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Analysis of Storage Stability of Intermediate Moisture Foods

CONTRACT NAS 9-10658

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Final Report
May 15, 1970 to July 15, 1971

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

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SUMMARY

Intermediate Moisture Foods (IMF) are generally unstable systems with respect to chemical and microbiological deterioration. This is due to the fact that over the range in water activity to which they are prepared many reactions occur at accelerated rates. However, the method of preparation of food can have some influence on stability. This is because in the moisture range used for IMF sorption hysteresis occurs, thus a food with a higher moisture content is made by adding enough humectant to lower the water activity (A_w) to a given value (desorption) as compared to drying that same food-humectant mixture to a low moisture content and then rehumidifying it back to the same A_w (adsorption).

With respect to lipid oxidation, it occurs in meat type IMF at a rate that is faster than found in freeze-dried foods. Desorption prepared foods oxidize 3 to 6 times faster than adsorption prepared foods. This is due to the increased catalyst mobility in the liquid environment. Even with antioxidants desorption foods have less than 3 months stability. The addition of BHA as an antioxidant into an adsorption prepared IMF, however, should give a food of one year shelf life.

Non-enzymatic browning also occurs quite rapidly in IMF systems. Fruit type systems were not stable for more than three months. Desorption (higher moisture) systems brown at a slightly slower rate than adsorption systems. This was attributed to the product inhibition of water.

Most significantly it was found that microbial growth in IMF systems is a strong function of the method of preparation. Minimum water activities

for growth in the literature bear out the results for desorption prepared IMF systems with molds and yeast being the only problem. It was found,however, that the minimum water activity for four different organisms is much higher than reported if the food is made by the adsorption process, even with elimination of any processing stresses. This means stable IMF systems can be made without concern for microbial growth and without addition of anti-growth agents.

Packaging requirements were found to be minimal for IMF systems. Protection against water loss can be met by currently available materials. Adequate protection can also be found for oxidizable systems by using vacuum sealing and low oxygen permeable films.

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I. INTRODUCTION AND LITERATURE SURVEY

A. Purpose of Study

Intermediate moisture foods will serve a very important purpose in future manned space missions from several points of view. Intermediate moisture foods (abbreviated as IMF in the text) will allow for a convenient food which can supply both high nutrition, pleasing texture and ease of eating for the astronaut without the need for rehydration. In addition, through control of the method of preparation, these foods can be made shelf stable to microbiological decay, i.e. no refrigeration. These factors are based on preparation of the food to a moisture content range of 15-40 g H_2O per 100 g solids and a reduced water activity usually in the range of 0.6 to 0.85.

Labuza et al. (1970a) have reviewed the basic chemistry and elaborated on the deterioration mechanisms of intermediate moisture foods. In Contract NAS 9-9426 (Labuza 1970) studies were made in model systems to elucidate these deteriorative reactions and in addition some preliminary foods were prepared and stored for a short period of time to verify the model system studies. Several important factors in terms of water activity and additives were found which could improve storage life. These findings will be discussed below.

In Contract NAS 9-9426 studies were made with model systems to evaluate the extent of chemical deterioration in the intermediate moisture range. It was found that lipid oxidation was accelerated in this range reaching a maximum. Further work by Labuza et al. (1971) showed that this occurred with either protein or carbohydrate based systems. In addition, the antioxidant BHA and the chelating agent EDTA worked to reduce oxidation under the IMF conditions but the effectiveness was very dependent on the overall system composition. Browning in lipid-protein systems was negligible.

Preliminary studies were carried out in several foods which confirmed the above results. In a freeze dried rehumidified chicken system the antioxidant BHA gave very good protection to oxidation. Oxidation in the intermediate moisture range was rapid but not enough data points were taken to compare accurately between moisture levels. In a carrot IMF system it was found that the carotenoids oxidize more rapidly than in a system nearer the monolayer moisture content. This is as would be expected from the model system studies. Finally, a chicken system was prepared with a cooked ground chicken-slurry to which cellulose and glycerol were added to reduce the water activity to 75% RH. This system oxidized very rapidly as compared to the freeze dried chicken. BHA was found to be very effective in stopping oxidation; however, EDTA was not.

In terms of non-enzymatic browning an applesauce IMF system at 95% RH was studied in which only a slightly measurable increase in color was found at either 37° or 45°C in 2 weeks. At 52°C the brown color

developed more rapidly but this could be due to a change in reaction mechanism pathway (S. Fan 1969, M.I.T. M.S. Thesis).

This present study was designed to carry out storage studies of at least 3 months duration of several IMF items including a meat, vegetable and fruit type product. These products were kept at 37°C and analyzed for chemical and microbiological deterioration and the influence of protective additives.

As an important aspect of this study, some aspects of the methods of preparation were considered. Essentially to get to the final state of an IMF, two routes can be taken. In the direct procedure, a food at its normal fresh moisture content is combined with the various additives and humectants so that it approaches the final state by going down the desorption branch of the moisture sorption isotherm as shown in Figure 1. This method is that described for several current processes (Hollis et al. 1968, Chen 1970) including the manufacture of breakfast tarts from fruit preserves. The other major way is to start with dry ingredients and add the water either by humidification or by mixing in with a humectant solution. This is the method used by Labuza for NAS 9-9426 and in the studies of Heidelbaugh (1970), Heidelbaugh and Karel (1970) and Labuza et al. (1971). This method essentially entails going up the adsorption branch of the isotherm to reach the final condition. It can be seen from Figure 1 that the two methods give entirely different final moisture contents at the same relative humidity or water activity (water activity $A_w = \frac{\%RH}{100}$).

How this difference affects stability of the food is not known. In addition, many of the methods for manufacture of IMF pet foods employ the mixing of ground meat and meat by-products with dry ingredients and humectants so that a combination of adsorption-desorption steps occur (Bone 1968, Burgess and Mellentin 1965, Coleman 1969, Pavey and Schack 1969, Pinkos 1968, and Sanderude and Ziemba 1968). This method most likely approaches the adsorption rather than the desorption branch. Table 1 shows a summary of the various methods of preparation of IMF systems. This study was designed to use one of each of the two methods, i.e. a desorption and adsorption method, to analyze the stability of several IMF systems as well as the effectiveness of various additives in controlling stability.

B. Intermediate Moisture Foods

1. Description

A new approach to solve the undesirable organoleptic characteristics of dried products and the inconvenience of rehydration is to make food systems characterized by a water binding agent as a major compound (sugar, glycerol, salt, etc.). The presence of the water binding agent permits in these foods an increase of the moisture content to a level which improves the texture and yet controls the water activity in order to inhibit the growth of microorganisms. These foods "Intermediate Moisture Foods" (IMF) have a water activity of from 0.6 to 0.85 and moisture content from 20 to 40%. The moisture content in some cases is at higher values, depending on the method of preparation

and the quantity of water binding agent added to the food system.

A qualitative definition of IMF gives a better description of the desirable characteristics of these foods. They are somewhat plastic and easily masticated. Their texture is much closer to normal food than the rigid structures of dehydrated products. The IMF are ready to eat avoiding the need for water reconstitution of dried products. Since they are plastic, they can be molded into cohesive blocks of uniform geometry to facilitate packaging and storage. As in the case of dehydrated products, they are considered shelf stable without refrigeration.

The characteristics mentioned above give to IMF systems obvious marketing advantages such as elimination of the refrigeration requirement for consumer foods. IMF systems are relatively low in moisture content and hence can be considered concentrated from the standpoint of weight and caloric content. They permit easy packaging and relief from the problem of package breakage and subsequent food spoilage. Therefore these foods have been gaining in importance in recent years as reflected in the commercial success in the pet food industry, (Pinkos 1968, Coleman 1969) and in the increasing research in aerospace and military feeding systems (Hollis et al. 1968, Brockman 1970, Labuza 1971). Furthermore the IMF represents a potential method of food preservation in developing countries where the food distribution is difficult and large amounts of foodstuffs are wasted due to the lack of refrigeration systems.

Although in the past there have been various kinds of IMF such as confectionery products with high sugar content, dried fruits and certain bakery products, the current interest in this area represents a new look at what possibly can be done in the creation or design of new products.

In designing an IMF system the first consideration should be in the selection of the water binding agent. The characteristic binding strength, which is evidenced by a depression of the water activity of the water present in the food, can be estimated by relating water activity to Raoult's Law (Equation 1)

$$\frac{p}{p_o} = A_w = \frac{N_{H_2O}}{N_{H_2O} + N_s} = \frac{\%RH}{100} \qquad (1)$$

Where

p = vapor pressure of food

p_o = vapor pressure of pure water

A_w = water activity

N_{H_2O} = moles of water

N_s = moles of solute

%RH = equilibrium relative humidity

Actually the solutions in the food system do not behave as ideal solutions, and therefore there are deviations from Raoult's Law: (1) not all the water is available as a solvent, (2) not every solute is accessible, and (3) because of the presence of interactions among the components of the system. These facts were confirmed by Heildelbaugh (1970) in IMF using glycerol as a water binding agent. However, equation (1) can be used as a good estimate especially in a simple single solute system.

Table 2 shows the theoretical depression of water activity of a single solute and the actual values found for salt, sucrose, and glycerol which have been used for IMF systems.

The general problem is to find a water binding agent that has a large water activity lowering effect, is of low molecular weight, has plasticizing effects and contributes very little to adverse flavors. It can be seen from Table 2 that the depression of A_w is a colligative property. Salt, NaCl, will yield two ions on solvation thus twice the effect. Table 3 shows some additional compounds which have been used or could be used in lowering A_w for IMF. Not very many compounds can be used in foods in great quantities without having an adverse flavor effect or a slight toxic effect. Sucrose can be put into dog food but humans are not as easy to feed very sweet meat or fish products. In addition sucrose can only reduce the A_w to about 0.85 at saturation concentrations (67%). Fructose is more soluble and has a lower molecular weight, but the problem is then one of sweetening power. Fructose is better than sucrose as its molecular weight is less, but this advantage is somewhat offset due to higher sweetening power. In addition it is a reducing sugar and may affect chemical stability through browning.

Another important characteristic to look at in the water binding agents is the water activity stabilization. It will be seen that exact control of A_w may not be important to stability. Thus 22 g of water are needed to raise a solution containing 100 g of sucrose from A_w 0.85 to 0.90. Solutions of glycerol and salt require far greater

amounts of water for the same rise of water activity. This steepness of the isotherm in the IMF range is important since it means that the packaging requirement may not have to be very stringent since a large water transfer would have to take place before a significant change in A_w occurs.

2. Preparation

Once having selected a water-binding agent, the next step is to determine the method to introduce it into the food system. Essentially there are two methods, differing primarily in the initial moisture of the food being used in the process.

One method starts with freeze-dried foods. They are soaked in an infusing solution containing the water binding agent (Hollis et al. 1968, 1969). The concentration of the solution depends on the desired level of water activity of the final product. It was observed that a dehydrated food soaked in a solution, whose viscosity is not high to slow internal diffusion, will absorb a volume of solution approximately equal to the volume of water it would normally absorb (Kaplow 1970). Hollis et al. (1968) assumed that the additives will diffuse into the food to an extent that the final concentration in the food and in the solution will be similar. Another possibility using this method is to start with dry ingredients (including the water binding agents) and add the water to the food by rehumidification to the desired level of water activity (Heildelbaugh 1968, Heildelbaugh and Karel 1970, and Labuza et al. 1970a). It should be observed that these methods

essentially take the food up the adsorption branch of the isotherm.

In the second basic method or direct mix procedure, a normal moisture food is mixed or soaked with the various additives and humectants. In this method equilibration in the food is obtained by losing water, therefore the desorption branch of the isotherm is followed. It can be seen from Figure 1 that the two methods give entirely different final moisture contents at the same water activity. This is an important factor in selecting the method because more water would give a better moist texture but higher moisture content could affect the storage stability of the IMF.

Another possible method, a combination of the two described above, consists of mixing different foods with different characteristic isotherms. Each component then follows either the adsorption or desorption isotherm depending on whether it gains or loses water. This is basically the method used for most pet food IMF (Burgess 1965, Bone 1968). In these foods,for example,the meat component goes down its desorption branch and the cereal components go up an adsorption branch.

Some examples of IMF foods either tested or presently in the consumer market are listed in Table 4. It is possible to observe that the two basic preparation methods are followed and that sugar is the most used water binding agent for market items and glycerol for test foods.

The theoretical basis of the phenomenon of sorption hysteresis which causes the difference noted above is very involved and will not be discussed in detail in this contract. A recent review has been made

by Hurtado (1971 M.S. Thesis M.I.T.). He has shown very large differences in moisture content between the two preparation methods. The reasons for the large differences in moisture contents are rather complicated and have been summarized by Adamson (1960) and Labuza (1968). In a study of model food systems, Labuza and Rutman (1968) felt the most likely reason for the adsorption-desorption hysteresis is due to the ink bottle pore structure as proposed by Rao (1941). Essentially this means that in desorption a large portion of the water is at a much higher activity but does not see the vapor space. Thus reaction rates would be expected to be quite different on the adsorption branch although no known data are available. Labuza et al. (1970a), Bone (1969) and Labuza (1970a) have discussed food deteriorative reaction mechanisms as a function of the state of water but have not eluded to the differences that might occur according to the method of preparation.

3. Storage Stability

The storage stability of IMF, due to the range of water activity (0.6 - 0.8) in which they are contained, is related to such problems as spoilage by microorganisms, enzymatic reactions and non-enzymatic chemical reactions i.e. browning and lipid oxidation. In Figure 2 is shown a general "map" of effects of water activity on the different food deterioration problems (Labuza 1970a).

a. Microbiological

Very little data are available as to the effect of processing

on viability of microorganisms in intermediate moisture foods. However, several studies have been made which have significance in terms of growth and destruction of microorganisms under the processing stress of lower water activity.

It has been well established that in food processing wet heat kills spores and vegetative cells faster than dry heat (Scott, 1957). However, little data have been collected for intermediate moisture values and the effects of humectants on cell viability. Barrile et al. (1970) showed for example that the D value (time to destroy 90% of the population) decreased with increasing moisture content up to 10% moisture for dry milk chocolate, but no attempt was made to correlate this with water activity. Goefert et al. (1970) found that at constant water activity (A_w = 0.96), and using different sugars to achieve this level, the D value varied by one log cycle depending on the sugar. Thus not only is both water activity and osmotic pressure important but probably composition of the system.

In preparing an IMF food by the direct mix procedure the organisms present suffer several stresses. First the osmotic gradient will cause the cells to lose cell water in order to equilibrate with the surrounding water. This in turn can cause the cell to shrink and break its cell wall. Simultaneously the salts in the cell sap are being concentrated and may affect cell stability (Merryman, 1970). Lastly, in any normal process the food will be subjected to a heating process so that the D value must be known as a function of water activity in order to predict the heat treatment necessary for a low viable count.

Many confusing results are present in the literature. Hoffman et al. (1968) found that for many bacteria spores, although resistance

to thermal death increases with decreasing relative humidity, certain selective media could reverse this. Goepfert et al. (1970) found that for pathogenic organisms the resistance also increased with decreasing humidity and that the amount of resistance was a strong function of media. In the most comprehensive work Murrell and Scott (1966) showed that, for spores at least, as the A_w is decreased from unity resistance goes to a maximum and then falls again but is still larger at $A_w = 0$ than at $A_w = 1$. This maximum in fact seems to occur in the intermediate moisture range. Thus intermediate moisture food should be heat treated for cell destruction prior to mixing to achieve the desired water activity. This would minimize the heat treatment needed.

For intermediate moisture foods prepared by the adsorption process some information is necessary as to the effect of drying on cells. Very little data are available but some generalizations can be made. For air-dried-products drying usually 2-4 log cycles of kill results. In continuous processes, such as drum drying and spray drying, 3-5 log cycles are possible depending on the conditions used. (Labuza et al. 1970b, 1970c, 1970d; O'Connor et al. 1969; Sieburth and Jensen 1967). Thus if one starts with relatively clean food components the amount of cells per gram of food at mixing should be small. Freeze drying of food components puts additional stresses on organisms and their survival is very species dependent. Kill will vary between 1-5 log cycles with molds being most resistant (Bennedict et al. 1958; Heckley 1961; Mazur and Schmidt 1968; Rose 1970; Vanderzant and Suarez 1967). One major

factor is the effect of the rate of freezing, which has a strong effect on cell viability (Mazur 1963a, 1963b, 1965, 1966). This is due to the concentration of solutes outside the cell, as in the preparation of intermediate moisture foods, which causes the cells to lose water and dehydrate. In general both very fast and very slow freezing are detrimental to cell viability with intermediate rates giving the best survival.

Several workers have studied survival of cells during storage of freeze dried foods up into the intermediate moisture range (Chipley and May 1968; Silverman and Goldblith 1965; Saleh and Goldblith 1966; Sinskey and Silverman 1970). In general for freeze dried foods which are humidified to high relative humidity cell viability decreases rapidly. This is quite important from the standpoint of intermediate moisture foods, as this gives evidence that the cells are less resistant on the adsorption branch of the isotherm. Sinskey and Silverman (1970) attribute this to lack of ability to repair dehydration damage as well as to possible chemical reactions causing death. At $A_w = 0.75$ they find 4-5 log cycles destruction occurs in 10-20 days. In terms of storage stability of IMF from Figure 2 it can be seen that decreasing the water activity from 1, the first problem evidenced is microbiological spoilage. The growth of microorganisms is limited to a rather small but characteristic range of water activity values. Each kind of organism apparently has its characteristic optimum water activity at which it grows best. As A_w is reduced below an optimum level there is an increase in the lag or latent period, a decrease in

the rate of growth, and a decrease in the amount of cell substance synthetized. These effects are similar to those lowering temperature from the optimum. Several studies and reviews are present in the literature for the determination of the lower limits of water activity at which growth can occur for various kinds of microorganisms. (Mossel and Ingram, 1955; Christian 1963, Scott 1957). Bone (1969) summarized studies, as shown in Table 5, where the approximate lower limits of water activity for each kind of microorganism are tabulated. In general there are some variations of these values with nutrients (Snow et al. 1944), temperature (Scarr 1951), oxygen (Scott 1953), pH (Schelhorn 1951) and other factors. Thus for specific food systems experiments have to be made to determine the optimum value to prevent the growth of microorganisms. Another possible factor influencing the water activity growth minimum could be the method utilized to get to the final activity, i.e. whether the desorption or adsorption branch of the hysteresis loop is followed. According to the above literature both ways have been used in microbiological studies to prepare the systems, decreasing the A_w with water binding agents in liquid systems (desorption) and rehumidifying the food (adsorption). Although differences in lower values of A_w for growth of microorganisms in solids and liquids systems have been reported (Scott 1957), it is not possible to make comparisons, due to differences in the composition of the systems utilized.

The only study on actual stability of microorganisms in intermediate moisture foods is the work of Hollis et al. (1968, 1969).

They found that for foods prepared by a direct mix (either cook-soak procedure or soaking freeze-dried in an excess of liquid, both with added sorbate) to A_w = 0.75 to 0.8, initial mold counts were less than 10 per gram. After four months the number was also less than 10 per gram. Yeast increased to 400 per gram in beef stew (A_w = 0.76) and all total plate counts were less than 60 per gram. No attempt was made to correlate these data with A_w or food type. In Phase II of the work (Hollis et al. 1969) all foods were prepared by the cook-soak procedure. Again molds and yeast were examined for and found to be less than 10 per gram at zero time, one month and four months. Total counts were much higher, however, ranging initially up to 1-2 x 10^3 per gram but decreasing by 50 to 90% in storage except for cheese, which remained stable (A_w = 0.83). It should be noted that it is most likely that organisms were not growing at these water activities but were damaged. Some of them are able to start growing again when transferred into media for plate counts. This could be due to the fact that potassium sorbate was added to all systems and then diluted out in plating, allowing growth to occur.

In an inoculation study by Hollis et al. (1969) using pathogens in a chicken a la king casserole (A_w = 0.85) the following was found.

(1) Staph. aureus decreased by one log cycle in one month and by more than 5 log cycles in 4 months.

(2) E. coli, Salmonella and Cl. perfringens decreased by more than 3 log cycles in 4 months.

Similar results were found for a ham in cream sauce casserole (A_w = 0.85). These results also indicate that the organisms are not able to grow at these humidities and in the presence of sorbate.

In the above discussed work no data are available on the same food prepared by both desorption and adsorption methods and without growth inhibitors. The literature suggests that death will be faster on the adsorption branch than on the desorption branch. This could be due to the lower water content available for growth on the adsorption side. A manufacturer could thus prepare a food by humidification to a higher moisture value and have a safer food product.

b. Enzymatic Reactions

In relation to the stability map (Figure 2) the other important biochemical deteriorative process is enzymatic activity. Enzymes have been found to have a great range of reaction in relation to the moisture content of a food. Activity even below the monolayer value of moisture content, has been observed by Lea (1943), and Acker (1969). Acker (1963) showed that the enzymatic reaction is accelerated in the region of intermediate moisture levels. Acker (1969), working with a hydrolitic enzyme, found that as moisture content increases, the rate and extent of the enzymatic reaction increases. This is because the water not only permits more diffusion, but is acting as a solvent capable of dissolving the necessary reactants. From these studies it is possible to conclude that enzymes can play an important role in the deterioration of IMF, therefore enzymes will have to be inactivated

for proper stability of IMF. However with certain food items because of nutrient degradation heat treatment may not be possible, so that a knowledge of reaction rate as a function of A_w as well as method of preparation is important.

c. Non-Enzymatic Browning

From Figure 2 it is possible to observe that in the intermediate moisture range non-enzymatic chemical modes of deterioration can predominate i.e. non-enzymatic browning and lipid oxidation.

If the storage stability of IMF in terms of the above deteriorative chemical reactions has to be described, some knowledge of the role of water is necessary. Water in these reactions has a multiple role:

- Water acts as a solvent permitting the dilution and mobilization of reactants, as well as the diffusion of reactants and products (Duckworth and Smith, 1963).
- Water may act as a reactant participating in specific reactions (Schobell et al.,1969).
- Water may modify properties of reactants by hydration (Maloney et al., 1966; Labuza et al., 1966; and Karel et al., 1967).

Non-enzymatic browning involves the reactions between carbonyl and amino compounds, resulting in an objectionable brown color, off-flavors, and loss of proteins. This reaction shows (Figure 2) an increased rate as humidity increases up to a maximum in the intermediate range after which it decreases again (Lea 1958, Labuza 1970a).

An important type of browning reaction, i.e. the Mallaird Reaction

which occurs between carbonyls and amino groups could predominate in IM Foods. This is because many humectants that would be used to lower water activity into the intermediate moisture range are sugars which have reducing carbonyl activity. These sugars most likely will have to be avoided in manufacture of IMF. However even non-reducing sugars such as sucrose can cause problems due to acid hydrolysis. Schobell et al. (1969) has studied the rate of sucrose hydrolysis and Karel and Labuza (1968) have shown how this reaction caused browning in protein systems.

As explained above, the rate of non-enzymatic browning increases as moisture content is increased. This is because water, acting as a solvent, increases diffusion and disolution of reactants. The value of water activity in which this maximum is observed depends on the composition of the systems and how water activity was depressed (by drying, addition of salt or glycerol or other water binding agents). The reason of the decrease of the rate of browning after this maximum is not clear. Loncin et al. (1968) explained that this decrease in the rate of browning is due to the fact that water, produced in the reaction, increases moisture content and produces product inhibition.

Recently Eichner (unpublished MIT 1971) has confirmed the inhibitory effect of water in liquid systems undergoing browning. He found that the presence of water slowed the rate. The observed maximum in rate for dry foods however does not occur until the moisture content reaches a certain level, in which the effect of water by increasing diffusion and solubilization of more reactants is diminished by product inhibition or by the dilution of the reactants. Since by the laws of mass action, the rate of reaction is proportional to concentration, a decrease in

concentration by dilution with water will also decrease the rate (Labuza 1970a).

Because of the multiple effect of water an interesting question may be asked. Since in direct mixed systems the moisture content is higher (desorption branch) than in dry systems obtained by rehumidification (adsorption), product inhibition or dilution might prevail so that browning should occur at a slower rate.

d. Lipid Oxidation

Lipid oxidation involves the reaction of oxygen with unsaturated fatty acids by means of a free radical mechanism, catalyzed by metals, resulting in rancidity, off-odors and off-flavors (Lundberg 1961, and Schultz et al., 1962). Besides, it can produce free radicals that can react with proteins to reduce solubility and biological value (Andrews et al., 1965), and destroy lipid-soluble vitamins in foods. Unsaturated carbonyls formed in the free radical mechanism can also directly react at the proper moisture content in the non-enzymatic browning pathway (Martinez and Labuza, 1968; Le Roux et al., 1969).

In the case of lipid oxidation the influence of water also creates a very complicated situation. At low values of water activities the water exerts an antioxidant property. It inhibits lipid oxidation by tying up hydroperoxides and preventing their decomposition to free radicals or by hydrating metal catalysts (Labuza et al., 1966) making them less effective. Another reason for the protective effect of water may be that it promotes radical recombination. The antioxidant behavior of water is also evident in protection of oxidation sensitive pigment of salmon (Martinez and Labuza,1968).

However, when the water activity is increased into the intermediate moisture range, water may promote oxidation. This effect is especially significant in the presence of catalysts at concentrations exceeding 100 ppm in the system. It is believed that solubilization and mobilization of these catalysts, counteracts and overshadows the antioxidant effect of water (Labuza et al., 1971; Heildelbaugh, 1970). Labuza et al. (1971) working with model systems based on protein (casein), studied the development of peroxides in the intermediate - moisture range. They found as the water activity increases from 0.6 to 0.75, the moisture content more than doubles, from 11 to 24 g/100 g solids, and the rate of oxidation falls. It is possible that, as in non-enzymatic browning, the addition of water dilutes the metal concentration, and the reaction thus becomes diffusion-limiting.

In summary for the two reactions of non-enzymatic browning and lipid oxidation, there exists a rate promoting level of water activity for each one, that in most cases is not located at the same water activity. Because foods are complex mixtures of lipids, carbohydrates, proteins, metals, and water, it is very difficult to predict the extent and rate of deterioration as a function of water content, especially in the intermediate moisture range. For IMF both oxidation and browning can occur simultaneously as indicated in model system studies. Another complicating factor is the effect of the method of depressing the water activity, by rehumidification of by direct mixing which would give different values of moisture content

at the same water activity, affecting the location of this optimum level. Therefore the complexity of IMF requires particular caution in predicting storage behavior. However, an understanding of the pattern of reactions and of their response to A_w, will allow improvement in design of storage studies and accelerated tests.

II. IMF SYSTEM PREPARATION

A. Basic Food Systems Method

Overall the method used was to take a semi-solid food (in general a 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 various additives were added and then the portions were divided into 3 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 were then frozen, freeze dried, and then rehumidified back to the same water activity. These were then placed in storage (about 3 days later) with the direct mixed systems usually at 37°C. Samples were then taken periodically for testing.

1. Direct Mixing (Desorption Process)

The food was removed from the jars as purchased, and combined in a Waring Blendor jar. To this the various additives such as glycerol, cellulose, antioxidants (dissolved in 1 ml ethanol) and sorbic acid or

propionate were added. This was then blended for 10 min, cooling with ice. The samples were then transfered into the storage flasks; either

(1) tared Warburg flasks

(2) 50 Erlenmeyer flasks

(3) or 3 oz. screw cap bottles

and the weight was recorded. Samples were then placed in storage at the desired temperature, usually 37°C. In the results section this procedure is referred to as the desorption method or the direct mix method.

2. Freeze-Dry-Rehumidification Method (Adsorption Process)

The samples were mixed and weighed as above and then were frozen at -40°C for 5 hours. The samples were then freeze-dried at 80°F, and 100 μHg for 48 hours. The vacuum was broken with nitrogen and the samples were rehumidified to the original water activity of the direct mix system by using desiccators containing the appropriate salt solutions (see NAS 9-9426). Humidification was carried out for 18-24 hours and then the samples were removed, capped and placed in storage at the desired temperature. Zero time for these samples was taken as the time out of the humidification process. In the results section this method is referred to as the adsorption method, the freeze-dried method or the freeze-dried rehumidified method.

B. Moisture Content

Moisture content of all sample treatments was measured by the vacuum oven procedure. Duplicate samples were placed in a vacuum oven at 29" Hg and 70°C for 24 hours. The weight change was recorded as the moisture content.

C. Fat Content

The fat content of each system was measured using the extraction procedure for peroxide determination. The method is outlined in the stability test methods section.

D. Water Activity Measurement

In order to measure the equilibrium vapor pressure or water activity of the food the apparatus described by Karel and Nickerson (1964) and Karel and Labuza (1967b) is used. Essentially this consists of a system in which the sample at 37°C in a flask is evacuated for 2 minutes to remove air and then connected to a sensitive oil manometer to measure the vapor pressure of water in equilibrium with the sample. Approximately ½hour is needed to reach equilibrium. In some cases a direct reading instrument was used (Hygrodynamics Electric Hygrometer).

E. Determination of Glycerol Level for Control of Water Activity

In this study glycerol was used as the sole humectant. Figure 3 shows the vapor pressure lowering effect of glycerol in water (Miner 1953, Newman 1968). It can be seen that glycerol is needed at about 50 to 70% by weight of water to reduce the activity to a level of 0.8 to 0.65. In a food however other soluble solids as well as effects of structural elements also help to lower the vapor pressure (Labuza, 1970a). Thus less glycerol will be needed, however in foods of very low soluble solids content conversion into an IMF by this method will require that glycerol comprises about 60-80% of the total solids. This type of product will have very little structure to it.

Basically to determine the desired humectant content, glycerol was added to each liquid food system in different ratios and the water activity was determined by the above method. Figures 4 and 5 show the curves determined for the various direct mixed IMF systems prepared using the semi-solid foods as a starting point. The diced chicken system will be described subsequently. Table 6 lists the composition of the original semi-solid foods used for most of the studies. In Figures 6-8 are presented the pseudo-isotherms for each of the food systems. It can be seen that in each case at a given water activity the direct-mixed (desorption) system has about double the moisture content. Thus this allows the study of reaction kinetics at the same water activity and solids composition, but very different moisture contents. The glycerol levels used to achieve the desired water activities for each run are listed in Table 7 for the chemical stability tests and in Table 8 for the microbiological storage tests. Potassium Sorbate was added to most of the chemical stability test systems to prevent microbial growth.

F. Special Test Systems Preparation: Chemical Studies

RUN 2

In Run 2 glycerol and microcrystalline cellulose (AVICEL^{R}) were mixed with Gerbers Baby Food Chicken in several batches as listed in Table 9. Both citric acid and butylated hydroxy amisole (BHA) were tested as anti-oxidants. Each of these systems were freeze-dried and rehumidified to several levels. Oxidation was followed by several techniques.

RUN 11

In Run 11 the cook-soak procedure of Hollis et al. (1969) was used to prepare chicken to various water activities. The procedure (outlined in Table 10) was to cut fresh raw chicken into 1/2 to 1 inch dice, and soak in solutions adjusted to water activities of A_w = 0.61, 0.75 and 0.85. The mixture was heated to 96 to 99°C, held for 15 min and then left overnight to soak in a refrigerator. The dice were then removed and patted dry. One half of these were then frozen at -30°C for 5 hrs and freeze-dried for 48 hrs at 100 μHg and 80°F platen temperature. They were then rehumidified to the various water activities of the soaked samples.

RUN 12 - Enzyme Study

A model system study consisting of cellulose as a base with corn oil, glycerol, water and the enzyme lipase was made to investigate the effect of sorption hysteresis on enzyme reactivity. Table 11 contains the compositions of the systems used. Basically the oil was added to the cellulose in a beaker, hand mixed, glycerol and the enzyme in solution were added and blended with the rest of the water by hand (10-15 min). This was then divided and one half was freeze-dried and humidified as individual samples as in previous studies. Six gram samples were used and stored at 37°C and tested periodically for free fatty acid production.

RUN 13 - Peroxides in Commercial IMF Pet Foods

An IMF dog and cat food was purchased from a local supermarket. The composition is shown in Table 12. Five gram samples were held at 37°C in

capped jars and tested for rancidity by determination of peroxides. The extent of browning in the extracted residue was also being measured. In addition samples of each were freeze-dried and rehumidified to the original water activity for comparison of the rates of deterioration.

G. Special Test Systems: Microbiological Studies

Most systems for microbiological studies were prepared in the same manner as for chemical studies. Addition of microorganisms to the test systems was made in several ways as will be explained in the methods section. Several special systems were prepared as follows:

RUN 2M - Infusion of Freeze-Dried Chicken System

Table 8 contains the composition of the infusion chicken system. In this case 10 g samples of freeze-dried chicken were mixed with exactly 3 grams of liquid per gram of chicken to give the desired water activities. This is different from the soak infusion procedure of Run 11, which uses fresh chicken. About 10^3 mold spores per gram chicken were added in the solution. One half of each batch was freeze-dried and rehumidified before storage at 37°C. The water activity of the systems was determined in the same manner as described above and is represented in Figures 4 and 8.

RUN 8M - Laboratory Dog Food System I

In Run 8M a study of a few different humectants was made in an actual dog food mixture. The compositions used are shown in Table 13. The procedure used was to combine in a blendor jar the dry ingredients, add in the liquid ingredients and mix. The hamburger was fried till brown (5 min) and added in, blending for 3 min. Then 10^3 mold spores per gram in 1 ml

water were added followed by blending for an additional 5 min. These were then transferred to storage at 37°C after weighing 10 g samples in 3 oz. bottles and capping.

RUN 9M - Laboratory Dog Food System II

In order to get samples of higher water activity the composition of proteins and meat was changed in Run 9M to that shown in Table 13. The procedure used was the same as for Run 8M.

III. STABILITY TEST METHODS

A. Test Procedures for Oxidation

1. Manometric Determination of Oxygen Absorption

Samples (in triplicate or duplicate) of the 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 Apiezon B oil (J. G. Biddle, Philadelphia, Pa.) as the manometer fluid. These were then placed in water baths at 37°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 4 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:

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

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

T_o = 273°K

T_1 = run temperature, 310°K

P_o = 1 atm. of manometer fluid = 11,880 mm oil

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 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).

2. 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 40 min on a rotary vacuum

evaporator. The weight of lipid was then calculated by weighing the flask. The residue remaining on the Buchner funnel was saved for determination of the extent of nonenzymatic browning.

The lipid in the flask was dissolved with 10 ml. of a mixture of glacial acetic acid: chloroform (3:2). The 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 is then titrated with 0.01 N $Na_2S_2O_3$ using 1 ml 5% starch solution as an indicator. The thiosulfate is standardized periodically against potassium dichromate. The peroxide value is calculated as:

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

3. Sniff Test

The IMF samples for oxidation studies were smelled daily by at least 2 persons to determine the onset of rancidity. This was evidenced by a stale, painty, or hay like odor, which occurs near the end of the induction period.

4. Carotenoid Procedure

a. Carrots

Samples prepared to 5.0 $\pm$ 0.1 g were extracted with 60 ml of 1:1 acetone: hexane (3 portions of 20 ml each for ½ hour on a rotary shaker) directly in the storage bottle (3 oz. screw cap). The optical density at 450 mμ was then reported as the carotenoid index, since measurements at other wave lengths were extremely low and variable.

b. Sweet Potatoes

The same procedure as above was used except:

(1) 6 gram samples were used

(2) 1:1 (v/v) hexane: chloroform was used as the solvent. The absorption curve is shown in Figure 9.

(3) The carotenoid index is reported as

$$\text{INDEX} = \frac{OD_{450} + OD_{475}}{OD_{350}(\text{g dry food solids})}$$

5. Polymerization (Browning) in Lipid Phase

An aliquot of the extract made above for the peroxide value determination was read on a Hitachi spectrophotometer at 420 mμ. Browning was reported as:

$$B_o = \frac{OD_{420} \times \text{wt. solvent}}{\text{total wt. sample solids} \times \rho_{\text{solvent}}}$$

B. Non-Enzymatic Browning Procedures

Method A. Aqueous Extraction

To the sample from storage, 30 ml of water are added and the sample is shaken for 1 hr at room temperature. The sample is filtered on a vacuum Buchner funnel and the filtrate collected. This is centrifuged for 20 min at 18000 rpm and the optical density is measured at 420 mμ. If the sample is initially high in fat, it is first extracted with 30 ml of 3:1 $CCHCl_3$:MeOH (v/v) and a representative sample (0.3-0.5 g)is taken. It should be noted that even with centrifuging with many model systems and food samples, the solution contains a dispersed phase which causes errors in the results.

Method B. (Trypsin Method)

(1) Weigh out 2.0 g samples.

(2) Add 20 ml of distilled H_2O and 2.5 ml of freshly prepared 10% trypsin solution. Prepare a blank also.

(3) Incubate samples for 1 hour at 45°C.

(4) Add 2 ml of 50% TCA solution and 0.1 g of Celite filter aid.

(5) Filter the samples through No. 576 Whatman filter paper and centrifuge as above if necessary.

(6) Read optical density at 450 mμ.

$$B.U. = \frac{OD_{450} \times 100}{\text{gms solids}}$$

Method C. Pronase Procedure

To the sample in the storage bottle 30 ml of water was added along with 20 mg pronase enzyme. The pH was adjusted to pH = 7.8 - 8.2 using 0.2 N sodium hydroxide (about 0.5-1 ml) and 1 ml isopropanol was added to inhibit mold growth. This was kept at room temperature for 24 hours. The optical density on this solution was measured at 420 mμ and was reported as

$$B = \frac{OD_{420}}{\text{g food solids}}$$

As in the other procedure if the sample is high in fat it is extracted first as for peroxide determination and browning is done on the residue. The amount of extracting water is adjusted for sample size but is kept constant for each test on one food system.

Method D. Pectinase Procedure

A 5 gram sample was suspended in 30 ml distilled water (final pH 4-5) and $\simeq$ 100 mg of pectinase (Nutritional Biochemicals Co.) was added. This was shaken for 1 hr at 25°C then centrifuged 40 min at 17,000 rpm. The supernatant was then filtered through 2 sheets of #589 Whatman filter paper, and the optical density at 420 mμ was read on the filtrate. The browning was reported as above.

Method E. Multiple Enzyme Procedure

1. Add 25 ml of buffer pH 8.0 solution* to each sample. Then add a small amount of the following enzymes: pectinase, pronase, and amylase (about 100 mg of each). Add 1 ml of isopropyl alcohol to the samples and shake well.

2. Store samples at 37°C for 4 hours. Shake once or twice during incubation to insure complete extraction.

3. Centrifuge samples for 20 minutes at 18,000 rpm and 5°C. Filter by gravity through 1 layer of No. 589 Black Ribbon S & S filter paper.

4. Centrifuge again for 40 minutes at 18,000 rpm and 5°C. Filter through 2 layers of filter paper.

5. If the solutions are still cloudy, centrifuge again for 40 minutes at 18,000 rpm and 5°C.

6. Read Absorbance at 420 mμ.

7.
$$B = \frac{(\text{OD at } 420\ m\mu) \times 100}{\text{gms solids}}$$

* Buffer pH 8.0 solution: 50 ml of 0.1 M KH_2PO_4 and 46.7 ml of 0.1 M NaOH.

C. Free Fatty Acid Analysis

1. Extract fat from sample as in peroxide procedure.

2. Dissolve in 50 ml benzene alcohol - phenolphtalein solution. (To 1 benzene and 1 95% ethanol add 0.4 g phenolphtalein.)

3. Titrate solution to distinct pink or orange pink color with 0.1 N KOH.

4. Make a titration blank on the benzene solution; subtract from sample value.

5. Report as FFA:

$$\text{FFA} = \frac{\text{N of KOH (ml sample - ml blank)}}{\text{gm fat}} \times 100$$

D. Microbiological Procedures

1. Initial Systems

Each system was prepared as outlined previously by first making a direct mixed IMF system. This was then split into various portions and the test organism was added in a minimum amount of water (less than 0.5cc/300 g food).

In Runs 1M and 3M no attempt was made to add an exact amount of mold spores (Aspergillus niger isolated from pumpkin pie mix left out on bench top overnight); however an estimate of about 10^3 to 10^2 spores were added per gram of food solids. In Run 2M the spores were added as part of the infusion water. In each of these cases the spores were added prior to freezing, freeze drying and rehumidification. Plate counts of a 1 g sample made on TSY agar after 24 hours incubation at 370°C.

2. Intermediate Systems

In Run 4M and 5M a quantitative addition of organisms was attempted.

Organisms of various types were grown in TSY broth and the Klett (optical density) value was determined as a function of cell concentration. The results are shown in Figure 10. After 48 hours, the culture was diluted to give about 10^7 organisms per cc. which when added to each food system gave about 10^3 to 10^5 organisms per gram.

Table 15 shows the type and amount of organisms added to each direct mixed batch for Run 4M. The amount added was calculated to give about 10^5 organisms per gram of food solids (excluding glycerol) so that comparisons could be made as a function of water activity. In the mixture 10^5/gram of each organism was also added to the samples containing 0.3% of potassium sorbate and 0.3% propionate, respectively. After thorough mixing 15 gram samples were weighed into 3 oz jars. Half of these were frozen at -40°C and then freeze-dried, as was done for the food stability tests. The remaining samples of each water activity were stored at room temperature (25°C). After drying the samples were humidified for 24 hours to the proper water activity (see Table 15). Plate counts of the freeze-dried samples were made either after drying, after humidification or both. The samples were then capped and stored along with the direct mixed samples. Plate counts were made of all systems at the initial time and at subsequent intervals after preparation using duplicate plates and the proper dilution. TSY agar plates were used and incubated at 37°C, counting after 24 hours.

In Run 5M only the mold was studied and a higher humidity system was made by adding water to the bananas. Addition of the mold was made in the direct mix system.

3. Advanced Studies

As will be discussed because of stress on the organisms several studies were made to eliminate the freezing, drying and rehumidification effects.

RUN 6M - Banana IMF

In Run 6M the same study as Run 5M was repeated. However instead of adding the mold to the humidified product before drying, it was added afterwards. Essentially the direct mix system was made and divided in half. To one half about 3 x 10^3 mold spores/gram solids was added and then this was divided into storage jars and held at room temperature. The other half was divided into storage jars, frozen, freeze dried and then to each sample about 0.1 cc. of water containing enough mold spores to give 10^3 organisms per gram was added. The samples were then placed in their respective desiccators for 48 hours to come to the final equilibrium and then an initial plate count was made. The jars were capped and then left in storage with the direct mixed samples for subsequent analysis.

RUN 7M - Pork IMF

A study similar to the procedure used in Run 6 was made on a pork IMF system. All the organisms studied in Run 4 (Table 15) were added to the freeze-dried samples, after drying, in a 0.1 cc. solution to give about 10^3 organisms per gram.

4. Laboratory Dog Food Studies

Systems described in Tables 13 and 14 were prepared and mold spores were added as indicated in the procedure described previously.

IV. RESULTS AND DISCUSSION

The results of this study will be discussed on the basis of each deteriorative type of reaction studied. It should be kept in mind that preliminary studies were made first to determine the probability of a particular reaction being important and that subsequent studies were made with a variety of treatments. Each run will be discussed individually.

A. Oxidative Rancidity:

1. Lipid Oxidation

Based on the results of the previous contract it was concluded that lipid oxidation would be the primary deteriorative chemical reaction in IMF systems. This would be especially true for both meat and vegetable type products. The studies presented below were designed to determine the stability of meat type IMF systems, prepared in several ways, and the effectiveness of the various antioxidants found suitable in NAS 9-9426.

RUN 2 - Cellulose/Chicken IMF

In Run 2 a chicken/cellulose/glycerol containing IMF system was prepared according to the composition shown in Table 9. It should be noted the product was freeze-dried and humidified to several levels and that besides the control, systems containing respectively 200 ppm BHA (fat basis) and 100 ppm citric acid (solids basis) were prepared. These latter antioxidants were found to be very effective in the model system studies of NAS 9-9426 at 75% RH.

Oxidative deterioration was followed by measurement of oxygen

uptake, peroxide value and organoleptically. The results of oxygen uptake carried over a 40 day period are shown in Figure 11 and the average rates of oxidation are presented in Table 16. The rates of oxidation are at least 1/10 slower than that found by Heidelbaugh for a pork IMF system prepared in the same manner (Heidelbaugh and Karel, 1970). This is probably due mainly to the differences in fatty acid composition between chicken and pork. The expected trend in oxidation with increased water activity was found for all treatments, namely humidification to 32% RH decreases the rates by 50-60% over that of the control. However, at 75% RH the rates are even faster than the dry system as had been found by Heidelbaugh and Karel (1970). This is as would be expected if catalyst mobility is important showing the pro-oxidant effects of water. The action of antioxidants on the system especially at 75% RH bears out the work in the model system studies (NAS 9-9426). Citric acid being a chelating agent gives about a 40% decrease in oxidation rate (Table 16) probably because it is partially tied up by the protein bound metal catalysts. On the other hand BHA decreases the rate over 4 times that of the untreated control.

Table 17 shows that the peroxides were just beginning to show up at 2 months and no real significant differences were apparent up to that time. However, it can be seen that with further storage the control freeze-dried system became rancid in about 4 months at both 32% and 75% RH, whereas the dry control was still acceptable. The systems with BHA and citric acid were both acceptable over the 4 month storage period.

Further samples were not available.

Table 18 compares the time to reach rancidity of the direct mix system (same composition) of the previous study (NAS 9-9426) with the 75% RH control of Run 2. It can be seen that in the direct mix system which contains twice as much water the product becomes rancid within 23 days and the peroxide values are very high. The adsorption system, however, oxidizes much slower not becoming rancid until 4 months.

These runs were prepared from the same batch of chicken so that the difference is real and is due to system preparation. Most likely the higher amount of water of the same relative humidity contributes to the solubilization and mobilization effects of water making it an effective pro-oxidant. This difference in rate of rancidity development would be quite important in terms of manufacture of IMF food items for space missions, but to confirm it further tests were designed and studied.

In Table 19 the browning extract from the lipid phase is shown. As in Run 23 (NAS 9-9426) no detectable aqueous extracted browning was found. Although the results are quite scattered they seem to follow the same trend as in oxidation indicating that the pigment comes from oxidative polymerization pathways.

RUN 4 - Chicken: Glycerol IMF System

In order to determine the validity of the results shown in Run 2 a test was made of a chicken IMF system prepared both by desorption and adsorption methods simultaneously. The test parameters are listed in Table 7. In addition to the control, both BHA (at 200 ppm fat basis)

and EDTA (100 ppm solids basis) were also studied.

Table 20 contains the results of the peroxide determinations, and Figure 12 illustrates the increase of PV with time for the control system. As with the chicken: cellulose: glycerol system (Run 2), the direct mixed samples in all cases oxidized faster than for the adsorption prepared systems. Table 21 lists the days to reach rancidity by organoleptic analysis for all conditions. The pattern with humidification,however, is not as clear as with the preparation method. With the controls the samples at 61% RH were oxidizing faster than 75% RH; however, with the samples containing EDTA the samples at 75% oxidized faster. The BHA treated samples did not oxidize significantly under any conditions over the 83 day study. Insufficient samples did not allow the study to be carried on any longer as more data points in the beginning were felt to be necessary.

The organic phase browning data are presented in Table 22. No obvious trend as found in Run 2 was observable.

It is obvious from the data for Run 4 that oxidation proceeds much faster in the direct mixed systems, supporting previous conclusions. Treatment with antioxidants does tend to minimize the differences between the two preparation procedures. The use of BHA gave very good stability but a longer storage study would be necessary to

see if stability would last over six months. This study shows, however, that with respect to oxidation a lower moisture at a given water activity gives better protection. In this case the degree of protection was about 3 to 4 times for the adsorption system without antioxidants added.

RUN 6 - Pork IMF System

This run was made to collect preliminary data on the development of rancidity in a meat product other than chicken. In the previous two runs it had been shown that chicken IMF systems oxidized from 3 to 6 times faster in the direct mix state than in the freeze-dried system.

The composition and moisture values for Run 6 were presented in Table 7. Both BHA and EDTA addition were also tested at the same levels as before. Table 23 contains the results of the one month study. It is obvious that the direct mixed samples are oxidizing much faster, which is similar to what occurred in the chicken samples. In fact both the control and EDTA treated samples were rancid, as determined by smell, in less than 20 days for the direct mixed system, whereas, no odor was evident in any of the freeze-dried rehumidified samples. Not enough samples were available to carry the study further, however.

RUN 7 - Pork IMF System

This run was made to extend the time scale of Run 6 for the Pork IMF System. The compositionsof the systems tested were shown in Table 7. Table 24 contains the peroxide values. Although oxidation went a little slower than in Run 6, the same trend as in Run 6 occurred for the direct mixed system. The control and EDTA treated samples have gone rancid in

just over 20 days, whereas the BHA treatment is just becoming unacceptable at 34 days. None of the freeze-dried systems showed any degree of rancidity for up to 3 months. Beyond that time the control at 84% RH showed slight rancidity at 105 days but this odor was not that prevalent beyond that time. At four months all the freeze-dried samples were still acceptable.

As in the previous tests it is seen that the method of preparation significantly affects storage stability for the pork system. The adsorption prepared food is stable for over 4 times longer without any antioxidant addition. Incorporation of EDTA or BHA as antioxidants increases this stability to at least 5 to 6 times greater and possibly longer. Thus an intermediate moisture food can be prepared with at least six months stability.

RUN 11

In Run 11 the method of soak-cooking fresh chicken meat was used to prepare the IMF direct mix system. This is the method developed by Hollis et al. (1969) and achieves the final A_w by a desorption process. As in the previous tests samples were taken after preparation and put through the freeze-drying rehumidification process to achieve the adsorption side of the hysteresis loop.

Table 25 contains the peroxide data for the chicken prepared to three relative humidities. It can be seen that results are quite similar to the previous systems using chicken and pork slurries. The

direct mix material oxidized faster than the freeze-dried system as measured by peroxide value, and for the two highest water activities became rancid first. The freeze-dried had only about an extra 25 days stability, considerably less than observed in the previous studies. In no case were any of the control samples in the 0.75 to 0.85 water activity range stable beyond 2-2½ months. This is unfortunate since the cook-soak system has the advantage of being solid pieces so it can be handled easier for eating. Very little difference was seen between the two methods at A_w = 0.61. This most likely is because they have about the same moisture content (Table 10) indicating that this was the closure point of the hysteresis loop.

RUN 13 - Commercial Pet Foods

Both a commercial dog food and cat food (Table 12) were studied for development of peroxides in storage. As outlined previously the foods were stored as is, 6 g in 2-3 oz. bottles, and portions were also freeze-dried and rehumidified back to the same water activity. Both peroxides and extent of browning were measured. For the cat food no peroxides could be determined because of the high food dye concentration. This interfered with the end point determination. Table 25A contains the data peroxides in the dog food held at both 37 and 44°C. It can be seen that with both preparation treatments the peroxides are increasing slowly. The values are higher at 37°C than at 44°C. This is another indication that in many cases high temperature accelerated storage tests may not give expected results (Mizrahi et al. 1970a). Most likely the peroxides

formed at 44°C are more reactive and disappear faster.

In no case did the samples smell rancid after the two months of storage. This is as should be since both BHA and citric acid are present as antioxidants. In addition the differences between treatment methods are not significant. This is probably because as indicated in Table 25A the moisture levels do not differ very much as compared to the differences found in the test systems for this study. The small difference is because the commercial foods contain much less soluble solids. In any case the dog food appears to be at least stable at 37°C for 2 months and possibly based on the time in transit before purchase; it has a shelf life of at least 4-6 months.

2. Pigment Oxidation

The oxidative stability of many foods especially of low moisture can be followed by loss of pigments (Martinez and Labuza, 1968; Labuza 1970a). Based on the work in the previous contract (NAS 9-9426), studies similar to those for oxidative rancidity described above were made on two vegetable type products utilizing carotenoid pigment loss as an index of oxidation.

RUN 5 - Carrot IMF System

Carrot IMF system (Run 5) was prepared according to the specifications given in Table 7 and additional systems containing 200 ppm BHA (fat basis) and 200 ppm EDTA (total solids including glycerol) were respectively tested. The optical density of the extract from 5 g samples

taken from storage was measured at 450 mμ and is shown respectively in Figures 13-15 for each system and treatment.

It is obvious that the pigments are oxidized very rapidly in all systems. Table 26 lists the approximate time to reach an OD of 0.1 in the extract which would be a highly bleached food and which would be unacceptable. The control systems follow the same pattern as was indicated in peroxide oxidation measurements made on chicken, i.e. the direct mixed samples oxidized faster but the degree of protection afforded by the freeze-dry process is not as large.

With the addition of BHA the results, however, are confusing at 75% RH and with EDTA the direct mixed system is the most stable. In all cases the variability in the analysis was large and the stability of the product low so that no real conclusions can be made except that EDTA has some protective effect in the direct mixed systems. In fact EDTA is better than BHA, as was found for high cellulose model systems in NAS 9-9426. No system, however, had much more than one month stability.

RUN 9 - Sweet Potato IMF System

In previous Run 5 the loss of carotenoids was followed in a carrot IMF system as a secondary method of measuring oxidation. A similar run was set up using sweet potato as a source of carotenoids. The results of up to 77 days storage are presented in Table 27. At a value of about 15 to 16 the sweet potatoes become quite bleached and would be unacceptable. It is obvious that no system gives much better than 32 days stability with or without antioxidants added. This is quite similar to the results of Run 5 for carrots and may indicate that some other mechan-

ism is affecting carotenoid stability. This may preclude the manufacture of these types of systems for intermediate moisture foods. Other vegetable products might be more stable, but even chlorophyll is degraded very rapidly at IMF conditions (La Jolla et al., 1971).

B. Non-enzymatic Browning

Non-enzymatic browning is the second major route of chemical deterioration of many foods in the IMF range. This has been found in NAS 9-9426 especially for fruit type products high in sugars, but low in protein. In that previous contract browning in high protein type systems (such as meat) was not significant because of the slower rate as compared to lipid oxidation. Based on this, the studies in this contract were confined to IMF systems of fruit type base.

RUN 1 - Applesauce IMF System

The composition used to prepare the initial applesauce IMF system was shown in Table 7. The direct mix procedure was used. Applesauce contains less than 0.3% fat so that it would not be expected to be subject to oxidation. The major route of deterioration most likely is non-enzymatic browning because of the high sugar content, the small amount of protein (0.3-0.4%) and the low pH (3-4). Samples were held at 37°C, 44°C, and 53°C after preparation to a water activity of A_w = 0.95 and a moisture content of 2.7 g/g solids.

The results of increase in brown color are shown in Figure 16 using the aqueous extraction method A for measurement of the pigment.

As would be expected an increase in temperature increases the rate. Measurement of the activation energy gives a value of 15 Kcal/°K mole; this is very low for non-enzymatic browning. Usually a value of 25-30 Kcal would be expected. In addition the rate is substantial even at 37°C. This could indicate that the browning may be enzymatic in nature due to the fact that enzymes may not have been destroyed during the milk heat treatment given commercial applesauce (less than 190°F). In addition, due to the introduction of sugar syrups, many precursors or intermediates to the browning reaction may have been added in the manufacture thus eliminating some limiting rate steps. In any case at a browning index of 4-5 the product was quite dark in color and most likely would be unacceptable. This means that even at 37°C stability is less than 30 days. This agrees with the poor stability found by Hollis et al. (1968) for a similar apple IMF product. For commercial success to eliminate these problems the sauce will most likely have to be precooked and some sulfite may have to be added. Since a very high A_w was used in this test, lower humidities were prepared in order to find the minimum rate moisture content for the IMF range.

It was also noted that after about 30 days the pigment was highly insoluble even when the enzymatic digestion procedure (Method B) was utilized. This may pose some problems for a longer range storage study.

RUN 3 - Applesauce IMF

Run 3 was made at lower water activities (0.61 and 0.75) which would

be more indicative of an IMF system than in Run 1. The compositions of the systems were shown in Table 7. Samples were prepared by both preparation techniques as in the lipid oxidation studies to determine the effect of moisture content on rate at constant A_w. The browning results using Method A for up to 50 days followed by Method D are shown in Table 28. It is obvious that for all samples very little increase in browning occurred over the first 40 days. This is as would be expected on the basis of previous tests done at the higher water activity which showed only a moderate increase in browning. It is interesting to note however that at the lower activity (61% RH) the rate was faster in all cases. This could be due to a maximum in the concentration of solutes at this point, thus increasing the rate. At 75% RH these solutes are diluted out, thus slowing the rate. Above this activity the previous run (Run 1) showed faster rates, as is evident in Table 29 in which the time to reach a browning value of 5 has been estimated. This faster rate is either due to increased solubilization of reactants or better mobility in the less viscous solution since much less glycerol is present. Ordinarily a maximum exists in the IMF range for browning (Labuza 1970a) but this is usually tested on a food of single composition. In the present study the amount of glycerol contained in the liquid phase is changing and two maxima may occur. From the standpoint of stability however the samples of Run 1 are at too high a water activity to be safe as an IMF food system. From Run 3 it can be surmised that the direct mix system

gives better stability than the freeze-dried at the same activity. This could be due to the higher dilution of reactants in the aqueous phase. This is also opposite to the effect found for lipid oxidation.

RUN 8

A study of a banana IMF system was made using the multiple enzyme browning procedure (Method E). The other methods (A through D) when tried in preliminary tests with a banana IMF gave very variable results because of cloudy extracts. With Method E the solution could always be made clear. The systems tested are shown in Table 7. The samples were held at two humidities, three temperatures and were prepared by both direct mix and freeze drying procedures.

Table 30 contains the results of the first 44 days of a study with the Run 8 banana IMF system based on total dry weight including glycerol. In Table 31 the results of up to 78 days are presented on a per gram of total dry weight excluding glycerol. From Table 30 it appears that at each temperature the rate is faster at 75% RH than 61% RH. This is opposite to what was found in Run 3 for applesauce, although very little increase took place for the applesauce system as compared to the bananas. With respect to the two preparation methods no large differences were apparent.

In Table 31 the data were recalculated on a per gram of banana solids basis. This is as was done for applesauce in Run 3. In this case glycerol is assumed to contribute only to the liquid phase. On this basis and using the time to when it was estimated that the pro-

duct had an unacceptable color (B 250 to 60), Table 29 shows that there is a small difference between the two preparation techniques as was found for applesauce. The freeze-dried adsorption samples show less stability, but overall it is on the order of only 6 to 10 days less. Since measurements were made weekly this may be due just to experimental error. It should be noted that after 44 days a change in personnel occurred and many of the values appear to be lower but this did not affect the trends established.

It can be seen that high temperature storage (57°C) causes the product to have less than one month stability, and storage at 44°C gives a stability of just over one month. Even at 37°C stability is less than three months for all conditions. Therefore, an acceptable IMF fruit product cannot be prepared with greater than 3 months stability unless sulfite is to be added. However, sulfite may be banned by the FDA in the future. This was not studied in this contract.

The following should be considered with respect to the reasons for finding only a small difference between the two preparation methods. According to the present theory water has several actions in non-enzymatic browning (Labuza 1970a).

1. Water dissolves and mobilizes reactants accounting for an increased rate as A_w increases.
2. Water dilutes the reactants once all the dissolvable substrate is in solution, thus slowing the reaction at a given

moisture content (Labuza 1970a).

3. Water is a product of the browning reaction and thus slows the reaction but does not manifest itself until the water concentration exceeds a certain value. (Eichner 1971, personal communication; Loncin et al. 1968).

According to the above, the amount of water at both humidities in both preparations is probably high enough to slow browning to the same rate. The activation energy calculated from the data of both preparation techniques yields a value of 25 Kcal/g mole. On the basis of the values of Mizrahi et al. (1971) this is a minimum for dried fruits and vegetables at high moisture content. The minimum activation energy thus shows that the water availability in both preparations is not very different in terms of banana browning as it was in the case of lipid oxidation or for applesauce browning.

RUN 10 - Applesauce IMF

In this run an applesauce IMF system was again studied because of the difficulty experienced in measuring browning in Run 3 before Method E was established. Table 7 listed the systems tested.

Table 32 contains the results of Run 10 for up to 37 days. The run was terminated as the test method did not correlate with the visual browning. At 37 days at humidities higher than 61% RH all samples were visibly dark yet the pigment was not extracted by the method used. In fact the 61% RH samples showed the lightest color and has the highest

optical density. It seems that the pigment produced becomes highly insoluble as indicated previously and thus cannot be measured accurately.

RUN 13 - Commercial Pet Foods

An attempt was made to follow browning development in the commercial pet foods (Table 12). Many problems evolved including interference with the food dye, very poor extraction of the samples, and extreme difficulty in solubilizing the samples even with enzymes. It was qualitatively noted that solubility decreased with storage at least indicating that non-enzymatic browning is occurring.

C. Enzymatic Activity

As previously noted enzymes would be most active in intermediate moisture systems unless the products were thermally treated. In Run 12 a model system containing lipase and a triglyceride oil was prepared to several water activities and by the two preparation methods (Table 11). If enzymes were active, hydrolysis of the fat would occur producing free fatty acids which could be titrated. The results of this test are shown in Table 33. It can be seen over the two month storage period the values in all systems were rising slowly. It was noted afterwards that the sample size used was too small, thus very little base was used in the titration causing some error. Thus the few very high data points are probably in error since they do not fit the trends of the data.

With respect to A_w and method of treatment no pattern could be found. It should be expected that the rate should increase with A_w as

was found by Acker (1969). However, because of dilution at the higher moisture contents the direct mix should proceed slightly slower. This could be counter balanced by the higher viscosity of the freeze-dried system and thus it is possible that no difference would occur. The minimal results reflect this but do not confirm the theoretical basis. More studies would be needed in enzyme systems.

D. Microbiological Stability

Microbiological decay of intermediate moisture foods could be a serious problem if antimicrobial agents cannot be added. The studies outlined below were designed to determine the degree of growth in IMF systems, the effectiveness of antigrowth agents, and the effect of preparation techniques on microbial growth.

RUN 1M

In a preliminary test to determine the effect of direct mix and freeze drying methods on cell growth in IMF systems, a pumpkin pie mix was prepared according to Table 8. The direct mix system was inoculated with a 1 ml solution of mold (Aspergillus niger) and then this was used to prepare the freeze-dried systems. All systems were held at 37°C and each of the systems from the freeze drier were humidified to four humidities to encompass the desired level of water content and water activity.

The results of mold growth observations are shown in Table 34. For the direct mix system growth appears at all humidities above A_w = 0.75,

as would be expected. In addition, as A_w decreases the growth rate decreases as a result of the osmotic stresses put on the organism. At an A_w = 0.70 no growth occurs, which is as expected, since this is below the minimum A_w for most mold growth (Bone 1968).

Each of the direct mix systems were freeze-dried and humidified to four levels, giving systems at each humidity with increasing glycerol concentration. It would be expected that with a higher glycerol content the moisture content of the sample at the same A_w would also be higher and might allow for faster growth. However, no growth occurred at any of the conditions, suggesting that either all the spores were killed in the freeze drying or that the combination of low pH stress (3.5-4.0 in the pumpkin alone) and low A_w prevents all growth even without any antimycotic added.

RUN 2M

In order to study a system without a low pH Run 2M (Table 8) was prepared by soaking freeze dried chicken dice in 3 volumes of various neutral solutions at given A_w's. A 1 ml solution of mold was added to give about 10^4 spores per gram chicken. After soaking at each A_w portions were freeze-dried and then each system was humidified to four levels corresponding to the range of A_w = 0.61 to 0.90. The pH's of these systems are about 6-6.5.

Table 35 contains the results of Run 2M. As with the pumpkin, growth appeared at the three highest A_w levels but was delayed as A_w decreased. After 14 days the chicken was plated out and it can be seen

that active growth occurred in all three levels. Also there were organisms present even at a water activity of 0.70. Most likely these organisms were not actively growing, since their number did not increase over what was added, but they did survive the low A_w stress. When plated out (A_w of medium $\simeq$ 0.98) they were able to grow again.

The freeze-dried rehumidified systems held at A_w = 0.9 and 0.84 show the presence of organisms after 14 days. It is obvious that at A_w = 0.84 the organisms have been dying out and are not growing, whereas at 0.90 they may be growing slowly. At the two lowest humidities all organisms are dead. This suggests that the mold is not able to survive the added stresses of drying, high osmotic pressure, and low water activity. The direct mixed sample at 0.75 did show growth but the rehumidified did not, suggesting also that water activity alone is not responsible for controlling growth and that water content may also be important.

RUN 3M

A more quantitative run with a pumpkin IMF system was made in Run 3M,(Table 8) adding a known amount of spores to the direct mixed system and making counts over several days. As can be seen in Table 36, the mold in the direct mixed sample at A_w = 0.84 is able to grow and multiply. The stress of freeze drying is seen to decrease the mold count by almost two log cycles, as observed by the initial counts from the freeze drier. Humidification to values that are not inhibitory to mold also causes more death. Finally, holding at these high humidities causes

the cells to die out within 10 days, similar to the results of Sinskey et al. (1970) for bacteria. This suggests that either the organisms are completely damaged by the drying process and cannot repair themselves or that the low pH prevents repair, since in the chicken system with a much higher pH organisms were able to survive for a longer time.

RUN 4M - Banana IMF

In Run 4M banana systems of intermediate pH were prepared so that pH effects would not be as prevalent in the freeze-dried humidified systems (Table 8). The pH ranged from 5.8 at A_w = 0.9 down to 4.7 at A_w = 0.65. Several classes of organisms were studied by quantitative methods, adding as close as possible to between 4 x 10^4 to 10^5 organisms per gram of food solids. This eliminates the dilution effect of glycerol on cell counts. The organisms used were: a mold (A. niger), a common yeast (Candida utilus), a common bacteria of prominence in food spoilage (Pseudomonas fragi) and a pathogenic organism of public health significance (Staphylococcus aureus). The latter two bacteria represent a gram negative organism and a gram positive organism, respectively. It should be noted that gram positive bacteria are usually more resistant to stresses and can usually grow at lower water activities. In addition to the above a mixture of all the organisms was made, a control with no organisms and the mixture again with potassium sorbate in one system and calcium propionate in another. The test conditions are also outlined in Table 15.

The results of a two week storage test for Run 4M are shown in

Table 37. With respect to each organism the following was found:

(1) Molds

The molds behaved as in the previous studies in that very slight growth occurred at all humidities, with the exception of A_w = 0.68 where the organisms died out in seven days although they were present at the start. Growth seemed to be slow even at the highest humidity, as found by Hollis et al. (1969). Freeze drying caused about 2 log cycles kill and no growth occurred after humidification to any of the A_w levels. Mold appeared to be stable at A_w = 0.68 but not at the higher humidities. The reason for this is unknown but could be due to protection by the high glycerol content.

(2) Yeast

The yeast appeared to be able to grow in the direct mix system at both A_w = 0.8 and 0.85 but was killed rapidly at lower humidities. No yeast was present in the adsorption prepared samples due to complete kill by freeze drying or to the fact that the damage caused by all the stresses is not repairable.

(3) Pseudomonas

The pseudomonads being gram negative cannot resist much stress and as is obvious from Table 37 they have disappeared in all systems, except at A_w = 0.85 for the direct mix. However, these die out in less than seven days.

(4) Staph

The staph organisms, being bacteria which are more resistant to stress, do survive both preparation to lower humidities and freeze drying and humidification. However, the damage occurring is irreparable and they disappear in 7 days. Hollis et al. (1969) found a greater survival of this organism even with sorbate added in his direct mix system (cook-soak method), although the organisms disappeared in 4 months. The pH of his systems (chicken and ham casserole) may have been higher, however, and may have been a better source of nutrients for the organisms.

(5) Mixture

The mixture of organisms showed the same results as the mold and yeast, which were the only organisms found on the plates. The survival at A_w = 0.75 for the freeze-dried system is not explainable and may be due to contamination.

(6) Control

The control shows no growth in any system, indicating adequate procedures were used to eliminate extraneous contamination.

(7) Sorbate and Propionate

Potassium sorbate is effective in killing off all cells after a few days storage. The initial high cell count occurs because the cells have only been in the food a short time before dilution and plating out. The

plating technique lowers the sorbate concentration below the inhibitory level. Calcium propionate has very little effect on the cells in the direct mix system, as indicated by growth at A_w = 0.80 and 0.85. The survival at lower activities and in the freeze-dried system seems to be greater than for either the molds or yeast alone. This might be due to a synergistic effect or some other unknown phenomenom.

In general this run shows that molds and yeast are probably the only real problem in intermediate moisture foods. By using the freeze-dry-humidification method molds become the only problem, but even in this case they do not grow but slowly die out. It is possible, as presumed, that on the adsorption branch the water activity must be higher before growth can occur, since no real growth occurred in any of the systems studied which were prepared under that condition.

RUN 5M - Banana IMF

This run was prepared in order to study molds further, as they were most resistant to the adsorption system preparation (freeze-dry-humidify). Also a wider range of relative humidity was used to determine if growth could occur using the above preparation procedure. Table 8 outlines the banana systems studied and the results are shown in Table 38.

The results of Run 5M corroborate the previous tests in that the mold in the direct mix system is able to grow slowly or survive from water activities of 0.68 up to 0.90, with the growth occurring at the higher humidities. With freeze drying and humidification survival

is better at the high humidities but growth is nottaking place, thus indicating damage. At A_w = 0.75 and 0.68 in these systems the organisms are all killed by the freeze drying-humidification procedure. It is obvious that K-sorbate is a very good preservative since all mold is killed in storage.

What is not possible to determine from this run is whether the mold can grow if prepared on the adsorption branch. Most likely it is irreparably damaged by the procedures used. These tests also have not established whether the limiting A_w is higher on the adsorption branch of the hysteresis loop than on the desorption branch. Subsequent studies were designed with this in mind.

RUN 6 - Banana IMF

In the previous tests, the organisms were added to the direct mix system, a portion of which was then freeze-dried and rehumidified to make the adsorption system at the same A_w. As noted, this kills or stresses most of the organisms so that very little or no growth occurred in the adsorption system. A question that was brought up,however, is whether an organism if introduced into an adsorption prepared system would be able to grow at the same limiting water activity as for the desorption system. Run 6M was prepared to test this hypothesis using the same conditions as Run 5M (Table 8) but the procedure of introducing the organisms by injection of a solution into the food samples after drying but prior to humidification. Mold was the only organism tested.

Table 39 contains the 4 month data of Run 6M and shows that for the direct mix system growth occurs very rapidly at 90, 85 and 80% RH but seems to oscillate. Growth occurs even at A_w = 0.68, but may have been due to water vapor leaking into the sample containers. However, for the freeze dried humidified samples even at 90% RH the mold spores are dying out. This indicates that the minimum water activity is greater than 0.9 for the adsorption branch but may be just less than 0.68 for the direct mixed system. Thus a high moisture product could be produced without sorbate by using the adsorption process. Table 39 also indicates that where growth is occurring sorbate is a very effective killer of the mold spores. It is interesting to note that at the high humidities for the freeze-dried system the mold survives the presence of sorbate but is not growing.

RUN 7M - Pork IMF

A study similar to Run 6 was made using all the test organisms (Run 4M) and adding to the freeze-dried product so as to eliminate the freezing and drying stress. Also a medium pH system, pork, was used. The compositions used are shown in Table 8.

The results shown in Table 40 indicate that as with the banana system a higher minimum water activity is found for samples prepared by the freeze-dried/humidified method. For each organism, in the adsorption process the minimum A_w is 0.9 or larger whereas it is much less in the desorption food as shown below:

ORGANISM	DESORPTION	ADSORPTION
Mold	minimum $A_w < 0.75$	0.9
Yeast	$A_w < 0.84$	> 0.9
Pseudomonads	$A_w < 0.84$	> 0.9
Staph	$A_w < 0.84$	0.9

It should also be noted that the sorbate has much less effectiveness in this system as compared to bananas because of the neutral pH (Table 8). This suggests that the method of preparation by adsorption would be useful for neutral pH systems. It is also noted that the Gram + Staph are more resistant at neutral pH to low A_w than in previous runs at lower pH.

In summary for the pork system it has been found that:

(1) Mold grows at about the same rate in the direct mix system as with the low pH bananas but seems to die out sooner at low A_w. For adsorption systems,however, the mold is much more stable and survives the low water activities.

(2) Yeast as in previous studies dies out in all the adsorption prepared systems indicating very poor resistance to stress.

(3) Psuedomonads exhibit a behavior very similar to that of yeast.

(4) The food poisoning organism Staph. aureus can grow down to an $A_w = 0.84$ and survives for at least two months at $A_w = 0.75$ for direct mixed systems. This indicates that introduction of this organism during manufaction of IMF will have to be monitored. However, for the adsorption prepared IMF, the organ-

ism dies out in two months in the intermediate moisture range. These results are similar to those of Hollis et al. (1969).

RUN 8M - Laboratory Dog Food System

It was decided to test a commercial dog food IMF system for survival of molds, and as well to try the normal humectants used in industry, i.e. sugars and proteins. In addition 1-3 butane diol, a new compound, studied by the Air Force and NASA for use in food systems and currently being tested by a commercial company as a food supplement was also tested. Table 13 contains the compositions of the systems tested. It was not evident when the mixture was formulated but after setting up the test, the A_w was found to be in the range of 0.60 to 0.65 with a moisture content of 18 g H_2O/100 g solids. This is a little low for a commercial pet food.

The results are shown in Table 41. It can be seen that no growth is occurring even in the control without sorbate. This is probably due to the low water activity that the food was made to, which is below the minimum growth activity. The presence of butane diol besides acting as a humectant seems to kill off the cells readily. This may be a better humectant than glycerol because of this antimycotic effect.

RUN 9M - Laboratory Dog Food System

In Run 9M the composition of the pet food was changed to increase the moisture content to 31 g/100 g solids and increase the water activity to 0.65 to 0.70 (see Table 14). Again the final A_w was lower than

expected but could not be changed once the system was in storage.

Table 42 shows the results of the mold counts over a one month period. They show the same trend as in Run 8M with the mold slowly dying out in the control system. It should be noted that the mold survives the sorbate much better than in Run 7M at low A_w. This most likely is due to the poorer mixing with the hamburger since the system without sorbate showed similar results. Again butane diol kills the organisms almost from the start suggesting that this will be a useful additive.

E. *Packaging Predictions*

With respect to packaging requirements for IMF systems some suggestions and calculations can be made. IMF systems by virtue of their definition occur in the region of the sorption isotherm characterized by a steep increase in moisture content as a function of A_w. This suggests that a large moisture change must take place before a large change in stability will occur.

Two possibilities exist in terms of packaging requirements with respect to moisture. The first and most logical is that, since under most conditions the outside relative humidity is less than the equilibrium humidity of IMF systems, the foods will lose water. Thus, most likely, according to the stability map (Figure 2), reaction rates will decrease and shelf life will increase. The only problem would be loss in palatability as the product becomes drier. Some estimation of the requirements for this situation can be made based on the studies of

Mizrahi et al. (1970), and the contract work of Karel and Labuza (1969) on space foods.

To make some estimates the chicken IMF system represented in Figure 6 was chosen as a model. The linear isotherm estimation method was used which defines the shelf life equation as:

$$(2)\quad \ln \frac{M_e - M_i}{M_e - M_c} = \frac{(k)(A)}{(x)(W_s)} \frac{P_s}{b} \quad \theta$$

where M_i = initial moisture content of food

M_e = moisture content in equilibrium with external humidity

M_c = critical moisture content of food at which is unacceptable

k = film permeability in $\frac{\text{gram } H_2O \text{ mil}}{M^2 \text{ day mm Hg}}$

x = film thickness in mil

A = area of package in M^2

W_s = weight of dry solids in grams

P_s = saturation vapor pressure of water at temperature of system

θ = desired shelf life in days

b = slope of linear isotherm for food

The assumptions necessary to use this equation and its limitations have been discussed in detail by Mizrahi et al. (1970b) and will not be gone into here.

To use this equation one must first make a linear approximation to the isotherm. For the chicken system the parameters of the isotherm

equation obtained for the desorption and adsorption systems are shown in Table 43. Because of hysteresis a different slope and intercept exists for each system. For simplicity based on the package systems for NASA and the MOL (Karel and Labuza 1969) all calculations were made for the same package weight and size (Table 43). Other configurations can be easily put into Equation 2. It was further assumed for calculation of weight loss that the temperature was constant at 80°F and the external relative humidity was 50%. For calculation of weight gain the external relative humidity was chosen to be 90%.

For any calculation a critical value at which the product is unacceptable must be picked. As discussed above under normal circumstances these products will lose water, so that they become dry and unpalatable. As a rough guess it was assumed that a 25% loss in water content would be critical; for the opposite condition, for example, where the packages may be shipped to a southern climate or held in a high humidity warehouse, the critical moisture can be evaluated as the point where microbiological growth could occur. For intermediate moisture foods this limit is close to an A_w = 0.80 to 0.90 depending on the method or preparation and composition of the system as was shown previously. For calculation purposes the critical value in this study was taken at an A_w = 0.85.

Figure 17 shows the results of the calculations. As would be expected the film permeance decreases as the maximum storage life requirement increases in a hyperbolic function. Table 44 lists some actual

film permeabilities for various commercial films. Compared with the data in Figure 17 it can be seen that the packaging requirement for one year stability can be easily met for any situation. In fact, the cheapest material, polyethylene, which is used extensively for bread, would give overprotection. More permeable materials such as nylon or mylar with various thicknesses could be used.

Figure 17 also suggests that if the foods are protected against a 25% water loss, then they will be well protected against infiltration of water and microbial decay. The latter requires less protection or conversely a more permeable film can be used. These same types of conclusions should be applicable to the other foods within the same order of magnitude.

With respect to oxidation, prediction of packaging requirements is not possible at this time. Simon et al. (1971) discussed the methodology necessary for this type of prediction. It was shown that present techniques available were not adequate to give the same type of analysis as for moisture. What can be concluded,however, is that since oxidation occurs rapidly, a vacuum packaged system, with a low oxygen permeability film, and low head space would be necessary.

V. CONCLUSIONS

A. Introduction

This study was designed to produce several intermediate moisture foods and test their stability. Both chemical and microbiological deterioration were presumed to be of importance to the storage of IMF.

With respect to chemical stability, lipid oxidation was studied in meat systems and non-enzymatic browning in fruit type systems. To eliminate interferences from other reactions, enzyme activity was followed in a model system. Microbiological growth was studied in various fruit and meat type products over a large pH range.

Of primary importance to the study, the method of preparation was tested. Two methods were used, one to bring a food down the desorption branch of the hysteresis loop on the sorption isotherm and another which took that same food of similar solids composition and brought it up the adsorption branch of the isotherm. The use of these two methods thus produced foods made simultaneously from the same batch that had similar solids composition, the same water activity but vastly different moisture contents. Thus this study also allowed the determination of the effect of water content as well as water activity on rate controlling deteriorative reactions in IMF systems.

Before discussing each reaction type it is important to reiterate the function of water in dry and intermediate moisture foods. Firstly water has the ability to dissolve solutes in it. Secondly these solutes are mobile in the water or aqueous phase even down to a monomolecular coverage. As a consequence of this, water also plasticizes large polymers such as proteins making them more soluble and opening spaces between them where they may have been in contact. Lastly water acts as a medium for chemical reactions as well as participating in reactions

as either a bound moiety, a reaction substrate or a product. As a consequence depending on the predominating mechanism water behavior in foods is very complex.

B. Lipid Oxidation

A study of lipid oxidation was made in several meat type products by following the development of peroxides and rancid off flavor. It should be remembered as discussed in the Literature Survey that water exerts several modes of action with respect to lipid oxidation. (Labuza 1970a). Water exerts antioxidant properties at low A_w through hydration of catalysts thus lowering their activity and by binding peroxides thus interrupting the chain reaction. As was found by Heidelbaugh and Karel (1970) and Labuza et al. (1971) in the intermediate moisture range water acts as a strong proxidant through increased catalyst mobility and placticization of polymers exposing new catalyst sites. The results of Run 2 with the chicken/cellulose system confirm these principles. As seen in Table 16, without or with antioxidants added, humidification to 32% RH, near the monolayer reduces the oxidation rate significantly as compared to the very dry state. This is the protective effect of water. However, humidification to 75% RH, in the IMF range, causes the pro-oxidant effects of water to predominate causing even faster oxidation than the dry state. This thus confirmed the previous work that lipid oxidation would be a primary deteriorative reaction in IMF systems making them of limited stability.

The most significant finding in this study was that in systems

without antioxidants, the method of preparation of the IMF greatly affected the rate of oxidation. Table 45 summarizes the results of the five runs in which lipid oxidation was studied. It can be seen that with the direct mix procedure, typical of most current manufacturing techniques for IMF, maximum stability for foods was usually less than one month. However, when the same food was prepared by the adsorption method to the same water activity, thus having a lower moisture content, the stability of the food increased from three to six times. Thus foods of at least 4 to 6 months stability can be made by the IMF techniques without antioxidants.

Several explanations for the unusual effect of moisture content are plausible. It is possible that the freezing and drying involved in the adsorption process causes the destruction of membranes protecting the lipids and releases them to become more susceptible to oxidation as what happens in spray-dried milk. However, as was shown, they are oxidized much more slowly thus eliminating this possibility. Secondly, it is also plausible that at the higher water content (direct mix system) diffusion to the site of interaction takes longer. Again, the direct mix system oxidizes faster so that this is not the case.

The opposite could also be true. It is possible that the presence of glycerol in the aqueous environment increases the viscosity of the liquid phase thus decreasing the rate of diffusion of oxygen to the reacting lipid site. Thus the adsorption systems with less water would oxidize slower because of the decreased diffusion coefficient of oxygen which is inversely proportional to viscosity. Table 46 lists

the viscosities of the aqueous phase for Runs 4 and 7 assuming only water and glycerol are involved. It is seen that the viscosity is about 2 times larger for all the freeze-dried systems as compared to the desorption systems. Thus this can account for a part of the differences shown, however, from Table 45 it was seen that the rates were from three to six times slower. Therefore other factors must also be of importance.

The other possibility accounting for the major difference in oxidation rates is based on the fact that the greater amount of water present acts as a pro-oxidant in the direct mixed system. As discussed above, the water can plasticize and expose new catalysts on polymer chains and mobility of catalysts may be enhanced especially in the lower viscosity system. With the greater water content swelling of the system would thus enhance the rate of oxidation as was observed.

With respect to antioxidants, the addition of either BHA or EDTA would enhance storage life of IMF systems thus making them of commercial significance. In Table 47 are comparisons of the times to reach rancidity for the IMF systems with and without antioxidants added. Only BHA, citric acid and EDTA were studied as these were found to have the best antioxidant effects in NAS 9-9426. In most tests as shown in Table 47 a guess had to be made as to the time for rancidity in most systems since not enough samples were available to carry the experiment through the long times. These estimates are based on relative times to reach the same peroxide value and relative rates of oxidation

No quantitative formula was used, however.

As seen in Table 47, BHA as a primary antioxidant is a very effective additive. Use of BHA would allow for over 6 months stability of chicken systems and possibly 5-6 months for pork systems. The effectiveness seems to be very dependent on the water activity-moisture content of the system but no general pattern can be found from the data collected. EDTA and citric acid as chelating agents are less effective antioxidants as was found in NAS 9-9426. This is most likely due to these compounds being tied up by protein bound metals.

In summary, lipid oxidation occurs at a significant rate in IMF systems and through the control of the method of manufacture and the addition of BHA as an antioxidant, at least six months stability should be expected. It should be noted that these studies were made with the samples stored in air, that is, no protective barrier was present. By the use of vacuum packaging and a film low in oxygen permeability, these foods would have the desired one year shelf life. However, further studies would be needed to confirm this.

It can be seen,however, from Table 25A that the commercially marketed dog food IMF system is stable for at least 2 months at 37°C, for both the direct mix and freeze-dried preparations. This stability most likely is due to the excellent choice of both BHA and citric acid as antioxidants in the system. These foods probably have at least 6 months shelf life and under good packaging conditions probably one year.

With respect to pigment oxidation as previously noted, (Martinez and Labuza, 1968), the patterns found in the past seem to parallel lipid oxidation. In these studies it was found that the carotenoid pigments in two vegetable IMF systems were destroyed rapidly no matter what treatment with antioxidants, method of preparation, or water activity was used. This did not follow the pattern shown for lipid oxidation as described above. No plausible explanations could be made from the data and further work would be needed. However, it would seem that storage life of vegetable IMF systems would be extremely short. Reduction of oxygen tension by packaging might increase the shelf life to 6 months but that is pure speculation. With green vegetables, as was studied by La Jolla et al. (1971) chlorophyll loss is solely dependent on moisture content and the absense of oxygen makes no difference. In that study at 75% RH chlorophyll was degraded in less than one month in both spinach and model systems.

C. Non-enzymatic Browning

Non-enzymatic browning in the intermediate moisture range was studied in several fruit type systems. As was discussed for many foods, a maximum in rate exists, in the IMF range (Labuza 1970a). This is attributed to the fact that at some A_w or moisture content, as many of the solutes as possible are dissolved and above that value further increases in moisture dilute the reaction thus slowing the rate. In addition both Loncin et al. (1968) and Eichner (MIT unpublished work) believe that water exerts a retarding influence on the reaction

because of product inhibition. Eichner found in studies of solutions of glucose and amino acid mixtures in various solvents mixed with water, the higher the concentration of water, the slower the rate.

In foods, as the water content-water activity is increased, the rate increases to a maximum then falls in the intermediate moisture range (Labuza, 1971). Thus it seems that water, which is a product of non-enzymatic browning, causes product inhibition. However, since water is necessary to dissolve and mobilize reactants in a solid food system, the product inhibition of water does not occur until the moisture content reaches a certain level. This results in the maxima found in the non-enzymatic browning curve for foods. Thus, since the direct mixed system is much higher in moisture content than the dried system, product inhibition should be greater and the rate of browning slower than for the adsorption prepared foods. This would be opposite to the effect described above for lipid oxidation. However, the presence of a greater concentration of glycerol or humectant in the adsorption system increases the viscosity and thus slows the diffusion rate of reactants. Thus this would counterbalance the enhanced rate due to a lower water concentration and no general trend can be predicted for any IMF system on an "a priori" basis.

The results found tend to confirm the above conclusions but are difficult to interpret because of the difficulty found in measuring the amount of brown pigment formed. In Run 3 (Table 29) with applesauce for

example it can be seen that at both an A_w = 0.61 and 0.75 the freeze-dried system (adsorption) browns faster by 2 to 3 times. It is also apparent that browning is faster at an A_w = 0.61. This supports the fact that it is concentration of reactants and of water that is controlling the rate. The viscosity effect does not seem to be apparent since the lower A_w and lower moisture contents all browned faster. It is obvious,however, from this test that overall stability is less than 4 months.

In Run 8 with bananas the differences were much smaller than for applesauce (Table 29). The same trend existed in that less water in the system accelerated the rate but in this case the amount of water may have been such that its concentration of reactants may have been high enough in both systems to cause about the same amount of product inhibition. As was noted stability of all products was poor with less than three months acceptability. An additional test on applesauce (Run 10) was inconclusive due to difficulty in analysis of the pigment.

In conclusion it has been found that the amount of water exerts the opposite influence on reaction rate for non-enzymatic browning as compared to lipid oxidation. Thus the adsorption systems brown faster due to less product inhibition by water. The extent of this is very product dependent as it was with lipid oxidation. Unfortunately browning even at 37°C was fast enough to limit stability to less than three to four months. If a reducing sugar humectant had been added it would

have been much less. It would seem therefore that these products will have to be heavily sulfited to give over six months stability.

D. Enzyme Activity

The results of the enzyme study were not very conclusive since most of the systems reacted at the same rate. This could be expected on the following basis. Theoretically the rate should increase with A_w due to mass action. However, as the moisture increases, the reactants get diluted thus slowing the rate. In addition the rate in the adsorption system should be faster due to the lower moisture level. However, the higher viscosity of this system might slow diffusion. Based on this there might be very little difference between either A_w or preparation method in the intermediate moisture range as has been found. Further studies would be needed to confirm this.

E. Microbiological Growth

The most significant findings of this study are the effects of processing methods on microorganism growth. The effect of water activity has been discussed in detail in the Literature Survey section. In brief there has been found for each type of organism a minimum water activity below which it will not grow. Because of this and the ranges of the growth minima, growth in intermediate moisture foods should be limited to yeasts and molds. Their extent of growth in turn should be effected by the pH of the system, nutrient availability, and presence of growth inhibitors.

In the preliminary tests (Runs 1M through 3M) using mold as the

test organism in various foods, the direct mixed food showed the expected pattern, with no growth occurring below an A_w = 0.70. When samples of these foods were frozen, freeze-dried and rehumidified after the addition of the spores, growth was either seriously hindered or the organisms were either totally killed or died soon in storage. This was attributed to the various osmotic and physical stresses the organisms underwent in the process. It also showed that this technique would be an adequate method to reduce spoilage of an IMF.

In Run 4M several different organisms were tested using the same methodology as in the previous tests. A banana system was used, however, to reduce the pH stress that might have been present. As in the previous tests the mold grew down to a level of A_w = 0.68 in the direct mix system. With the adsorption process even at a higher pH (~ 4.5-5) about 2 log cycles of kill occurred reflecting the stresses involved. Yeast, because of a shorter generation time, grew rapidly down to A_w = 0.8 somewhat lower than would be expected from Table 5. They were completely killed in the adsorption preparation system thus indicating as above that this would be a useful technique to obtain initial low counts. Pseudomanas organisms, which might constitute a natural contaminant in meat products, were unable to grow at any conditions and in fact completely disappeared in seven days. This is as would be expected for a gram negative organism. The only public health significant organism tested was Staph. aureus which showed no growth under

the conditions tested. The growth inhibitor, potassium-sorbate, was very effective in all systems but propionate was not, probably because of the low pH.

The final test in the preliminary series (Run 5M) was run with molds only because they showed the most resistance of any of the organisms. The results were similar to the previous tests in that the molds can multiply in the direct mixed IMF system down to an A_w = 0.68 but do not grow in the freeze-dried rehumidified system even at A_w = 0.9. These studies thus suggested that there was severe damage occurring to the organism as studied by Mazur (1966), Merryman (1966), and Sinskey et al. (1970) which under the lower than optimum growth A_w could not be repaired. Thus the adsorption technique although expensive would be a useful technique for making IMF systems stable to microbiological deterioration.

Another important factor could also be responsible for the differences observed. It is possible that A_w alone is not the critical factor in cell multiplication as suggested by Scott (1957) but that water content may be as important. The results of the chemical deterioration studies support this fact. It is obvious that for microbial growth the properties of water such as dissolution of solutes, mobility of chemical constituents, chemical reactivity and as a media for reaction are just as important. Thus the remaining experiments were designed to test microbial growth minima but without subjecting the or-

ganisms to the stresses of freezing, drying and rehumidification.

In these final studies (Runs 6M and 7M), it was found that there is, in fact, a controlling influence of water content on microorganism growth. Systems prepared to similar water activity but different moisture contents using the desorption and adsorption techniques were inoculated with various test organisms. The organisms were added by injection of a minimum volume of cell suspension to the dried IMF system. The conclusions drawn from studies in banana (pH 4-4.5) and pork (pH ~7) IMF systems show that for all types of organisms including a mold, yeast, pseudomonad species and staph type species, the minimum A_w for the adsorption system is greater than 0.9 whereas the minimum growth A_w for the desorption systems is near the values predicted by Scott (1957) and Bone (1969).

These results are listed in Table 46. These tests also showed that at the IMF water activities many organisms can be present but will not grow. This may be important in assessing the microbiological quality of IMF systems especially with respect to public health organisms. If foods are prepared by the adsorption process, however, they should pose no microbiological problem even without addition of a growth inhibitor. On the other hand desorption prepared foods either need a growth inhibitor or must be kept at the low A_w end of the intermediate moisture range. Thus as was shown in Runs 8M and 9M with a laboratory prepared dog food, at low A_w (0.6 to 0.7) no measurable growth occurs even without K-sorbate present. However, the organisms do not disappear com-

pletely, about a loss of 1 log cycle occurs over a 4 to 6 week period, similar to the data of Hollis et al. 1968. The presence of 1-3 butane diol, however, as a humectant in place of sugar completely killed all the organisms. This could have been due to increased permeation of the cell with bursting.

F. Packaging Requirements

It has shown that packaging requirements for maximizing shelf life could be easily predicted based on standard procedures. Moisture loss with a resultant loss in palatability is of primary concern. Films currently available in the market can meet the needs adequately for a one year shelf life requirement. With respect to oxidation, films of low oxygen permeability would have to be used as well as sealing of the package under vacuum.

G. General Conclusions

Overall it has been found that intermediate moisture foods are fairly unstable systems as was suggested by the stability map (Figure 2). With respect to lipid oxidation, rancidity occurs in under three months unless antioxidants are present. Non-enzymatic browning also takes place causing unacceptability in under three months. These reactions thus limit storage life unless special procedures are used. Microbial decay is basically limited to molds and yeast, since most bacteria die out rapidly at the water activities used. Potassium sorbate is useful in controlling the growth of the molds and yeast and thus increase storage life.

The most significant finding of this study is that the method of manufacture has an important influence on the rate at which the deteriorative reactions take place. It was shown that, because of sorption hysteresis, a food of similar solids composition, similar water activity, but vastly different water content could be prepared by following a desorption vs. an adsorption process. With respect to these techniques, lipid oxidation proceeds 3 to 6 times faster in the higher moisture system (desorption), non-enzymatic browning about 1.5 to 2 times slower in the desorption system and microbial growth minima are much higher for the adsorption prepared systems. From a technological standpoint these tests suggest that by using the adsorption process an IMF food with a shelf life of over one year can be made for human consumption. However, further tests are needed to confirm this. Packaging requirements for foods with one year shelf life can be met easily with materials and methods presently available.

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VII. T A B L E S

TABLE 1

IMF MANUFACTURE

DIRECT MIX - DESORPTION PROCESS

Moist Food + Dry Ingredients + Humectant------>Cook Extrude

or

Semi-liquid Food Blend + Humectant---------->Semi-solid

or

Moist Food + Soak Infusion Solution---------->Cook-soak

ADSORPTION PROCESS

Dried Food + Humectant Soak Solution---------->Semi-solid

Dried Food + Dry Humectant----->Humidified---->Semi-solid

TABLE 2

Molalities of Some Solutes for Various Values of A_w at 25°C

A_w	Ideal Value	Na Cl	Sucrose	Glycerol
0.995	0.281	0.300	0.272	0.277
0.990	0.566	0.600	0.534	0.554
0.980	1.13	1.214	1.03	1.11
0.960	2.13	2.406	1.92	2.21
0.940	3.54	3.44	2.72	3.32
0.920	4.83	4.62	3.48	4.44
0.900	6.17	5.66	4.11	5.57
0.850	9.80	8.06	5.98	8.47
0.800	13.90	10.30		11.5
0.750	18.50			14.8
0.700	23.8			18.3
0.650	30.0			22.0

TABLE 3

Solutes Used as a Water Binding Agent

Solutes	M_w	Minimum A_w Achievable Solubility (25°C)	Reference
NaCl	58	0.75	(Olynk and Gordon 1943)
Sucrose	354	0.85	(Scott 1957)
Fructose	180	0.63	
Glucose	180		
Glycerol	92	miscible limited by taste	(Miner and Dalton 1953)
Amino Acids	100-200	0.01-0.2	
Ethanol	46	miscible limited by taste	
1-3 Butane Diol	90	miscible limited by taste	

TABLE 4

Some Commercial and Tested Intermediate Moisture Food Systems

Food	A_w	m.c.	Method	Binding Agent	References
Chicken	0.78	27.8	adsorption	glycerol	Hollis et al. (1968)
Carrots (slices)	0.77	39.3	adsorption	glycerol	Hollis et al. (1968)
Apple (slices)	0.72	25.0	adsorption	glycerol	Hollis et al. (1968)
Tuna	0.81	38.8	desorption	glycerol	Brockman (1970)
Carrots	0.81	5.15	desorption	glycerol	Brockman (1970)
Pork	0.81	42.5	desorption	glycerol	Brockman (1970)
Celery	0.83	39.6	desorption	glycerol	Brockman (1970)
Meat Pet Food	0.80	25	mixed	sucrose	Burgess (1965)
Pie	0.82	30	mixed	sucrose	Toast'em
Peanut Butter	0.70	20	mixed	sucrose	Space Food Sticks
Cat Food	0.85	34	mixed	sucrose	Tender Vittles

TABLE 5

Approximate Lower Limits of A_w for Microorganism Growth

Organism	Minimum A_w
Bacteria	0.91
Yeasts	0.88
Molds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

TABLE 6

Semi-solid Foods Used

A. Mott's Applesauce	79% H_2O
B. Gerber's Baby Food: Carrots	89.5% H_2O 0.3% fat
C. Gerber's Baby Food: Chicken	77% H_2O 10% fat
D. Swift's Baby Food: Pork	78.1% H_2O 11.8% fat
E. Gerber's Baby Food: Bananas/Tapioca	77.6% H_2O
F. Gerber's Strained Sweet Potatoes	83.3% H_2O 0.6% fat
G. Lucky Leaf Pumpkin Pie Mix	89% H_2O - pH 4.3

TABLE 7

IMF Food Stability Test Systems

RUN #	COMPONENTS	% USED IN PREPARATION	
1.		A_w = 0.95	
	Applesauce	97.2	
	Glycerol	2.8	
	M = 2.7 g H_2O/g solids		
	Tested at 37°, 44°, and 53°C		
2.	Gerber's Chicken	27.4 - (see Table 9)	
	Microcrystalline Cellulose	53.4 - (" " ")	
	Glycerol	19.2 - (" " ")	
3.		A_w = 0.61	0.75
	Applesauce	36	58
	Glycerol	64	42
	Moisture Content(g H_2O/g solids)		
	Direct Mix	0.39	0.84
	*FD - Rehumidify	0.25	0.40
4.		A_w = 0.61	0.75
	Gerber Chicken	41.1	62.3
	Glycerol	58.6	37.7
	Moisture Content(g H_2O/g solids)		
	Direct Mix	0.47	0.93
	FD - Rehumidify	0.26	0.48
5.		A_w = 0.61	0.75
	Carrots	35.5	52.8
	Glycerol	64.5	47.2
	Moisture Content(g H_2O/g solids)		
	Direct Mix	0.47	0.90
	FD - Rehumidify	0.20	0.36

* Refers to adsorption system - freeze-dried and rehumidified.

TABLE 7
(Continued)

IMF Food Stability Test Systems

RUN #	COMPONENTS	% USED IN PREPARATION			
6.		A_w = 0.75	0.84		
	Pork	64.1	79.3		
	Glycerol	35.9	20.7		
	K-Sorbate	0.2	0.2		
	Moisture Content(g H_2O/g solids)				
	Direct Mix	1.00	1.55		
	FD - Rehumidify	0.38	0.58		
	Treatments include 200 ppm BHA (fat basis), 100 ppm EDTA (solids basis), control.				
7.	Pork Same as Run 6 except 0.3% K-Sorbate used.				
8.		A_w = 0.61	0.75		
	Bananas/Tapioca	50	79.5		
	Glycerol	50	20.5		
	K-Sorbate	0.3	0.3		
	Moisture Content(g H_2O/g solids)				
	Direct Mix	0.69	1.74		
	FD - Rehumidify	0.27	0.42		
	Treatments include storage at 37°C, 44°C, and 55°C				
9.		A_w = 0.61	0.75		
	Sweet Potato	21.3	36.0		
	Glycerol	78.7	64.0		
	Moisture Content(g H_2O/g solids)				
	Direct Mix	0.36	0.72		
	FD - Rehumidify	0.18	0.34		
10.		A_w = 0.90	0.84	0.75	0.61
	Applesauce	87.8	74.7	57.7	35.7
	Glycerol	12.2	25.3	42.3	64.3
	K-Sorbate	0.3	0.3	0.3	0.3
	Moisture Content(g H_2O/g solids)				
	Direct Mix	2.1	1.43	0.90	0.46
	FD - Rehumidify	0.96	0.69	0.57	0.33
11.	Infusion Diced Chicken System (See Table 10)				
12.	Enzyme Study (See Table 11)				
13.	Commercial Pet Foods (See Table 12)				

TABLE 8

IMF Composition - Microbiological Studies

RUN #	COMPONENTS	% USED IN PREPARATION			
1M		A_w = 0.85	0.80	0.75	0.70
	Pumpkin Pie Mix	76	67	60	55
	Glycerol	24	33	40	45
	System #	A	B	C	D
	Moisture Contents(g H_2O/g solids)				
	Direct Mix	2.06	1.48	1.14	0.96
	FD - Rehumidify	A_w = 0.90	0.84	0.75	0.61
	System A	1.08	0.69	0.48	0.32
	System D	0.84	0.64	0.52	0.33
2M		A_w = 0.85	0.80	0.75	0.70
	Freeze-dried Chicken Dice (Eastern Freeze-dried Foods)	10g	10g	10g	10g
	Water*	25	23	20.7	18
	Glycerol	5	7	9.3	12
	Salt	2	2	2	2
	MBT Chicken Soup Base	3	3	3	3
	Moisture Contents(g H_2O/g solids)				
	Direct Mix	1.25	1.05	0.85	0.67
		A_w = 0.90	0.84	0.75	0.61
	FD - Rehumidify	1.15	0.84	0.55	0.29
	System*	A	B	C	D

*When mold suspension added this is subtracted from total water.

3M		A_w = 0.85	
	Pumpkin Pie Mix	75.5	
	Glycerol	24.5	
	Freeze-dried held at	A_w = 0.84 and 0.90	

4M		A_w = 0.85	0.80	0.75	0.68
	Gerber's Banana/Tapioca	100	88.7	78.4	66.7
	Glycerol	0	11.3	21.6	33.3
	Moisture Contents(g H_2O/g solids)				
	Direct Mix	3.46	2.20	1.55	1.07
	FD - Rehumidify	0.7	0.5	0.4	0.3

TABLE 8
(Continued)

IMF Composition - Microbiological Studies

RUN #	COMPONENTS	% USED IN PREPARATION				
		A_w = 0.90	0.85	0.80	0.75	0.68
5M	Banana/Tapioca	83.3	100	88.7	78.4	66.7
	Glycerol	0	0	11.3	21.6	33.3
	Water Added	16.7	-	-	-	-
	Moisture Contents(g H_2O/g solids)					
	Direct Mix	4.36	Same as Run 4M			
	FD - Rehumidify	0.90	"	"		
6M	Same as Run 5M					
		A_w = 0.68	0.75	0.84	0.9	
7M	Pork	57.2	64.1	79.3	100	
	Glycerol	47.8	35.9	20.7	0	
	pH	6.85	6.95	7.25	7.0	
	Moisture Contents(g H_2O/g solids)					
	Direct Mix	0.81	1.00	1.63	3.57	
	FD - Rehumidify	0.32	0.45	0.70	0.90	
8M	Laboratory Dog Food System (See Table 13)					
9M	Laboratory Dog Food System (See Table 14)					

TABLE 9

Special Systems Run 2

RUN #	COMPONENTS	TREATMENTS
2	Chicken 27.4%	A - Control
	Cellulose 53.4%	B - 200 ppm BHA/g fat
	Glycerol 19.2%	C - 100 ppm citric acid/gram solids
	Moisture Contents g H_2O/100 g solids)	A_w = 0.01 / 0.22 ; 0.32 / 2.67 ; 0.75 / 15.2

TABLE 10

Diced Chicken. Infusion Method. Run 11.

I. Fresh Raw Chicken Meat 75% H_2O ½ to 1" dice

II. Infusion solution (grams)

	A_w = 0.85	0.75	0.61
Glycerol	39	157	514
Water	514	392	39
NaCl	15	15	15
MBT Dry Chicken Broth	27	27	27
K-Sorbate	3.6	3.6	3.6

III. Combine 200 g chicken with total infusion solution. Heat to 96-99°C and cook for 15 min. Soak overnight in solution in refrigerator.

IV. Moisture Contents (g H_2O/g solids)

A_w	Direct Mix	Freeze-dried
0.85	1.70	0.52
0.75	1.17	0.36
0.61	0.20	0.19

TABLE 11

Enzyme Study. Run 12

COMPONENTS	A_w=0.84	0.75	0.61	0.51
Corn Oil (Wesson)	5 gms	5 gms	5 gms	5 gms
Cellulose (Avicel)	50	50	50	50
Glycerol	40	57.5	75	80
Water	59	41.5	24	19
Enzyme Solution*	1	1	1	1

*(0.016 g lipase in 1000 ml H_2O pH 8.5)

Moisture Content				
Direct Mix (g H_2O/g solids)	0.63	0.38	0.19	0.15
Freeze-dried (g H_2O/g solids)	0.46	0.37	0.20	0.15

TABLE 12

Pet Food Compositions. Run 13

Gaine's Prime General Foods

Dog Food

A_w = 0.75 for sample as is

m = 0.27 g H_2O/g solids

Ingredients:

Beef and Beef by products,
Soy Grits, Sucrose, Soy Meal,
Propylene Glycol, $Ca(PO_4)_2$, Soy Hull 2%,
Animal fat with BHA, Dried Whey, Salt 1.2%,
Mono-Di-Glycerides with BHA and citric acid in glycol,
$CaCO_3$, Chicken by products, K-Sorbate,
Artificial Coloring, Vitamin Mix, Garlic Powder,
Fat Content 7%.

Cat Food - Purina Tender Vittles Liver Flavor

A_w = 0.83 for sample as is

m = 0.51 g H_2O/g solids

Ingredients:

Poultry by product meal, Ground yellow corn, Corn gluten,
Soy meal, Chicken, Wheat flour, Propylene glycol, Animal
fat with BHA, Liver, Dried yeast, Whey, Phosphoric acid,
Salt, Glycerol monostearate, Onion powder, Artificial color,
Vitamin mix, Fat Content 7%.

TABLE 13

IMF Dog Food Composition I Run 8M

A. Control — A_w = 0.60

	grams	
Hamburger	32	
Soy Flour	20	
Non-Fat Dry Milk	15	
Calcium Phosphate	3	
Crisco	1	Basic Ingredients
NaCl	1	
FDC #2 Red Lake Dye	0.006	
Garlic Powder	0.2	
Sucrose	24.5	
K-Sorbate	0.3	Additives
Propylene Glycol	2.0	
Atmul 80	1.0	

B. Control - No Sorbate — A_w = 0.62

Same as A but no sorbate.

C. BD — A_w = 0.63

Same as A except 27.8 grams 1-3 butane diol replaces sucrose, sorbate, propylene glycol and Atmul.

D. Sucrose - BD

Same as A except 12 grams sucrose and 15.8 grams butane diol replaces additives.

m = 0.18 g H_2O/g solids in all systems.

TABLE 14

IMF Dog Food Composition II Run 9M

		grams	
A.	Control		A_w = 0.68
	Hamburger	47	
	Soy Flour	10	
	Non-fat dry milk	10	
	Calcium Phosphate	3	
	Crisco	1	Basic Ingredients
	NaCl	1	
	FDC #2 Red Lake Dye	0.006	
	Garlic Powder	0.2	
	Sucrose	24.5	
	K-Sorbate	0.3	Additive
	Propylene Glycol	2.0	
	Atmul 80	1.0	
B.	Control - so sorbate		A_w = 0.68
	Same as A but no sorbate added.		
C.	BD -		A_w = 0.65
	Same as A except 28.8 g 1-3 butane diol replaces all additives.		
D.	Sucrose - BD		A_w = 0.66
	Same as A except 14.4 g sucrose and 14.4 g 1-3 butane diol replaces additives.		
	m = 0.31 g H_2O/g solids.		

TABLE 15

Test Conditions. Run 4M

A_w	Saturated Salt Solution	ml of Organism Solution Added to 300 g Food
0.85	K_2CrO_4	0.66 ml of 10^7 organism/cc - $\approx 10^5$/g food solids
0.80	$(NH_4)_2SO_4$	0.60 ml " " " "
0.75	NaCl	0.53 ml " " " "
0.68	$SrCl_2$	0.45 ml " " " "

Organisms Tested

Code

mold - Aspergillus niger

yeast - Candida Utililus

bacteria - Pseudomonas fragi

pathogen - Staphylococcus aureus

mixture - each of the above four

control - no organisms added

sorbate - mixture of each plus 0.3% K-sorbate on total weight basis.

propionate - mixture of each plus 0.3% Ca-propionate on total weight basis.

TABLE 16

Run 2 Chicken/Cellulose/Glycerol System

Oxidation Rates

($\mu 10_2$/gram) Per Day

	<0.1%RH	32%RH	75%RH
Control	0.588	0.368	1.00
100ppm citric acid	0.395	0.289	0.737
200ppm BHA	0.167	0.100	0.233

Protective Factors*

	<0.1%RH	32%RH	75%RH
Citric Acid	1.5	1.3	1.4
BHA	3.5	3.7	4.3

*$\frac{\text{Rate of Control}}{\text{Rate of Additive}}$

TABLE 17

Run 2

Peroxide Values

Freeze-dried Humidified Chicken System at 37°C

Pv = MEQ/Kg Fat

Days	Control			200ppm BHA			100ppm Citric Acid		
	Dry	32%	75%	Dry	32%	75%	Dry	32%	75%
7	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0
41	0	0	2.04	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0
56	0	3.77	3.12	0	0	0	0	0	2.63
76	0	5.88	5.36	2.80	1.53	2.86	0	0	5.38
90	0	3.77	3.42	0	2.18	2.52	2.12	4.22	5.88
100	-	-	*	-	-	-	-	-	-
121	2.70	32.6*	96.9	2.66	7.16	3.17	0	1.53	11.37
137	9.29	214.5	43.1	-	-	-	-	-	-

* Time at which rancid by sniff test

TABLE 18

Comparison of Oxidation

Freeze-dried Humidified vs Direct Mixing at 75% RH

Run 2 Chicken/Cellulose/25% Glycerol System at 37°C

Peroxide Value meg/Kg

Days	Freeze-dried Humidified*	Direct Mix System** (Run 23 NAS 9-9426)
0	0	0
7	0	99
23	0	120 Rancid
37	2	50
48	0	29
56	3	5
76	5	-
90	3	-
121	97 Rancid	-

* 15.15 g H_2O/100 g solids at 75% RH

** 30 g H_2O/100 g solids at 75% RH

TABLE 19

Run 2

Browning of Freeze-Dried-Humidified Chicken System @ 37°C.

$B = (OD_{420}/g \text{ solids}) \times 100$

Days	Dry	32%	75%	Dry	32%	75%	Dry	32%	75%
7	.315	.188	.319	----	----	----	----	----	----
23	.238	.316	.200	----	----	----	----	----	----
37	.432	.404	.285	.223	.127	.213	.333	.230	.267
41	.268	.248	.199	.530	.142	.364	.504	.373	.286
48	.354	----	.934	.357	----	.359	.416	1.236	.478
56	.825	.122	.970	.207	.317	.269	1.587	1.101	1.432
76	.836	.631	.954	.664	----	.688	.671	.917	2.292

TABLE 20

Run 4. Chicken IMF System at 37°C. Peroxide Values

	Control				200 ppm BHA				100 ppm EDTA			
	Freeze Dry		Direct Mix		Freeze Dry		Direct Mix		Freeze Dry		Direct Mix	
Days	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH
0	0.44	0.32	0.0	0.0	0.60	0.21	0.0	0.0	0.12	0.40	0.0	0.0
7	----	----	5.71	11.91	----	----	0.0	0.0	----	----	0.0	0.0
8	0.0	0.0	----	----	0.0	0.0	----	----	6.23	0.0	----	----
12	0.0	5.44	11.07	11.00	0.0	3.37	4.01	0.0	9.63	2.23	0.0	0.0
19	----	----	21.19	35.43	----	----	2.32	0.0	----	----	0.0	0.0
22	1.25	8.43	54.13[r]	35.04	6.08	4.19	4.76	1.48	6.43	0.0	0.0	17.34
26	----	----	64.09	50.02[r]	----	----	2.55	0.0	----	----	17.24	13.86
27	4.79	7.49	107.8	81.11	6.41	2.10	----	----	2.38	0.0	----	----
29	----	----	----	----	----	----	10.38	3.22	----	----	5.85	64.5
34	----	----	----	----	----	----	----	----	----	----	----	76.96[r]
35	----	6.07	----	----	6.07	0.0	0.0	1.86	2.56	0.40	----	----
37	----	----	142.6	42.4	----	----	2.58	6.59	----	----	2.63	102.7
41	----	----	184.5	101.6	----	----	----	----	----	----	----	177.8
42	3.69	5.49	----	----	3.66	3.26	----	----	2.52	7.40	----	----
43	----	----	----	----	----	----	3.88	1.53	----	----	2.86	----
49	----	----	----	----	----	----	10.68	----	----	----	4.86	----
50	5.50	4.99	----	----	3.14	0.0	----	----	0.0	----	----	----
56	----	----	----	----	----	----	11.58	2.95	----	----	4.77	----
57	6.62	4.16	----	----	0.0	0.0	----	----	4.16	18.7	----	----
63	----	----	----	----	----	----	3.72	7.01	----	----	1.04	----
64	5.23	3.94	----	----	0.0	0.0	----	----	0.0	8.69	----	----
72	33.7	17.7	----	----	6.60	1.52	----	----	2.24	52.4[r]	----	----
76	41.5[r]	20.4	----	----	7.07	5.03	----	----	----	107.4	----	----
82	15.1	18.9	----	----	15.1	13.10	----	----	1.86	24.6	----	----

[r]Indicates rancid odor detected

-Indicates not done on that day

TABLE 21

Run 4 Chicken/Glycerol IMF

Time to Reach Rancidity

Days

	Freeze-Dried	Direct Mix
Control		
61% RH	76	22
75% RH	>82	26
200 ppm BHA		
61% RH	>82	>63
75% RH	>82	>63
100 ppm EDTA		
61% RH	>82	>63
75% RH	72	34

TABLE 22

Run 4. Chicken IMF System at 37°C. Chicken Browning (Organic)

OD_{420}/g solids

	Control				200 ppm BHA				100 ppm EDTA			
	Freeze Dry		Direct Mix		Freeze Dry		Direct Mix		Freeze Dry		Direct Mix	
Days	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH	61%RH	75%RH
0	.079	.499	0.290	0.60	.204	.351	0.51	0.30	.224	.123	0.23	0.30
7	----	----	0.500	2.38	----	----	1.46	0.58	----	----	0.59	0.78
8	.213	.501	-----	----	.437	.422	----	----	.217	.456	----	----
12	.257	.542	-----	1.13	.504	.643	0.36	----	.311	.475	0.57	0.60
19	----	----	0.410	1.91	----	----	0.95	----	----	----	0.62	1.19
22	.489	.644	1.10	1.66	----	.566	0.90	----	.384	.432	0.69	1.64
26	----	----	0.98	1.27	----	----	1.26	1.23	----	----	0.54	----
27	.296	.705	-----	----	.328	.697	----	----	.329	.451	----	----
29	----	----	1.05	1.00	----	----	0.25	0.98	----	----	0.34	1.34
34	----	----	0.29	0.60	----	----	0.67	0.96	----	----	0.49	0.83
35	.417	.864	-----	----	.285	.717	----	----	.340	.551	----	----
37	----	----	0.82	1.04	----	----	0.54	0.67	----	----	0.53	0.94
41	.461	.719	0.60	1.03	.388	.881	----	----	.332	----	----	----
44	----	----	-----	----	----	----	.397	.827	----	----	.490	----
49	.482	.839	-----	----	.436	.779	----	----	----	1.069	----	----
50	----	----	-----	----	----	----	----	.829	----	----	.570	----
56	.683	1.085	-----	----	.568	.947	----	----	.354	.878	----	----
57	----	----	-----	----	----	----	.460	.762	----	----	.360	----
63	.677	1.384	-----	----	.452	.972	----	----	.578	2.032	----	----
64	----	----	-----	----	----	----	.377	1.354	----	----	.498	----
72	.573	1.283	-----	----	.612	1.828	----	----	.449	2.726	----	----
82	1.703	.871	-----	----	.441	.606	----	----	.548	1.137	----	----

TABLE 23

Run 6 - Prok IMF System

Peroxide Values (meq/kg fat)

	Direct Mix						Freeze Dried					
	84% RH			75% RH			84% RH			75% RH		
Days	Control	BHA[a]	EDTA[b]	Control	BHA	EDTA	Control	BHA	EDTA	Control	BHA	EDTA
0	0	0	0	0	0	0	0	0	0	0	0	0
5	7.7	0	6.7	9.3	0	13.5	0	0	0	0	0	0
10	11.8	0.6	3.8	8.6	0.89	35.7[R]	9.4	0.2	0	0	0	0
15	14.6	0	0	1.6	0	37.3	-	-	-	-	-	-
18	-	-	-	-	-	-	0.9	0.5	1.4	0.4	1.7	1.4
19	14.2[R]	2.4	16.4[R]	11.1[R]	2.14	40.2	-	-	-	-	-	-
23	189.2	4.4	25.2	26.0	-	156.9	-	-	-	-	-	-
24	-	-	250.5	-	-	-	1.5	0.9	0	0	0	0
29	32.6	8.0	-	10.7	1.05	107.2	-	-	-	-	-	-

a - 200 ppm fat basis
b - 100 ppm food solids basis
R - onset of rancidity by odor

TABLE 24

Pork IMF System - Run 7

Peroxide Values (meq/kg fat)

	Direct Mix						Freeze Dried					
	75% RH			84% RH			75% RH			84% RH		
Days	Control	BHA[a]	EDTA[b]	Control	BHA	EDTA	Control	BHA	EDTA	Control	BHA	EDTA
0	0	0	0	0	0	0	0	0	0	0	0	0
3	1.2	0	4.1	0.6	0	3.7	-	-	-	-	-	-
6	2.5	0	7.7	0.6	0	5.1	0	0	0	0	0	0
9	1.6	1.1	13.0	3.3	0	3.8	-	-	-	-	-	-
14	-	-	-	-	-	-	0	0.8	1.3	0	0	0
17	8.4	1.9	-	11.9	2.4	-	-	-	-	-	-	-
21	9.0	0.8	17.2	4.7	1.1	25[R]	-	-	-	-	-	-
25	-	-	-	-	-	-	0.9	0.2	1.0	-	1.8	1.5
27	18.7[R]	12.7	64.2[R]	14.6[R]	5.1	56	-	-	-	-	-	-
34	8.1	6.5[s]	102	12.6	6.9[s]	147	-	-	-	-	-	-
39	-	-	-	-	-	-	1.8	1.7	1.9	0	3.2	0.7
54	-	-	-	-	-	-	0.2	0.8	-	1.1	1.8	0.7
67	-	-	-	-	-	-	4.2	3.4	3.8	4.2	6.3	5.3
74	-	-	-	-	-	-	1.3	3.8	0	4.2	5.2	2.1
81							2.6	4.1	2.5	4.3	2.1	1.1
89							-	5.4	2.6	3.2	3.2	1.1
96							6.2	5.2	5.3	4.2	2.1	2.1
105							2.6	2.5	5.2	6.3[s]	6.3	5.3
112							2.5	6.2	5.4	4.2	6.3	5.4
120							2.6	2.6	2.5	3.2	3.2	3.2

a - 200 ppm fat basis; b - 100 ppm solids basis; R - onset of rancidity;
s - v, slight rancid odor

TABLE 25

Infusion Chicken System. Run 11

Peroxide Value (meq/kg fat)

Days	A_w =	Direct Mix 0.61	0.75	0.84	Freeze Dried 0.61	0.75	0.84
0		0	0	0	0	0	0
10		0	3	6	0	0	0
24		0	3	8	0	0	0
30		-	-	-	0	1	1
35		0	15	24	-	-	-
49		-	-	-	0.2	1.2	2.7
54		2.4	6.6[R]	14.6[R]	-	-	-
63		-	-	-	1.3	2.1	2.8
68		2.6	6.0	10.0	-	-	-
78		-	-	-	3.0	4[R]	7.2[R]
83		2.5	5.8	9.0	-	-	-
93		-	-	-	2.6	4.6	6.5
109		-	-	-	3.9	6.9	7.4
134		9.9	-	-	8.9[R]	5.7	4.8

[R] = rancidity

TABLE 25A

Peroxide Values of Commercial Dog Foods

PV = meq/kg fat

DOG FOOD

Days	Direct Mix 37°C	Direct Mix 44°C	Freeze-Dried 37°C	Freeze-Dried 44°C
0	2.9	2.9	2.4	2.8
7	3.8	2.7	3.9	2.6
16	-	-	7.1	2.7
20	8.2	4.4	-	-
24	-	-	7.4	2.9
28	6.9	4.1	-	-
43	-	-	11.7	4.1
47	7.9	4.9	-	-
59	-	-	11.5	-
63	11.2	7.9	-	-
Moisture content (g H_2O/g solids)	0.27		0.20	

TABLE 26

Run 5. Carrot IMF System

Time to Reach
An Unacceptable Color Level (Days)

$OD_{450} = 0.1$

	Direct Mix	Freeze-Dried
Control		
61% RH	12	21
75% RH	21	28
200 ppm BHA (fat basis)		
61% RH	9	27
75% RH	34	25
200 ppm EDTA (total solids basis)		
61% RH	37	33
75% RH	42	28

TABLE 27

Sweet Potato - Run 9

Carotenoid Index

Days	Control				BHA (200 ppm fat basis)				EDTA (200 ppm solid basis)			
	Freeze Dry		Direct Mix		Freeze Dry		Direct Mix		Freeze Dry		Direct Mix	
	0.75	0.61	0.75	0.61	0.75	0.61	0.75	0.61	0.75	0.61	0.75	0.61
0	28.1	26.0	39.6	54.6	26.8	27.1	52.1	36.5	27.6	21.6	49.4	45.0
3	----	25.8	----	----	29.1	24.3	----	----	29.6	27.6	----	----
7	22.5	26.5	26.5	32.9	27.5	20.4	23.9	32.7	26.7	28.1	26.2	39.5
11	----	----	21.7	29.1	----	----	17.5	30.9	----	----	22.4	29.2
13	18.6	20.2	----	----	22.3	19.8	----	----	20.8	25.1	----	----
17	----	----	15.2	18.3	----	----	17.4	23.0	----	----	20.1	24.6
21	17.6	16.1	----	----	25.7	----	----	----	21.7	17.1	----	----
25	----	----	19.7	17.1	----	----	25.9	15.2	----	----	17.4	18.6
32	12.3	16.3	----	----	17.4	23.0	----	----	11.3	----	----	----
36	----	----	4.0	12.2	----	----	11.5	14.9	----	----	11.4	12.9
52	4.8	5.1	----	----	5.2	9.9	----	----	4.8	8.8	----	----
56	----	----	4.6	4.8	----	----	4.9	5.4	----	----	4.9	7.1
73	3.4	8.4	----	----	3.5	6.8	----	----	3.5	8.0	----	----
77	----	----	2.9	4.0	----	----	4.9	4.5	----	----	4.3	6.9

TABLE 28

Run 3 Applesauce IMF System

Browning (OD/gram) x 100

Days	61% RH	75% RH	61% RH	75% RH	61% RH	75% RH	61% RH	75% RH
0	4.2	2.8	2.8	2.1	4.2	1.9	0.9	1.4
8	---	3.4	---	---	---	1.9	---	---
12	---	---	2.1	2.8	---	---	2.6	3.0
15	---	3.7	---	---	---	1.5	---	---
19	---	---	3.5	2.8	---	---	2.7	3.5
23	5.2	2.4	3.3	2.3	2.9	1.0	1.7	---
27	---	2.9	---	---	4.3	2.7	---	---
42	6.4	1.8	2.2	3.0	6.9	4.9	3.8	---
50*	6.2	1.7	2.0	---	7.9	3.1	2.9	5.9
57	5.6	2.1	3.8	2.3	7.1	3.2	5.6	3.8
64	6.4	2.4	4.1	2.3	7.8	3.4	7.6	3.5
74	6.5	2.7	4.4	3.4	10.5	4.9	9.3	4.8
83	5.8	2.8	4.6	2.7	10.5	4.1	7.7	3.8

*Pectinase added after this time

TABLE 29

IMF Systems for Browning

Kinetic Values

Run		A_w	% Glycerol in Liquid Phase	Time to Reach B = 5
Run 1(Applesauce)	37°C	0.95	3.5	34 days
(Direct Mix)	45°C	0.95	3.5	20 days
Run 3(Applesauce)	37°C	0.75	52	>100 days
(Direct Mix)		0.61	69	85 days
	45°C	0.75	52	>85 days
		0.61	69	55 days
	37°C	0.75	66	>100 days
(Freeze Dry)		0.61	78	≃20-30 days
	45°C	0.75	66	≃85 days
		0.61	78	30 days
Run 8(Bananas)	37°C	0.61	68	>80 days
(Direct Mix)		0.75	50	>80 days
	44°C	0.61	68	58
		0.75	50	36
	57°C	0.61	68	30
		0.75	50	23
(Freeze Dry)	37°C	0.61	77	>80
		0.75	63	78
	44°C	0.61	77	55
		0.75	63	30
	57°C	0.61	77	20
		0.75	63	20

TABLE 30

Banana IMF - Run 8

Browning Value = (OD_{420}/total dry weight) x 100

Days	Direct Mix						Freeze-Dried					
	61% RH			75% RH			61% RH			75% RH		
	37°C	44°C	57°C	37°C	44°C	57°C	37°C	44°C	57°C	37°C	44°C	57°C
0	3.4	3.4	3.4	5.7	5.7	5.7	3.6	3.6	3.6	4.3	4.3	4.3
6	5.3	6.6	4.8	5.1	7.0	12.1	5.2	8.5	7.1	13.6	12.0	12.8
9	5.9	6.3	6.8	10.6	10.1	12.4	---	---	---	---	---	---
12	---	---	---	---	---	---	5.1	4.6	8.1	12.0	16.6	18.1
15	5.3	5.5	8.0	8.0	12.4	13.1	---	---	---	---	---	---
20	---	---	---	---	---	---	5.4	6.0	10.0	13.7	14.3	30.2
23	4.8	7.7	8.3	10.3	17.1	33.0	---	---	---	---	---	---
27	---	---	---	---	---	---	5.4	8.9	11.8	18.6	19.1	39.2
30	8.1	9.1	9.5	16.0	17.6	36.5	---	---	---	---	---	---
33	---	---	---	---	---	---	9.9	10.4	12.2	19.1	30.8	56.9
36	7.4	8.0	19.0	14.5	24.5	37.1	---	---	---	---	---	---
41	---	---	---	---	---	---	6.8	7.9	18.8	13.1	22.2	75.0
44	6.3	10.0	25.8	11.8	27.3	67.0	---	---	---	---	---	---

TABLE 31

Banana IMF - Run 8

Reduced Browning Value = (OD_{420}/dry weight banana solids) x 100

Days	Direct Mix											
	61% RH			75% RH			61% RH			75% RH		
	37°C	44°C	57°C	37°C	44°C	57°C	37°C	44°C	57°C	37°C	44°C	57°C
0	18.9	18.9	18.9	13.0	13.0	13.0	19.8	19.8	19.8	9.5	9.5	9.5
6	29.6	36.6	24.9	11.5	15.7	27.1	28.6	47.4	39.5	30.2	26.8	28.5
9	32.7	34.7	37.7	23.7	22.4	27.8	----	----	----	----	----	----
12	----	----	----	----	----	----	28.4	25.5	42.5	26.8	37.1	40.4
15	29.5	30.6	44.6	17.9	27.7	29.3	----	----	----	----	----	----
20	----	----	----	----	----	----	30.0	33.3	55.7[u]	30.7	31.9	67.4[u]
23	26.8	42.8	46.3	23.0	38.3	73.8[u]	----	----	----	----	----	----
27	----	----	----	----	----	----	29.8	49.8[u]	65.6	41.6	42.7	87.4
30	45.0	50.4[u]	52.9[u]	35.6	39.3	81.5	----	----	----	----	----	----
33	----	----	----	----	----	----	----	57.9	68.1	42.6	68.8[u]	128.0
36	41.1	44.2	105.5	32.5	54.7	83.1	----	----	----	----	----	----
41	----	----	----	----	----	----	37.7	44.0	115.1	29.3	49.7	167.8
44	35.2	55.5	143.6	26.3	61.0[u]	149.8	----	----	----	----	----	----
48	----	----	----	----	----	----	25.9	48.0	93.7	30.1	61.9[u]	265.0
51	28.8	39.9	181.1	23.3	26.0	----	----	----	----	----	----	----
55	----	----	----	----	----	----	48.5	60.3[u]	----	39.7	97.2	----
58	46.0	48.9[u]	----	36.9	39.7	----	----	----	----	----	----	----
61	----	----	----	----	----	----	45.0	63.9	----	50.9	85.0	----
64	54.4	69.7	----	33.6	40.0	----	----	----	----	----	----	----
68	----	----	----	----	----	----	62.3	69.5	----	57.6	87.7	----
71	50.9	92.8	----	45.1	----	----	----	----	----	----	----	----
78	----	52.5	----	27.0	78.1	----	----	90.5	----	60.0	93.9	----

[u]-Unacceptable color

TABLE 32

Applesauce IMF. Run 10

(OD/gram applesuace solids) x 100

Days	Direct Mix RH = 61%	75%	84%	90%	Freeze-Dried 61%	75%	84%	90%
0	36.4	38.4	20.5	20.3	109.9	58.4	19.1	19.4
5	77.8	28.6	37.5	35.4	----	----	----	----
6	----	----	----	----	97.9	41.7	31.5	30.4
13	65.7	33.8	38.6	40.7	82.3	35.8	35.5	19.1
17	----	----	----	----	69.3	34.1	34.7	24.4
22	50.5	35.2	32.0	21.8	----	----	----	----
30	----	----	----	----	79.5	52.2	44.8	36.5
35	57.5	34.8	26.1	35.4	----	----	----	----
37	----	48.8[u]	34.0[u]	37.3[u]	68.7	51.6[u]	42.9[u]	39.4[u]

[u] = Unacceptable

TABLE 33

Run 12 Lipase. Free Fatty Acid Determination.

Acidity = (N x ml_{KOH}/grams fat) x 100

	DIRECT MIX				FREEZE-DRIED			
DAYS	A_w = 0.51	0.61	0.75	0.84	0.51	0.61	0.75	0.84
0	3.7	0.8	1.2	0.4	3.9	1.9	1.5	1.2
5	---	---	---	---	3.7	2.3	1.6	0.8
6	4.1	2.8	1.6	0.8	---	---	---	---
14	3.6	3.2	1.6	1.9	---	---	---	---
15	---	---	---	---	4.2	3.0	2.0	1.5
25	4.0	3.0	3.0	2.4	---	---	---	---
27	---	---	---	---	4.0	2.2	3.6	2.4
34	3.7	3.4	3.1	2.3	---	---	---	---
41	4.4	4.5	3.3	2.8	3.8	4.3	2.7	2.0
55	4.8	16.0	3.9	3.4	---	---	---	---
60	---	---	---	---	9.0	7.4	16.0	11.2
69	---	---	---	---	---	2.36	28.27	3.16
70	5.4	3.8	3.5	59.3	---	---	---	---
76	---	3.32	4.81	42.6	---	---	---	---
83	---	---	---	---	8.65	23.7	34.4	17.6
90	8.10	8.14	22.7	43.1	---	---	---	---

TABLE 34

Run 1M - Pumpkin IMF System

Mold Growth

Direct Mix System

System	A_w
A	0.85 - Growth in 48 hrs
B	0.80 - Growth in 120 hrs
C	0.75 - Growth in 2 weeks
D	0.70 - No growth

Freeze-Dried System

System	%Glycerol	A_w = 0.90	0.84	0.75	0.61
A	24	No growth in three weeks			
B	33		"	"	
C	40		"	"	
D	45		"	"	

TABLE 35

Run 2M - Chicken Dice Infusion IMF System

Direct Mix

System	A	B	C	D
A_w	0.85	0.80	0.75	0.70
Observation	Visible growth in 5 days	Visible growth in 6 days	Visible growth in 12 days	No visible growth in 14 days
14 days # organisms/g	10^9	3.4×10^8	1.2×10^6	4.6×10^4

Freeze-Dried (14 day count - # organisms/gram)

A_w	0.90	0.84	0.75	0.61
System				
A	1.3×10^4	8×10^2	No growth	No growth
B	2×10^2	1×10^2	"	"
C	1.7×10^4	1×10^2	"	"
D	6×10^2	1×10^2	"	"

TABLE 36

Run 3M - Pumpkin IMF - Cell Counts

(# mold/gram food solids)

Direct Mix Control	84% RH
Initial	1×10^3
7 days	10^5
14 days	$>10^7$

Freeze-Dried	84% RH	90% RH
Initial (from drier)	183	183
5 hr Rehumidified	130	100
3 days	30	20
5 days	20	20
11 days	10	0

TABLE 37

Banana IMF - Cell Viability - Run 4M

(cell counts in #/gram food solids)

Treatment	Days =	68% RH			75% RH		
		0	7	14	0	7	14
Mold							
Direct Mix		1.7×10^5	0	0	1.7×10^4	1.1×10^4	1.9×10^5
Freeze Dry		10.7×10^3	5.3×10^3	3.3×10^3	2.3×10^3	0	0
Yeast							
Direct Mix		1.5×10^5	0	0	1.3×10^4	1.7×10^3	0
Freeze Dry		0	0	0	0	0	0
Pseudomonas							
Direct Mix		0	0	0	0	0	0
Freeze Dry		0	0	0	0	0	0
Staph							
Direct Mix		2.4×10^4	0	0	0	0	0
Freeze Dry		6.6×10^3	0	0	0	0	0
Mixture							
Direct Mix		4.0×10^4	0	0	2.9×10^4	1.1×10^5	7.9×10^5
Freeze Dry		2.0×10^4	0	0	2.3×10^3	5.7×10^2	1.7×10^2
Control							
Direct Mix		0	0	0	0	0	0
Freeze Dry		0	0	0	0	0	0
Sorbate (Mixture)							
Direct Mix		6.7×10^3	0	0	1.1×10^4	0	0
Freeze Dry		6.7×10^3	0	0	5.7×10^3	0	0
Propionate (Mixture)							
Direct Mix		3.3×10^4	4.7×10^3	1.0×10^4	4.0×10^4	2.3×10^3	5.7×10^2
Freeze Dry		1.3×10^4	0	0	3.4×10^3	5.7×10^2	1.7×10^3

TABLE 37
(continued)

Banana IMF - Cell Viability - Run 4M

(Cells Counts in #/gram food solids)

Treatment	Days =	80% RH 0	80% RH 7	80% RH 14	85% RH 0	85% RH 7	85% RH 14
Mold							
Direct Mix		3×10^4	1.2×10^4	6×10^5	6.7×10^4	4.5×10^5	5.3×10^5
Freeze Dry		9×10^3	2×10^3	0	4.5×10^3	0	0
Yeast							
Direct Mix		2×10^5	10^7	10^6	6.8×10^5	8.9×10^6	8.9×10^5
Freeze Dry		0	0	0	0	0	0
Pseudomonas							
Direct Mix		0	0	0	4.5×10^3	0	0
Freeze Dry		0	0	0	0	0	0
Staph							
Direct Mix		9×10^4	0	0	7.5×10^4	0	0
Freeze Dry		4×10^3	0	0	3.1×10^3	0	0
Mixture							
Direct Mix		9.5×10^4	8.3×10^5	6×10^5	12.5×10^4	4.9×10^5	1.6×10^5
Freeze Dry		2.1×10^4	5×10^2	0	2.2×10^3	0	0
Control							
Direct Mix		0	0	0	0	0	0
Freeze Dry		0	0	0	0	0	0
Sorbate (Mixture)							
Direct Mix		2.5×10^4	0	0	4.5×10^4	0	0
Freeze Dry		8.5×10^3	0	0	1.1×10^4	0	0
Propionate (Mixture)							
Direct Mix		6.5×10^4	10^7	10^6	8.0×10^4	8.9×10^6	8.9×10^5
Freeze Dry		3.4×10^4	7×10^3	6.8×10^2	2.7×10^3	8×10^3	8×10^2

TABLE 38

Banana IMF - Cell Viability - Run 5M

Treatment	# Cells/gram food solids MOLD Direct Mix	MOLD Freeze Dry	CONTROL Direct Mix	CONTROL Freeze Dry
A_w = 0.90				
initial	3.2×10^4	5.6×10^4	0	0
after humidification	-	3.2×10^4	-	0
4 days	4.4×10^4	-	0	-
7 days	9.6×10^4	6.4×10^4	0	0
10 days	-	3.7×10^4	-	0
12 days	2.9×10^5	-	0	-
19 days	1.7×10^4	-	-	-
30 days	1.9×10^4	8.5×10^4	0	0
A_w = 0.85				
initial	6.2×10^4	1.3×10^5	0	0
after humidification	-	1.3×10^5	-	0
4 days	4.5×10^5	-	0	-
7 days	8.9×10^4	8.5×10^4	0	0
10 days	-	6.8×10^4	-	0
12 days	3.6×10^5	-	0	-
19 days	1×10^5	-	-	-
30 days	8.6×10^5	4.5×10^3	0	0
A_w = 0.80				
initial	4.5×10^4	4.7×10^4	0	0
after humidification	-	5×10^2	-	0
4 days	1.4×10^5	-	0	-
7 days	2.3×10^5	5×10^2	0	0
10 days	-	8×10^3	0	0
12 days	2×10^5	-	0	-
19 days	2.4×10^4	-	-	-
30 days	1.1×10^5	2.5×10^3	0	0
A_w = 0.75				
initial	3.4×10^4	5.7×10^3	0	0
after humidification	-	0	-	0
4 days	1.7×10^3	-	0	-
7 days	-	0	0	0
10 days	-	0	-	0
12 days	1.1×10^3	-	0	-
19 days	5.7×10^2	-	-	-
30 days	8.6×10^5	0	0	0

TABLE 38 (Continued)

Banana IMF - Cell Viability - Run 5M

Cells/gram food solids

Treatment	MOLD		CONTROL	
	Direct Mix	Freeze Dry	Direct Mix	Freeze Dry
A_w = 0.68 initial	4.6×10^4	0	-	0
after humidification	-	0	-	0
4 days	7.3×10^3	-	0	-
7 days	1.3×10^3	0	0	0
10 days	-	0	-	0
12 days	2.6×10^4	-	0	-
19 days	-	-	-	-
30 days	3.9×10^4	0	0	0

TABLE 38 (Continued)

Banana IMF - Cell Viability - Run 5M

Cells/gram food solids

Treatment	Sorbate (Plus Mold)* Direct Mix	Freeze Dry
A_w = 0.90		
initial	3.1×10^4	5.4×10^3
after humidification	-	5.4×10^2
4 days	0	-
7 days	0	0
10 days	-	0
12 days	0	-
19 days	0	-
30 days	0	0
A_w = 0.85		
initial	6.2×10^4	7.1×10^3
after humidification	-	0
4 days	0	-
7 days	0	0
10 days	-	0
12 days	0	-
19 days	0	-
30 days	0	0
A_w = 0.80		
initial	3×10^4	5×10^3
after humidification	-	0
4 days	5×10^3	-
7 days	0	0
10 days	0	0
12 days	0	-
19 days	0	-
30 days	0	0
A_w = 0.75		
initial	2.5×10^4	0
after humidification	-	0
4 days	0	-
7 days	0	0
10 days	-	0
12 days	0	-
19 days	0	-
30 days	0	0
A_w = 0.68		
initial	1.3×10^4	0
after humidification	-	0
4 days	1.6×10^4	-
7 days	0	0
10 days	-	0
12 days	0	-
19 days	0	-
30 days	0	0

TABLE 39

Banana Run 6M Plate Counts

Mold Counts/g Banana Solids x 10^{-3}

A_w =	0.9		0.85		0.8		0.75		0.68	
Time	D.M.*	F.D.**	D.M.	F.D.	D.M.	F.D.	D.M.	F.D.	D.M.	F.D.
pH	5.78		4.40		4.36		4.52		4.68	
Initial	3.2	4.2	3	4	2.6	2.5	2.8	3.9	2.4	0
3 days	4.8	2.1	45	3.1	10	4.5	9.1	0	5.3	0
1 week	33	2	27	1.7	35	1.8	3.8	.74	1.3	0
2 weeks	48	.63	36	1.7	24	1.5	4.6	.11	3.3	0
3 weeks	21	1.4	54	2.7	31	0.5	6.8	.17	15	0
5 weeks	53	0.4	39	0.8	--	C.1	9.7	0	27	0
7 weeks	21	1.2	48	1.3	40	0	42	0	35	0
9 weeks	79	.79	76	1.1	19	0	27	0	93	0
10 weeks	44	.53	110	1.2	35	0	--	0	--	0
12 weeks	25	.57	15	2.0	30	0	97	0	45	0
	90% + sorbate		85% + sorbate		80% + sorbate		75% + sorbate		68% + sorbate	
Time	D.M.	F.D.	D.M.	F.D.	D.M.	F.D.	D.M.	F.D.	D.M.	F.D.
Initial	1.7	4.2	3.1	4.9	2.5	2	2.4	2.9	3.7	0
3 days	0	.53	0	1.3	0	0	1.1	0	3.3	0
1 week	0	2.8	0	1.9	0	0	0	0	0	0
2 weeks	0	1.4	0	1.2	0	0	0	0	0	0
3 weeks	0	.85	0	2.2	0	0	0	0	0	0
5 weeks	0	0	0	1.3	0	0	0	0	0	0
7 weeks	0	0	0	1.2	0	0	0	0	0	0
9 weeks	0	.05	0	.67	0	0	0	0	0	0
10 weeks	0	.74	0	.58	0	0	0	0	0	0
12 weeks	0	0.3	0	.45	0	0	0	0	0	0

*Direct Mix
**Freeze Dried

TABLE 40

Pork IMF. Run 7M

(# cell/gram solids)

		MOLD		YEAST		PSEUDOMONADS	
A_w = 0.90	pH	Direct Mix	Freeze Dry	Direct Mix	Freeze Dry	Direct Mix	Freeze Dry
initial	7.0	7.3×10^3	5×10^3	1.5×10^4	0	8×10^3	0
5 days		3.9×10^4	9.1×10^3	1.1×10^5	0	2.2×10^7	0
2 weeks		3.8×10^5	5.5×10^3	5.1×10^7	0	3.7×10^8	0
1 month		2.8×10^5	5.5×10^3	1.8×10^8	0	1.4×10^8	0
2 months		3×10^5	3.7×10^3	5.9×10^7	0	2.7×10^8	0
A_w = 0.84							
initial	7.25	8×10^3	5.5×10^3	1.2×10^4	0	8×10^3	0
5 days		1.2×10^4	-	2.5×10^4	0	9.2×10^3	0
2 weeks		2×10^5	5.2×10^3	3.4×10^5	0	2.3×10^4	0
1 month		4.1×10^5	1.7×10^3	8×10^5	0	3.5×10^6	0
2 months		5×10^5	2×10^3	2.2×10^7	0	1.6×10^7	0
A_w = 0.75							
initial	6.95	7×10^3	6.6×10^3	8.6×10^3	0	8×10^3	0
5 days		1.4×10^4	2.9×10^3	0	0	0	0
2 weeks		7.9×10^3	1.4×10^3	0	0	0	0
1 month		5×10^3	5.4×10^3	0	0	0	0
2 months		8.6×10^5	2.9×10^2	0	0	0	0
A_w = 0.68							
initial	6.85	5.6×10^3	7×10^3	8×10^3	0	8×10^3	0
5 days		7.2×10^3	1.1×10^4	0	0	0	0
2 weeks		4×10^3	-	0	0	0	0
1 month		0	-	0	0	0	0
2 months		0	3×10^3	0	0	0	0

TABLE 40
(Continued)

Pork IMF. Run 7M

(# Cells/gram Solids)

	Staph		Mold + Sorbate	
	Direct Mix	Freeze Dry	Direct Mix	Freeze Dry
A_w = 0.90				
initial	1×10^4	1.1×10^5	8.7×10^3	3.4×10^3
5 days	1.4×10^5	4.5×10^5	7.3×10^5	4.6×10^2
2 weeks	4.2×10^8	5×10^4	5×10^4	-
1 month	2.4×10^9	1.5×10^5	7.3×10^3	1.4×10^2
2 months	1×10^6	3.4×10^3	1.9×10^5	0.9×10^2
A_w = 0.84				
initial	8.6×10^3	3.1×10^4	5.3×10^3	3.9×10^2
5 days	2.8×10^7	6.9×10^3	8×10^3	9.2×10^3
2 weeks	5.5×10^8	-	1.7×10^4	5.8×10^3
1 month	5.2×10^9	1.7×10^2	1.6×10^6	1.7×10^3
2 months	3.7×10^9	0	4.7×10^7	-
A_w = 0.75				
initial	1.3×10^4	3.6×10^4	4.4×10^3	10^4
5 days	1.4×10^3	2.9×10^3	2.1×10^3	2.9×10^3
2 weeks	2.5×10^4	0	2.1×10^3	-
1 month	3.7×10^4	0	0	2.2×10^3
2 months	5.6×10^4	0	0	1.1×10^3
A_w = 0.68				
initial	6×10^3	1.8×10^3	4.9×10^3	4×10^3
5 days	1.8×10^3	8×10^2	1×10^4	3.2×10^3
2 weeks	5.6×10^3	0	3.2×10^3	0
1 month	0	0	0	0
2 months	0	0	0	0

TABLE 41

Dog Food Stability. Run 8M

Mold/gram Mixture

A_w = 0.60-.65

Sample	Control	Con/No Sorbate	BD	Sucrose/BD
Initial	1.2×10^4	9.4×10^3	0	2.1×10^3
4 days	8.8×10^3	8.8×10^3	0	0
2 weeks	5×10^3	8×10^3	0	0
1 month	7.6×10^3	1.5×10^4	0	0
6 weeks	3.1×10^3	9.4×10^3	0	0

TABLE 42

Dog Food Stability Run 98

Mold/gram Mixture

A_w = 0.65-0.70

Sample	Control	Con/No Sorbate	BD	Sucrose/BD
initial	1.4×10^4	1.7×10^4	5.3×10^3	6.7×10^3
3 days	8.3×10^3	1.2×10^4	0	0
1 week	6.4×10^3	8.1×10^3	0	0
2 weeks	2.9×10^3	3.9×10^3	0	0
1 month	5×10^2	1.7×10^3	0	0

TABLE 43

Packaging Analysis

Chicken IMF System

Food Factors	Desorption Isotherm	Adsorption Isotherm
b isotherm slope gram H_2O/g solid	3.8	1.4
C isotherm intercept	-1.85	-0.6
m_i g H_2O/g solid	0.80	0.42
A_w initial	0.7	0.75
m_e at 50% RH	0.05	0.10
m_e at 90% RH	1.7	0.66
m_c (25% loss of water)	0.6	0.3
m_c (gain of water to A_w = 0.85)	1.3	0.6

Storage Factors

T = 80°F P_s = 30 mmHg

Package contains 25 g solids including glycerol

Package area 0.025 m^2

$\therefore$ $A/w_s = 10^{-3}$ m^2/gram

For weight loss external % RH = 50

For weight gain external % RH = 90

TABLE 44

Typical Values of Water Permeability

for Commercial Films

(@ 80 - 100°F)

Film	K_{H_2O} = grams mil/m^2 day mm Hg
1. Saran	0.0025
2. Polyethylene	0.025
3. Scotch Pak Laminate #104 (7.5 mil)	0.07
4. Scotch Pak Laminate #5 (3 mil)	0.15
5. Mylar	0.5
6. Teflon	0.7
7. Nylon 6	2.0
8. Polystyrene	4.0
9. Cellulose Acetate	25

TABLE 45

Summary of Time to Reach Rancid Off Flavor

Run #	System	Direct Mix	FD-Rehumidified
2.	Chicken/Cellulose/Glycerol A_w = 0.75	23	121
4.	Chicken/Glycerol A_w = 0.61	22	76
	0.75	26	>82
6.	Pork/Glycerol A_w = 0.75	19	>29
	0.84	19	>29
7.	Pork/Glycerol A_w = 0.75	27	>120
	0.84	27	~105
11.	Infusion Chicken A_w = 0.75	54	78
	0.84	54	78

TABLE 46

Viscosities of Aqueous Phase Due to Glycerol

Run	A_w	Method of preparation	Glycerol concentration[a] (%)	Viscosity[b] (cp)
4	0.61	direct mix	63	6.8
		freeze-dried	75	13.6
	0.75	direct mix	43	2.1
		freeze-dried	60	5.1
7	0.75	direct mix	41	2.1
		freeze-dried	60	5.1
	0.84	direct mix	27	1.5
		freeze-dried	44	2.1

[a] $\frac{\text{weight of glycerol}}{\text{weight of glycerol + weight of water in food}}$

[b] Newman (1968)

TABLE 47

Antioxidant Effectiveness

Days to Rancidity

Run #	System	Control	200 ppm BHA	100 ppm EDTA
2	Chicken A_w = 0.75	121	(500)*	(175)*
4	Chicken			
	Direct Mix A_w = 0.61	22	(150)	(100)
	A_w = 0.75	26	(150)	34
	FD-Rehumidify			
	A_w = 0.75	76	(180)	(200)
	A_w = 0.75	(100)	(200)	72
7	Pork			
	Direct Mix A_w = 0.75	27	(40)	27
	A_w = 0.84	27	(40)	21
	FD-Rehumidify			
	A_w = 0.75	(150)	(150-200)	(150-200)
	A_w = 0.84	105	(150-200)	(150-200)

(a) In Run 2 citric acid was used instead of EDTA.

* Numbers in brackets are estimates since not enough samples were available to carry the test through these times.

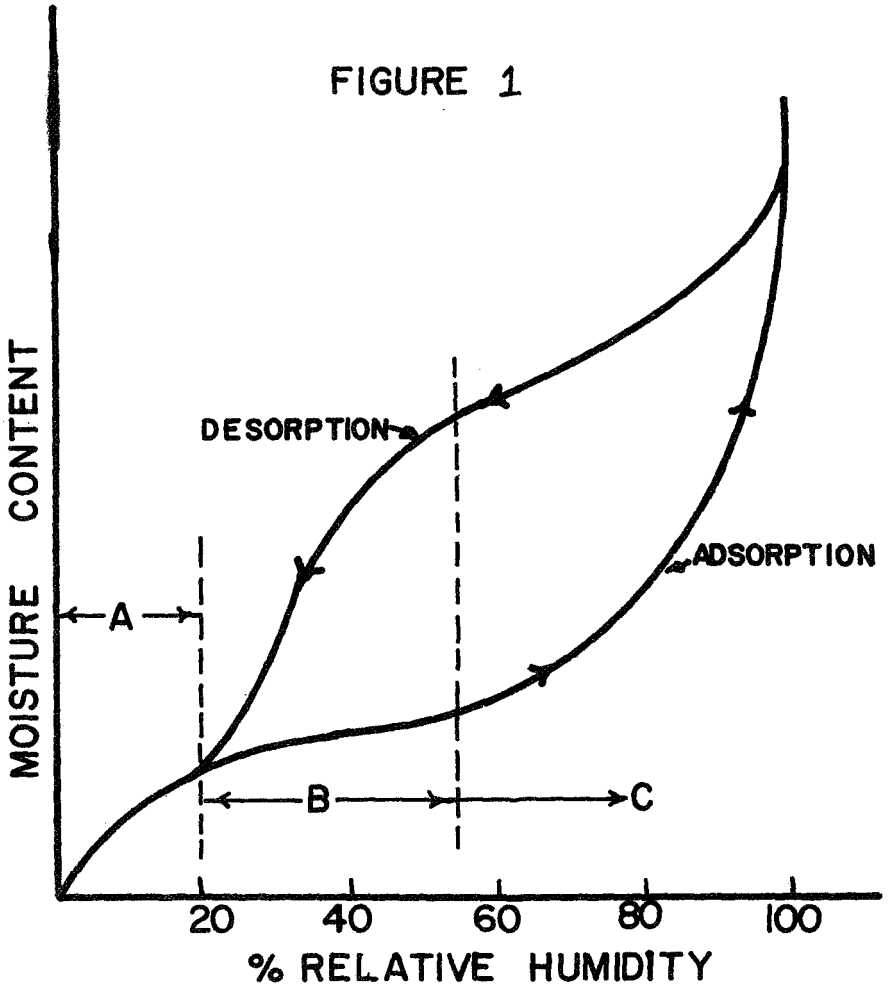
TABLE 48

Summary of Minimum Growth A_w in IMF Systems

SYSTEM	DESORPTION	ADSORPTION
Banana IMF		
Mold	~ 0.68	>0.9
Pork IMF		
Mold	0.75-0.68	>0.9
Yeast	0.84-0.75	>0.9
Pseudomonads	0.84-0.75	>0.9
Staph	0.84-0.75	>0.9

VIII. F I G U R E S

FIGURE 1


MOISTURE CONTENT
DESORPTION
ADSORPTION
A
B
C
20
40
60
80
100
% RELATIVE HUMIDITY

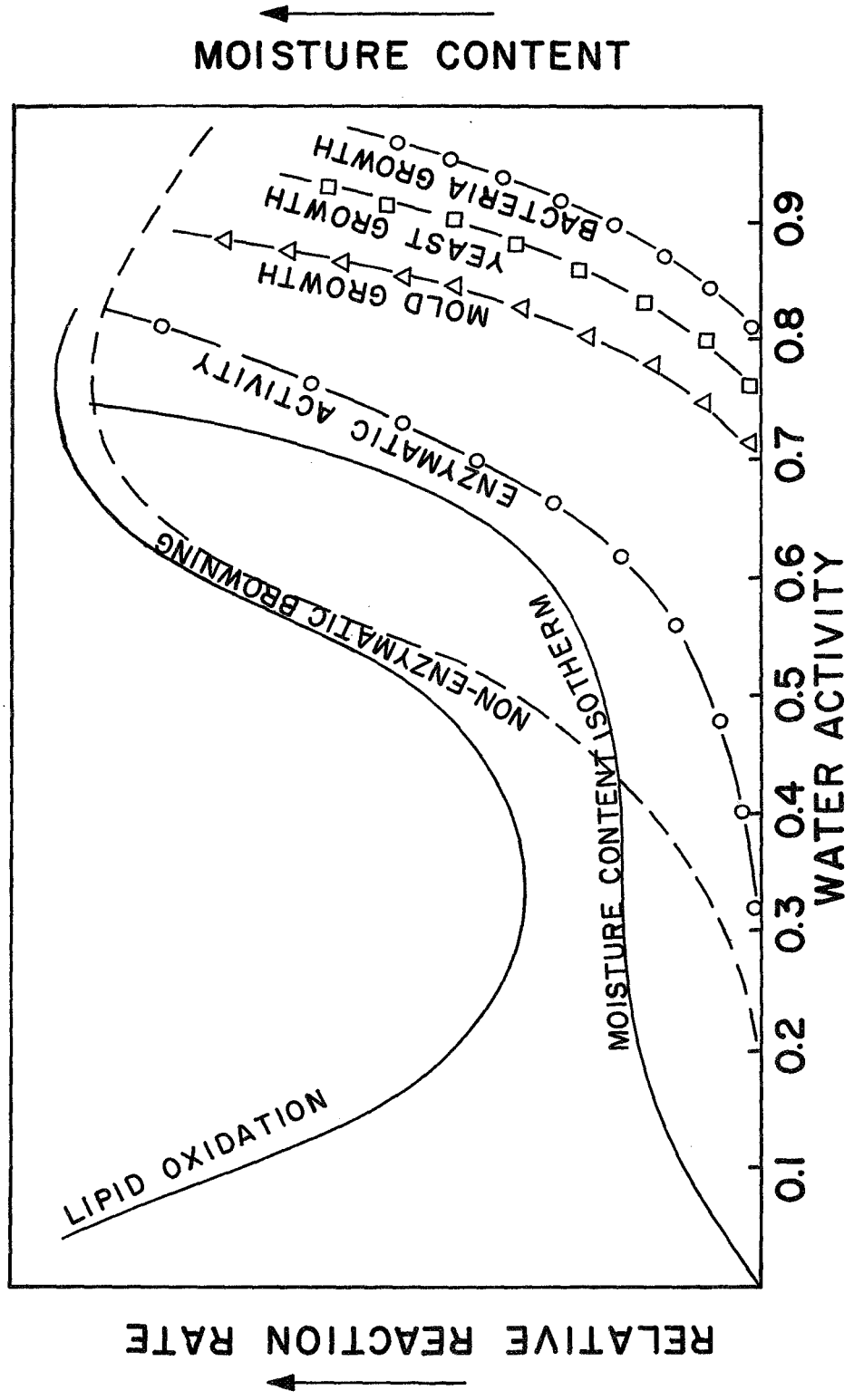


FIGURE 2 STABILITY MAP OF FOODS

FIGURE 3
WATER ACTIVITY LOWERING BY GLYCEROL

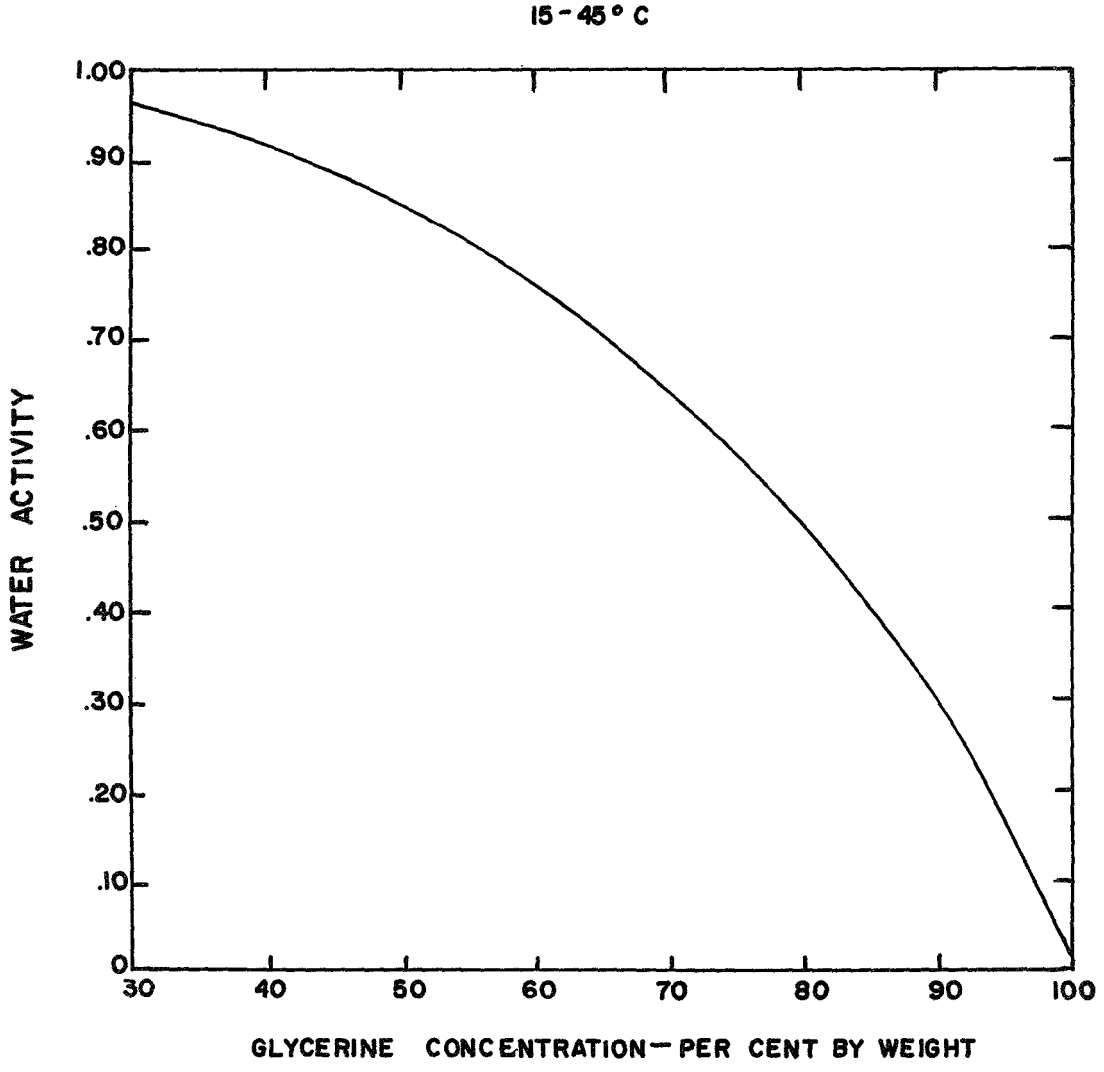
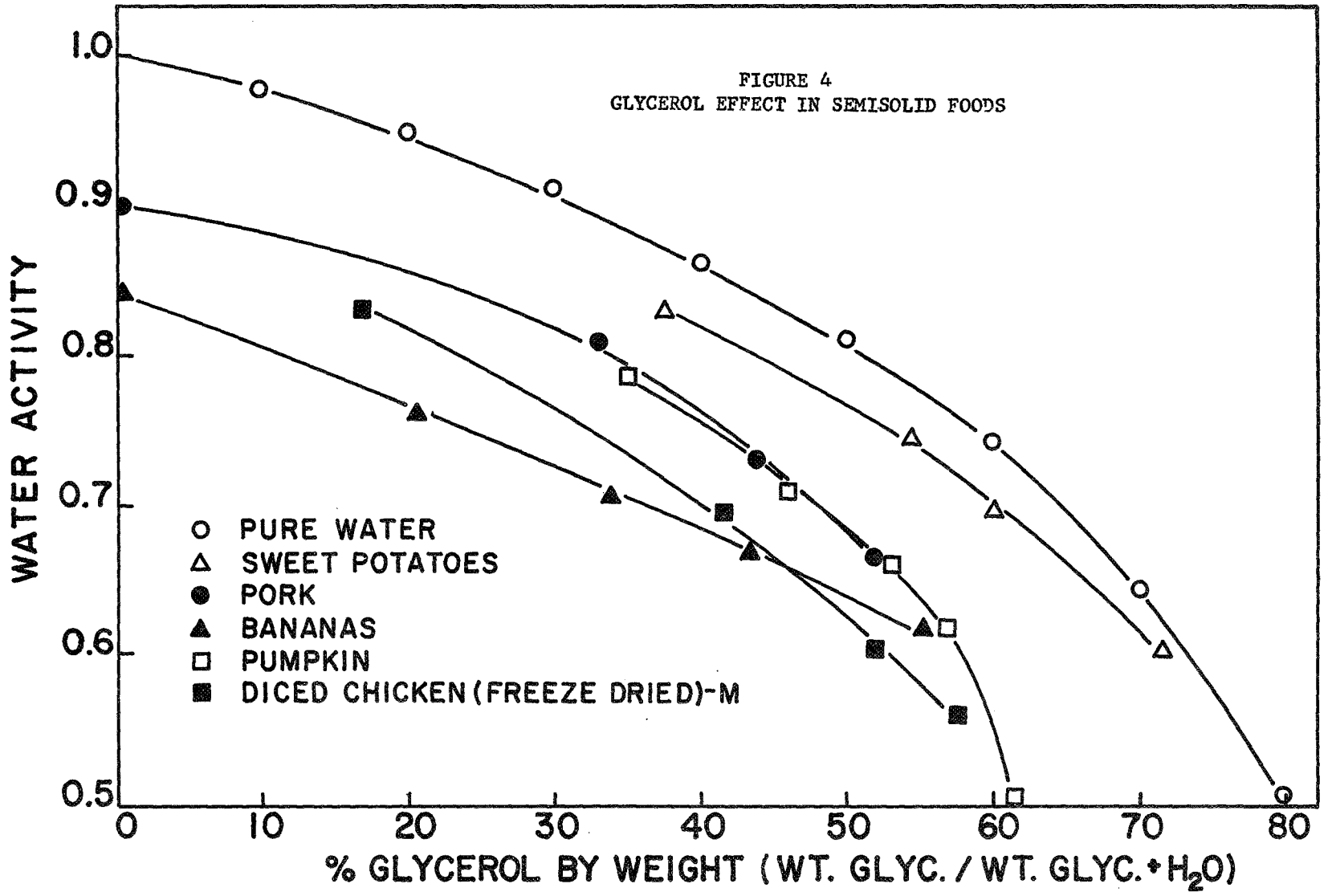


FIGURE 4
GLYCEROL EFFECT IN SEMISOLID FOODS



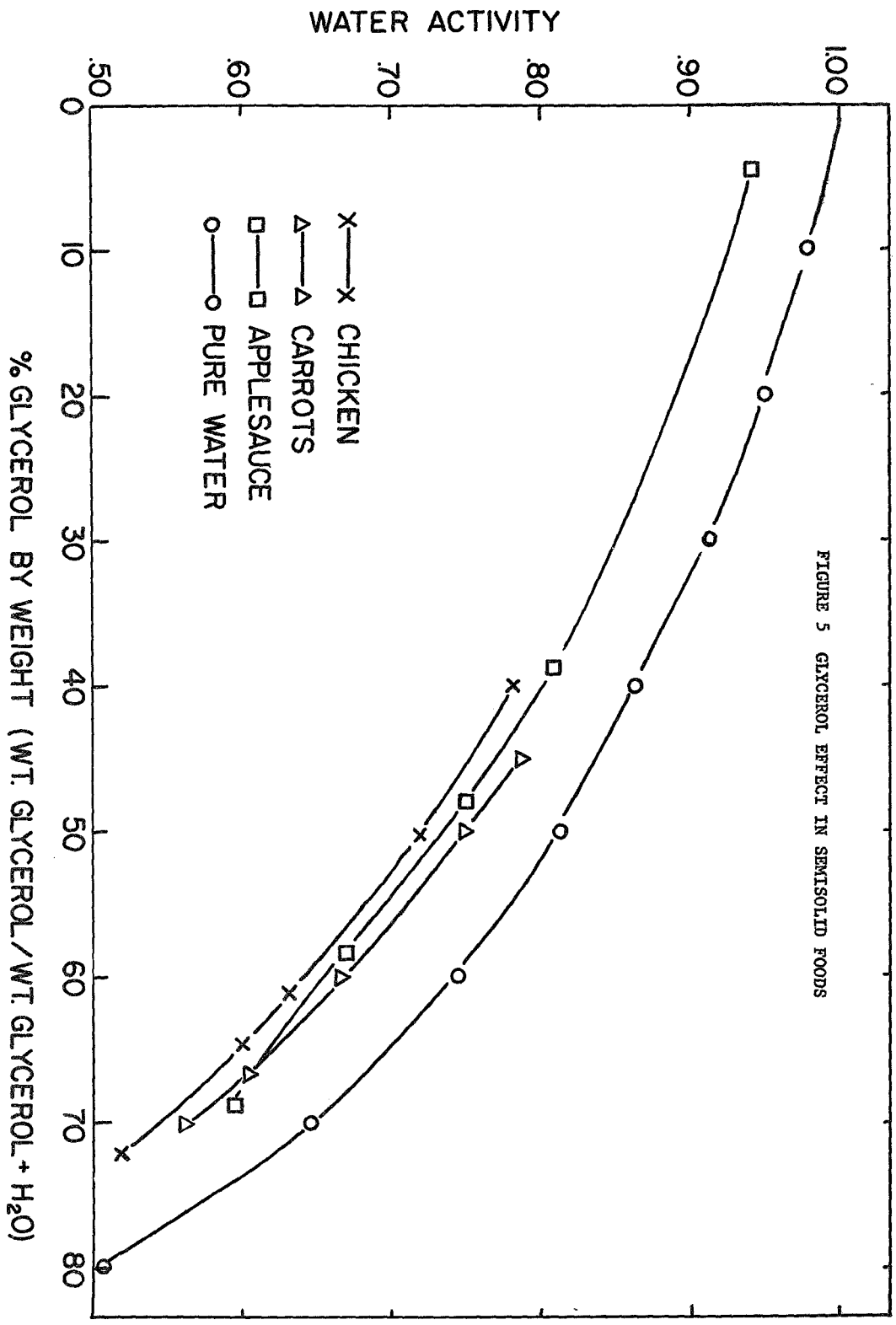


FIGURE 5 GLYCEROL EFFECT IN SEMISOLID FOODS

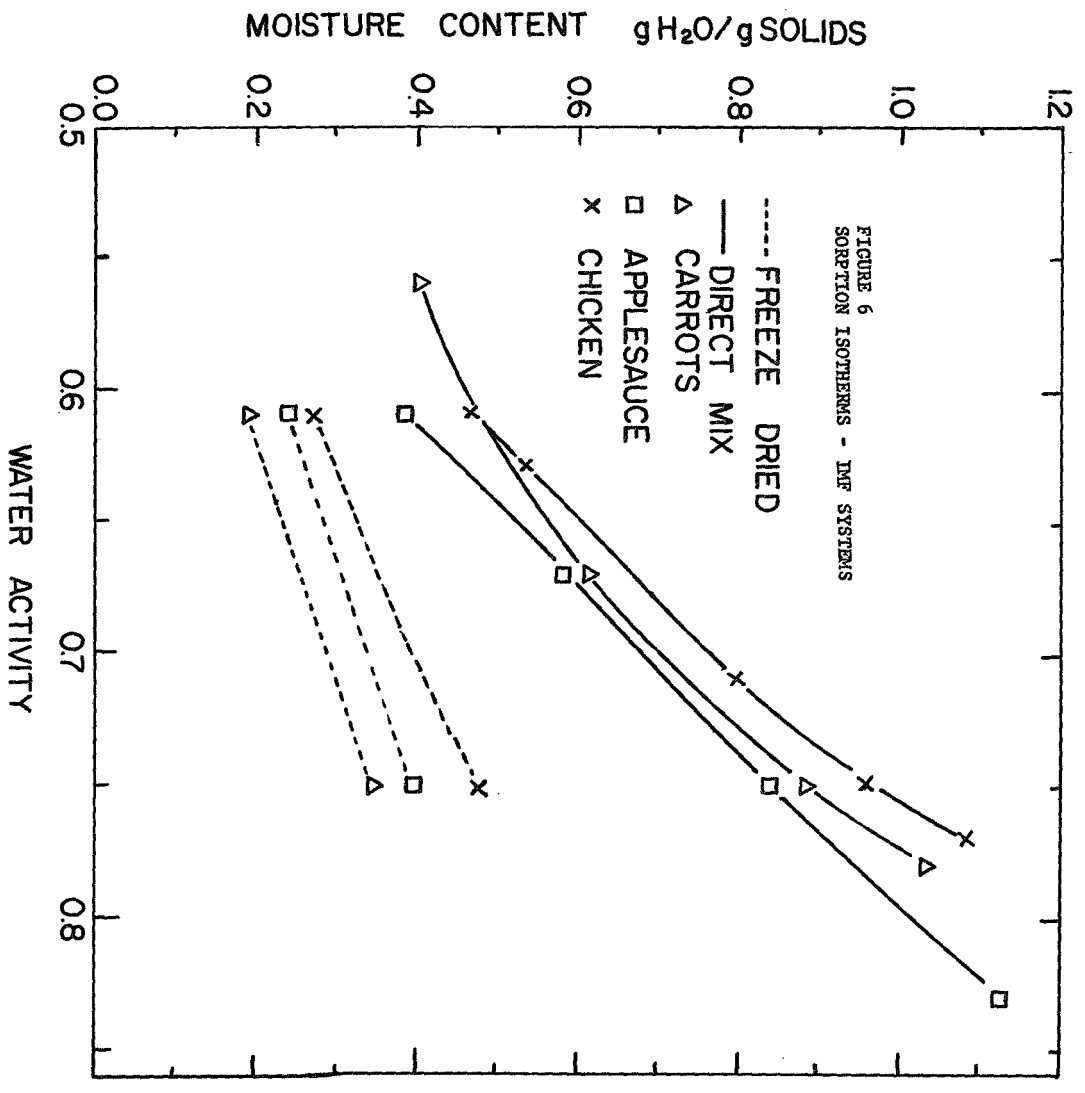
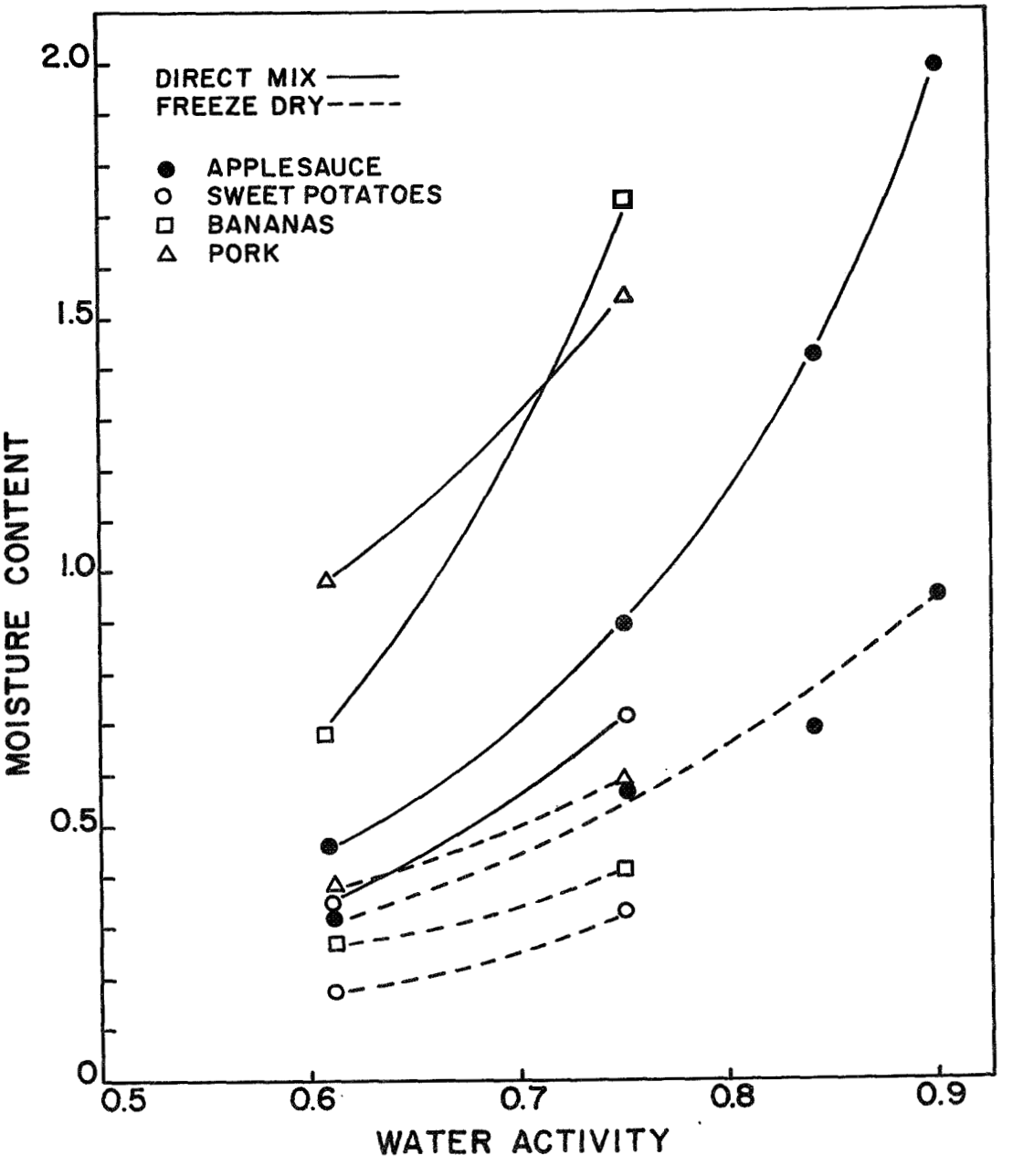


FIGURE 6
SORPTION ISOTHERMS - IMF SYSTEMS

FIGURE 7 SORPTION ISOTHERMS - IMF SYSTEMS



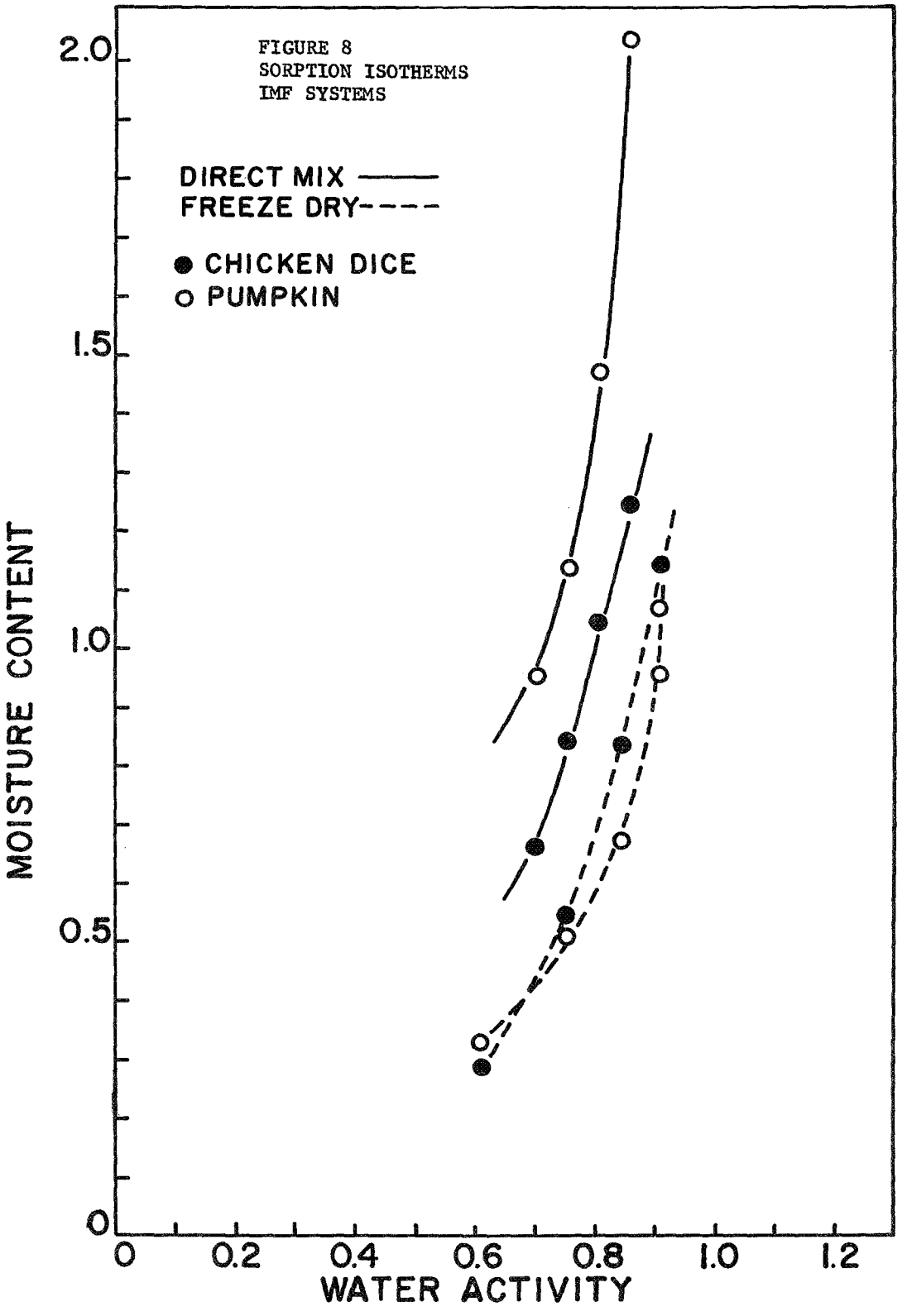


FIGURE 8
SORPTION ISOTHERMS
IMF SYSTEMS
DIRECT MIX
FREEZE DRY
CHICKEN DICE
PUMPKIN
MOISTURE CONTENT
2.0
1.5
1.0
0.5
0
0
0.2
0.4
0.6
0.8
1.0
1.2
WATER ACTIVITY

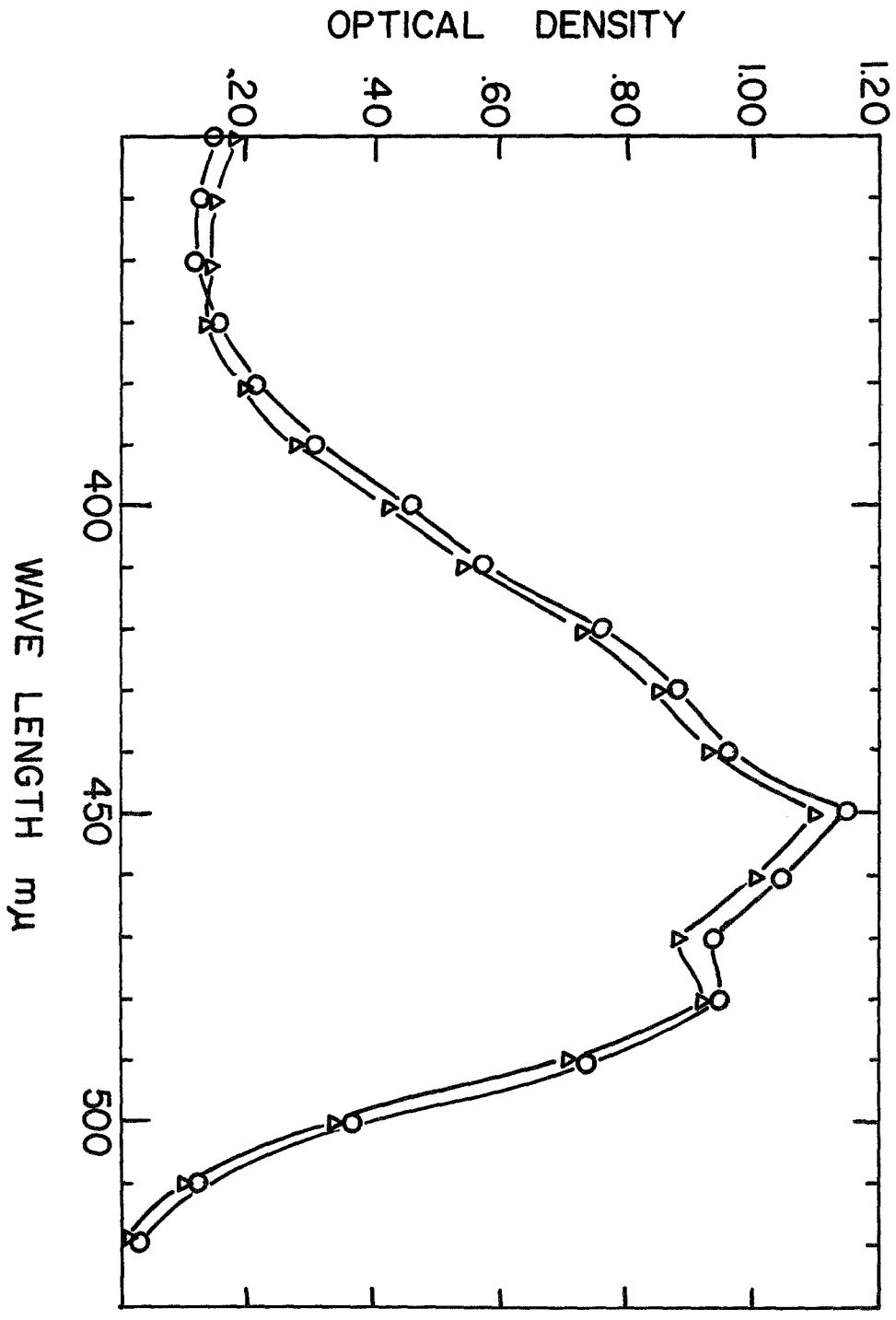


FIGURE 9
CAROTENOID ABSORPTION: SWEET POTATOES

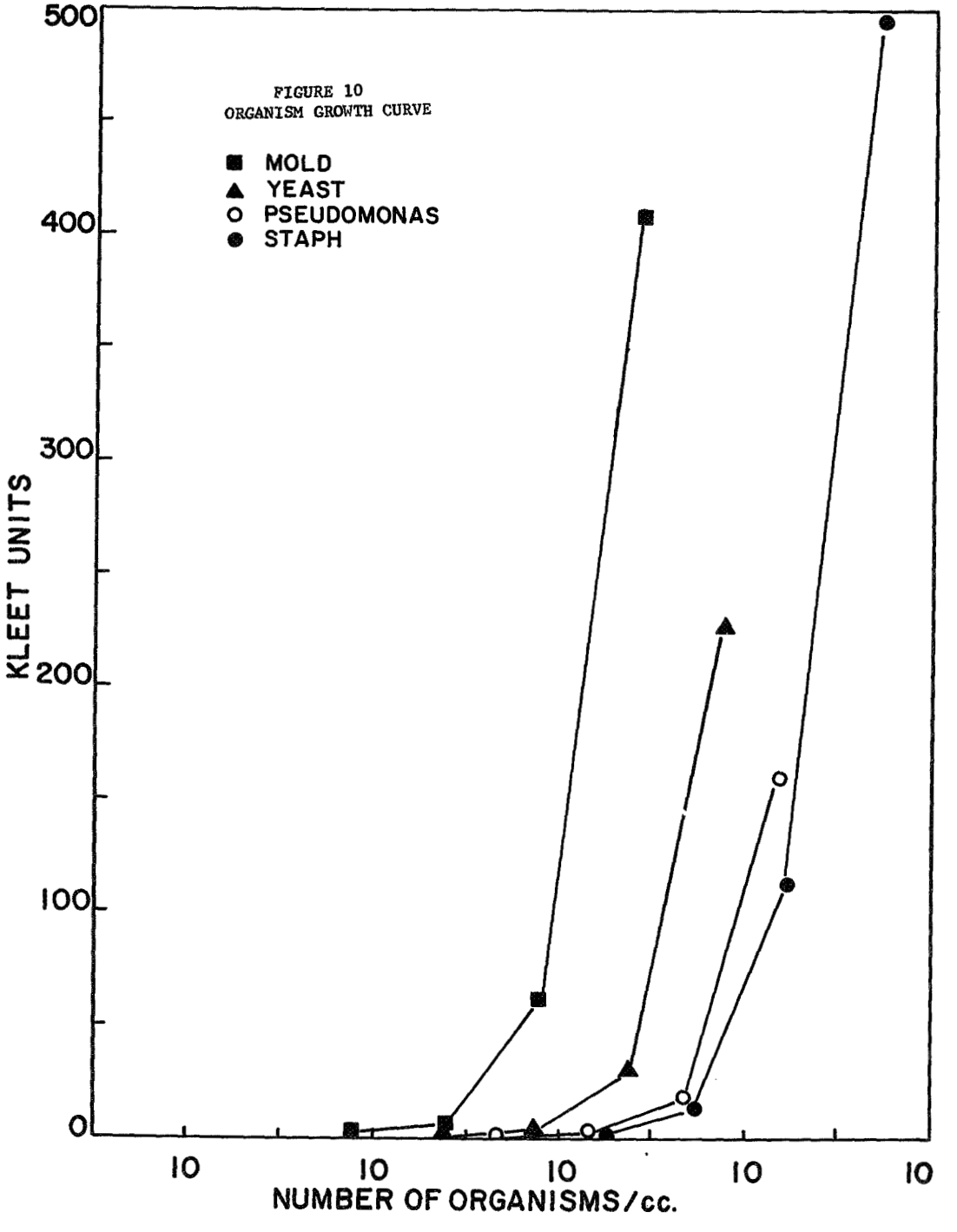


FIGURE 10
ORGANISM GROWTH CURVE

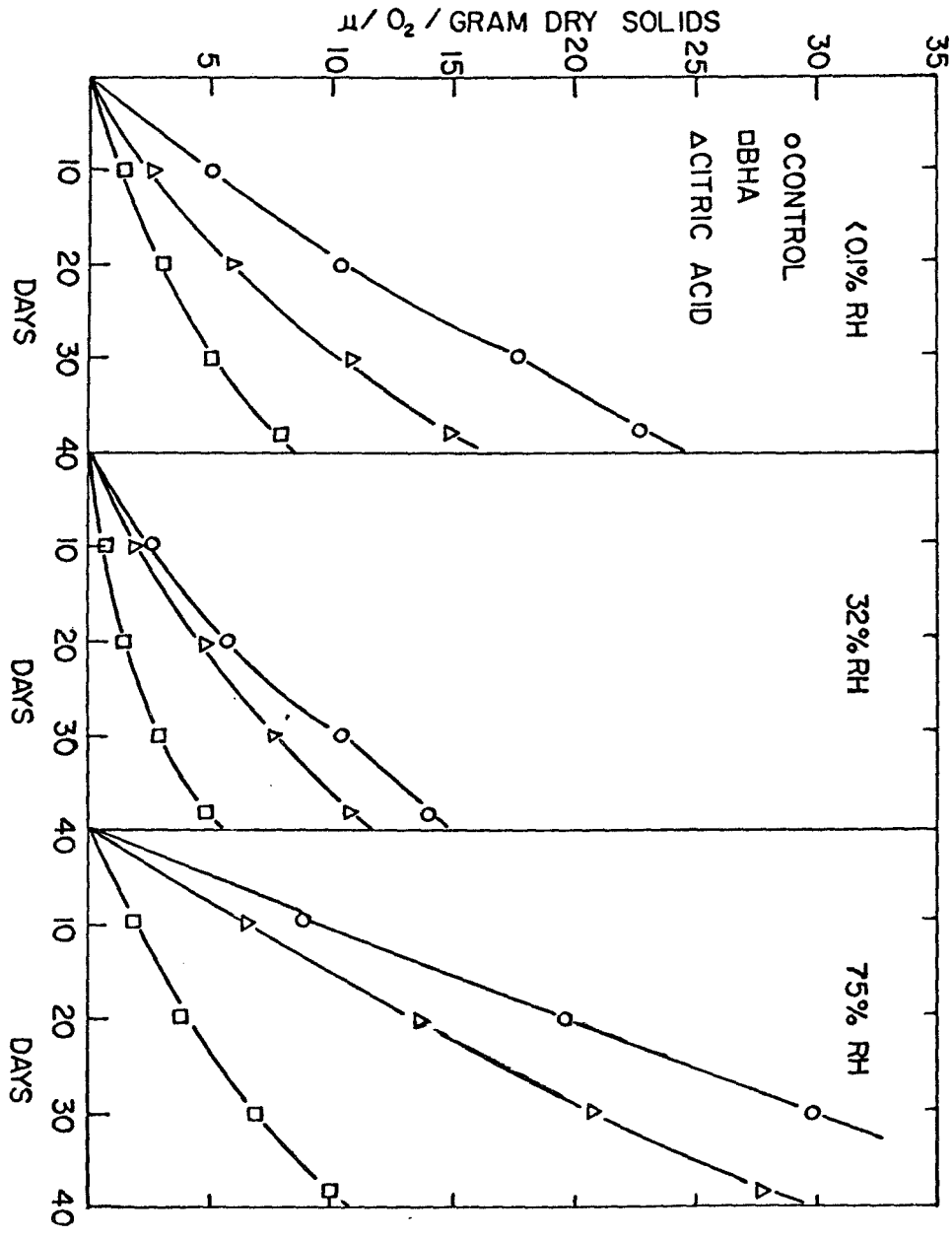


FIGURE 11 RUN 2 OXYGEN ABSORPTION FREEZE DRIED CHICKEN

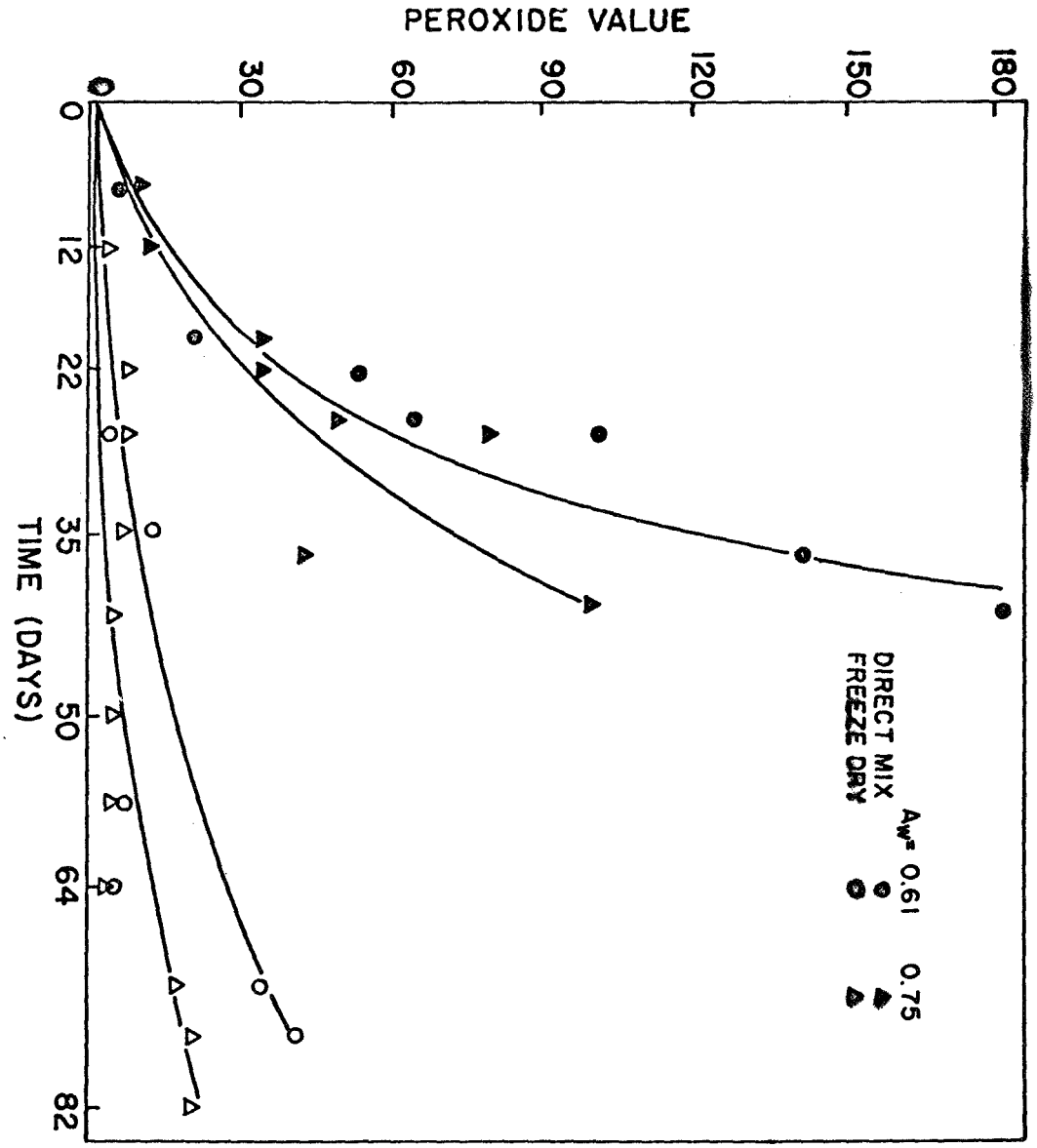


FIGURE 12 RUN 4 PEROXIDE VALUES IMF CHICKEN SYSTEMS

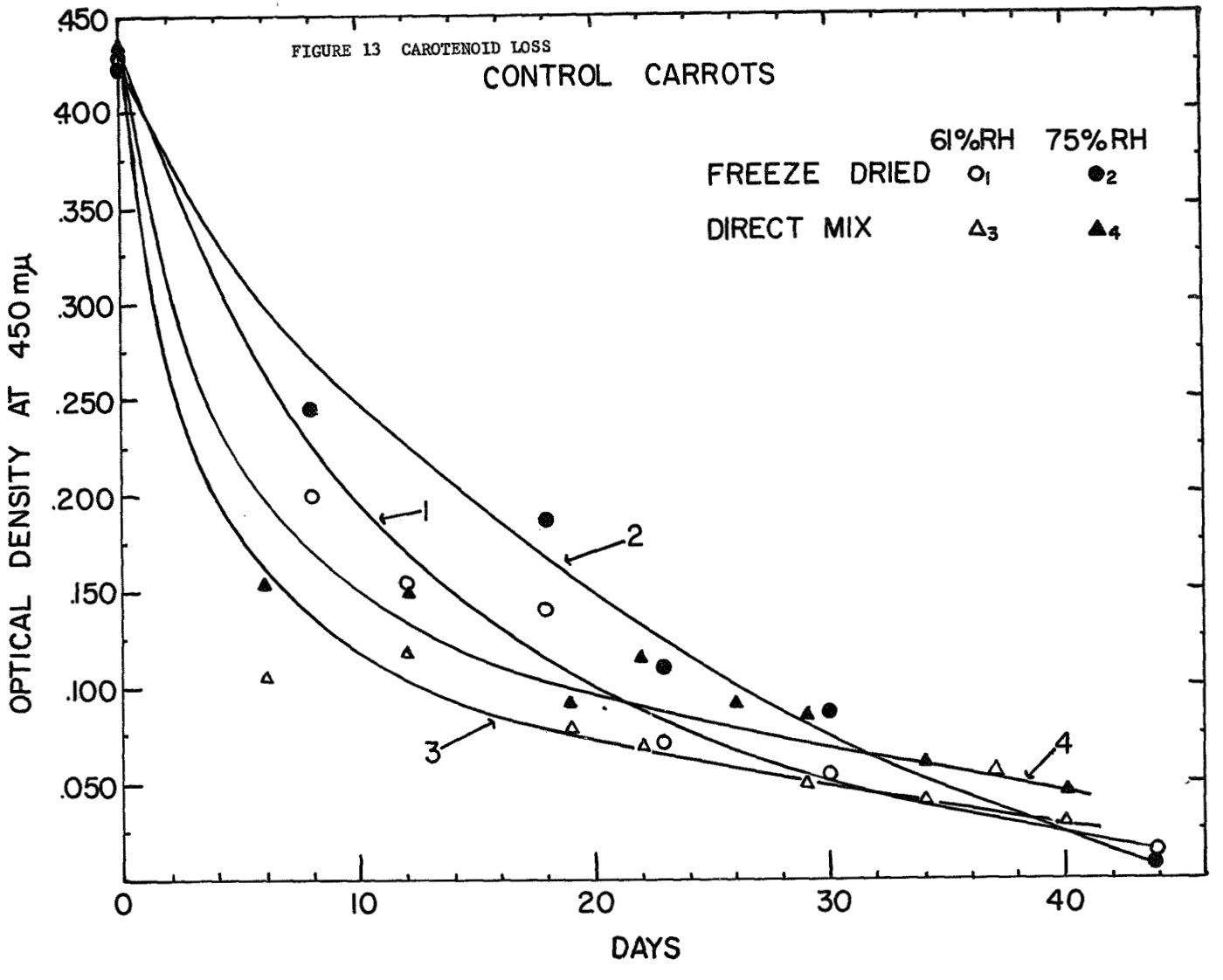


FIGURE 13 CAROTENOID LOSS

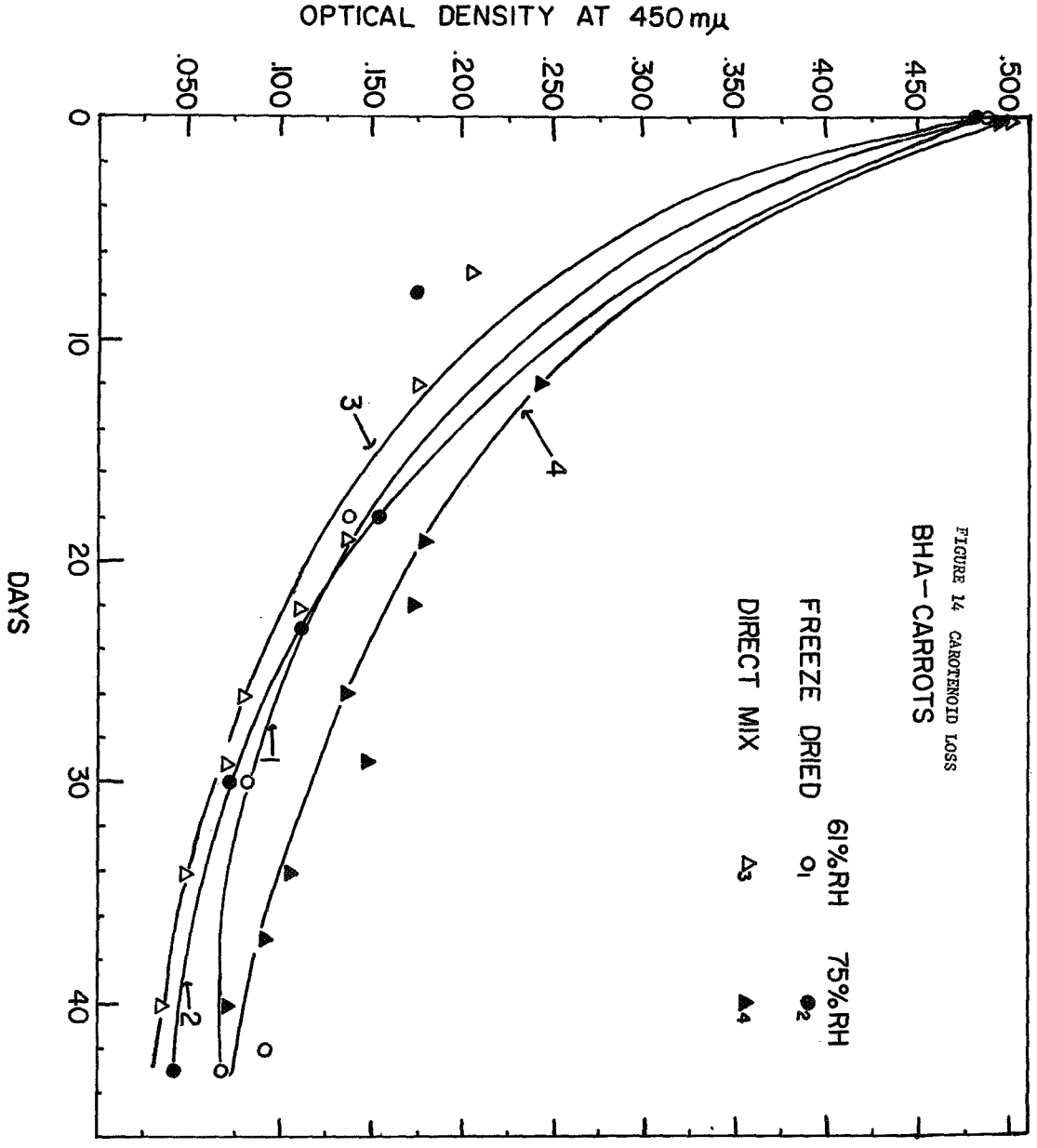


FIGURE 14 CAROTENOID LOSS

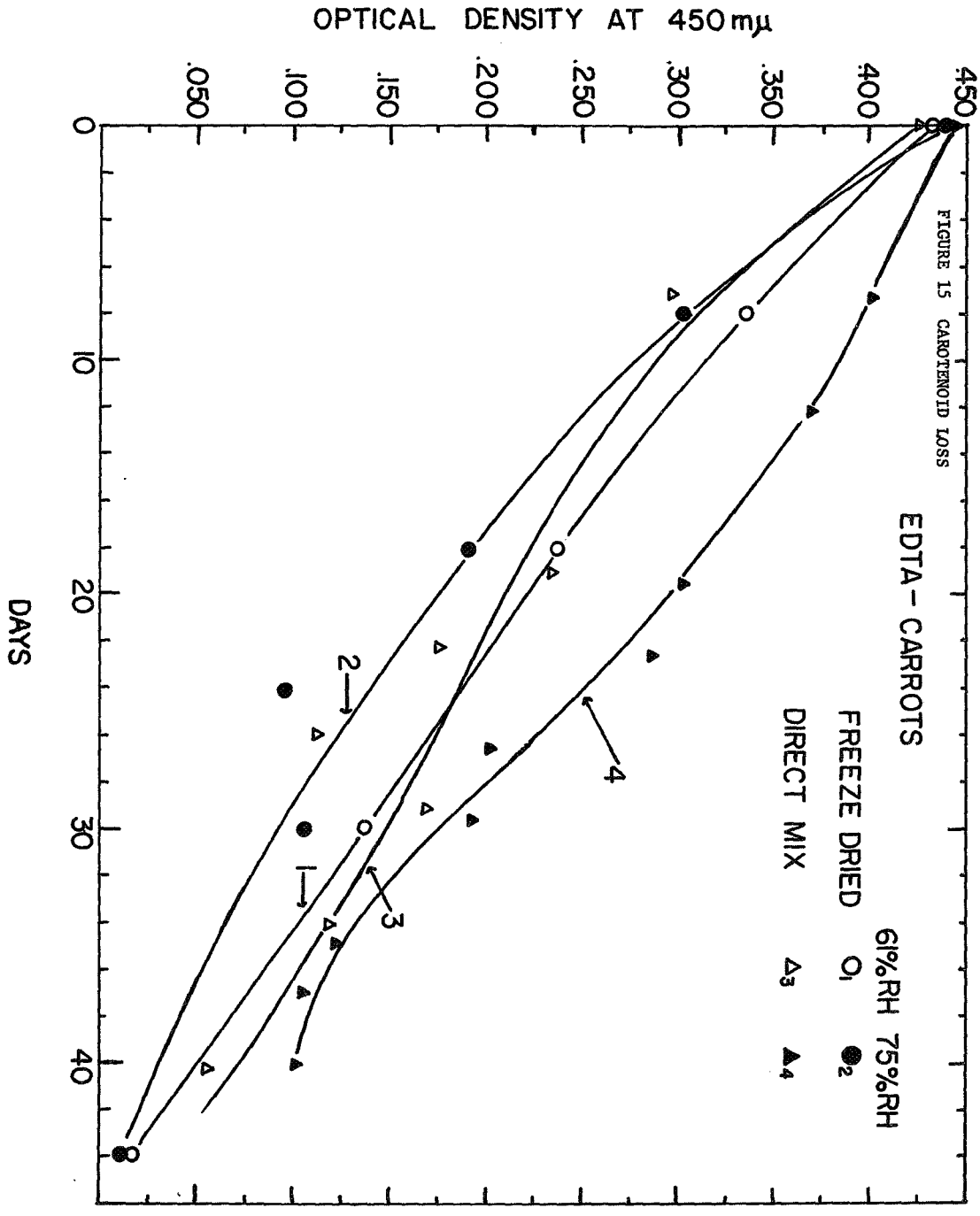


FIGURE 15 CAROTENOID LOSS

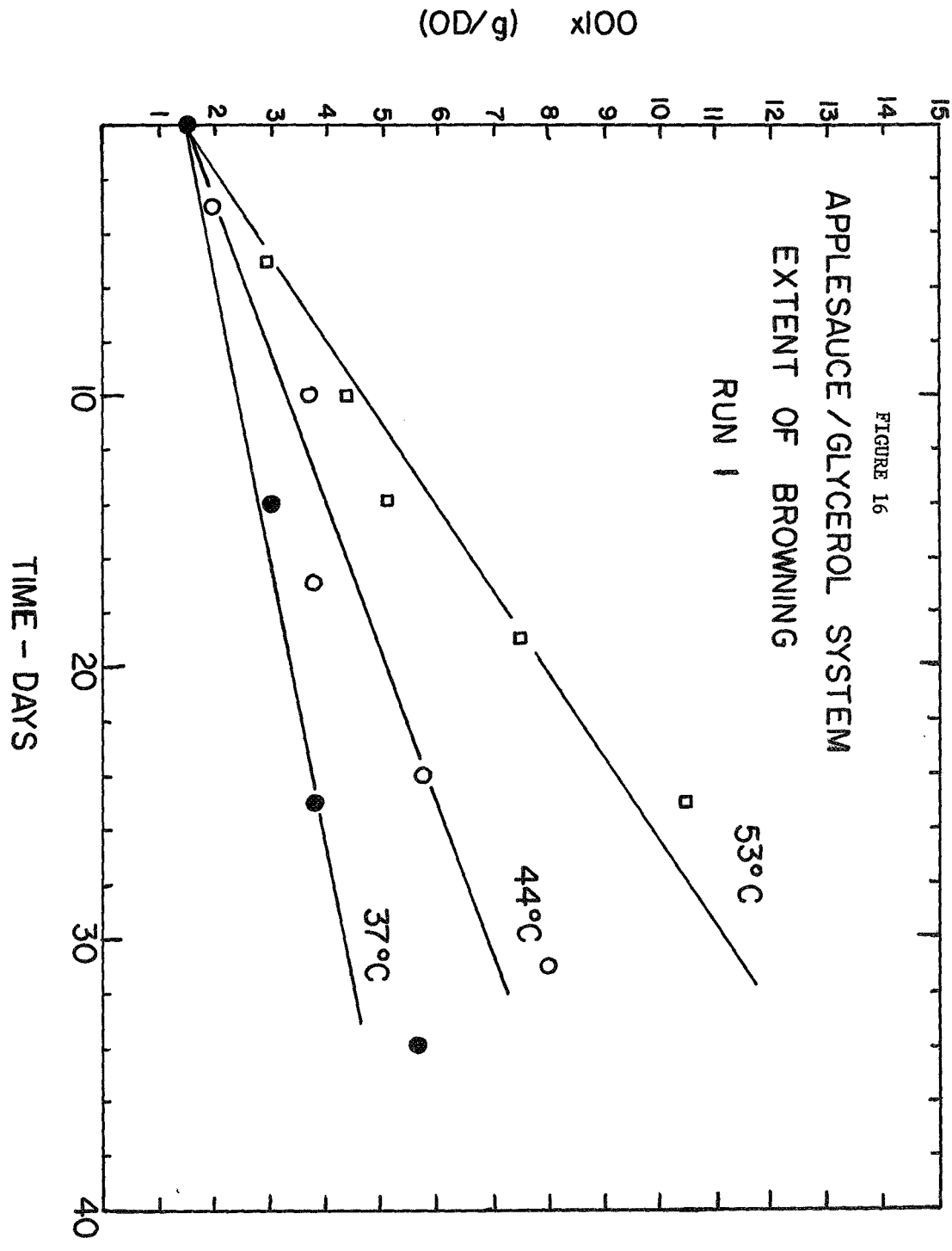

(OD/g) x100
APPLESAUCE / GLYCEROL SYSTEM
EXTENT OF BROWNING
RUN I
53°C
44°C
37°C
TIME - DAYS

FIGURE 16

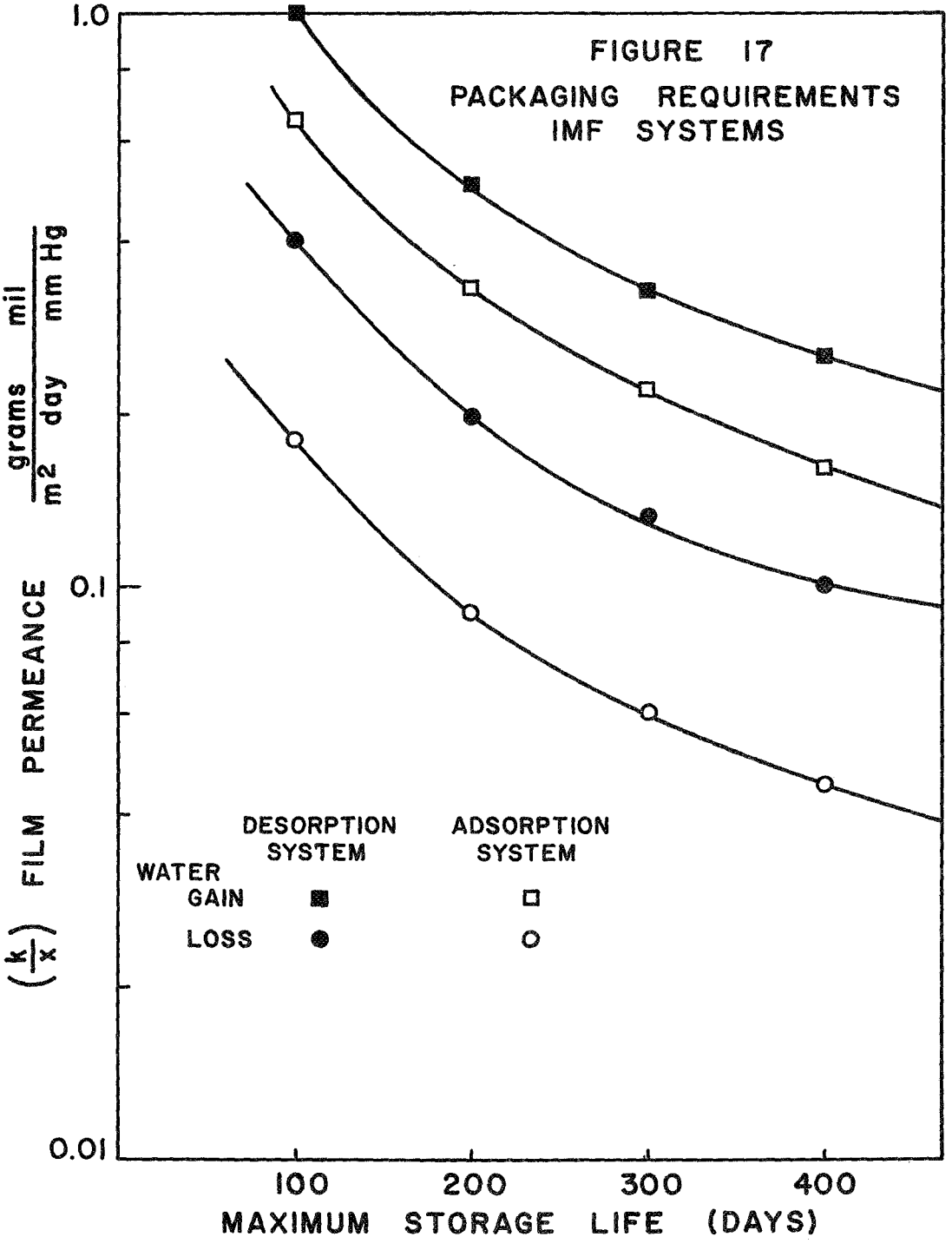


FIGURE 17
PACKAGING REQUIREMENTS
IMF SYSTEMS
1.0
0.1
0.01
100
200
300
400
MAXIMUM STORAGE LIFE (DAYS)
(k/x) FILM PERMEANCE grams / m2 day mm Hg mil
DESORPTION SYSTEM
ADSORPTION SYSTEM
WATER
GAIN
LOSS