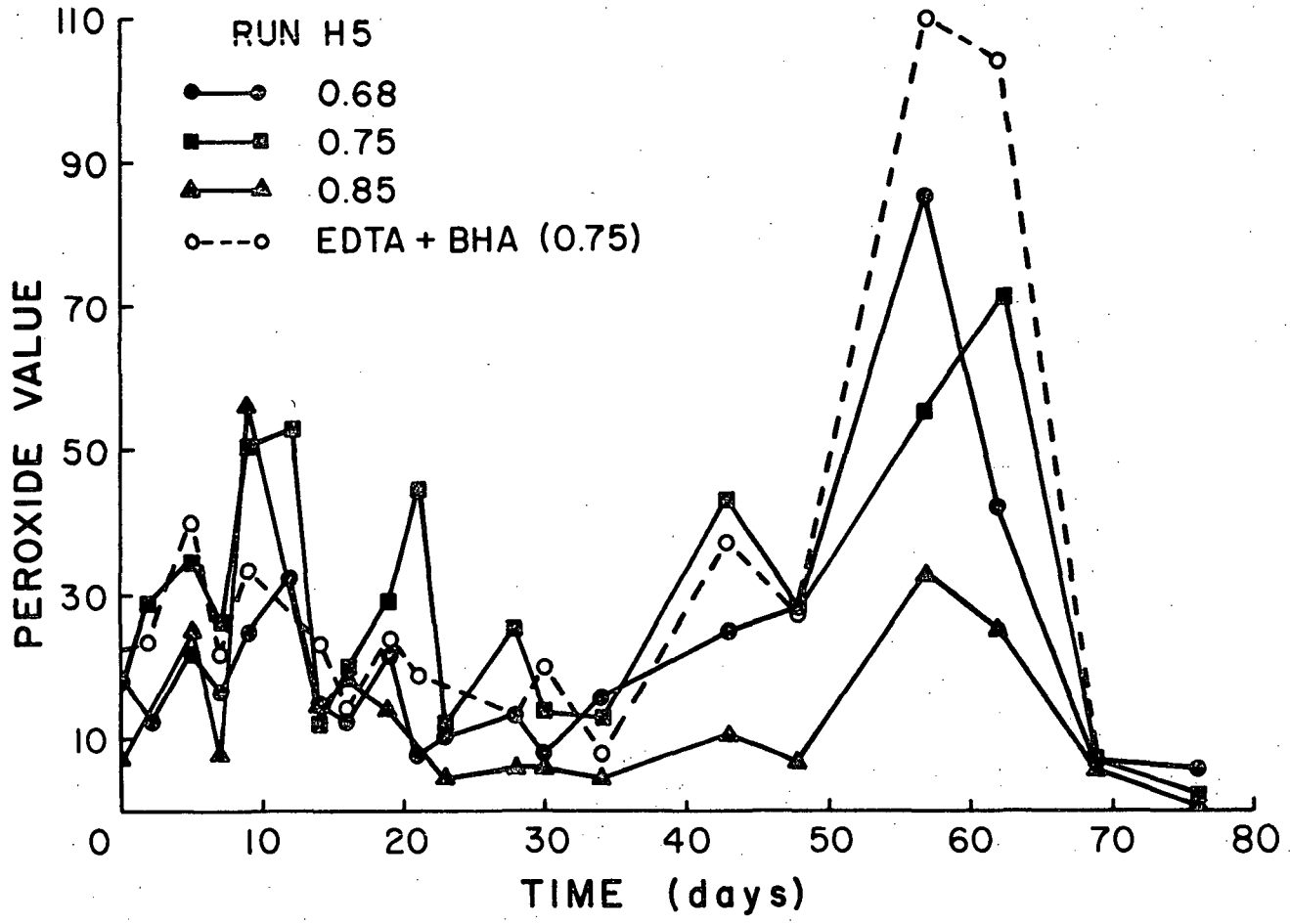
Figure 51 shows the results of the peroxide determination. As compared to Run H4 the initial values are all lower as would be expected based on the precautions used. The results show a small maximum at around 5-12 days storage and another at 57-62 days. After the latter time the peroxides drop to low values. At 100 days all peroxides were below 2. The cause of the double maximum is not known, however, it could be due to sample variability. In Figures 52 and 53 are shown the results of oxygen uptake by Warburgs. The results parallel the peroxide data in that the slowest oxidizing system is at A_w 0.85 and the antioxidant system is the fastest. As seen, the oxidation rate decreases as humidity increases as predicted by the results of the cellulose system. It would

TABLE 89

Hennican Microbiological Quality

Component	Counts/gram
Peanuts	200
Freeze-dried chicken	0
Raisins	300
Peanut butter	100
Honey	30
Pepper	1.5×10^4
NFD milk	1.2×10^3
Water	1.8×10^3
Hennican Run H5	
21 days @ 35°C	
$A_w = 0.68$	300
$A_w = 0.75$	200
$A_w = 0.85$	1.1×10^3

FIGURE 51. PEROXIDE VALUE AS A FUNCTION OF A_w FOR HENNICAN STORED IN VACUUM PACKAGES



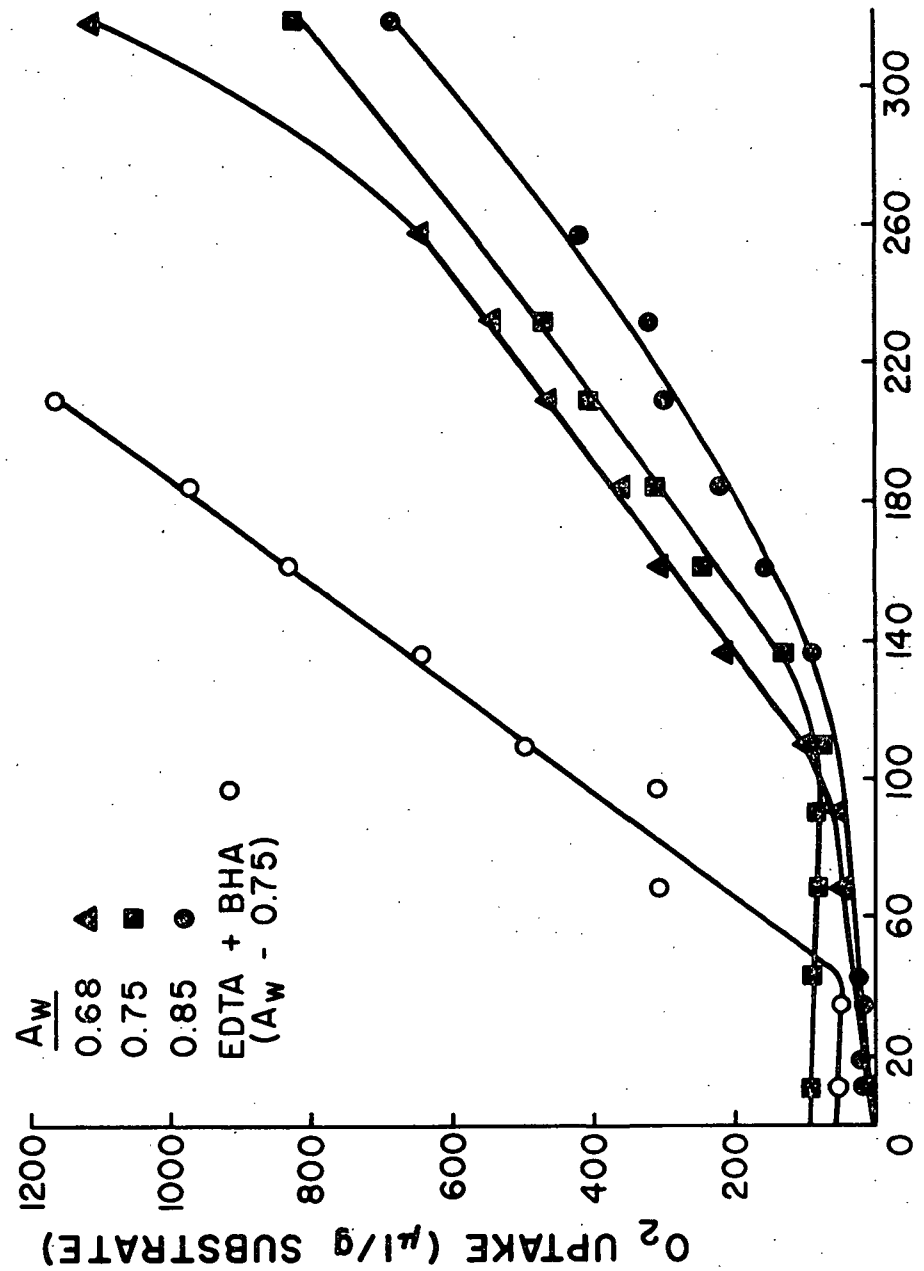
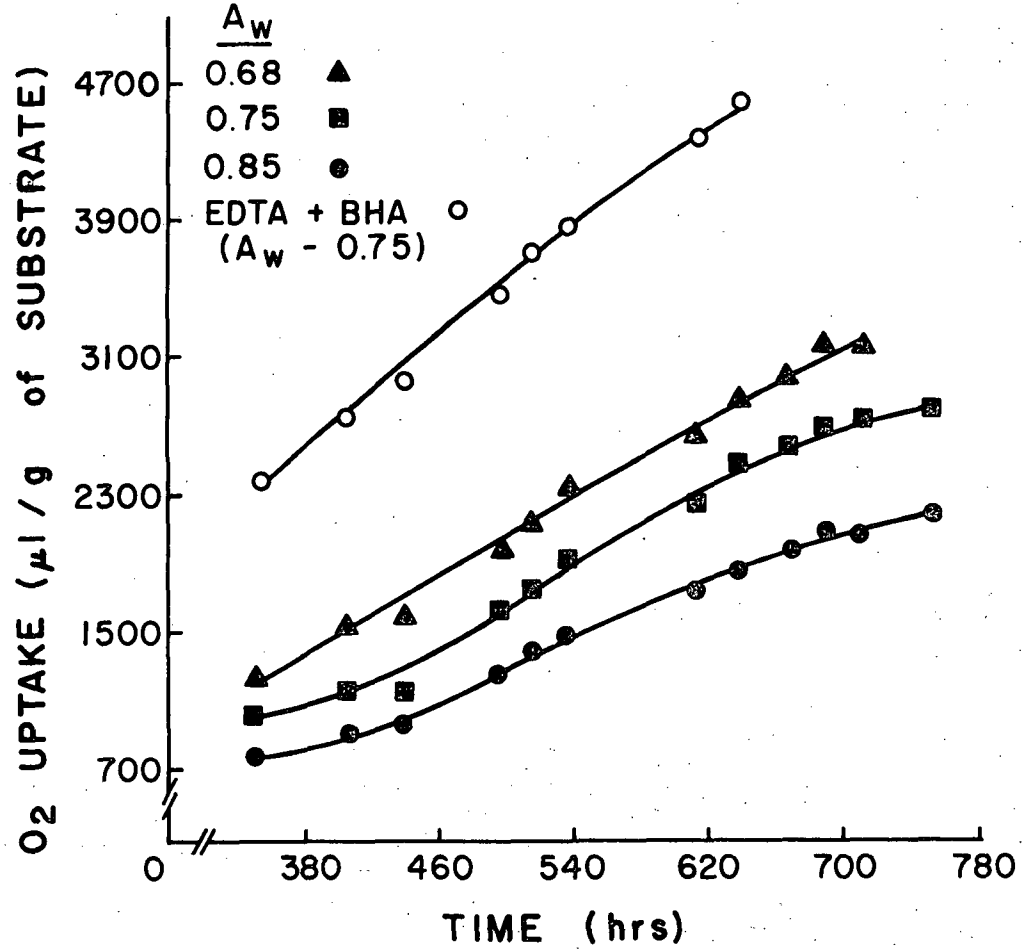


FIGURE 52. RUN 5H - INITIAL OXIDATION EXTENT AS A FUNCTION OF A_w FOR HENNICAN @ 35°C

FIGURE 53. RUN 5H - FINAL STAGE OXIDATION EXTENT AS A FUNCTION OF A_W FOR HENNICAN @ 35°C



be expected that there would be a lot of free metals present due to the chicken. The antioxidant combination, however, did not work in reducing the rate, possibly for the same reasons as in Run H4. Because of the special precautions taken in all cases an induction time was observed which did not occur in the previous run. Table 88 lists the oxidation rate constants for Run H5. The data were split into two segments over which a change in rate was apparent. Except for the antioxidant treatment the rates were not much different than in Run H4. The results confirm the effect of A_w on decreasing the rate for systems with a high metal content. It is also interesting to note that even though the product was vacuum packaged, the rancidity development by PV determination followed the Warburg data which was carried out in air.

The main complaints about the product during storage were an off-odor, a bitter taste and a toughening even though there was no significant change in moisture as seen in Table 91. The toughening and bitter flavor could be due to non-enzymatic browning. Samples were extracted with water and checked for non-enzymatic browning by measurement of the OD at 420 nm. The results are shown in Figure 54. A definite pattern can be seen in that a maximum in rate occurs at A_w 0.75 with the rates being slower at 0.68 and 0.85. The antioxidant treatment had no effect on the rate of browning whereas storage at freezer temperature (-20°C) eliminated browning. The available lysine content decreased in all samples as compared to the frozen control (Figure 55), however, the loss after 3 weeks did not increase significantly over the following 8 weeks. Again, the product at 0.85 showed the slowest change. The low loss of lysine is unusual since the amount of browning increased by a factor of

TABLE 90

Run H5

Moisture Changes before and after Storage

	Theoretical A_w			
Measured A_w	0.68	0.75	0.85	EDTA & BHA 0.75
Direct mix				
Initial	0.64	0.74	0.82	0.75
19 days storage	0.68	0.75	0.83	0.77
34 days storage	0.67	0.77	0.84	0.80
Moisture (g H_2O/100 g solids) (1)				
Direct mix				
Initial	17	23	38	22
8 days storage	16	21	34	21
19 days storage	14	19	34	18
34 days storage	17	22	36	22
pH (2)	5.5	5.5	5.6	5.5
Fat content (%)	9.25	7.40	9.61	7.40
Protein content (%)	28			

(1) by vacuum oven, 18 hrs, 65-75°C
(2) 3 grams slurried with 5 ml water

FIGURE 54. RUN 5H - NON-ENZYMATIC BROWNING AS A FUNCTION OF A_W FOR HENNICAN @ 35°C - STORED IN VACUUM PACKAGES

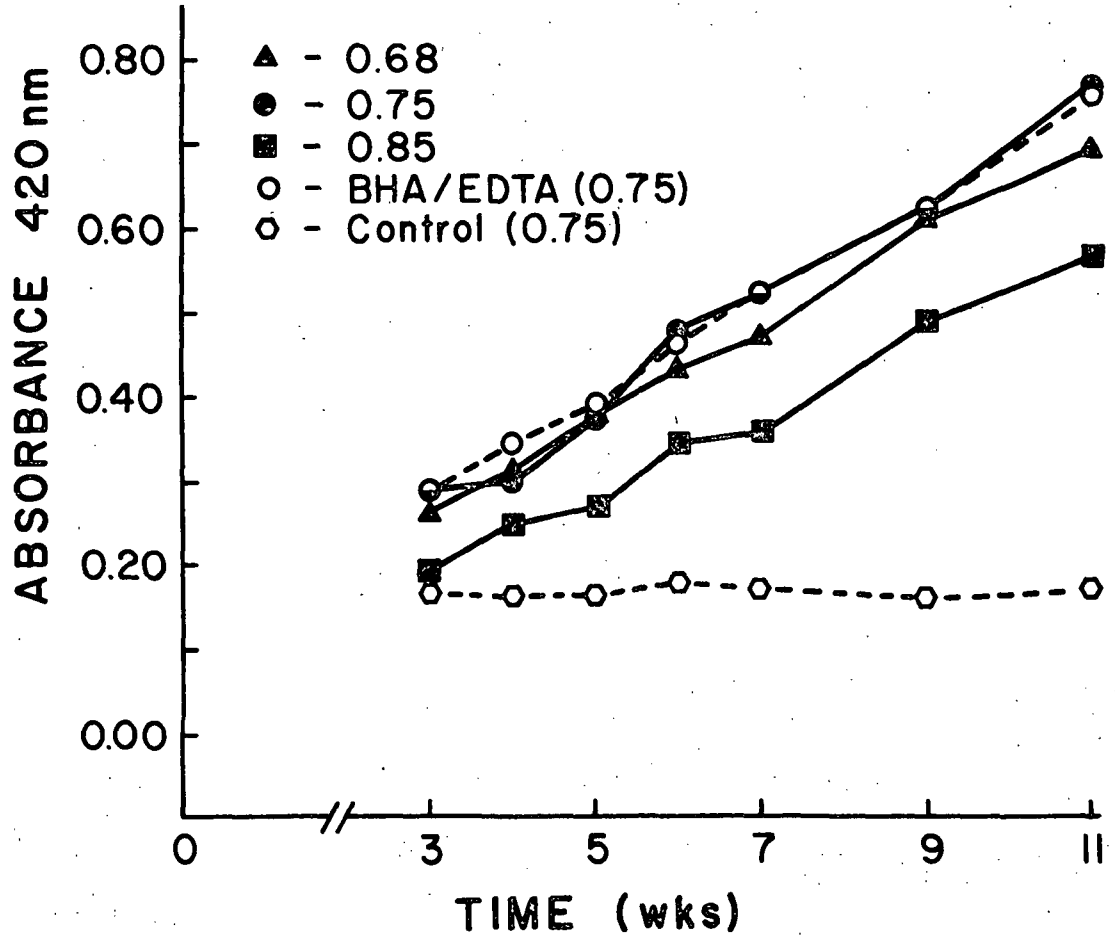
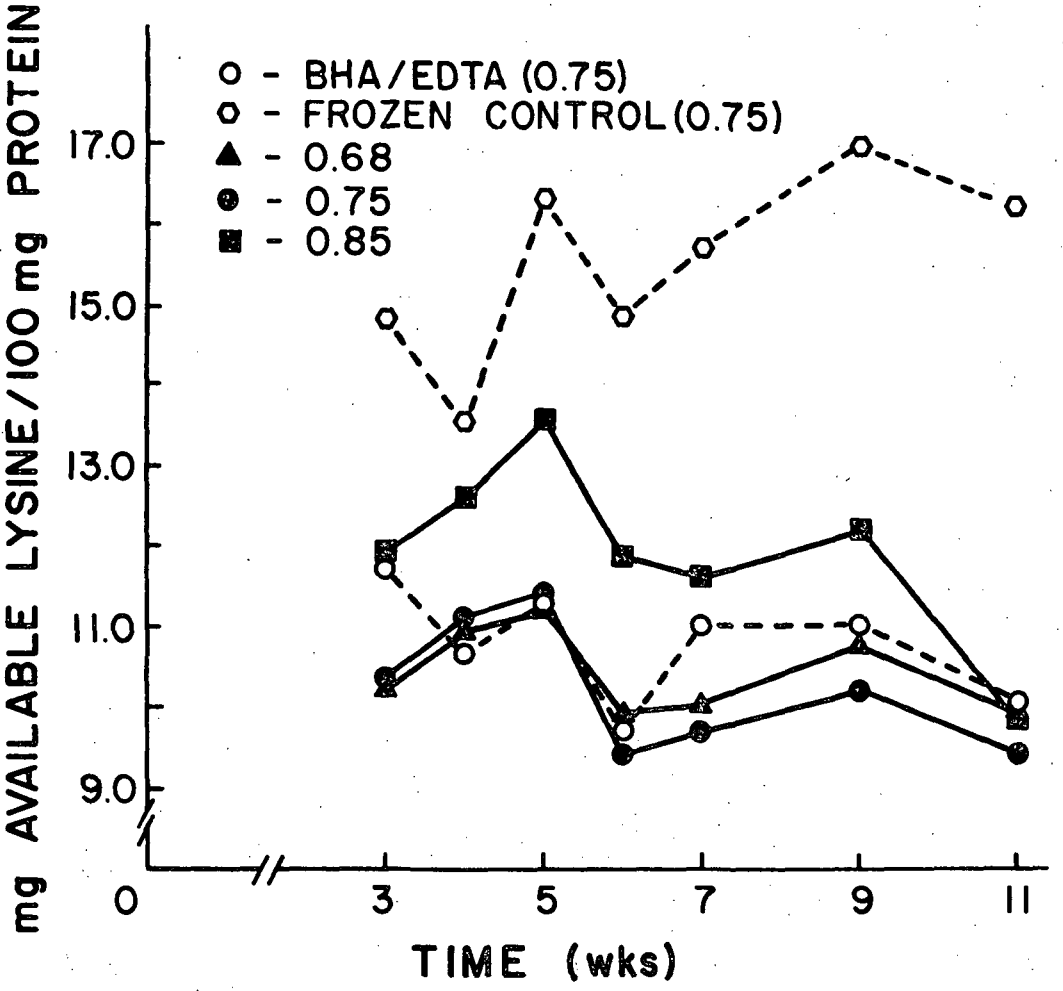


FIGURE 55. RUN 5H - LYSINE STABILITY AS A FUNCTION OF A_W FOR HENNICAN @ 35°C - STORED IN VACUUM PACKAGES



2 to 2.5. The small change could be due to the problem of arginine interference in the TNBS test procedure which masks any lysine losses or the fact that lysine is not the significant amino group reacting in this product.

Table 91 summarizes the organoleptic scores for the product during storage. As seen, the antioxidant product was the worst due both to the flavor of BHA and the faster oxidation rate. The other systems showed similar results except that at A_w 0.85 the product had a little longer acceptability. As compared to Run H4, these systems had about 3 times longer shelf life or about 42-48 days. Still this is not long enough for an acceptable product.

The overall results of Run H5 indicate that the Hennican product has the best stability at the highest A_w (0.85) from the standpoint of lipid oxidation, nonenzymatic browning, lysine loss and organoleptic score. The problem with using this high A_w is based on the microbiological considerations. Since Staphylococcus organisms could possibly grow and produce toxin at this A_w a lower A_w product would be safer unless acid is added. Acid, however, would create flavor problems.

In Run H6 a longer range storage study was made on Hennican at an A_w of 0.75 for a control and a system at A_w 0.83 with a combination of antioxidants was prepared including 100 ppm EDTA, 0.1% citric acid, 100 ppm BHT, 50 ppm BHA and 50 ppm BHA. The product (about 10-15 g) was vacuum sealed into Scotchpak polyester film #20 (3M Co.) in 5¼" x 7½" bags. The bags were stored at high humidity $\geq$ 75% and 35°C. Citric acid was added both as an antioxidant and to increase the effectiveness of the sorbate. Similar precautions were taken as in Run H5 using only

TABLE 91

Organoleptic Score Summary*

Run H5

Days	0.68	0.75	0.85	EDTA & BHA 0.75
0	8	8	8	7
2	8	8	8	8
5	8	7	7	6
7	6	7	8	4
9	6	7	7	4
12	6	5	5	2
14	6	6	6	5
16	4	5	6	5
19	6	6	7	5
34	5	6	7	4
43	4	4	5	4
48	4	4	4	2
57	4	3	4	2

*average of 3-5 scores

fresh material.

Table 92 shows that during storage for 62 days at 35°C, 80% RH in sealed bags, the A_w did not change, however, there was some loss of water. This also occurred in Run H5. This loss of water could be due to chemical reaction or permeation through the package.

Table 93 and Figure 56 indicates the degree of oxidative rancidity development. As seen, the peroxides start out much lower than in Runs H4 and H5 and do not reach the initial maximum in 8-13 days as seen in these two runs. The oxygen uptake rate is similar to that for both Runs H4 and H5 for the direct mix system at A_w 0.75 (Table 88). The antioxidant treated system in this case did not oxidize faster probably due to the low initial peroxides, thus chain propagation was not enhanced. However, the antioxidants gave no protection in terms of rate as compared to the control.

Since the method used for lysine determination in Run H5 was not acceptable, lysine was not followed in Run H6 The results for non-enzymatic browning are shown in Figure 57 along with a measurement for texture. As was noted above and in previous runs the product hardens in storage. Although this could be due to moisture losses these losses are slight. Most likely the hardening is due to the non-enzymatic browning reaction. To test for texture the following procedure was tried using an Instron Universal Tester Model TM.

(a) initial thickness of sample (2 g) measure = L

(b) sample compressed with square plunger through an undirectional grid to 1 mm thickness

(c) from instrument, force measured to compress to one mm

TABLE 92

Moisture - A_w Relationship

Run H6

	Theoretical	
Measured A_w	0.75	0.83 (combination)
Direct mix		
Initial	0.73	0.82
28 days	0.74	0.81
39 days	0.74	0.82
62 days	0.74	0.83
Moisture Content (g H_2O/100 g solids)		
Direct mix		
Initial	21	21
29 days	17	18
40 days	14	13
62 days	18	18

TABLE 93

Run H6

Peroxide Value

Summary

Day	0.75	0.83 (combination)
0	16.6	14.1
4	9.6	6.9
7	3.5	2.5
10	3.6	2.2
13	3.9	6.7
20	0.7	N.C.C.
26	1.9	N.C.C.
41	1.7	4.6
49	9.6	7.2
62	13.0	9.2

FIGURE 56. RUN 6H - OXYGEN UPTAKE AS A FUNCTION OF HENNICAN SYSTEM

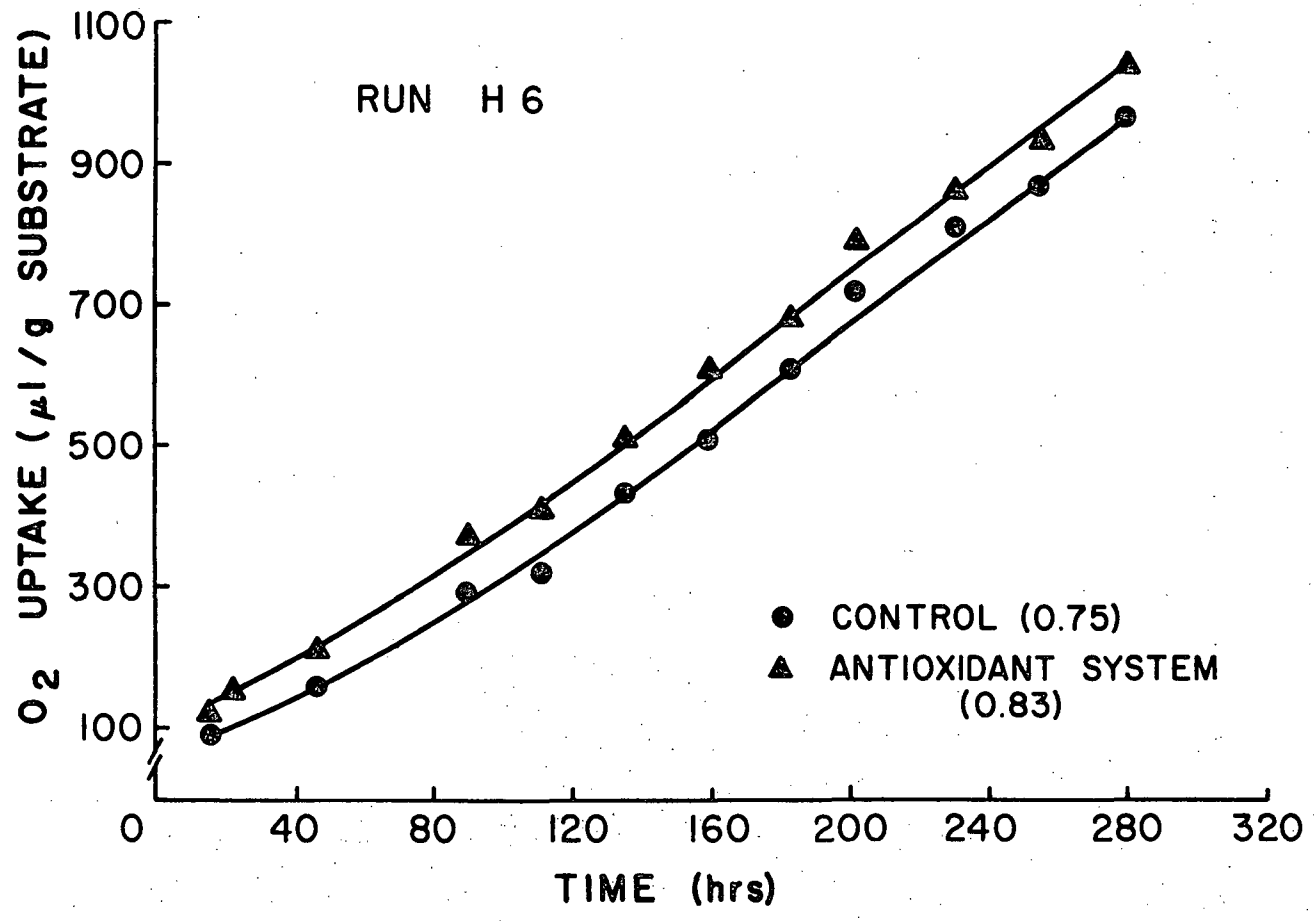
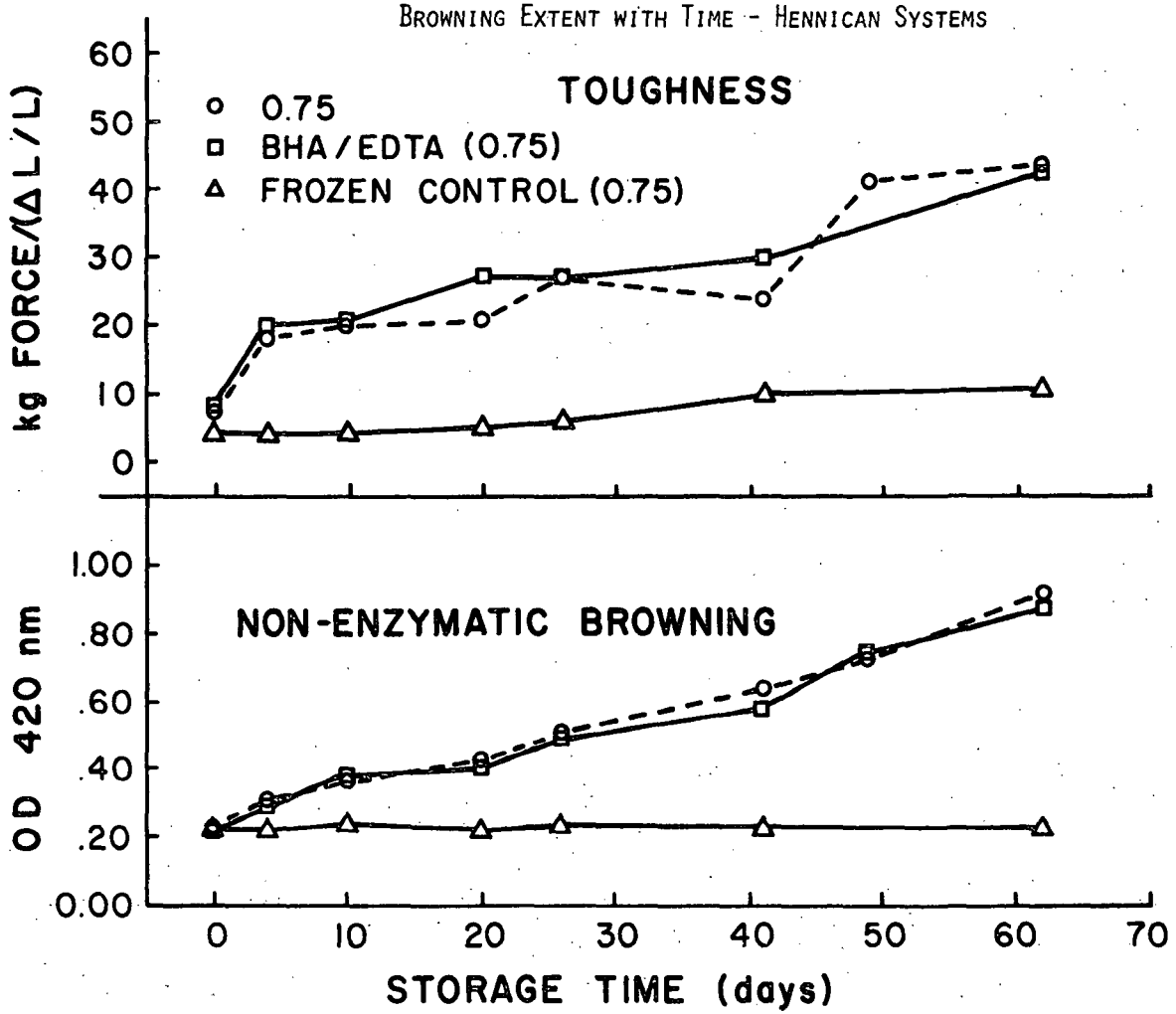


FIGURE 57. RUN 6H - TOUGHNESS AND NON-ENZYMATIC BROWNING BROWNING EXTENT WITH TIME - HENNICAN SYSTEMS



(d) toughness reported as = $\frac{\text{Force (Kg)}}{\Delta L/L}$

where $L = L - 1$ in mm

As seen in Figure 57, the force necessary to bite through the Hennican squares increased during storage paralleling the non-enzymatic browning. It should be noted that the visual color of the product was extremely brown after two months storage correlating well with the measured browning. Figure 57 also shows that the frozen control showed no change during storage.

The organoletpic data in Table 94 show that both products had about the same rate of loss of acceptability. Shelf life at 35°C would be about 2 months for this product, an improvement of about three weeks as compared to Run H5. At room temperature of 21-25°C the product would then have close to 6 months stability based on an average activation energy of 25 Kcal/mole. Thus, this product is very acceptable in meeting the needs of the flight qualifiable food.

V. Future Experiments Necessary to Study Improvement of Shelf Life

A. Vitamin Studies

1. Test for both C and B_1 in hysteresis system
   (a) effect of pH
   (b) effect of EDTA
   (c) three temperatures to get E_a
   (d) determine separate effect of moisture and A_w
2. Prepare Hennican and Granola Bar with B_1 and C. Test in accelerated storage.

TABLE 94

Organoleptic Summary*

(35°C)

Run H6

Day	0.75	0.83 (combination)
0	9	9
4	8	8
7	7	7
10	8	8
13	6	5
20	7	6
26	7	6
41	6	6
49	5	5
62	4	4

*Average of 5 scores

B. Lipid Oxidation Model Systems

1. Study amylopectin at various metal levels to see if dilution effect occurs

C. NEB-Model System

1. Prepare a system with hysteresis
2. Establish a satisfacotry procedure for lysine
3. Study browning, available lysine and hardening in this system as a function of
   (a) several A_w's and moistures
   (b) temperature

D. Microbiological Studies

1. Determine more accurately the limiting A_w range for Staphylococcu aureus and Aspergillus niger in the soak-infusion chicken cubes.
2. Infect Hennican with mold and study the effectiveness of several inhibitors at different levels including:
   (a) sorbate
   (b) propionate
   (c) 1-3 butanediol
   (d) propylene glycol
   (e) benzoic acid

E. Hennican Improvement

1. Prepare Hennican at A_w 0.83 with new combination of antioxidants
2. Study effect of temperature on stability
3. Study air packaging vs vacuum

4. Do following tests in these studies

   (a) peroxide value

   (b) Warburg

   (c) organoleptic

   (d) lysine

   (e) NEB

   (f) texture

   (g) vitamins

5. Determine total trace metals content in Hennican and degree of boundness

F. Granola Bar

1. Determine extent of sorption hysteresis

2. Prepare system at several A_w's by both sorption methods and test stability

3. Study

   (a) control vs antioxidants

   (b) direct mix vs humidification

   (c) effect of A_w and moisture

   (d) air vs vacuum packaging

   (e) influence of temperature

4. Do the following tests in these studies

   (a) peroxide values

   (b) Warburg

   (c) organoleptic

   (d) lysine

   (e) NEB

(f) texture

(g) vitamins

G. Extrusion Systems

1. Develop a formulation to produce an acceptable IMF product made by extrusion processing

2. Study the kinetics of microbial death and vitamin destruction in this product during process as a function of:

(a) temperature

(b) pressure

(c) A_w

(d) residence time

3. Develop mathematical models to predict product quality factors for industrial extrusion processing

H. Renal Diet Systems

1. Develop a low protein, low potassium, low sodium IMF food system

2. Shelf life test this food under accelerated conditions based on previous studies

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