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MECHANISMS OF DETERIORATION OF INTERMEDIATE MOISTURE FOOD SYSTEMS

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16. Abstract The use of intermediate moisture foods has become of increased interest in the past few years. These foods are fabricated so that they can be shelf-stable without canning, refrigeration, or freezing, by maintaining the water activity below a level where microorganisms can grow through the use of added humectants. Because of this, the chemical environment can be inducive to increased rates of deteriorative reactions including lipid oxidation and non-enzymatic browning. In order to determine means of preventing these reactions, model systems were developed having the same water activity content relationship of intermediate moisture foods. Models were based on a cellulose-lipid and protein-lipid system with glycerol added as the humectant. Experiments with both systems indicate that lipid oxidation is promoted significantly in the intermediate moisture range. The effect appeared to be related to increased mobility of either reactants or catalysts, since when the amount of water in the system reached a level where capillary condensation occurred and thus "free" water was present, the rates of oxidation increased. With added glycerol, which is water soluble and thus increases the amount of mobile phase, the increase in oxidation rate occurs at a lower relative humidity. The rates of oxidation were maximized at 61% RH and decreased again at 75% RH probably due to dilution. This will be important in the design of stable food items. No significant non-enzymatic browning occurred in the protein-lipid systems. Prevention of oxidation by the use of metal chelating agents was enhanced in the cellulose system, whereas, with protein present, the lipid soluble chain terminating antioxidants (such as BHA) worked equally as well. Preliminary studies of foods adjusted to the intermediate moisture range bear out the results of oxidation in model systems.

It can be concluded that for most fat containing intermediate moisture foods, rancidity will be the reaction most limiting stability. By the use of a combination of EDTA ($\sim$ 200 ppm on solids basis) and BHA (200 ppm on lipid basis) these foods should be much more shelf stable. It is likely that if protected from oxidative rancidity, the intermediate moisture foods may deteriorate due to non-enzymatic browning, however, this may not occur until after the normal shelf life. Experimental evidence for this in foods has not been obtained.

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Summary

The use of intermediate moisture foods has become of increased interest in the past few years. These foods are fabricated so that they can be shelf-stable without canning, refrigeration, or freezing, by maintaining the water activity below a level where microorganisms can grow through the use of added humectants. Because of this, the chemical environment can be inducive to increased rates of deteriorative reactions including lipid oxidation and non-enzymatic browning. In order to determine means of preventing these reactions, model systems were developed having the same water activity-water content relationship of intermediate moisture foods. Models were based on a cellulose-lipid and protein-lipid system with glycerol added as the humectant. Experiments with both systems indicate that lipid oxidation is promoted significantly in the intermediate moisture range. The effect appeared to be related to increased mobility of either reactants or catalysts, since when the amount of water in the system reached a level where capillary condensation occured and thus "free" water was present, the rates of oxidation increased. With added glycerol, which is water soluble and thus increases the amount of mobile phase, the increase in oxidation rate occurs at a lower relative humidity. The rates of oxidation were maximized at 61% RH and decreased again at 75% RH probably due to dilution. This will be important in the design of stable food items. No significant non-enzymatic browning occured in the protein-lipid systems. Prevention of oxidation by the use of metal chelating agents was enhanced in the cellulose system, whereas, with protein present, the lipid soluble chain terminating antioxidants (such as BHA) worked

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MECHANISMS OF DETERIORATION OF "INTERMEDIATE MOISTURE FOOD SYSTEMS"

I. Introduction and Literature Survey

A. Purpose of Study

The role of water activity in the control and mechanisms of deterioration of dehydrated food systems has been of prime importance ever since the beginning of World War II, when the need for unrefrigerated stable rations increased immensely. Since that time, foods of depressed water activity have become of increased significance. Examples of this application of depressed water activity in foods are: a) in the home and institutional markets where convenient quick meal preparation was desired; b) in modern military food systems in which the soldier may prepare a hot meal out in the field simply by adding hot water to a flexible pouch containing the food; c) in space flight where conservation of water has been critical to the mission because of thrust weight limitations; and d) in future long duration space flights in which recycling of water will be available.

It can be stated that with a water activity of one, most unprocessed foods spoil rapidly due to enzymatic-biochemical reactions or through microbiological attack. The basis of food processing is for the insurance of the safety of a food for later consumption by creating conditions under which these degradative reactions do not occur. Heat processing destroys the destructive microorganisms and enzymes but requires packaging designed to prevent any subsequent contamination. Refrigeration or freezing lowers the reaction rates, but requires the need for

cooling capacity. Development of dehydrated foods eliminated the cooling capacity need and reduced packaging weights. Disadvantages of most dry foods are that they must be rehydrated to be eaten and that their texture deteriorates during storage. By processing of foods to bring them to the intermediate moisture range, they have the advantage that the food contains enough water so that it possesses a soft texture and can be eaten directly, but is still low enough in water activity so that refrigeration is not necessary to prevent microbiological growth. No studies in the past have examined the chemical deteriorative reactions occuring in the intermediate moisture range of foods. This study was designed to investigate model systems of intermediate moisture content according to methods developed for dehydrated foods. With these results, a better design of intermediate moisture foods could be made.

B. Reactions in Dehydrated Foods

1. Oxidative rancidity

The major problems associated with dehydrated foods at the low water activities are usually chemical reactions which limit their stability. At low water activities (< 0.01 to 0.6), the reaction most associated with deterioration of dehydrated food products is lipid oxidation with production of undesirable flavors and odors (Karel, 1963). This reaction not only makes the food organoleptically undesirable, but results in products which may interact with proteins (Carpenter *et al*., 1962; Toyomizu *et al*., 1963; Desai and Tappel, 1963) and vitamins. This results in decreased nutritional value and may include the formation of toxic compounds (Kaunitz, 1962; Kummerow, 1962; Baker and Wilson, 1966).

The mechanism of the interaction of water and lipid oxidation has been studied extensively both in model systems (Maloney *et al*., 1966; Labuza *et al*., 1966; Karel *et al*., 1966; Karel *et al*., 1967; Karel and Labuza, 1968a) and in foods (Labuza and Martinez, 1968; Karel and Labuza, 1967; Karel and Labuza, 1968b). It has been found that the following factors are of importance in the control of the reaction:

1) Water acts in purified systems, as well as in foods, as an antioxidant by interfering with the normal bimolecular decomposition of the peroxides produced during oxidation.

As seen in Figure 1 (from Maloney *et al*., 1966), as the water activity and thus the water content is

increased, the extent of oxidation of methyl linoleate deposited on micro-crystalline cellulose is decreased. In this model system at 22% RH, the hydrophillic sites have one molecule of water each attached to them according to the BET monolayer concept. It can be seen from Fig. 1 that the inhibition of oxidation can occur above and below this monolayer. In Figure 2 (Labuza and Martinez, 1968) is presented the data for the extent of oxidation of salmon held in air at 37° C and different relative humidities (same conditions as for Fig. 1). The results indicate the same trend as in the linoleate-cellulose model system verifying the mechanism proposed for water inhibition.

2) Water has an additional inhibiting effect through hydration of the trace metals present, rendering them ineffective as catalysts. The results in Figure 3 for the cobalt catalyzed oxidation of methyl linoleate deposited on micro-crystalline cellulose illustrate this phenomenon (Labuza _et al._, 1969).

3) Water acts by rapidly destroying the free radicals produced in the drying operation. Munday _et al._ (1962) has shown that there exists about 10^{15} radicals/g solids in freeze-dried foods with the amount being proportional to the fat content. These radicals can rapidly react and cause deleterious reaction such as loss in texture or color. Simatos (1966) and Rockland (1969) have shown that humidification of the dried food rapidly decreases the radical content probably due to recombination reactions.

4) In systems containing trace metals of limited solubility and at high water contents (water activity = a_w = 0.6 to 0.95) water may have an accelerating effect on the oxidation rate. This is probably due to solubilization or increased mobility of the catalysts (Karel, 1960; Tjhio, 1968). This poses a serious problem in the storage stability of foods maintained at these water activities. Figure 4 shows that for the same model systems as presented in Figure 3, when the moisture content is increased to the level where capillary condensation occurs, the inhibitory effect of water disappears at 75% RH (Yeh, 1969) with an acceleration over rates at lower moisture contents.

5) Water has an important effect on the reaction between oxidation products and proteins. At low water activity the protein is degraded into lower molecular weight fragments while at higher water activity aggregation reactions predominate (Zirlin and Karel, 1969; Takahashi, 1970).

Antioxidants are affected by the presence of water in that those which are water soluble show increased antioxidant activity as the water activity increases. Antioxidants which are non-polar work more effectively at moisture contents below the monolayer (Karel and Labuza, 1967). In addition, certain complexes of amino acids and trace metals become effective antioxidants at increased water activity (Tjhio et al., 1969).

The findings of these above studies have elucidated the mechanism of the interaction of water and oxidizing lipids which allow useful and meaningful predictions to be made as to the stability of dehydrated foods (Labuza *et al.*, 1970).

2. Non-enzymatic browning

Foods held at higher water activities (a_w = 0.2 to 0.85) deteriorate through additional reactions, the major one involving the interaction of amino groups and carbonyl compounds. This non-enzymatic browning mechanism results in loss of texture due to reduced solubility, formation of dark pigments, the production of off-flavors and odors, and loss in nutritional value. The complex nature of this reaction (Maillard Reaction) has been extensively reviewed by Hodge (1953), and Ellis (1959).

In general, the rate of browning increases as the moisture content or water activity increases up to about 0.5 to 0.6 and then falls again due to dilution of the reaction components. This has been observed both in model systems (Lea, 1958) as well as in dehydrated food systems (Karel and Labuza, 1968b; Labuza *et al.*, 1970). Figure 5 illustrates the effect of water activity on the rate of browning of a freeze-dehydrated pea soup mixture as done in the latter study. In general, the reaction proceeds only when enough water becomes available, i.e., above the monolayer adsorption region where multilayer formation and capillary condensation occurs. Karel and Labuza (1968a) have shown browning to occur in sucrose-citric acid-egg albumin systems

in the multilayer region. These results show the importance of the control of water content on activity in the reaction. It was found that the acid present was enough to hydrolyse the sucrose into reducing sugars. Regier and Tappel (1956), and Thomson et al. (1962) have shown browning to be the major reaction limiting stability of freeze-dried beef, even down to low moisture contents supporting the above work.

A complicating factor in the prediction of stability of intermediate moisture and dehydrated foods is the interaction that occurs between lipid oxidation and non-enzymatic browning mechanisms. Products of lipid oxidation such as the aldehydes and ketones having a carbonyl group, can initiate browning especially in fish (Tarr and Gadd, 1965). In addition, certain end products of browning have antioxidant properties slowing down lipid oxidation (Lea, 1958). Martinez and Labuza (1968) showed that these interactions occurred during storage of freeze-dried salmon at 37° C. In that study, the kinetics of the reactions were determined as a function of water activity, and it was shown that lipid oxidation could initiate browning. Leroux (1968) studied this problem extensively in model systems containing methyl linoleate and casein. It was shown that at high water activities (85-95% RH), oxidation occurred very rapidly and browning was extensive. Part of the brown pigment also became bound to the protein, although most of the brown pigments were present in the lipid phase. This, however, made the determination of total browning difficult.

3. Enzymatic degradation

Enzyme activity at low water content or water activity can also occur in dehydrated foods. Acker (1969) has extensively reviewed this area. Figure 6 shows a typical result in which the rate and extent of a hydrolytic enzymatic reaction increased as moisture content or relative humidity increased. It can be seen that some activity occurs even down to the monolayer value for water adsorption and that the rate is fastest in the intermediate moisture range. This is due to better solubility of the enzymes in the food capillaries. In terms of processing of actual intermediate moisture foods, blanching to destroy enzyme activity, before dehydration or infusion of the humectant solution will be imperative to insure stability.

4. Microbiological deterioration

In addition to the reactions discussed above which lead to deterioration of dried foods, microbiological deterioration can also occur. Bacteria, molds, and yeast each have their own specific water activity below which growth cannot occur. In most cases, foods with equilibrium relative humidities below 70-75% RH (% RH = 100 a_w, where a_w = equilibrium water activity of the food) are stable to microorganism destruction since not enough water is available for their growth. Scott (1957) and Christian (1963) have reviewed this area extensively, and Mossel and Ingram (1955) have suggested guidelines for lower limits of water activity, especially in terms of microbes which might be a public health hazard for

dehydrated foods. Table 1 lists the water activity below which microorganism growth is minimized (Scott, 1957; Mossel and Ingram, 1955).

C. Intermediate Moisture Foods

Dehydrated foods are prepared in such a manner as to eliminate the above mentioned deteriorative reactions. Normally, foods are dried to low moisture contents (2-5% water) so that either oxidation or browning is the only limiting factor for stability with enzymatic and microbiological decay prevented. However, with "intermediate moisture foods" all above mechanisms of deterioration may be operative. Foods of this class include such items as dried sausage, soft candies, and dried fruits such as dates and figs. The "intermediate moisture foods" usually range from 15 to 30% water on a dry solids basis, with water activities between 0.6 to 0.85. Under these conditions, enough water is present for non-enzymatic browning to occur, and possibly lipid oxidation if metal catalyst solubility and mobility is increased, as was discussed previously. Microbial growth can occur, but is prevented, especially in the foods with water activities greater than 0.75 by the use of inhibitors such as sorbic acid or potassium sorbate. Enzymatic activity will occur at a maximum rate unless the food is blanched to destroy the enzymes.

The impact of "intermediate moisture foods" on commercial processing is only in its infancy. These items are attractive

because they present a plastic feel and taste without the sensation of dryness. In terms of the present market for new food items of this category, the pet food industry has played the major role (Anon., 1965). The typical items, such as Ken-L-Ration Special Cuts (U. S. Patent 3,380,382, April 1968), are in the range of water activity of 0.70 to 0.90 having a moisture content of 20 to 40%. In order to maintain the desired water activity, the major component, other than meat and cereal, is some form of sugar such as sucrose or dextrose. These carbohydrates allow for more water adsorption than protein because of the larger number of hydrogen bonding groups per unit of chain length. Sorbic acid or potassium sorbate is added to inhibit any mold growth since molds may grow down to an $a_w = 0.8$. Propylene glycol is also added to contribute both to water adsorption and to the forming of the desired plastic texture. This type of product eliminates the need for refrigeration although it contains meat and can have the texture of an actual meat piece. Because it already contains the necessary water, there is no rehydration problem associated with all dried foods of this type.

The attractiveness of an item of this type has led to the initial development of several items, including meats, vegetables and fruit for aerospace food systems (Hollis *et al.*, 1968). In these items, the water content is controlled independently of the water activity by the use of humectants such as glycerol or their glycols. Several

techniques are used to produce these foods. In general, the food is freeze-dried and then soaked in glycerol, followed by humidification to the desired water content; or the food is cooked in a glycerol solution which displaces the desired amount of water. The latter process produces an inferior product. Other methods are used especially for the pet foods, e.g., comminuting all the ingredients together with enough added dry binding agents, such as milk solids and soy bean flakes, to reduce the moisture content.

This latter method is used also for the fruit paste filled breakfast pastries of the toaster variety currently on the consumer market. As with pet foods, these items use various sugars as humectants and sorbate salts for mold inhibition. They generally have a very sweet taste because of the sugar content. Glycerol can be substituted for a large part of the sugar (Hollis _et al_., 1968) which would reduce this high sugar taste, but may lead to other undesirable reactions as well as impart a bitter metallic taste. The advantage of using either glycerol or other glycols is their high hygroscopicity and their better plasticizing nature as compared with dextrose or other sugars. In addition, they can be used directly as a liquid instead of an aqueous solution, as with the sugars. The only factor presently prohibiting their use on a larger basis is their much higher cost than sugars.

In general, there has been no specific work done on the stability of "intermediate moisture foods", but it is

recognized that they present an environment suitable for deterioration (Labuza et al., 1970; Hollis et al., 1968). Microbial destruction is prevented by the addition of growth inhibitors and the product is blanched or cooked to destroy enzymes. The use of packaging materials to prevent moisture gain above the critical water activity for microbial growth is also imperative. The prevention of browning and lipid oxidation has, however, not been solved. It is the purpose of this study to investigate in model systems, as well as in actual food items intended for use in space feeding, the role and mechanism of deterioration through lipid oxidation and browning. It is further intended to use the results to predict methods of prevention of the above reactions.

This final report presents the methods developed to prepare the model systems for intermediate moisture contents and, in addition, results are presented on the effect of water content in the intermediate moisture range on the extent of oxidation and nonenzymatic browning of these systems.

II. <u>Methods</u>

A. Model Systems

1. Composition of glycerol systems

In order to understand the complex reactions that occur in intermediate moisture foods, model systems resembling foods were utilized. Two basic systems were used, one with the protein, casein, as the base, and one with microcrystalline cellulose as the base. This would correspond to a meat type food and a fruit or vegetable type food respectively.

Other proteins were not used because of the length of time required to get oxidation data in the presence of protein and the fact that previous information with casein showed that oxidation-browning interactions could be studied easily with this system (Leroux and Tannenbaum, 1969).

For study of lipid oxidation, an unsaturated fatty acid ester, methyl linoleate, was mixed in with the model. This fatty acid is very common in foods and its kinetics of oxidation are easy to follow (Maloney _et al._, 1966). Other lipids such as oleate which oxidizes very slowly, or Apeizon B oil (a high molecular weight hydrocarbon) could have been used, but these oxidize so slowly that useful data could not be obtained in a reasonable time span. In order to attain the 20-30% moisture content needed, glycerol was chosen as the major humectant. The reasons for the use of glycerol are that it is a polyhydric alcohol which is infinitely soluble in water and acts as a strong water binding agent giving an isotherm characteristic of intermediate moisture foods. In terms of space flight, glycerol is a major product that can be chemically formed from reclaimed CO_2 and water and may be practicle for

long range missions. It is available in highly pure form and is generally recognized as a safe (GRAS) food additive. Various levels were tested in order to determine the concentration needed to give the desired moisture content. The quantities of glycerol necessary to prepare the three model systems according to the procedures following are shown in Table 2.

All storage studies used those models with the concentration of glycerol giving the desired water activity of 0.6 to 0.85 and 15-30% moisture content. In this way using a constant glycerol content, water content could be varied by humidification at a different % RH. This allows for a better understanding of the different reactions. The effect of additives on the stabilities of the systems can be easily tested since they can be added either dissolved in the preparation water, the glycerol or the fatty acid if they are lipid soluble. Iron as myoglobin and cobalt as the nitrate salt were used as metal catalysts. Table 4 lists the various antioxidants used in this study and their approved levels of additions. Amino acids can also be used as antioxidants, but their cost is not worth the protection they afford. Karel et al. (1966) have shown that they act mainly as chelating agents and that EDTA has much more effectiveness.

2. Composition of special systems

Four experiments were performed using humectants other than glycerol to control water content and water activity. These included sucrose, sorbitol and propylene glycol all added to the basic 6:1 cellulose:methyl linoleate model system. Table 3 contains the compositions used for each system. Runs 10, 11 and 12 were made by freeze-drying, while Run 13 was prepared by a direct mix method

as will be described. The humectants were added at the 30% level based on dry solids including lipid except for Run 13 where propylene glycol was added at the 25% level.

3. Chemical suppliers

a. Microcrystalline cellulose (Avicel) - American Viscose Corporation, Maryland.

b. Casein (Hammersten) - Nutritional Biochemicals, Cleveland, Ohio.

c. Glycerol, propylene glycol, sorbitol (Spectro grade) - Eastman Organic Chemicals.

d. Methyl linoleate - Hormel Institute, Austin, Minnesota.

e. Antioxidants BHA, BHT, PG, tocopherol - Eastman Kodak Organics, Rochester, New York.

f. Water - Glass distilled.

g. All other reagents - Laboratory grade - Mallikrodt, St. Louis, Mo.

4. Model system preparation for freeze-drying

a. Protein model systems - The amount of water needed for adequate mixing (Table 1) was added to a 200 ml. cup of a Sorvall Omni-Mixer specially equipped with a screw port on the bottom for extrusion of the sample. The glycerol and methyl linoleate were added into the water in the cup. Casein was then added with slow mixing by hand to avoid losses due to dusting. The cup was then covered with the mixer top and attached to the power unit. The cup was submerged in an ice bath and the mixer was turned on for 10 min. at high speed (20-25,000 rpm). After mixing, the stirrer top and the cap on

the bottom port were removed, the plunger was placed into the cup and desired quantities were extruded into preweighed flasks for subsequent drying and testing. The first 10 grams were always discarded. The flasks containing the desired quantity of the system were covered with a small piece of filter paper over the opening, secured by a rubber band and then frozen in liquid nitrogen for 10-15 mins. These samples were then placed in a VIRTIS laboratory freeze-drier and were dried at room temperature for 48 hours @ 50-100 μHg. After freeze-drying, the samples were humidified according to the procedure described subsequently.

b. Cellulose model systems - The same method as for the protein system was used with the following method of addition of components into the cup: Glycerol and water containing any additives such as cobalt catalyst or buffer; stir by hand; methyl linoleate; hand stirred; cellulose; and 30 min. blending at high speed in the mixer. After some trouble with the initial runs in getting a smooth and consistent paste, as well as reproducible oxidation results, the sequence of addition was modified as follows: cellulose; methyl linoleate; stirred by hand into uniform paste; water solution containing all other components such as glycerol and cobalt catalyst; 20 min. blending at high speed in the mixer.

5. Humidification of model systems

a. Humidification - Upon removal from the freeze-drier, the samples were humidified in vacuum desiccators at 37° C to the desired water activity by using saturated salt solutions. The salts necessary to achieve these water activities are shown in Table 5. The procedure involves removing the filter paper cover from the sample, placing it in the desiccator, pulling a vacuum until the salt solution boils, leaving the sample in the desiccator under vacuum for 12-24 hrs., then breaking the vacuum and removing the samples for subsequent storage and analysis.

b. Moisture isotherms - An isotherm at 37° C was measured for each model, humidifying the samples for 12 to 24 hrs. The moisture content at each relative humidity was measured either gravimetrically (measuring on an analytical balance the increase in weight after humidification) or by gas chromatography when the instrument was available. The latter procedure gives results that are comparable to the gravimetric method (Karel and Labuza, 1967) and is as follows: Twenty ml. of dry MeOH were added to the sample after removal from humidification (usually a 50 ml. Erlenmeyer flask was employed). The flask was covered with a rubber serum cap and shaken at room temperature (rotary shaker) for 1/2 hr.

A sample of 5-100 μl. is taken from the supernatant using a calibrated microliter syringe and injected into a Perkin-Elmer Gas Chromatograph at the following conditions:

(1.) Column: 1' x 1/4" diameter copper tubing packed with Poropak Q (Waters Assoc., Framingham, Mass.) @ 180° C.

(2.) Gas: Helium, 10 lbs. pressure, 40 cc/min.

(3.) Detector: Thermal conductivity @ 100° C.

A standard was made using 1 cc H_2O to 49 ml. of the dry methanol. The moisture content was calculated from the ratio of the sample peak area to that of the standard.

6. Direct mixture procedure for Run 13

The amount of water as determined from the cellulose/glycerol linoleate isotherm was mixed together with the propylene glycol and the linoleate in a 250 ml. beaker by hand. The cellulose was then added a little at a time with thorough mixing until uniform. Two gram protions were weighed directly into 50 ml. flasks for subsequent storage and analysis.

B. Preparation of Intermediate Moisture Foods

It was initially assumed that intermediate moisture foods would be obtained from processors as soon as manufactured. In the case of vegetable items, none were currently being made.

For meats, other than sausage products which do not fulfill all the objects because of their higher water activity and bacteriological problem, the only meat products currently made were the dog food items. In analyzing some products and talking to the manufacturer, it was indicated that scrap meat and fairly old fat are used so that even initially the products were quite rancid. Fortunately, many dogs do not object to this product and acceptance of intermediate moisture foods among dogs and cats has been reported to be over 50% (personal communication, J. Schempp, Howes Pet Products). With the fruit products, the items on the market are either jams or the breakfast tarts which have a baked crust on them. In this case, we were advised it would be simpler to make our own. Based on these facts, it was decided to make intermediate moisture foods using baby foods as the base. In this case, a very homogeneous mixture would be started with into which one could mix in all the additives directly and store at the required humidity. In addition, after mixing, the product could be freeze-dried and then humidified. Results of Heidelbaugh (Ph.D. Thesis, M.I.T., 1970) showed this method to be very satisfactory.

1. Chicken freeze-dried food system - Run #21

A batch of Gerber's Baby Food "Chicken and Chicken Broth" was obtained and analyzed showing a moisture content of 77%. The fat content was 9.8% on a wet basis or 43% of the dry solids. To prepare the freeze-dried intermediate moisture food, the following procedure was used:

In a 1 liter beaker, 500 g. chicken was added, to which

enough glycerol was added to be 20% glycerol on a solids basis. This was stirred by a glass rod and the antioxidants (BHA and PG respectively in each system) were added by first dissolving in 1 ml. of ethanol. After thorough mixing, approximately 5-8 g. of wet sample were extruded into tared 3 oz. bottles, which were then weighed. After freezing in LN_2, the samples were freeze-dried and then humidified in the same manner as for the model systems and then stored covered with a rubber serum cap. Moisture content was measured at each humidity using the GLC method. For Run 21 the moisture contents shown in Table 6 were obtained.

2. Carrots freeze-dried system - Run #22

Gerber's Strained Carrots were used having a solids content of 10.5% with less than 0.3% lipid on a wet basis. The samples were prepared in the same manner as the chicken sample using, however, 23.1% glycerol on a solids basis for the test sample and no glycerol for a different control. A sample containing 400 ppm of BHA (on a solids basis) was also prepared. The moisture contents obtained after humidification are shown in Table 6. As can be seen, the addition of glycerol only changes the moisture content slightly, mainly due to the high cellulose content of the carrots. The values at 61% and 75% RH are well within the intermediate moisture range.

3. Chicken-cellulose system: direct mixing - Run #23

In order to eliminate the problem of volatile antioxidant loss during freeze-drying, a method was developed to prepare a chicken mixture in the intermediate moisture range

with at least a 30% water content. In a 500 ml. beaker to 130 g. of chicken (Gerber's baby food) were added 253 g. microcrystalline cellulose and 91 g. of glycerol. This results in a system with 25% glycerol at a moisture content of 33.6 g H_2O/100 g solids. Table 6 shows that the water activity measured for the sample by the method of Karel et al. (1967) was 75% RH, well within the intermediate moisture range.

After preparation, the batch was divided into three parts and samples were prepared with 200 ppm BHA (fat basis) and 200 ppm EDTA (solids basis). Each sample was then transferred to a Waring blender and mixed for 10 minutes. This produced a free flowing granular powder which although containing more water than the freeze-dried system, has a much drier and less sticky appearance. This is due mainly to the addition of the cellulose powder. This powder can then be weighed directly in to the storage flasks. Samples of each treatment were stored at 37°, 45° and 52° C. for determination of activation energy effects.

4. Applesauce food system - Run #24

A fruit type intermediate moisture food was prepared by mixing Mott's sweetened applesauce (24% solids) with various amounts of glycerol to reduce the water activity into the intermediate moisture range. Table 6 shows the humidities obtained vs. the glycerol addition. Because of the extreme sweetness of the mixed systems, the 10% glycerol level was tested in storage. Two gram samples after thorough mixing of the applesauce and glycerol were transferred to 50 ml.

Erlenmeyer flasks, stoppered and placed in storage at 37°, 45° and 52° C. It should be noted that because of the high soluble sugar content, this system contains much more water than other systems (2.7 g H_2O/g solids). This would be suitable for a dessert filling or a pastry filling, but not for direct hand held eating as with the other systems.

C. Oxidation Procedures

1. Manometric determination of oxygen absorption

Samples (in triplicate or duplicate) of the model system were prepared for oxidation studies by extrusion directly into 30 ml. Warburg manometer flasks. These were specially made without the standard side arms and center well. After humidification, 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 flaks 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 7 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:

$$\frac{\mu l\ O_2 \text{ absorbed (STP)}}{\Delta \text{ mm manometer reading}} = k_f = \frac{T_0}{T_1} \times \frac{1}{P_0} \times V \times 10^3$$

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

T_0 = 273° K

T_1 = run temperature, 310° K

P_0 = 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 were 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). The calculations are made using either a PDP-8S DEC computer (Digital Equipment Corporation, Maynard, Mass.) or a Wang 380 programmer (Wang Laboratories, N. Tewksbury, Mass.). Results are presented graphically either as moles or μl oxygen absorbed. In any case, only samples of one batch can be compared to each other in absolute rate terms. However, the trend of effect between treatment batches can be determined from the results.

2. Lipid oxidation kinetics

In order to measure differences between samples and their treatments, the data of oxygen absorbed per

gram of oxidizable lipid (methyl linoleate) can be treated according to the methods of Heidelbaugh, 1969.

Initially, oxidation takes place by monomolecular decomposition of hydroperoxides up to a level of about 0.5 to 1% moles oxygen absorbed per mole of linoleate. Since most foods are considered rancid near the end of this stage of oxidation due to production of volatiles, the time to reach a given extent of oxidation can be used as a comparison between treatments. In this study, the time to reach 1% oxidized on a molar basis was used. In addition, the time to reach 3% oxidized gives a further picture of the accelerated phase of oxidation in terms of the protection antioxidants afford.

3. Peroxide value (A.O.C.S. Method Cd. 8:53)

Samples for peroxide determination were extruded or placed directly into 50 ml. Erlenmeyer flasks during model system preparation. These were dried, humidified and stored at 37° C at the desired relative humidity. At each time interval, a sample was removed after headspace analysis was performed on the flask and an amount containing between 0.1 to 0.25 g lipid was weighed into a 125 ml. Erlenmeyer flask and 110 cc of chloroform:methanol (3:1 v/v), were added; the flasks shaken for 1/2 hr. 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. Since glycerol is also extracted, a ratio of the fat/glycerol weight is used to determine the lipid content. The residue remaining on the Buchner funnel was saved for determination of the extent of non-enzymatic browning. Since glycerol is also totally extracted by the solvent, the lipid is calculated as a % of the extracted weight.

The residue in the flask was dissolved with 10 ml. of a mixture of glacial acetic acid:chloroform (3:2). Then 0.5 ml of freshly prepared potassium iodide (saturated solution) was added and after exactly 2 min., 15 ml distilled water were added to stop the reaction. The mixture is then titrated with 0.01 N $Na_2S_2O_3$ using 1 ml 5% starch solution as an indicator. A lower normality is used at the start (0.001 to 0.0001). The thiosulfate is standardized periodically against potassium dichromate. The peroxide value is calculated as:

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

4. UV measurement

Before each model system preparation a sample (5-10 µl) of methyl linoleate is dissolved in 10 ml of methanol. The optical density on this is then measured at 233 mµ on a Hitachi-Perkin Elmer spectrophotometer. The amount of oxidized linoleate is calculated according to the method of Privett and Blank (1962). If the % oxidation (moles Φ_2 per mole lipid) exceeded 0.2%, the linoleate was further purified by distillation.

5. Headspace analysis for specific carbonyl compounds

Samples of 2-3 grams size in 25 ml. Erlenmeyer flasks after humidification from each treatment were covered with rubber serum caps and stored at 37° C. At suitable intervals (usually one a week), 1 ml. of the headspace was injected into an F & M 1609 gas chromatograph equipped with a flame ionization detector. The following conditions were used:

Column: 8 ft. x 1/4" 10% Lac 728 on 60-80W 609 Carbowax

Gas Flow: N_2 40 cc/min. 40 psi

Temperature: Column 80° C

Hexanal, a major oxidation product of methyl linoleate as well as some foods, shows up as a narrow peak with a retention time of 30 sec. The results are presented as peak height (mm.) divided by lipid weight (grams).

As was found by Karel and Labuza (1968b), no differences that could be quantitatively used were found for treatments at different humidities. Since samples and amount of personnel were limited, this test was dropped.

6. Organoleptic analysis: Sniff test

With the food samples prepared at different conditions daily, organoleptic analysis was made by at least two persons. The day was noted when the sample just began to get a stale painty odor indicative of the onset of rancidity.

7. Carotenoid pigment loss (Lime, 1969)

For the carrot food system, 10 grams of sample is extracted with 3 portions (40 ml. each) of 1:1 acetone:hexane by blending for 2 min. in a Micro-Waring blender cup.

The extracts were filtered, combined, and made up to 250 ml. The spectral curve obtained on the extract is shown in Figure 7. The carotenoid content correcting for the sample size by using an OD ratio is then based on the following because of the double peak shown:

$$C_I = \frac{OD_{450}}{OD_{350}}$$

$$C_{II} = \frac{OD_{480}}{OD_{350}}$$

$$C_{Total} = C_I + C_{II} = \text{carotenoid index}$$

D. Non-Enzymatic Browning

1. Increase in color value

Several methods were tested for increase in colored complexes as caused by non-enzymatic browning deterioration. The methods were:

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. Simple Enzyme Method (According to Choi *et al.*, 1949)

After extraction of the fat, a weighed amount (~ 0.5 g) of the solids residue is dispersed in 20 ml of water and 10 ml of a 10% suspension of the enzyme trypsin were added. The solution was held at 45° C for 2 hr. for complete hydrolysis, 2 ml of 50% Trichloro actetic acid were added, and the sample was filtered under vacuum and the optical density measured at 420 mμ. In most cases, the solutions were opaque causing erroneous results. The results were presented as : Browning value = B = $\frac{OD \times 100}{g \text{ solids}}$.

Method C. Complete Browning Procedure (According to the method of LeRoux and Tannenbaum, 1969)

In order to determine the total browning occurring in the protein model systems and some of the foods, the following procedure was developed and used.

To the sample from storage, 30 ml. of 3:1 (v/v) $CHCl_3$:MeOH were added and the mixture was shaken for 30 min. The sample was then filtered on a Buchner funnel using 30-40 ml. of the $CHCl_3$:MeOH solution. The optical density of the filtrate was measured at 420 mμ after weighing to determine the amount of solvent used (A $CHCl_3$:MeOH blank was used). The browning in the organic phase was determined from

$$B_0 = \frac{OD_{420} \times \text{wt. solvent}}{\text{total wt. sample solids} \times \rho_{solvent}}$$

$\rho_{solvent} = 1.3$ g/cc.

The residue was then removed and dried under vacuum over dessicant overnight and weighed. To this sample, in a 50 ml. flask, 20 ml. 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, which, in most cases, produced a completely clear solution for optical density measurement. The optical density on this solution was measured at 420 and 600 mμ to correct for light scattering due to suspended solids (A. Clark, Ph.D. Thesis, 1969, M.I.T.).

The browning was calculated from:

$$B_{corrected} = 1.27\ OD_{420} - 2.66\ OD_{600} \text{ (from Clark)}$$

$$B_A = \frac{B_{corr} \times \text{Volume of Sample Solution (cc)} \times \text{wt. fraction casein}}{\text{grams residue from extraction}}$$

A blank of 21 ml. H_2O, 1.5 ml. NaOH, 1 ml. 1-propanol and 15 mg. pronase was used in all cases.

The total browning is then $B_T = B_A + B_O$.

2. Carbon dioxide evolution

Carbon dioxide is a product usually found being produced during non-enzymatic browning of foods of low moisture content (Cole, 1967). In order to measure CO_2, the flasks that were prepared for peroxide, headspace or browning measurement were used (50 ml Erlenmeyers capped with a rubber serum

stopper containing 2-3 g sample). One ml of the headspace was injected into an Aerograph Model A-90-P Gas Chromatograph and analyzed according to the methods of Karel et al. (1963). The chromatograph was equipped with a dial disc integrator to measure area under the peak directly. This was calibrated with mixtures of CO_2 in the flask headspace as long as sample weights were similar. The results are presented as % CO_2 in the headspace with time.

3. Solubility

Because of the time limit of each run, and the amount of sample available, specific solubility tests could not be made. Qualitatively, solubility in the enzyme digestions were noted if any differences were observed.

III. Results and Discussion

A. Moisture Isotherm Relationships

Isotherms were prepared for each of the glycerol model systems shown in Table 2 in order to determine the percentage of glycerol necessary to make each system approximate the water activity curve of actual intermediate moisture foods. Figure 8 shows the results for the cellulose System C as a function of the amount of glycerol present. As can be seen at water activities below $a_w = 0.3$, there is little change in water adsorption, however, above 50% RH ($a_w = 0.5$), the increase in glycerol content significantly increases the moisture contents over the system without glycerol. The criterion set by intermediate moisture foods of 20 to 40% moisture content on a solids basis with water activities

between a_w = 0.6 to 0.85 is met by the systems containing either 30% or 40% glycerol. It should be noted that the solids basis includes the microcrystalline cellulose, methyl linoleate and the glycerol. Heidelbaugh and Karel (1970) discuss the significance of the vapor pressure lowering effect of glycerol at high activities (see appendix).

Figure 9 shows the moisture isotherms after 12 hours equilibration for the protein model System A. At low water activities, more water is adsorbed than for the cellulose model systems because of the bonding of water to the amino acid residues along the protein chain. The presence of glycerol at 10% and 20% seems to inhibit water adsorption in this range possibly due to reaction and bonding of the glycerol itself to these residues as was found for glycerol on cellulose. The 20% glycerol system meets the necessary criterion of moisture content and water activity for intermediate moisture foods as in the case of the cellulose system. In Table 7, the rate of equilibration of the 20% glycerol Protein Model System A is shown. In the range of 60% to 84% RH for the glycerol containing systems, there is an increase in the water absorbed when held for up to 48 hours. However, this increase is usually less than 2-3% over the value at 12 hours. Thus, in order to minimize the pre-treatment time of samples for subsequent chemical stability studies, it was felt that 12-18 hours equilibration was adequate to attain the desired moisture contents. This was the same case for the other model systems of this study. However, it should be noted that at lower humidities in Table 7, a loss in water content occurs when held over this time period. This is probably due to a relocation of water with glycerol on specific sites. Because of this it became necessary to standardize the procedure for humidification so that exactly 18 hours was used for the humidification time for all systems.

Figure 10 contains the results of the isotherms of the 20% and 30% glycerol containing model systems of Protein Model System B (3:2 protein-lipid). As with the other two basic model systems, the use of glycerol at 20 to 30% meets the necessary criterion imposed in this study for moisture content between the water activities of a_w = 0.6 to 0.85. These isotherms can be superimposed over the ones of Model System A. In Figure 11, isotherms of the 30% glycerol model system of 6:1 cellulose:lipid (Model C) and the 20% glycerol system of 4:1 protein:lipid (Model A) are shown in comparison to an isotherm prepared on Gainesburgers, a highly successful pet food of the intermediate moisture type. It can be seen that the two model systems and the pet food have isotherms that are very similar. In addition, nine out of ten intermediate moisture foods developed by Pavey and Schack (1969) for the Air Force were found to be located on the isotherms of the cellulose model system developed here. These observations led to the selection of the 20 to 30% glycerol systems as the basis for study fo the oxidative and browning reactions of intermediate moisture model food systems.

The composition for the non-glycerol cellulose based systems was shown in Table 3. The isotherm at 37° C for Run 10, the system containing a sucrose sorbitol mixture is shown in Figure 12. In comparison with Figure 8 for the 30% glycerol system, it can be seen that sucrose is a much poorer humectant on a weight for weight basis. For example, at 75% RH, the sucrose system holds only 14 g H_2O/100 g solids. Thus, the soluble solids may be almost twice the concentration

in the sucrose system at the same water activity. In Run 11 a 50/50 mixture of sucrose:propylene glycol was used. The isotherm as shown in Figure 13 shows an even lower amount of water absorbed even though the glycol should increase adsorption. A thorough check of the samples indicated a weight loss during drying greater than the amount of water present. To elucidate this, a system according to the composition shown for Run 12 (Table 3) was prepared using only propylene glycol. It was found that all the glycol was lost during freeze-drying because its vapor pressure is quite large under the conditions used for freeze-drying. This run was discarded since no useful data could be obtained. In order to obtain a system with propylene glycol as the humectant, the direct mix procedure of Run 13 was used. As can be seen from Table 3, the method gave a treatment which was ~20 g H_2O/100 g solids at 75% RH, a useful level for the intermediate moisture range.

B. Oxidation in Cellulose Model Systems

Experiments were performed to measure the effect of glycerol and different water contents on the extent of oxidation in the 30% glycerol-cellulose model systems as a basis for the effect in intermediate moisture foods.

Table 8 lists the run parameters. In Run 1 (Figure 14), the cellulose model system containing 30% glycerol with 100 ppm. cobalt added as a catalyst (10^{-4} g cobalt per g linoleate or 5 x 10^{-4} moles Co/mole linoleate) was prepared at five different relative humidities and oxidation was carried out in manometers. It can be seen that the results are

almost opposite to that found in similar model systems without glycerol (see Figure 1) in that, as the moisture content (i.e., relative humidity) was increased, oxidation proceeded faster. This was presumed to be due to the possibility that glycerol acts like water in that it mobilizes the catalyst and allows for more extensive initiation of hydroperoxide breakdown. A similar effect was found by Yeh (1969) for cobalt catalyzed oxidation of linoleate in the non-glycerol containing system held at 75% RH. (See also Heidelbaugh et al., 1970, Appendix.) In his study, when no trace metal catalyst was present, the samples at 75% RH oxidized even slower than at 52% RH, thus supporting the supposition that glycerol or water affects catalyst mobility when present together. Problems were found, however, in repeating this first run as the gel formed during mixing never had the same consistency. In addition, it was felt that possibly the pH of the system might have an effect on the catalyst activity as was found by Tjhio et al. (1969). Several runs using various buffers were performed, but variable results occurred. This was possibly due to the trace metal content, amounting to about 10 ppm., of possible catalysts in the buffer solutions. Run 2 is shown as an example of the variability found in the data (run parameters in Table 8). In this test, iron as myoglobin was used as the catalyst since it is the predominant catalyst in fleshy foods and a buffer solution was used instead of distilled water to prevent formation of iron hydroxide precipitates. It should be noted, however, that

the pH of the humidified system cannot be determined by any means available and cannot really be defined at these low water activities.

The results of Run 2 are shown in Figure 15 for the system without glycerol and in Figure 16 for the glycerol model system. Results of the non-glycerol system are similar to results obtained previously by Yeh (1969), except that humidification to 75% RH which is in the middle of the intermediate moisture range increased the rate of oxidation over that of the dry controls. This phenomenon occurred in Run 1, but was presumed to be due to the presence of glycerol. The results of glycerol addition to 30% is shown in Figure 16. Glycerol acted as a catalyst inhibitor in both the dry state and at 75% RH in comparison to the treatment with no glycerol. However, since these are different batches, it is quite possible that some artifact occurred to cause this,unless the glycerol acts by chelating the metal. Table 9 shows the results of the time to reach 1% oxidized for three other runs prepared in the same manner as Run 2. It was felt that the time to reach this extent of oxidation was indicative of the data and eliminated the need for presentation of oxidation curves for each run. The times presented are the average of duplicate or triplicate manometric measurements. Based on these results, it can be seen that when glycerol is not present, humidification to 75% RH promotes the rate of oxidation to a level that is usually faster than the control or is very close to the control.

This is in basic contrast to humidification to lower moisture contents which tends to act by inhibiting oxidation as reported in previous studies reported in the introduction. When glycerol is present, the results are very similar in trend to the model system without glycerol, i.e., a faster rate of oxidation at the high intermediate moisture content. However, in any one run (in which the same batch of methyl linoleate would be used) the effect of glycerol itself is variable. In the dry state, the presence of glycerol tends to promote the rate of oxidation, whereas at 75% RH, glycerol either promotes or inhibits oxidation in comparison to samples similarly treated but not containing glycerol. These variables could have been due to the very variable nature of the gel formed during mixing, suggesting different interactions occurring between the various components. Secondly, the glycerol is probably acting as a mobilizer for the trace metal catalysts to help promote oxidation, especially in the dry state, as well as a chelating agent to modify their catalytic behavior.

The variable results obtained in the first runs reported here showed that a very specific, defined method of preparation was necessary to get reproducible results. All subsequent runs therefore were performed using the modified mixing procedure in which the linoleate was first mixed into the cellulose and then an aqueous solution containing all the additives including glycerol was blended in. Cobalt was used as the catalyst and no buffer was used, to eliminate

further trace metal contamination. All runs were performed with two treatments using the same linoleate for each run:

1. No glycerol - catalyst
2. Glycerol - catalyst.

Samples were humidified to 20% RH, 52% RH and 75% RH as well as being held in the dry state.

The times to reach 1% oxidation for Runs 6-9 are shown in Table 10 and in Figures 17 through 20 the data for Runs 6 and 7 are shown. The results were very reproducible, thus justifying the use of the modified mixing procedure. It can be seen that in systems without glycerol, oxidation proceeds as expected. There is a substantial decrease in the rate of oxidation with increased humidification up to 52% RH. This is exactly as predicted by the theories of Maloney et al. (1966) and Labuza et al. (1966) which suggests water acts by hydrating hydroperoxides, preventing their breakdown, by chelation of trace metal catalysts, and by destruction of free radicals. Above this point, further increases in the moisture content causes water to condense in capillaries (Labuza, 1968) and in multilayers so that a more continuous network is formed. Thus, at 75% RH, the increased rate of oxidation can be explained most likely by increased mobility of both reactants and trace catalysts in the continuous network. This has been suggested by Labuza et al., 1969. These results suggest that humidification of any food to the intermediate moisture level will present a serious problem in that it becomes more susceptible to oxidation as well as being a good environment for non-enzymatic browning.

When 30% glycerol is added, the pattern is very similar to the nonglycerol systems except that at 52% RH, the protective effect of water has been decreased significantly as compared to no glycerol. This is explained, as was supposed in earlier runs, to be due to the network set up by the glycerol-water mixture which would promote catalyst mobility since the water is not held tightly. Thus, glycerol, or any other humectant of similar type, added to adjust a food to the intermediate moisture level will tend to promote oxidation by this mechanism. This supposition was tested in Runs 10 to 13 in which different humectants were used according to the compositions shown in Table 3. Oxidation was followed by measurement of peroxide value which, according to Martinez and Labuza (1968) and Heidelbaugh (1970), compares equally well with oxygen uptake for cellulose systems up to about a PV of 1000. Above this level of oxidation, the peroxide concentration peaks out and then decreases slowly.

The measured peroxides are shown in Table 11. It can be seen that for all the runs, the samples at 75% RH oxidized as fast or faster than the dry (< 0.1% RH) samples. In all cases, oxidation was very rapid exceeding a peroxide value of 120 within 2-4 days. This level is comparable to about 1% oxidized on a molar basis. It should also be noted that even at 32% RH, the samples oxidized fairly rapidly. It is possible that the humectants

used for these systems are better promoters of catalyst mobility so that the critical water activity at which protection against oxidation occurs is shifted to a lower level. Heidelbaugh (1970) found this for several systems containing glycerol and other additives. This data thus supports the fact that by adding a humectant which tends to bind more water, the added water overcomes the inhibitory effects against oxidation by promoting mobility of catalysts or other reactants.

C. Oxidation in Protein Model Systems

The first set of experiments showed that at intermediate moisture contents in model systems based on cellulose, water can act as a prooxidant. The systems used were comparable to many fruit and vegetable products which would be low in protein content. The following experiments were carried out in protein systems, which would be indicative of a meat or fish food, to determine whether similar effects occurred.

In Run 14, the oxidation of protein-glycerol systems A and B from Table 2 was studied at the 20% glycerol level. To all systems, 100 ppm of cobalt was added as a catalyst. The extent of oxidation as a function of time is shown respectively in Figures 21 and 22. This test was repeated with Run 15 and the results are shown in Figures 23 and 24. As with the cellulose-glycerol systems, oxidation is fastest in the dry state (< 0.1% RH) and is accelerated when the humidity reaches the intermediate moisture level. As can

be seen in Table 12, humidification to 50% RH had a protective effect as with the cellulose systems and increasing the moisture content to the 75% RH level caused a prooxidant effect of water. This was consistent for both protein to lipid ratios in both runs. The increased amount of protein in System A for Run 14 seems to have an antioxidant effect compared to System B; however, the differences in Run 15 are much smaller. The results of these two runs correlate very well with the previous work and show that oxidative rancidity will most likely take place in all types of intermediate moisture foods.

In Table 13 are presented the peroxide value data obtained from Run 14. Several results are apparent in conjunction with the manometric data. The peroxide values when multiplied by a factor of about 2 for System A and 4 for System B convert the data into oxygen absorption in μl O_2/gram system. A comparison of the data indicates that the peroxides account for much less than 50% of the oxygen absorbed in System A and this especially decreases as the humidity increases to 50% RH. In System B, the difference is even greater. Martinez and Labuza (1968) found perfect correlation of peroxide value to oxygen absorption for cellulose systems, but the same large difference in a real food. This makes the use of PV difficult in determining the extent of oxidation; however, in the present study, it should be noted that the same trend of oxygen absorption with respect to humidity is borne out by the peroxide values.

In the rapidly oxidizing systems, the normally expected peak in peroxide value is also seen. The time at which the maximum PV is reached is also indicated in Table 13. At 50% RH for System A, a maximum was never reached. However, for the other systems, a maximum was clearly determined. The time at these peaks is much longer than the time to either 1% or 3% oxidation, but is still indicative of the rate of oxidation of a particular system.

The major effect as seen in Table 13 is that in the presence of water, the peroxide values are much less than in the dry state at high extents of oxidation. This indicates that possibily the hydroperoxides are broken down very rapidly, or are more mobile, so they react with the protein and disappear. Humidification to the intermediate moisture level, however, does not enhance this effect, but rather the PV values become higher than at 50% RH. Other mechanisms that can account for the large discrepancy between PV and oxygen absorption are oxidation of other components and non-enzymatic browning due to reaction of end products of hydroperoxide decomposition. Heidelbaugh (1969) has shown that glycerol itself does not oxidize under these conditions, but Karel and Labuza (1967) have shown that protein oxidized at an increasing rate as moisture content increases. This latter effect can account for some of the difference; however, the previously mentioned work was done at 55° C. This would not be expected to occur at 37° C so that most likely the

presence of protein causes a more rapid decomposition of the peroxides.

Table 14 contains the results of the peroxide determinations of Run 15 for both protein systems. These data also confirm the manometric oxidation showing that the dry oxidizes fastest followed by 75% RH with 50% RH the slowest. The data are quite variable at low peroxide values, probably due to the fact that each point represents a different sample and the catalyst concentration may not be entirely uniform, especially for those metals present in the casein itself. In Table 14, is also shown the times found from manometric data for 1 and 3% oxidized (mole basis). It can been seen that the time when the peroxide value starts to go above 15-20 is close to the 1% oxidization level, however, the results are not completely quantitative. This level for rancidity development is much lower than the peroxide value of 100-120 for the cellulose systems again indicating an interaction of oxidizing lipids with the protein. This may have serious consequences on long term storage especially at high relative humidity since under these conditions protein aggregation is enhanced. This is as has been found by Zirlin and Karel (1969) for gelatin linoleate systems at 61% RH. The reaction is a consequence of forming protein free radicals which interact and can cause loss in good textural characteristics. The typical peak in peroxide values is also seen for the more rapidly oxidizing systems. In conclusion, the

peroxide data in the protein system correlate with the manometric data and indicate that, if enough data points are taken, peroxide determination in high protein foods may be an easy method to follow rancidity development.

Measurements of CO_2 evolution and presence of browning pigments were made for up to two weeks on both systems of Run 14 (same samples as used for PV). Both tests showed negligible results thus indicating that non-enzymatic browning was not a major mechanism in these oxidizing systems up to 2 weeks.

Table 15 contains the browning data for the samples of Run 15 stored over a ten week period. Total browning in both the organic and inorganic phases was followed. In all cases, browning of the organic phase (B_O) is negligible with most of the brown pigment going into the aqueous extract. No differences in browning were found between 50 and 75% RH, whereas, at the dry condition, the amount of browning was larger. This shows that end products of oxidation such as carbonyl compounds probably enter into the non-enzymatic browning pathway to react.

In retrospect, however, it can be concluded that in foods which contain both protein and oxidizable lipid, if lipid oxidation is enhanced such as by storage at intermediate moisture levels, rancidity development will proceed first and cause deterioration. Since at 37° C, browning is very slow, it will not be the major cause of deterioration. However, if antioxidants are added to stop oxidation,

browning could be the major pathway of deterioration unless it is dependent on oxidation for reaction intermediates. Most likely, under these conditions, end products of browning could also act as antioxidants, however, in rapidly oxidizing foods, their production would come too late in the storage life of the food.

D. Antioxidant Effectiveness in Intermediate Moisture Model Systems

In order to decrease the rate of oxidation of foods, antioxidants of two types are generally used. Table 4 listed the most commonly available antioxidants. Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), propyll gallate (PG) and α-tocopherol are considered Type I antioxidants in that they react with free radicals produced in the chain reaction and thereby yield a faster chain termination reaction. The others listed, citric acid, ascorbic acid, and ethylene diamine tetra acetic acid (EDTA), act by chelating metal catalysts thereby slowing chain initiation. These antioxidants are usually more water soluble than the former so that in intermediate moisture foods, they may be better antioxidants if catalyst mobility is an important factor. Studies were carried out in both protein and cellulose model systems using glycerol as the humectant. Three runs (16, 17 and 18) were made with the cellulose-glycerol model systems. In Run 16, BHA, BHT and PG were tested at 200 ppm (on a lipid basis) and the oxygen uptake is shown in Figures 25 - 27 for three relative humidities. It

should be noted that 20% RH is near the monolayer coverage of water for this system and would be most like a dry food. 51% RH is just at the start of the intermediate moisture range and should oxidize the slowest, with 75% RH being in the intermediate moisture area and should oxidize the fastest according to the above results. In Run 17, the same antioxidants (phenolic Type I, which cause chain termination) were tested at the same level as well as one system was prepared with EDTA at a level of 10 moles of EDTA per mole of added cobalt (50 ppm on a linoleate base). This gives 400 ppm EDTA on a total solids basis. Systems were tested at both 20% RH and 75% RH. The oxygen uptake for these models is shown in Figures 28 through 30. In Run 18 additional chelating agent antioxidants were tested including EDTA, citric acid and ascorbic acid. The phenolic antioxidant, α-tocopherol, was also tested. Samples were humidified to 61% and 75% RH, both within the intermediate moisture range. The oxidation results are shown in Figures 31 to 34. All run parameters are listed in Table 16 and the times to reach 1% and 3% oxidized in Table 17.

In analyzing the oxygen uptake curves and the times to reach 1% oxidized in Table 17 for Run 16, it can be seen that all antioxidants gave good protection in the dry state (20% RH) with PG the most water soluble, being the best. For the control treatment, humidification to 51% RH added some further protection, but for all treatments with

additives, oxidation at 51% RH was faster than the drier system. This has been also found by Heidelbaugh et al. (1970) and indicates that catalyst or reactant mobility is becoming predominant at the beginning of the intermediate moisture range in the presence of an added humectant. The water present is more mobile. This is further evidenced by the fact that the PG, the most water soluble of the antioxidants, exerts its best effectiveness at this humidity. When held at 75% RH, all samples oxidized faster than at 20% RH, as would be expected. It should be noted, however, that neither BHT or PG (the water soluble antioxidant) exerted any protective effect and BHA only gave about a 20% increase in time. It is possible the former antioxidants are much more reactive and are used up sooner than BHA. Unusual effects of BHT in cellulose and protein model systems have been reported by Labuza et al. (1969) with similar results. The results at a level of 3% oxidized also bear out the findings at 1%.

In Run 17, a lipid which was initially at a higher extent of oxidation was used as compared to Run 16, so that it reached 1% oxidized in 1/5 of the time. Under these conditions, neither BHT or PG are good antioxidants (Scott, 1965), but instead enhance the rate of radical decomposition and can cause a pro-oxidant effect as observed. In all cases, oxidation was accelerated at 75% RH as compared to 20% RH. BHA seemed to give better protection than the other phenolic antioxidants (as in Run 16), probably because it forms a more

stable radical especially in the dry state. At 75% RH, BHA gives some protection, but does not significantly delay oxidation. The best antioxidant properties are shown by the chelating agent EDTA for both humidities, although at 75% RH, the time to reach 1% oxidized is increased by 95 x as compared to 23 x at 21% RH. This suggests that at 75% RH the solubilized mobile catalysts are tied up more easily in the aqueous environment. The few peroxide value determinations made in Run 17 also show similar results (Table 18).

In Run 18 several other chelating agents were tested against EDTA at two intermediate moisture levels. This test gives a moisture content of 14 g H_2O/100 g solids at 61% RH compared to about 24 g H_2O/100 g solids at 75% RH or nearly twice the water content. Except for ascorbic acid from Table 17, it can be seen that all samples oxidized much faster at 61% RH as compared to 75% RH. This condition also occured for the protein systems studied which are reported later. It is possible that at 61% RH, all the reactants and catalysts present have been solubilized, and are mobile enough to overcome the antioxidant properties of water at lower humidities. By increasing the humidity to 75% RH, these same components are now diluted out so that by mass action and diffusion limitations, the rate of oxidation decreases. This would be very important in choosing the final moisture content for intermediate moisture foods.

As in Run 17, EDTA shows the best antioxidant properties followed by citric acid which is not as good a chelating agent. The citric acid might also affect the rate due to a pH difference. Both gave much better protection at the higher humidity. It can be seen that ascorbic acid, a non-toxic chelating agent proposed for inhibition of oxidation, has a pro-oxidant effect at both moisture contents. This is probably due to both oxidation of the ascorbic acid itself (Weissberger, et al., 1943), and the fact that at high moisture levels in chelation with metals, the chelated form of the metal may be an even better catalyst (Haase and Dunkley, 1969). These results rule out the addition of ascorbic acid as an antioxidant. Tocopherol is only slightly effective at 61% RH, but at 75% RH gives somewhat better protection, however, not as good as either EDTA or citric acid. Its effectiveness is probably due to the low initial extent of oxidation as found in Run 16.

In Runs 19 and 20, the effects of the antioxidants used in the cellulose systems were tested at two intermediate moisture levels (61% RH and 75% RH) for the 4:1 protein: lipid system using 20% glycerol (see parameters in Table 16). The results of oxygen uptake are shown for Run 19 in Figures 35-36 and for Run 20 in Figures 37-38 with the times to reach 1 and 3% oxidized in Table 19.

It can be seen from Table 19 that again for all treatments, oxidation was faster at 61% RH than at 75% RH, even for ascorbic acid which still acts as a catalyst. In Run 19, the ineffectiveness of BHT is duplicated as was found for the

cellulose systems. PG has good antioxidant properties at 61% RH which are negated at 75% RH again as found in cellulose systems. The best antioxidants are BHA and EDTA; however, neither were effective as in the cellulose system, and BHA seems to be more effective than EDTA at the higher moisture content. It is possible that the EDTA may be chelated with metals held in the protein structure thus tieing it up.

In Run 20 with BHA at half the concentration used previously, very little protection was evidenced in the time to reach 1% oxidized. However, the slower initial rate was maintained as evidenced by the time to reach 3% oxidized. Unfortunately, most foods would be rancid at the lower level of oxidation. In combination with half the amount of EDTA used previously, some protection is offered at 75% RH (about a 25% increase in time) for up to 1% oxidized. Much more protection was afforded at the 3% oxidized level (an increase of 200%). Ascorbic acid as in the cellulose systems acted as a prooxidant as well as citric acid at 61% RH. When diluted to 75% RH (about 2 x the water content), the citric acid seemed to afford some antioxidant effectiveness. It is possible that some pH affect is occuring at the lower relative humidity.

The results found above for oxygen uptake are also borne out by measurements of peroxides as shown in Tables 20 and 21 for Runs 19 and 20 respectively. As before, the peroxides are much lower because of the presence of protein. The peak value occurs sooner at 61% RH than at 75% RH indicating faster

oxidation and the general level of peroxides is much lower for the systems showing good antioxidant effectiveness. Thus, the peroxides could possibly by used as indicators of storage as long as a reasonable number of tests were made during the storage period. This is shown in Table 22 for Run 13 in which propylene glycol was used as the humectant and BHA at 200 ppm and EDTA at 10 moles/mole cobalt were tested as antioxidants. In this system, BHA gives the best protection as compared to the controls. EDTA afforded slight protection up to 8 days when it smelled rancid and the peroxide value rose. The controls were rancid at 4 days whereas with BHA, rancid odor notes were not discernable until 21 days. These results support many of the above conclusions as to relative humidity and antioxidant effectiveness.

As was stated previously, it is possible that if protected against oxidation, non-enzymatic browning could become an important route of deterioration. This would not be the case in the cellulose systems where no proteins or amino groups are present, but could certainly occur in the protein systems, especially since the browning reaction shows a maximum in the intermediate moisture range. In Run 19 and Run 20, browning was measured by Method C and the results are shown in Tables 23 and 24 respectively. The samples for Run 20 were held for up to 4 months.

The data for Run 19 indicate that the major browning pigments were extractable in the aqueous phase. This means

that the pigments formed were not lipid oxidation polymers, but were reaction end-products of carbonyl compounds and proteins. The increase in B_0 is very small for all systems and is quite variable probably because of the high error at low concentrations. Table 23 indicates that much of the browning may be due to reactions between glycerol and casein (the last system shown) alone, but the data are very variable and probably indicate very little browning for over one month. Measurements of CO_2 evolution showed less than 0.1% CO_2 at the end of 43 days in all samples with the results being very variable.

The results of Run 20, however, show that with much longer storage time, non-enzymatic browning becomes evident. Only the total browning values are shown since, again, the organic soluble component was very negligible and variable. It looks like from Table 24 that the slower oxidizing samples showed a lower amount of browning after about 75 days. This would indicate that the browning was coming from carbonyl compounds formed during lipid oxidation and that their concentration must be built up high enough before browning becomes significant. Thus, if a food system can be kept free of reducing sugars, and other carbonyls, and oxidation is inhibited, the food should be stable to both deteriorative reactions. This would be impossible to do, however, with meat or fish type foods which already contain sugars and may have a low enough pH to cause hydrolysis of sugars into reactive intermediates as found by Karel and Labuza (1968a).

E. Deterioration of Intermediate Moisture Foods

1. Run 21 - Freeze-dried chicken system

This system was prepared according to the methods described previously and was stored at 32%, 61% and 75% RH. A system containing 200 ppm of BHA and 200 ppm of PG on a fat basis were tested along with a system without glycerol. It should be noted that these antioxidant levels are much higher than in the model systems because all the lipid is not oxidizable fat. Oxidation was followed by measurement of peroxide value (Table 25) and browning was measured by Method B (results in Table 26).

In respect to rancidity development, all samples including a non-glycerol control were stable for up to almost 4 months except for the glycerol control systems at 61% RH and 32% RH. At the last measurement, both showed high peroxide values and were noted to have a faint painty rancid odor. The 75% RH sample did not show this. The antioxidants BHA and PG both gave protection with BHA showing slightly lower peroxide values. All treatments seem to show slower oxidation at 75% RH as compared to 61% RH as found in the model systems. Unfortunately, since these runs were started at the same time as the model system work, the effectiveness testing of EDTA was not done.

Table 26 shows a high degree of scatter in the browning data probably due to the inability to get a clear solution using the method. The increases in browning are variable so that no conclusions can be drawn.

2. Run 22 - Freeze-dried carrot intermediate moisture system

Three systems were prepared for the carrot system; a control containing 23% glycerol; one without glycerol; and a glycerol system with 384 ppm of BHA on a solids basis. This latter may be an unexceptionally high value in terms of FDA acceptance. Since the fat content was too low for effective peroxide measurement, the absorption curve for the carotenoid pigments was measured as a function of time. Figure 39 shows the total absorption curve for all three systems stored at 32%, 61%, and 75% RH. It can be seen that the total absorption decreases with holding time and that in the 61% RH sample, the pigments were oxidized the fastest.

In Figure 40 is presented the carotenoid index as a function of time for all treatments held for over 10 weeks. The antioxidant gives some protection, but not as much as would be desired. This could be due to the unfortunately high level tested. According to Scott (1965) and Karel _et al_. (1966), Type I antioxidants used at too high a concentration do not show good antioxidant properties. Very little difference is seen in the loss of pigment between the controls with and without glycerol. It can also be noted that in all cases, the samples held at 61% RH showed the faster rate of pigment loss. This is directly correlated to the rates of oxidation as a function of relative humidity as found in the model systems.

3. Run 23 - Chicken/cellulose/glycerol direct mix system

Using this method, the fat of the chicken would be expected to be spread out over a much larger surface area and thus more susceptible to oxidation. In addition, because of the higher total carbohydrate content including cellulose, more water could be present at 75% RH as is shown in Table 6. It is interesting to note, however, that this method produced a free-flowing powder, and that in Run 21 the system seemed to be a moist spongy matrix.

The antioxidants BHA (at 200 ppm fat basis) and EDTA (200 ppm total solids basis) were compared to a control and samples were placed at 37°, 45° and 52° C for determination of the temperature effect on the systems. As was shown by Karel and Labuza (1967) determination of reaction mechanisms as a function of temperature in model systems usually does not give a true indication of the effect on an actual food. Thus, in this study, the activation energy was measured in the actual food system.

Figure 41 shows the development of peroxides as a function of time for all systems studied. It should be noted that this occurred much faster than in Run 21. The samples were sniffed daily for development of rancidity and, as noted, this occurred under all conditions at a peroxide value of 120. As can be seen, BHA gives excellent protection against rancidity for all temperatures, whereas EDTA is

not as effective, but still gives a much longer storage life than the control. This is as occured in the protein systems and indicates a tieing-up of the chelating agent. The data also points out the need for multiple measurements with time because of the peak in the peroxide number with an eventual large decline.

The extent of browning was determined by Method C for all treatments. It was found that all the pigment was present in the organic extract with negligible browning in the aqueous extraction. Figure 41 shows the increase in browning in the organic phase as a function of time. It is obvious that the browning development follows exactly the same pattern as shown for rancidity development. The pigment formed here is probably polymerization products of lipid oxidation. This is borne out by the measurements of the activation energies as presented in Table 27.

The activation energy for peroxide development for the control system of 10.2 Kcal/°K mole is close to the value expected for lipid oxidation (Lundberg 1961, Schultz et al. 1962, Yeh 1969). In addition, the increased value in the presence of EDTA would be expected since the initiation rate activation energy is increased by removing the activating metal catalysts. The low value for BHA is reasonable based on the different reaction mechanism kinetics in the presence of a Type I antioxidant. No published values are available for comparison. The low value indicates, however, that the antioxidant protected food is able to withstand excursions to high temperatures without any substantial quality loss.

Finally, the activation energy for brown pigment formation is much lower than expected for non-enzymatic browning. Mizrahi et al. (1970) report values of 25-40 Kcal/° K mole for most dehydrated foods. This further indicates that the browning occuring was due to lipid oxidation end-product polymerization. This would also account for the slightly higher values than for peroxide formation because of the more complicated reaction steps.

4. Run 24 - Applesauce/glycerol direct mix system

Applesauce containing 10% glycerol (85% RH) was stored at 37°, 45° and 52° C for study of the development of non-enzymatic browning. Browning was followed by Method A, a direct aqueous extraction procedure, because of the absence of lipids and the low protein content (less than 2% on a dry basis). No measurable browning was observed for up to 2 weeks at 37 and 45° C; however, a slight increase in browning occured at 52° C. This test was not long enough to make any useful observations. However, it would seem that browning could occur, but would not become noticeable in terms of quality until after 3-4 months.

IV. Conclusions

A. Oxidative rancidity can be studied in model systems at intermediate moisture contents by using glycerol as a humectant to reach desired water content.

B. Rancidity development is accelerated in the intermediate moisture range as compared to dehydrated systems at low water contents.

C. A peak in rancidity development may occur in the intermediate moisture range with higher moisture contents decreasing the rate.

D. In protein and cellulose based model systems, the antioxidants BHA and EDTA seem to be the most effective.

E. Development of non-enzymatic browning to a significant extent does not seem to be the limiting reaction for storage of lipid containing model systems in the intermediate moisture range.

F. Similar results were found in prepared intermediate moisture foods indicating that they are very susceptable to development of rancidity.

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Table 1

Water Activity below which Microorganism Growth is Minimized

Organism	Water Activity
Bacteria	0.91
Yeasts	0.88
Molds	0.80
Halophilic Bacteria	0.75
Xerophilic Fungi	0.65
Osmophilic Yeasts	0.60

Table 2

Basic Model System Compositions (Grams)

PROTEIN SYSTEM A (4:1 Protein:Lipid)**

System	0A	10A	20A	30A
Water*	100	90	99.6	99.6
Methyl Linoleate	5	6	6	6
Casein	20	24	24	24
Glycerol	---	3.33	7.5	12.85

PROTEIN SYSTEM B (3:2 Protein:Lipid)

System	20B	30B
Water*	84.7	78.5
Methyl Linoleate	14	14
Casein	21	21
Glycerol	8.75	15

CELLULOSE SYSTEM C*** (6:1 Cellulose:Lipid)

System	0C	10C	20C	30C
Methyl Linoleate	3	3	3	3
Glycerol	0	2.34	5.34	9
Microcrystalline cellulose	18	18	18	18
Water*	90	90	90	90

* Amount necessary for preparation of system before drying.

** All protein systems contained 100 ppm of cobalt as a catalyst (on a lipid basis)

*** Contained catalysts as listed under particular run parameters.

Table 3

Composition of Non-Glycerol Special Systems

Grams

Component	Run 10	11	12	13
Microcrystalline cellulose	30	30	30	30
Methyl Linoleate	5	5	5	5
Sucrose	13.75	7.5	-	-
Propylene Glycol	-	7.5	15	11.7
Sorbitol	1.25	-	-	-
Water	150*	150*	150*	
a				.01**
b				1.4 **
c				5.6 **

* Amount necessary for blending prior to freezedrying.

** Amount water directly added to give respectively

(a) 1.08 gH_2O/100 g solids at < 0.1% RH

(b) 5.31 g H_2O/100 g solids at 32% RH

(c) 19.5 g H_2O/100 g solids at 75% RH

Table 4

Antioxidants Used

in Intermediate Moisture Model Food Systems

Antioxidant	Abbreviation	Maximum Level Approved as an Antioxidant by FDA
Butylated Hydroxy Anisole	BHA	200 ppm on fat basis
Butylated Hydroxy Toluene	BHT	200 ppm on fat basis
Propyl Gallate	PG	200 ppm on fat basis
α -dl-tocopherol	-	300 ppm on fat basis
Ethylene Diamine tetra-acetic acid	EDTA	100-350 ppm on total wt. basis (depends on food)
Citric Acid	CA	100 ppm on total wt. basis (depends on food)
Ascorbic Acid	AA	GRAS (used at safe level)

Table 5

Constant Humidity Solutions

Saturated Salt Solution	Water Activity 37° C
Drierite (Dry $CaSO_4$)	< 0.10
$LiCl$	0.11
CH_3COOK	0.23
$MgCL_2$	0.32
K_2CO_3	0.41
$Mg(NO_3)_2$	0.52
$NaNO_2$	0.62
$NaCl$	0.75
K_2CrO_4	0.84
K_2SO_4	0.96
$K_2Cr_2O_7$	0.98

Table 6

Moisture Contents of Intermediate Moisture Food Systems

Run 21 — Freeze Dried Chicken System

32% RH	4.02 g H_2O/100 g solids
61% RH	12.9 g H_2O/100 g solids
75% RH	21.9 g H_2O/100 g solids

Run 22 — Freeze Dried Carrots System

	No Glycerol	23.1% Glycerol
32% RH	7.44 g H_2O/100 g solids	7.9 g H_2O/100 g solids
61% RH	22.1 g H_2O/100 g solids	26.3 g H_2O/100 g solids
75% RH	38.3 g H_2O/100 g solids	45.0 g H_2O/100 g solids

Run 23 — Chicken/Cellulose — Direct Mix System

33.6 g H_2O/100 g solids — 75% RH

Run 24 — Applesauce/Glycerol — Direct Mix System

% Glycerol (solids basis)	% RH Obtained
0	89.5
10	85.0
20	80.4
30	79.2

Table 7

Isotherm Data

20% Glycerol 4:1 Protein:Linoleate System

MOISTURE CONTENT: GMS WATER/100 GMS SOLIDS

%RH	Time	12	36	48 hours
11		3.85	3.62	3.58
11		3.11	2.97	2.86
23		6.61	6.44	6.41
23		6.83	6.69	6.70
32		6.14	6.50	6.54
32		5.79	6.03	5.99
41		8.66	8.58	8.61
41		8.59	8.35	8.44
51		9.85	9.98	10.32
62		14.08	13.96	14.25
62		13.48	13.89	14.15
75		22.10	22.08	22.40
75		21.87	21.92	21.99
84		35.78	35.99	36.91
84		34.98	35.85	36.21
96		85.32	118.21	137.90
96		69.10	100.77	124.43

Table 8

Parameters for Study of Oxidation of Cellulose Model

Systems Containing 30% Glycerol. (37° C)

Run #	% RH	Catalyst	Buffer*	System Preparation
1	<0.1 32 52 75 84	(moles ion/ mole linoleate) 5×10^{-4} Cobalt	None	Original procedure: glycerol + water + cobalt solution stirred, add linoleate stir, cellulose stir, 30 min. blending
2	<0.1 75	2.1×10^{-3} Iron**	pH 5.8[1] 8.2×10^{-1} mole KH_2PO_4 5.9×10^{-2} mole NaOH	Buffer solution, glycerol linoleate stirring, iron solution, cellulose, 30 min. blending.
3	Same as Run 2		----------	----------
4	Same as Run 2		----------	----------
5	Same as Run 2		----------	----------
6	<0.1 20 75	5×10^{-4} Cobalt	None	Modified procedure: cellulose linoleate-stirred-aqueous solution containing all other additives - 20 min. blending.
7	Same as Run 6		----------	----------
8.	Same as Run 6		----------	----------
9	Same as Run 6		----------	----------

* Moles per mole linoleate

** as Myoglobin

Table 9

Time to Reach 1% Oxidized (Moles O_2/Mole Linoleate)

(Hours)

	No Catalyst		Catalyst (400 ppm Fe)			
	Run 2	3	2	3	4	5
WITHOUT GLYCEROL						
Dry	127	62	35	19	13	23
75% RH	19	18	24	5	11	28
WITH GLYCEROL (30%)						
Dry	56	31	60	11	9	18
75% RH	20	46	25	6	8	25

Table 10

Time to Reach 1% Oxidized (Moles O_2/Mole Linoleate)

(Hours)

	No Glycerol				30% Glycerol			
	Run 6	7	8	9	6	7	8	9
Dry	3	2	7	12	2	2	2	2
20% RH	24	53	33	63	60	125	6	42
52% RH	45	51	42	68	46	65	34	34
75% RH	8	24	30	36	16	24	14	12

Table 11

Peroxide Values of Non-Glycerol Special Systems

PV = meq/Kg lipid

	Run 10			Run 11			Run 13		
Days	%RH < 0.1%RH	32%RH	75%RH	< 0.1%	32%	75%RH	<0.10%	32%	75%
0	44	44	44	7.4	7.4	7.4	13	13	13
2	87	39	219	78	18	88	-	-	-
4	-	-	-	156	32	130	172	264	2947
7	-	-	-	2233	662	2112	-	-	-
8	934	1400	925	-	-	-	2890	2578	2771
9	1132	1222	788	-	-	-	-	-	-
10	847	1218	694	-	-	-	-	-	-
11	936	855	457	1251	2238	934	2783	2646	2604
14	740	825	376	-	-	-	2346	2751	2070
17	-	-	-	-	-	-	-	-	-
18	521	277	468	-	-	-	-	-	-
21	-	-	-	-	-	-	2103	1780	1967

Table 12

Oxidation Constants Protein Glycerol Model Systems

(20% Glycerol)

		Time To Reach in Hours					
		1-1/2% Oxidized			3% Oxidized		
		<0.1%RH	50%RH	75%RH	<0.1%RH	50%RH	75%RH
Run 14							
	System A	65	99	56	126	319	176
	System B	19	26	21	33	169	47
Run 15							
	System A	95	172	37	230	526	251
	System B	78	160	104	153	304	217

TABLE 13

Peroxide Values Run 14

Protein-Glycerol Model Systems

Days	System A			System B		
	Dry	50% RH	75% RH	Dry	50% RH	75% RH
0	7.8	6.1	10.5	9.1	11.8	14.1
1	9.5	-	9.9	19.9	15.9	27.6
2	9.3	-	17.2	52.0	32.9	49.1
3	5.6	10.9	13.0	160.3	74.2	95.1
4	38.7	5.7	14.0	956.8	93.5	161.7
5	32.5	19.3	29.0	1721.3	165.9	657.2
6	44.1	29.8	20.6	-	672.4	1348.9
7	381.2	17.0	33.7	1682.0	-	1487.2
8	351.8	32.1	-	1682.9	1314.2	1585.7
9	466.2	15.4	29.6	2175.6*	-	1596.0
10	1647.0	17.7	38.9	1392.6	-	1519.0
11	1730.4*	12.2	32.8	1615.8	-	1639.0
15	1261.9	37.0	72.9	1569.7	1514.5*	1682.0*
18	890.7	20.6	58.9	1193.0	1263.4	1145.2
24	858.2	35.0	110.9*	1138.9	1358.0	1087.5

* Time at which maximum PV is reached

Table 14

PEROXIDE VALUES Run 15 (meq/Kg)

Days	System 20 A Dry	50% RH	75% RH	System 20 B Dry	50% RH	75% RH
1	11	6	---	8	12	13
2	--	--	---	12	7	9
3	16*	4	12*	11*	14	4
5	--	--	---	19	5	6*
6	13	6	17	18**	12*	11
7	17	6*	17	30	11	10
8	26	12	17	24	4	24**
9	17	4	20	31	16	23
10	28**	4	17	49	13	9
11	48	5	18**	138	12	22
12	61	12	25	381	16	37
13	55	4	20	---	9**	17
18	1399	4	30	1865	10	21
22	1312	7**	20	1517	28	23
26	670	8	30	1096	35	239
40	441	6	89	710	103	560
52	399	64	312	950	589	777
67	201	69	933	794	643	397

* Time at which manometric data shows 1% oxidized

** Time at which manometric data shows 3% oxidized

Table 15

Non-Enzymatic Browning Deterioration

Run 15 37° C

Days	System 20 A Dry			System 20 A 50% RH			System 20 A 75% RH			System 20 B Dry			System 20 B 50% RH			System 20 B 75% RH		
	B_O	B_A	B_T	B_O	B_A	B_T	B_O	B_A	B_T	B_O	B_A	B_T	B_O	B_A	B_T	B_O	B_A	B_T
0	1.1	1.2	2.3	0.2	0.4	0.6	0.2	1.0	1.2	1.3	0.4	1.7	0	1.2	1.2	0	0.9	0.9
7	1.8	1.5	3.3	0	1.3	1.3	0	3.0	3.0	0	0.2	0.2	0	2.9	2.9	0	1.7	1.7
11	0.1	0.3	0.5	0	0.3	0.3	0	1.7	1.7	0.5	1.1	1.6	0	5.3	5.3	1.4	1.5	2.9
14	0.8	2.3	3.1	0.5	2.5	3.0	0.1	7.3	7.4	0	4.2	4.2	1.0	6.3	7.3	0.5	5.3	5.8
30	1.0	5.5	6.5	0	1.4	1.4	0.4	4.3	4.7	0.9	16.1	17.0	0	4.8	4.8	1.1	1.5	2.6
52	1.7	20.8	22.6	0	4.0	4.0	0	3.4	3.4	2.3	16.1	18.4	0.5	6.8	7.3	1.3	9.4	10.7
67	2.3	22.2	24.5	0.2	7.1	7.3	0.4	6.4	6.8	1.1	16.9	18.0	1.9	10.7	12.6	1.4	11.3	12.7

Table 16

Run Parameters - Antioxidant Studies*

Run 16 Cellulose/30% Glycerol System

Treatments - Control
BHA - 200 ppm (linoleate basis)
BHT - 200 ppm (linoleate basis)
PG - 200 ppm (linoleate basis)

Storage at 37°C and 20%, 52%, 75% RH

Run 17 Cellulose/30% Glycerol System

Treatments - Control
BHA - 200 ppm (linoleate basis)
BHT - 200 ppm (linoleate basis)
PG - 200 ppm (linoleate basis)
EDTA - 10 moles/mole cobalt (400 ppm solids basis)

Storage at 37°C and 20%, 75% RH

Run 18 Cellulose/30% Glycerol Systems

Treatments - Control
EDTA - 10 moles/mole cobalt (400 ppm solids basis)
Citric Acid - 10 moles/mole cobalt
Ascorbic Acid - 10 moles/mole cobalt
α-tocopherol - 200 ppm (linoleate basis)

Storage at 37 C°and 61%,and 75% RH

Run 19 Protein A/20% Glycerol System

Treatments - Control
BHA - 200 ppm (linoleate basis)
BHT - 200 ppm (linoleate basis)
PG - 200 ppm (linoleate basis)
EDTA - 10 moles/mole cobalt (1000 ppm solids basis)

Storage at 37°C and 61%, 75% RH

Run 20 Protein A/20% Glycerol System

Treatments - Control
BHA - 100 ppm (linoleate basis)
BHA (100 ppm) EDTA (10 moles/mole cobalt)
Ascorbic Acid (10 moles.mole cobalt)
Tocopherol (200 ppm linoleate basis)
Citric Acid (10 moles/mole cobalt)
Storage at 37 C and 61%RH, 75%RH

* All cellulose treatments have 50 ppm cobalt added.
All protein treatments have 100 ppm cobalt added.

Table 17

Kinetic Oxidation Constants

Cellulose-Glycerol-Linoleate Systems

	Time to Reach 1% Oxidized* (hrs.)			Time to Reach 3% Oxidized* (Hrs.)		
Run 16 Systems	20% RH	51% RH	75% RH	20% RH	51% RH	75% RH
Control	120	144	85	271	286	183
BHA	212	183	109	502	416	316
BHT	209	127	78	359	245	158
PG	297	243	94	444	410	229
Run 17 Systems						
Control	21	---	4	42	---	9
BHA	170	---	10	270	---	19
EDTA	480	---	380	570	---	510
BHT	14	---	4	31	---	9
PG	18	---	3	38	---	7
Run 18 Systems		61% RH	75% RH		61% RH	75% RH
Control	---	245	396	---	330	448
Ascorbic Acid	---	65	46	---	91	71
Tocopherol	---	340	530	---	438	600
Citric Acid	---	480	800	---	540	>1000
EDTA	---	802	>1000	---	>900	>1000

*moles oxygen/mole linoleate

Table 18

RUN 17. CELLULOSE MODEL SYSTEM

			PEROXIDE VALUES (MEQ/Kg FAT)							
System	Control		BHA		BHT		PG		EDTA	
%RH	20%	75%	20%	75%	20%	75%	20%	75%	20%	75%
Days										
1	68	285	20	51	84	385	54	537	9	8
2	803	---	--	46	207	---	225	---	-	-
14	---	---	2309	--	---	---	---	---	-	-
17	---	998	1537	952	---	---	755	1025	35	-

Table 19

Kinetic Oxidation Constants

Protein-Glycerol-Linoleate Systems

	Hours to Reach 1% Oxidized		3% Oxidized	
Run 19 Systems	61% RH	75% RH	61% RH	75% RH
Control	23	120	98	300
BHA (200 ppm)	90	318	425	860
BHT (200 ppm)	16	76	63	213
PG (200 ppm)	[illegible]	64	288	220
EDTA	161	277	710	808
Run 20 Systems				
Control	150	193	281	449
BHA (100 ppm)	132	187	449	684
BHA (100 ppm)/EDTA (5m/m)	154	256	768	1154
Citric Acid (10m/m)	15	200	76	674
Ascorbic Acid (10 m/m)	37	45	100	152
α-dl-tocopherol (200 ppm)	49	220	145	550

Table 20

PEROXIDE VALUES Run 19 System 20A 37° C

System	Days/ 0	8	17	22	29	36	43
Control							
61% RH	35	56	135	145	316	505	695
75% RH	36	13	140	164	138	360	652
BHA (200 ppm)[a]							
61% RH	14	6	41	24	16	60	164
75% RH	0	5	18	19	7	7	8
BHT (200 ppm)[a]							
61% RH	40	70	147	110	164	463	594
75% RH	19	28	73	40	187	168	287
PG (200 ppm)[a]							
61% RH	6	6	58	90	45	261	377
75% RH	9	19	33	18	--	341	37
EDTA (1000 ppm)[b]							
61% RH	11	5	21	49	21	6	11
75% RH	3	1	14	22	16	3	4

(a) ppm on linoleate basis (32 ppm solids basis)

(b) ppm on solids basis

Table 21

Peroxide Values Run 20

System 20A 37°C

System	Days 0	7	29	54	75	96	119
Control							
61% RH	1	2	92	592	658	484	397
75% RH	1	1	18	585	568	523	558
BHA							
61% RH	0.7	0.7	52	180	723	514	354
75% RH	0.8	4	7	23	16	236	282
BHA/EDTA							
61% RH	0.7	0.7	34	5	13	16	—
75% RH	0.7	3	~~21~~ 3	-	3	4	0
Citric Acid							
61% RH	3	5	148	813	493	257	510
75% RH	0.6	1	15	8	9	20	612
Ascorbic Acid							
61% RH	0.4	1	27	5	17	18	73
75% RH	2	2	76	327	536	257	516
Tocopherol							
61% RH	0.6	2	137	757	481	324	609
75% RH	0.8	0.6	32	209	288	750	411

Table 22

Peroxide Values (Meq/Kg)

Run 13 Direct Mix System Cellulose/Propylene Glycol

System	Days 0	4	8	11	14	17	21
Control							
< 0.1% RH	13	171	2890	2733	2346	-	2100
32% RH	13	264	2580	2646	2752	-	1780
75% RH	13	2950	2771	2600	2069	-	1970
BHA							
< 0.1% RH	13	9	13	19	38	19	49
32% RH	9	12	19	15	22	26	26
75% RH	8	16	80	24	36	27	36
EDTA							
< 0.1% RH	13	44	250	1282	2330	-	2100
32% RH	10	39	208	2524	2181	-	2181
75% RH	13	50	419	2211	1950	-	1735

Table 23

NON-ENZYMATIC BROWNING/Run 19/37° C

Days		0	7	16	22	29	36	43
Control								
61% RH								
	B_O	0	---	0.3	0	0.4	0.3	--
	B_A	4.0	---	5.7	5.1	2.3	5.3	--
	B_T	4.0	---	6.0	5.1	2.7	6.1	--
75% RH								
	B_O	0	0.2	0.5	0	0.2	0	0.7
	B_A	5.0	---	2.9	3.8	4.9	6.6	6.6
	B_T	5.0	---	3.4	3.8	5.1	6.6	7.3
BHA								
61% RH								
	B_O	0.1	0	0.3	0	0.4	0.5	0
	B_A	4.8	6.4	4.9	4.9	5.6	6.5	3.9
	B_T	4.9	6.4	5.2	4.9	6.0	7.0	3.9
75% RH								
	B_O	0	0	0	0	0.7	0	0
	B_A	4.5	---	4.4	2.5	3.3	3.7	3.1
	B_T	4.5	---	4.4	2.5	4.0	3.7	3.1
BHT								
61% RH								
	B_O	---	0	0.1	0	---	0	0.8
	B_A	---	3.4	3.7	4.0	---	6.3	6.5
	B_T	---	3.4	3.8	4.0	---	6.3	7.3
75% RH								
	B_O	---	0	0	0	---	0.3	1.7
	B_A	---	2.8	4.8	4.8	---	4.7	5.3
	B_T	---	2.8	4.8	4.8	---	5.0	7.0

Table 23 (Cont.)

PG							
61% RH							
B_O	0.8	0.2	0	0.2	0.2	0.3	0
B_A	5.0	---	3.3	4.5	7.8	6.3	6.7
B_T	5.8	---	3.3	4.7	8.0	6.6	6.7
75% RH							
B_O	0	0.8	0	---	---	---	1.3
B_A	3.7	5.1	4.2	---	---	---	4.0
B_T	3.7	5.9	4.2	---	---	---	5.3
EDTA							
61% RH							
B_O	1.5	1.0	2.6	0.7	1.0	0.8	1.1
B_A	5.6	9.7	5.1	6.8	4.1	6.2	6.3
B_T	7.1	10.7	7.7	7.5	5.1	7.0	7.4
75% RH							
B_O	0.2	0	0	0	0	---	0
B_A	3.7	---	3.4	1.2	5.7	---	2.0
B_T	3.9	---	3.4	1.2	5.7	---	2.0
Glycerol Casein*							
61% RH							
B_O	0	0.6	0	0	0	0	---
B_A	1.0	0	6.7	4.7	5.8	6.9	---
B_T	1.0	0.6	6.7	4.7	5.8	6.9	---
75% RH							
B_O	0	0.2	0	0.4	0	0.9	0.8
B_A	1.6	0	0	0	1.9	0.9	0.3
B_T	1.6	0.2	0	0.4	1.9	1.8	1.1

*No linoleate present

Table 24

Non-Enzymatic Browning Run 20

B_t (total browning value)

System	Days	0	7	29	54	75	96	119
Control								
61% RH		10.9	6.4	7.2	12.2	14.7	21.8	14.2
75% RH		6.9	7.5	5.7	11.7	12.1	12.5	11.2
BHA								
61% RH		8.4	8.5	6.8	8.0	16.8	16.0	13.9
75% RH		7.3	6.7	-	13.2	5.9	5.1	10.4
BHA/EDTA								
61% RH		11.5	8.9	5.8	-	4.8	2.7	-
75% RH		7.1	2.2	5.4	5.6	7.4	3.5	3.5
Citric Acid								
61% RH		10.2	11.8	17.8	8.9	17.8	17.4	17.3
75% RH		8.1	5.4	5.6	11.4	4.7	4.7	11.1
Ascorbic Acid								
61% RH		11.0	10.7	7.1	9.5	10.3	9.4	8.6
75% RH		8.5	9.7	6.3	8.5	12.6	14.1	13.5
Tocopherol								
61% RH		10.5	10.2	6.7	9.3	20.0	17.6	13.1
75% RH		7.7	6.5	10.7	6.0	6.0	8.2	9.0

Table 25

Peroxide Values Freeze-Dried Chicken System

Run 21 37°C

[PV=meq/Kg Fat]

System	Days	5	11	18	26	33	47	61	82	95	116
Control											
32% RH		1.0	0.1	1.3	2.7	3.3	4.0	4.5	5.0	7.3	*57.8
61% RH		0.1	0	1.6	3.5	3.4	3.2	5.3	5.7	9.3	*16.1
75% RH		4.4	0.1	1.7	2.2	4.2	2.5	4.1	6.4	5.9	5.8
BHA (200 ppm)[a]											
32% RH		0	0	2.6	0	1.3	1.6	1.9	3.0	2.6	3.8
61% RH		0	0	0	2.6	3.5	2.5	3.1	3.9	2.1	5.6
75% RH		0	0	2.4	2.7	2.3	1.2	2.9	2.6	0.9	2.7
PG (200 ppm)[a]											
32% RH		0	0	0.1	0.9	2.6	2.3	3.3	4.9	5.5	5.7
61% RH		0	0	1.4	1.9	1.1	2.0	2.8	4.3	7.4	4.9
75% RH		0	3.2	1.4	2.0	2.6	1.6	4.3	3.0	4.3	4.3
Control-NG[b]											
32% RH		0.8	3.2	---	3.6	---	4.4	4.2	---	6.5	---
61% RH		0.7	0	---	3.0	---	4.5	4.2	---	7.3	---
75%		0.8	0	---	3.9	---	6.1	9.9	---	7.8	---

(a) On fat basis

(b) No glycerol

* Noted slight rancid odor.

Table 26

Non-Enzymatic Browning Freeze-Dried Chicken System

Food Run 21 37° C

	B = (OD x 100)/gram					
Days	5	11	18	26	33	47
System						
Control						
32% RH	11.3	10.1	43.0	24.0	28.0	11.0
61% RH	2.6	6.9	----	26.6	17.7	11.0
75% RH	16.3	24.0	30.0	17.2	15.2	6.9
BHA						
32% RH	5.5	13.5	28.0	19.4	15.5	15.6
61% RH	9.4	8.4	27.0	30.5	40.1	34.2
75% RH	3.7	9.0	18.0	35.8	20.1	10.7
PG						
32% RH	12.1	8.3	17.0	27.1	26.5	34.1
61% RH	7.2	12.2	36.0	29.0	27.6	9.8
75% RH	7.2	9.5	19.4	42.8	16.5	17.8
Control-NG						
32% RH	8.8	7.4	----	55.4	----	49.0
61% RH	7.0	9.7	----	69.0	----	21.5
75% RH	7.7	7.6	----	33.6	----	55.7

Table 27

Activation Energies (K cal/ g mole °K)

Run 23 Chicken/Cellulose/Glycerol System

	Peroxide Development	Browning Development
Control	10.2	15.9
EDTA	16.7	17.4
BHA	1.5	17.6

VII. Figures

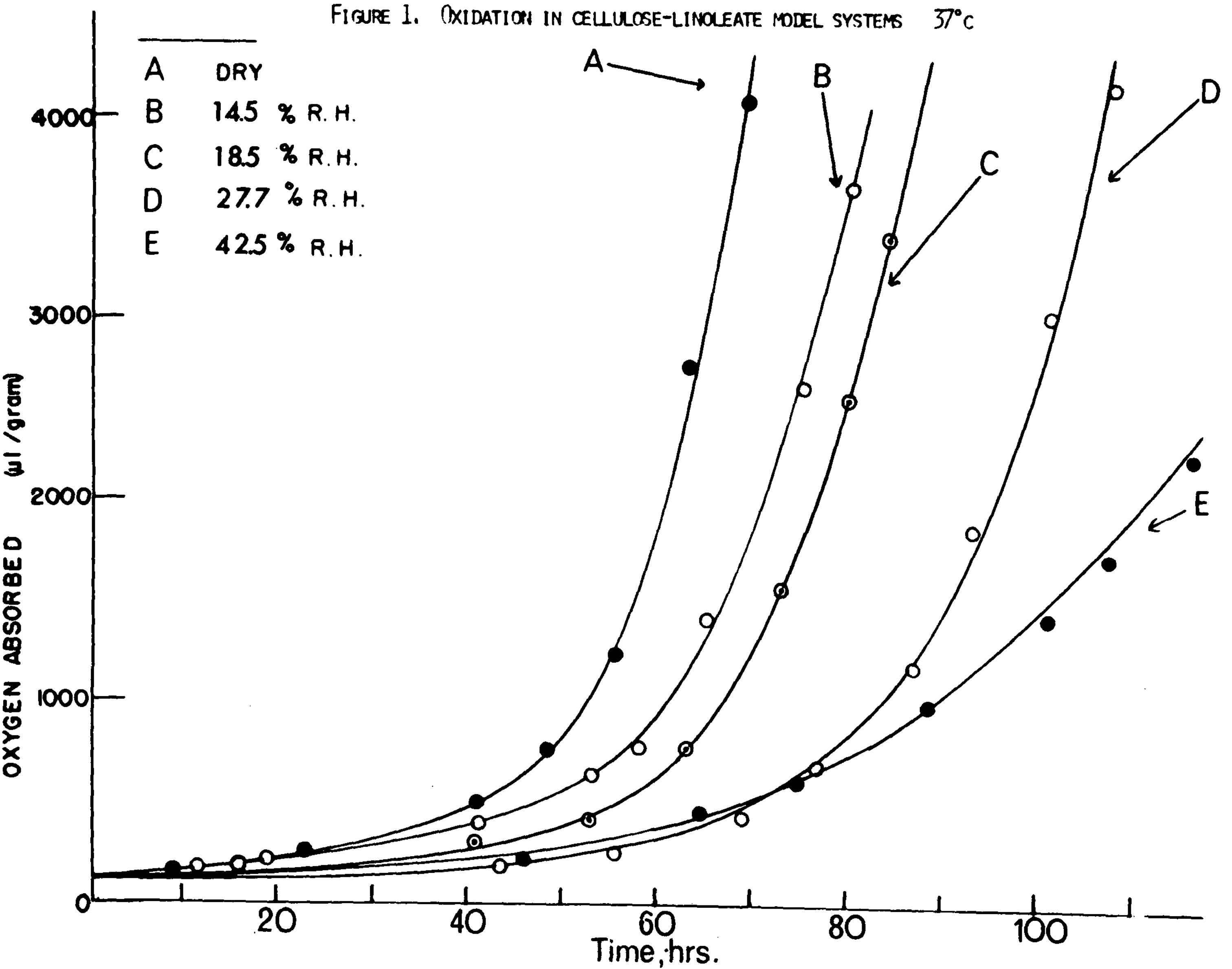


FIGURE 1. OXIDATION IN CELLULOSE-LINOLEATE MODEL SYSTEMS 37°C

Figure 2. Oxidation of freeze-dried salmon humidified to different levels 37°C.

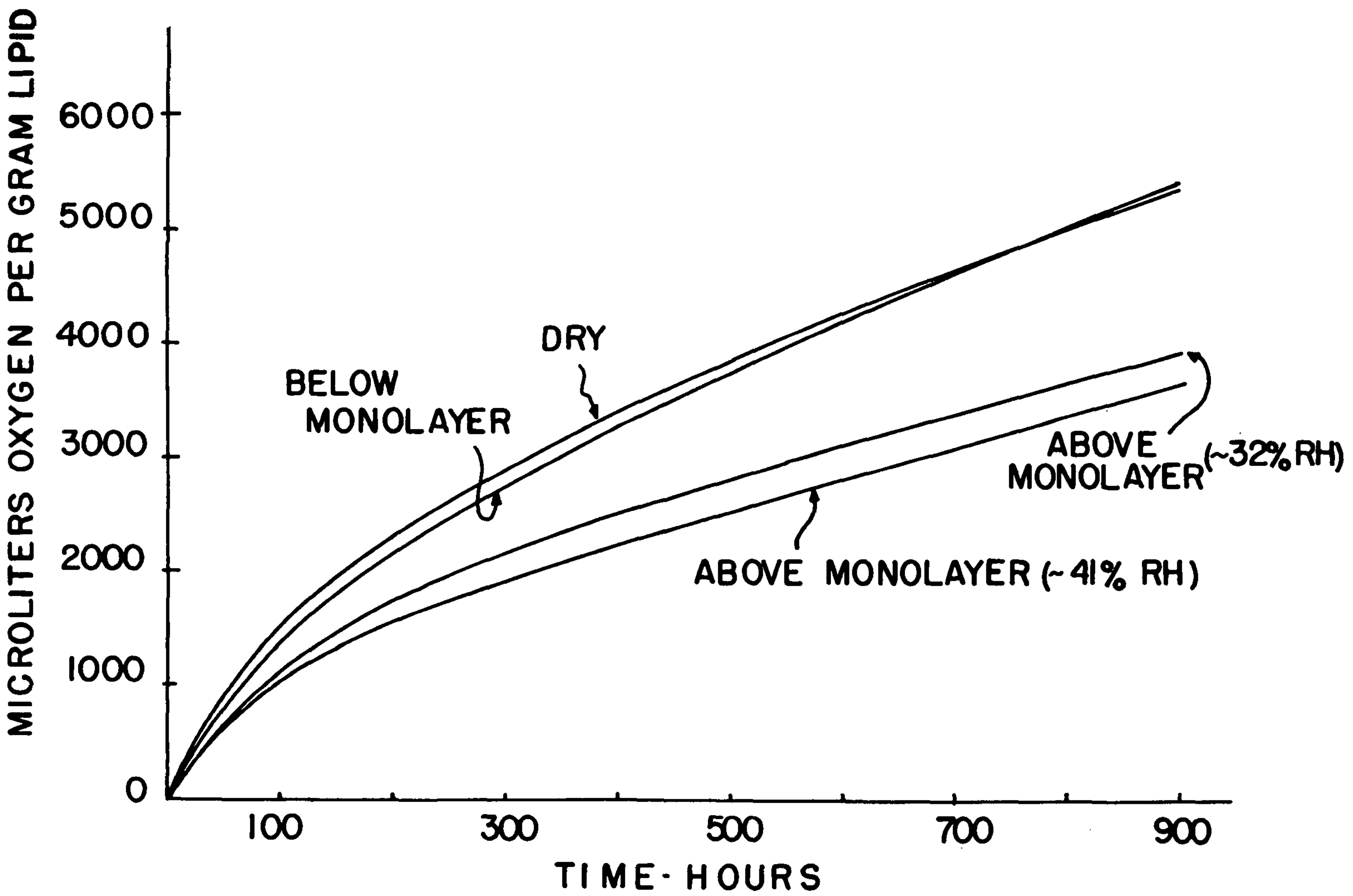
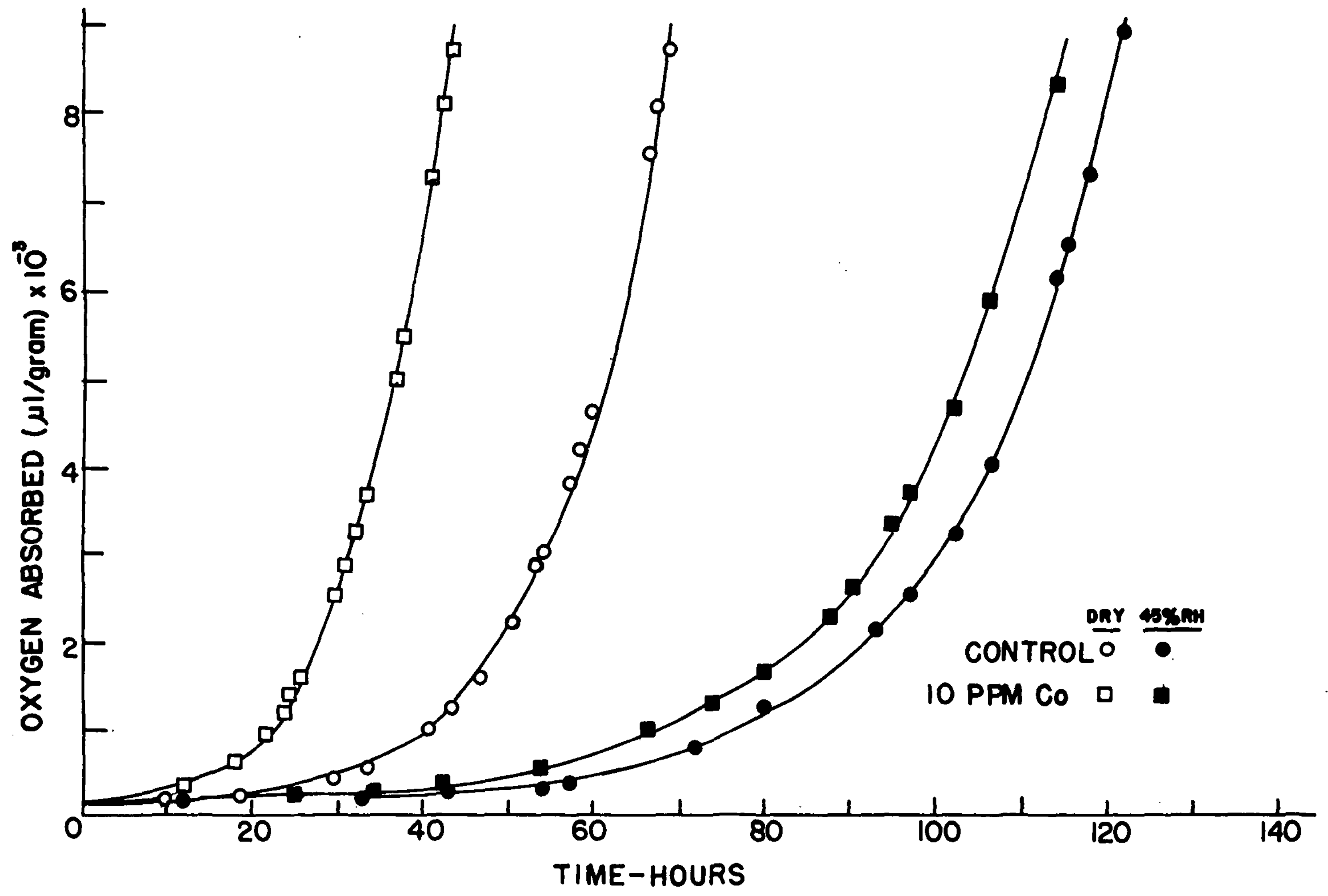


FIGURE 3. EFFECT OF HUMIDIFICATION ON COBALT CATALYZED OXIDATION OF CELLULOSE-LINOLEATE MODEL SYSTEMS.



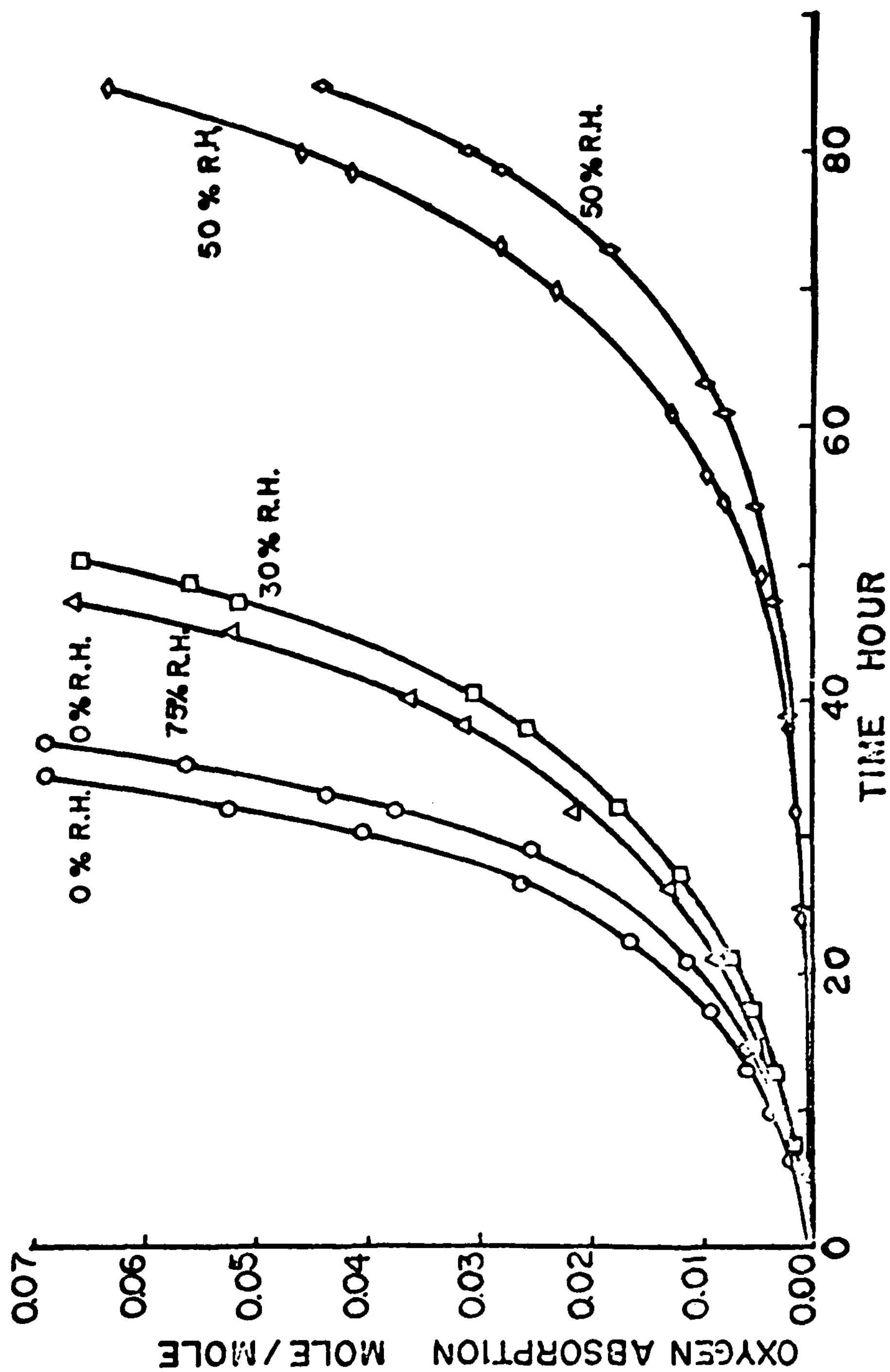


FIGURE 4. EFFECT ON HUMIDIFICATION IN THE INTERMEDIATE MOISTURE RANGE ON OXIDATION IN THE PRESENCE OF COBALT.

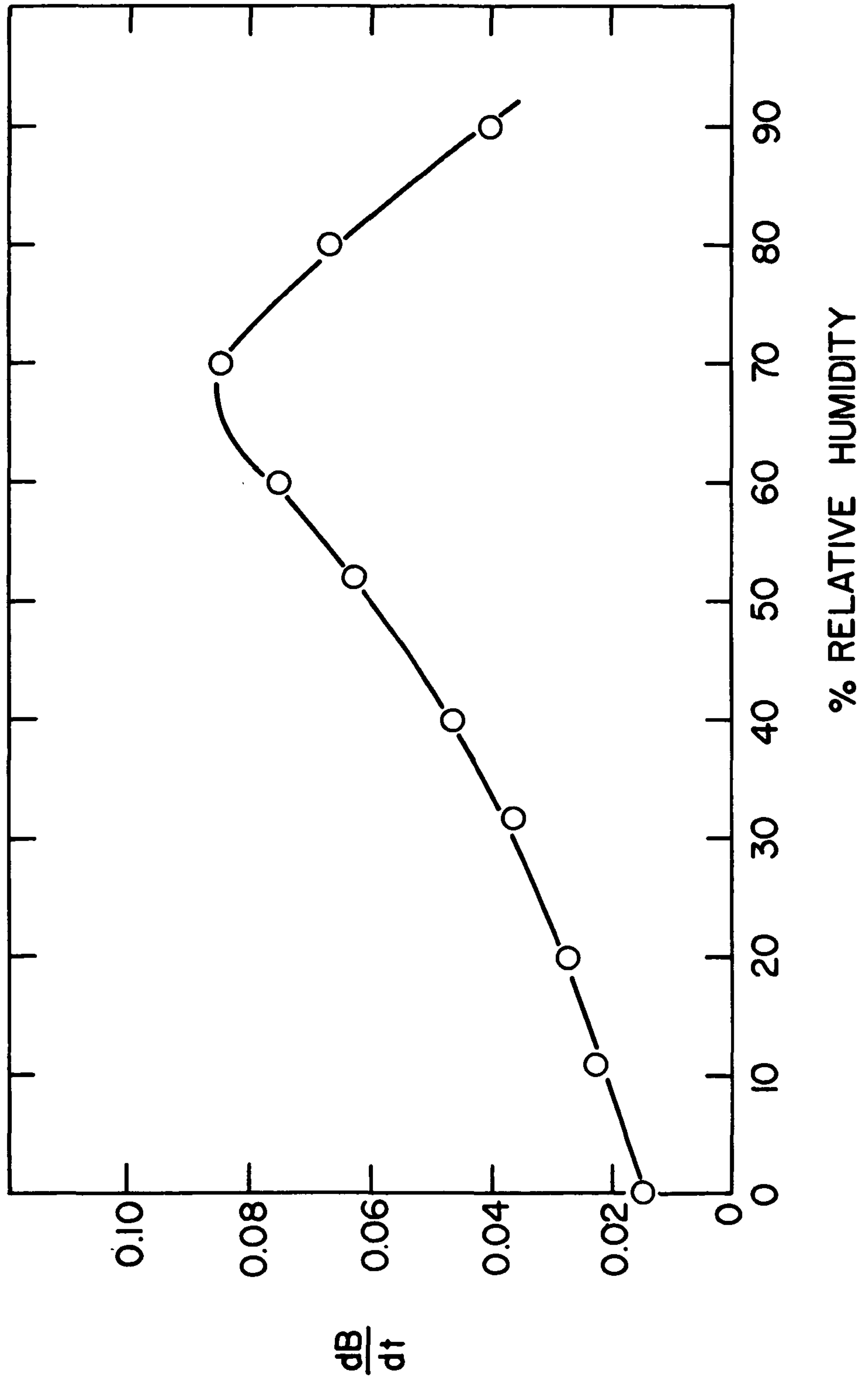


FIGURE 5. RATE OF BROWNING OF PEA SOUP AS A FUNCTION OF RELATIVE HUMIDITY.

FIGURE 6, ENZYMATIC HYDROLYSIS OF LECITHIN AS A FUNCTION OF RELATIVE HUMIDITY.

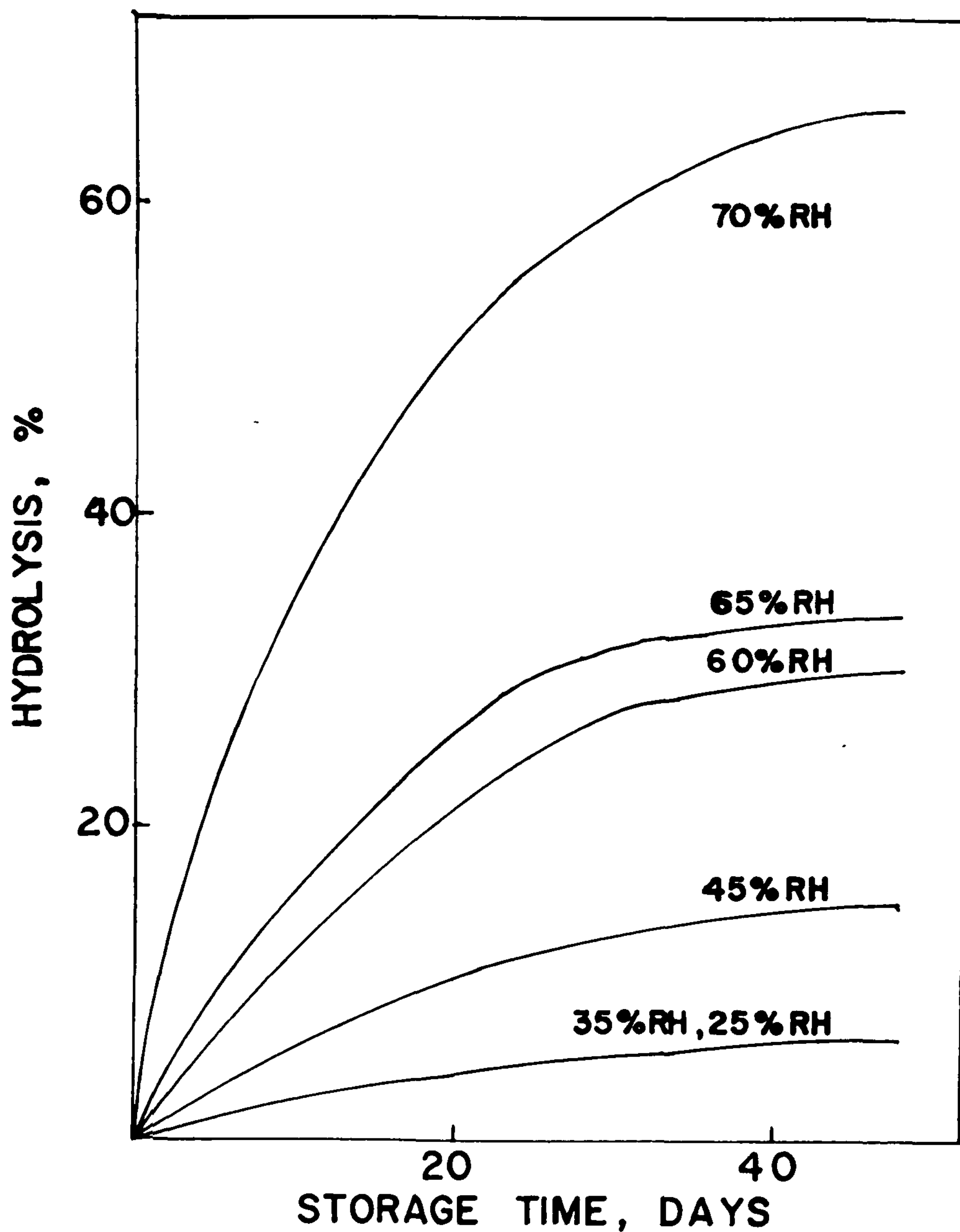


FIGURE 7, ABSORPTION CURVE FOR CAROTENOID PIGMENTS IN CARROTS.

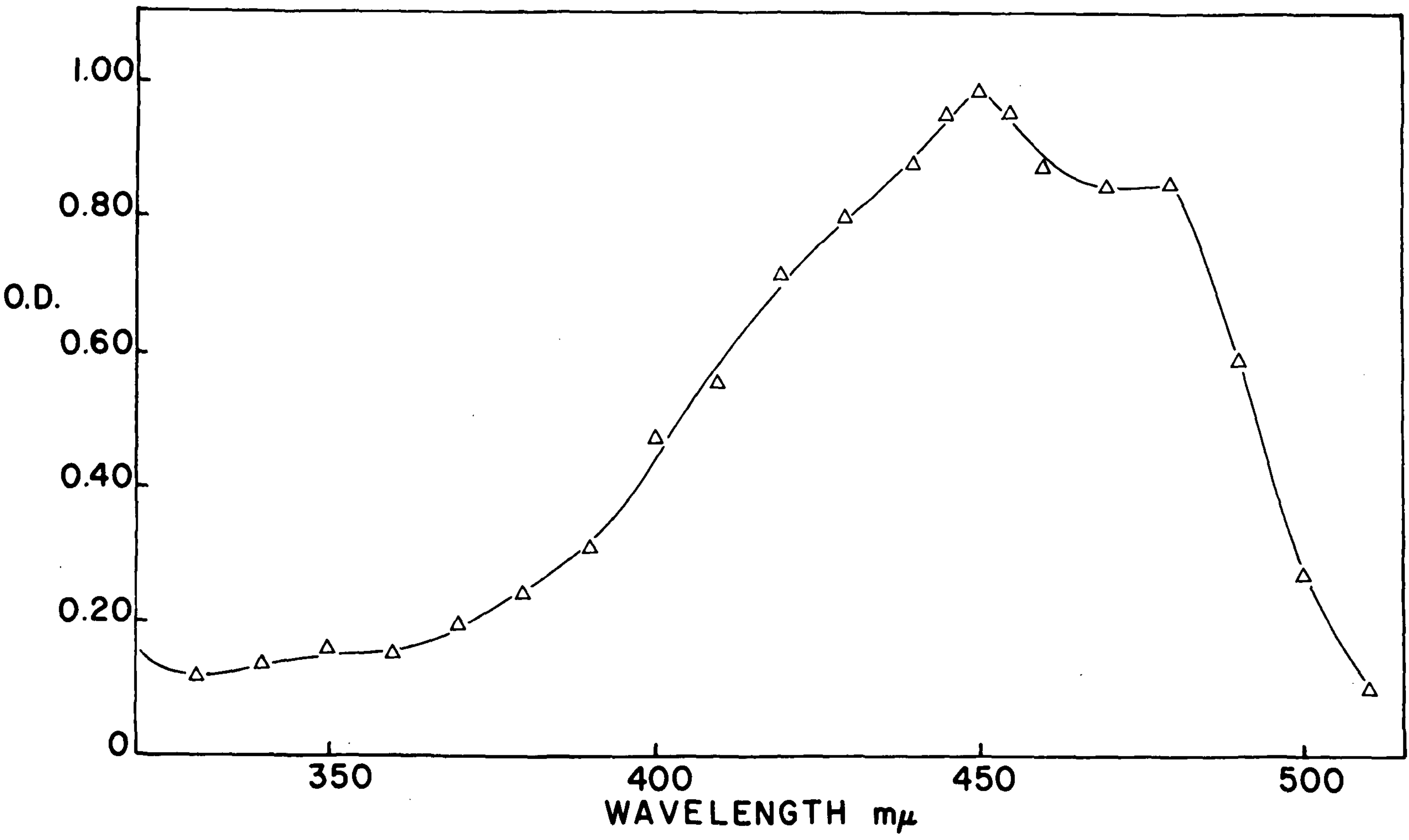


FIGURE 8, MOISTURE ISOTHERMS OF CELLULOSE MODEL SYSTEMS FOR VARIOUS AMOUNTS OF GLYCEROL. 37°C.

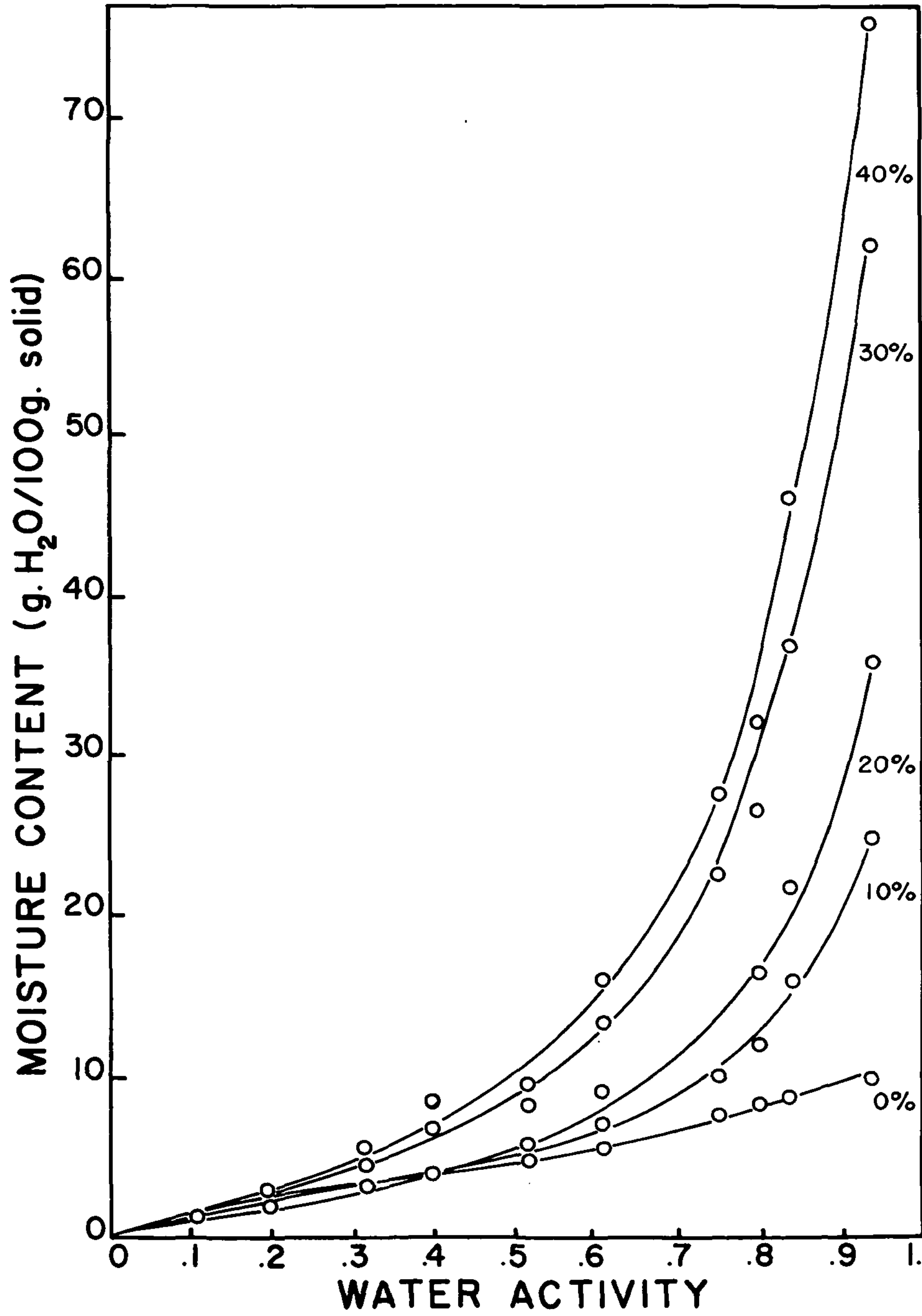


FIGURE 9, MOISTURE ISOTHERMS OF PROTEIN MODEL SYSTEM A FOR VARIOUS AMOUNTS OF GLYCEROL 37°C.

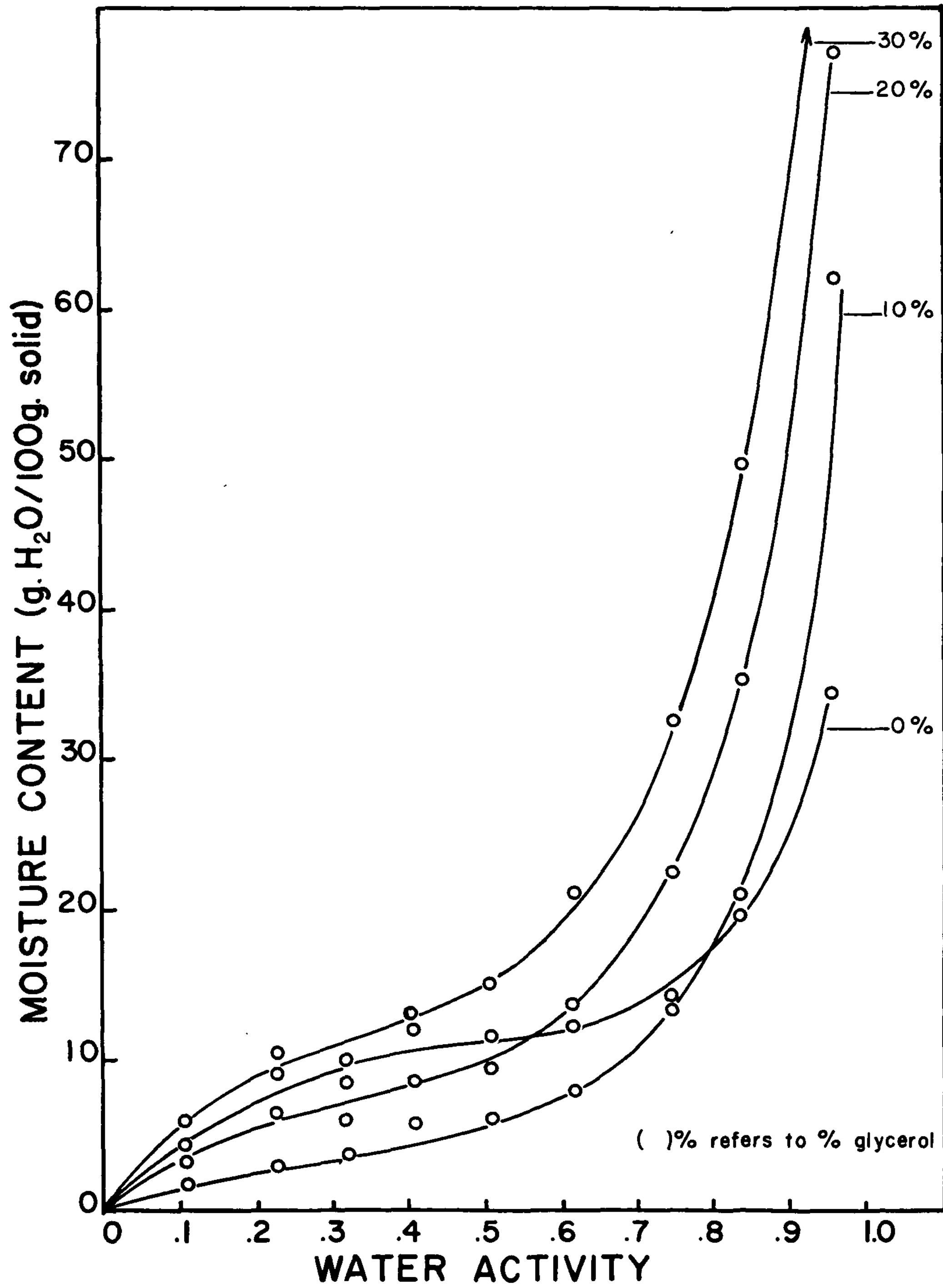


FIGURE 10, MOISTURE ISOTHERMS OF PROTEIN MODEL SYSTEM B FOR VARIOUS AMOUNTS OF GLYCEROL 37°C.

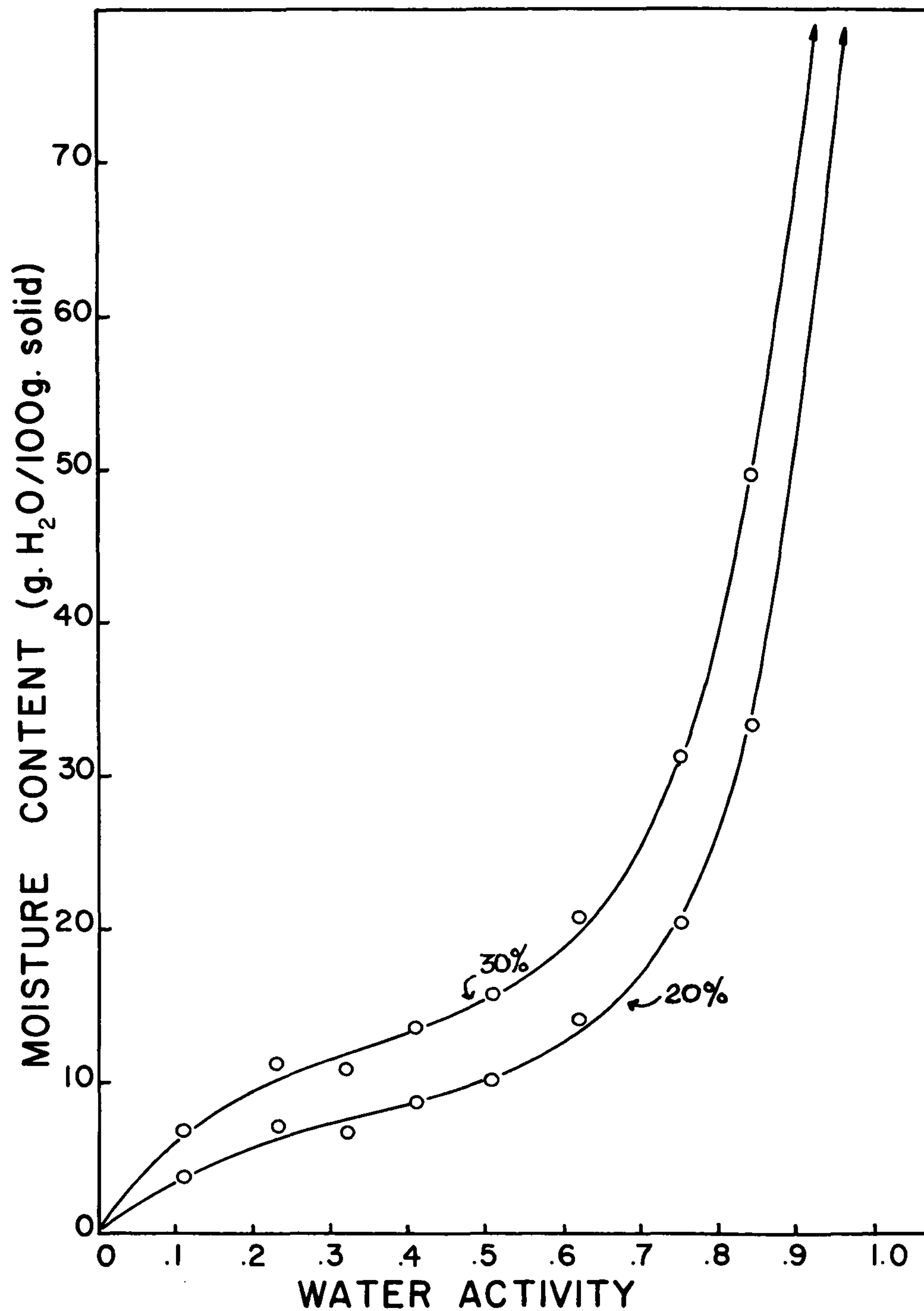


FIGURE 11. COMPARISON OF MOISTURE SORPTION ISOTHERMS 37°C.

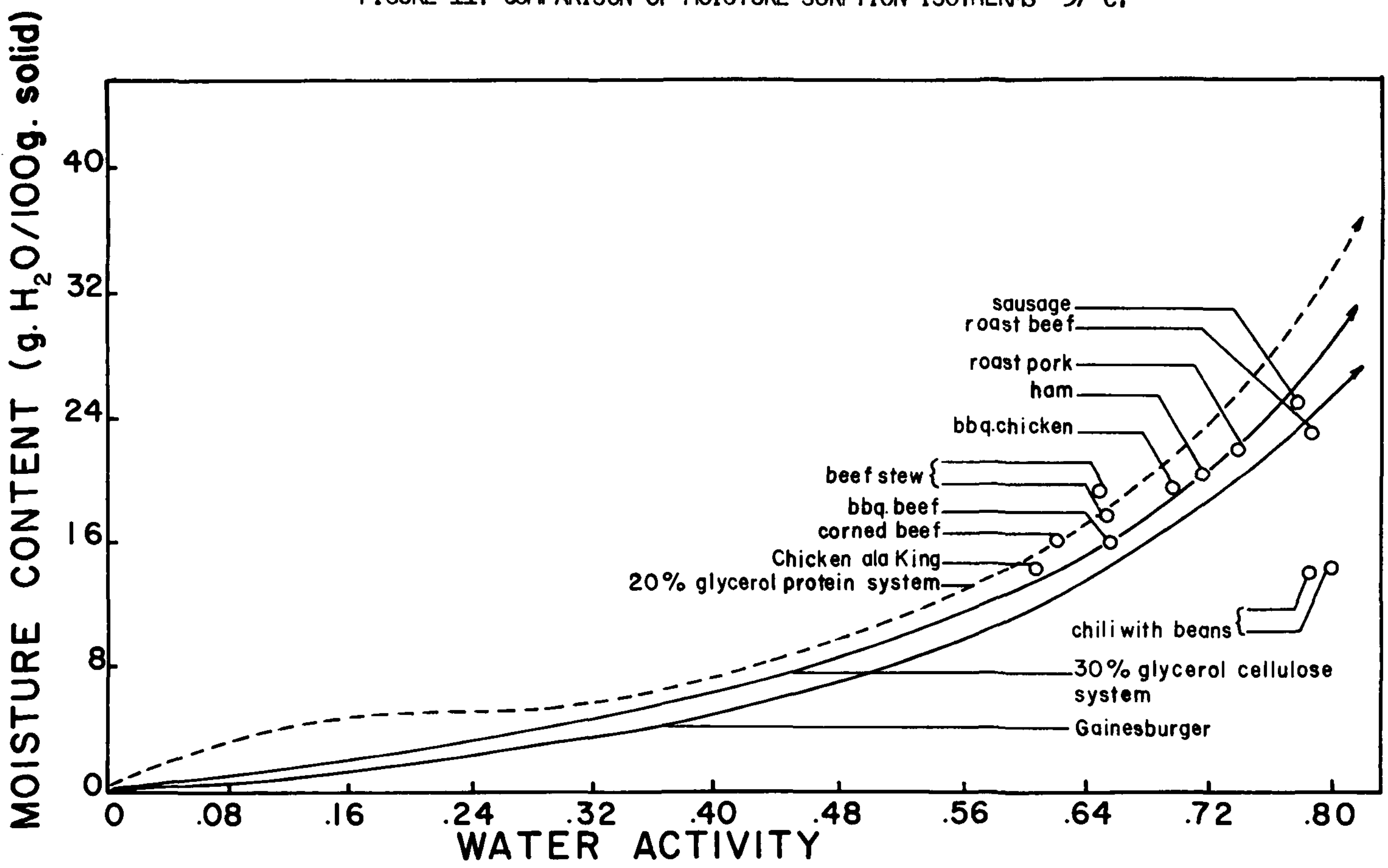


FIGURE 12. MOISTURE SORPTION ISOTHERM - SUCROSE/SORBITOL SYSTEM.

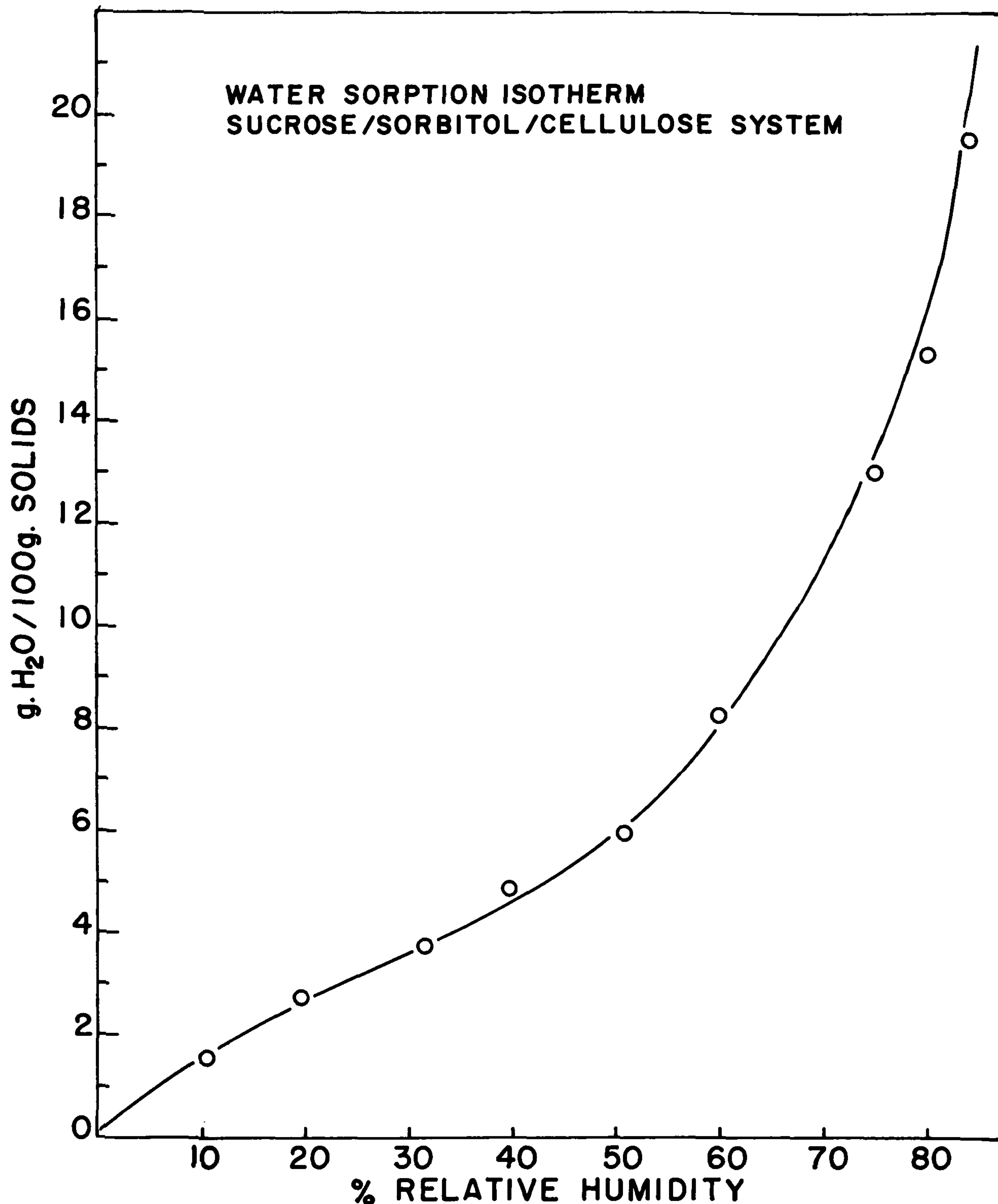


FIGURE 13. MOISTURE SORPTION ISOTHERM - SUCROSE/PROPYLENE GLYCOL SYSTEM 37°C.

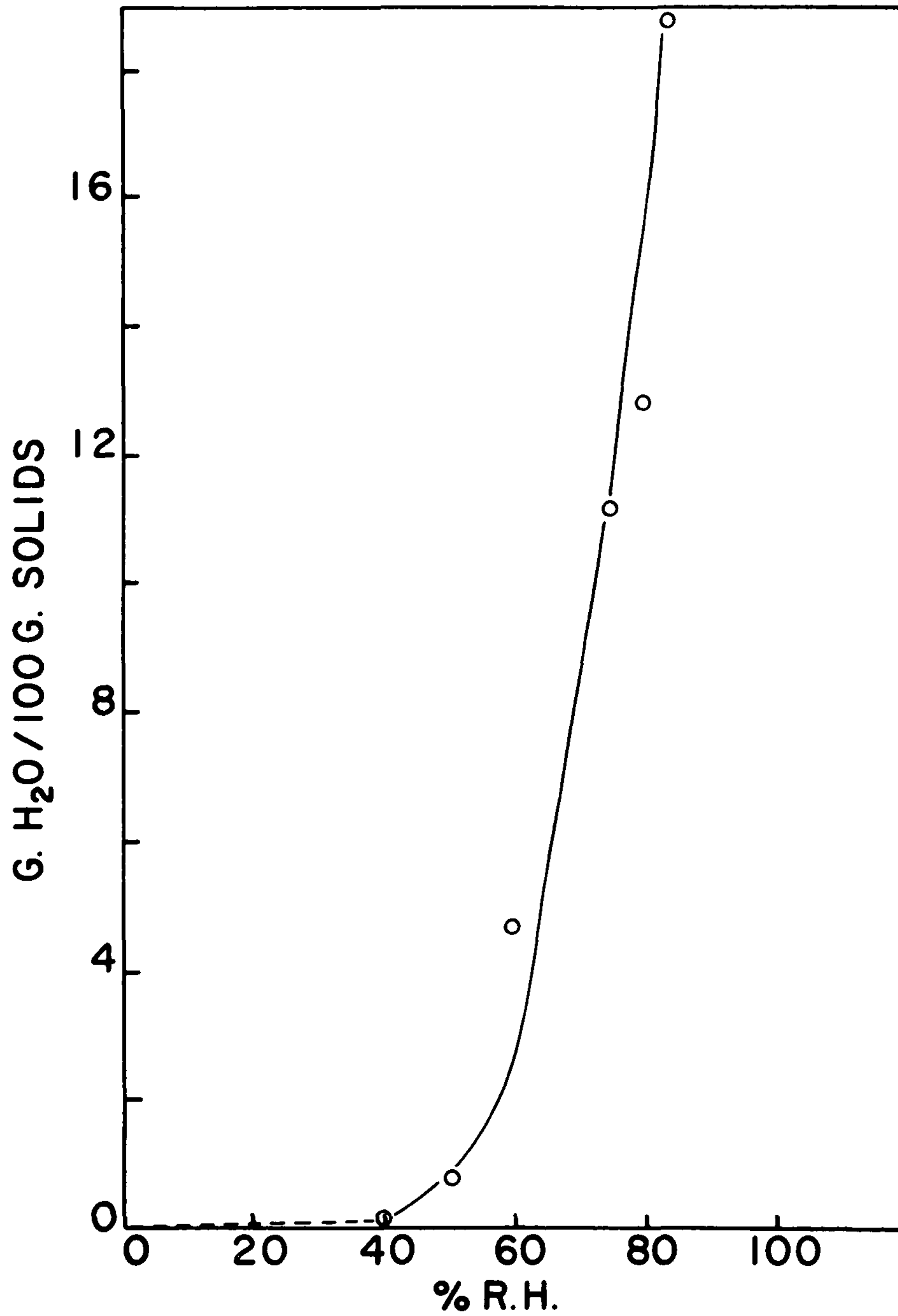


FIGURE 14. RUN # 1 - OXYGEN UPTAKE AT 37°C BY METHYL LINOLEATE-GLYCEROL CELLULOSE SYSTEM. CATALYST: 100 PPM OF COBALT AS $Co[NO_3]_2$

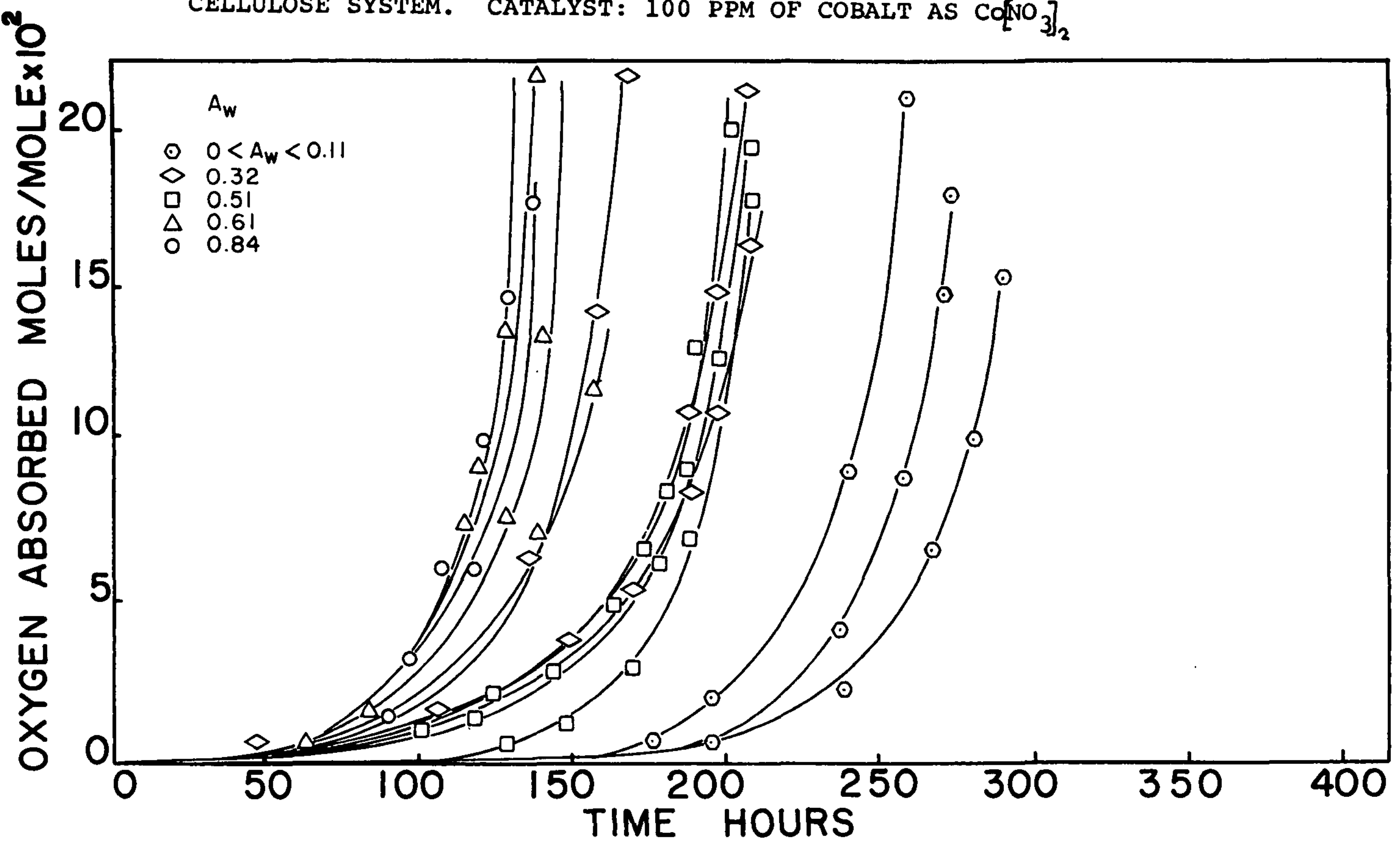


FIGURE 15. RUN #2 - OXIDATION OF CELLULOSE MODEL SYSTEM WITHOUT GLYCEROL EFFECT OF 75% RH VS. DRY

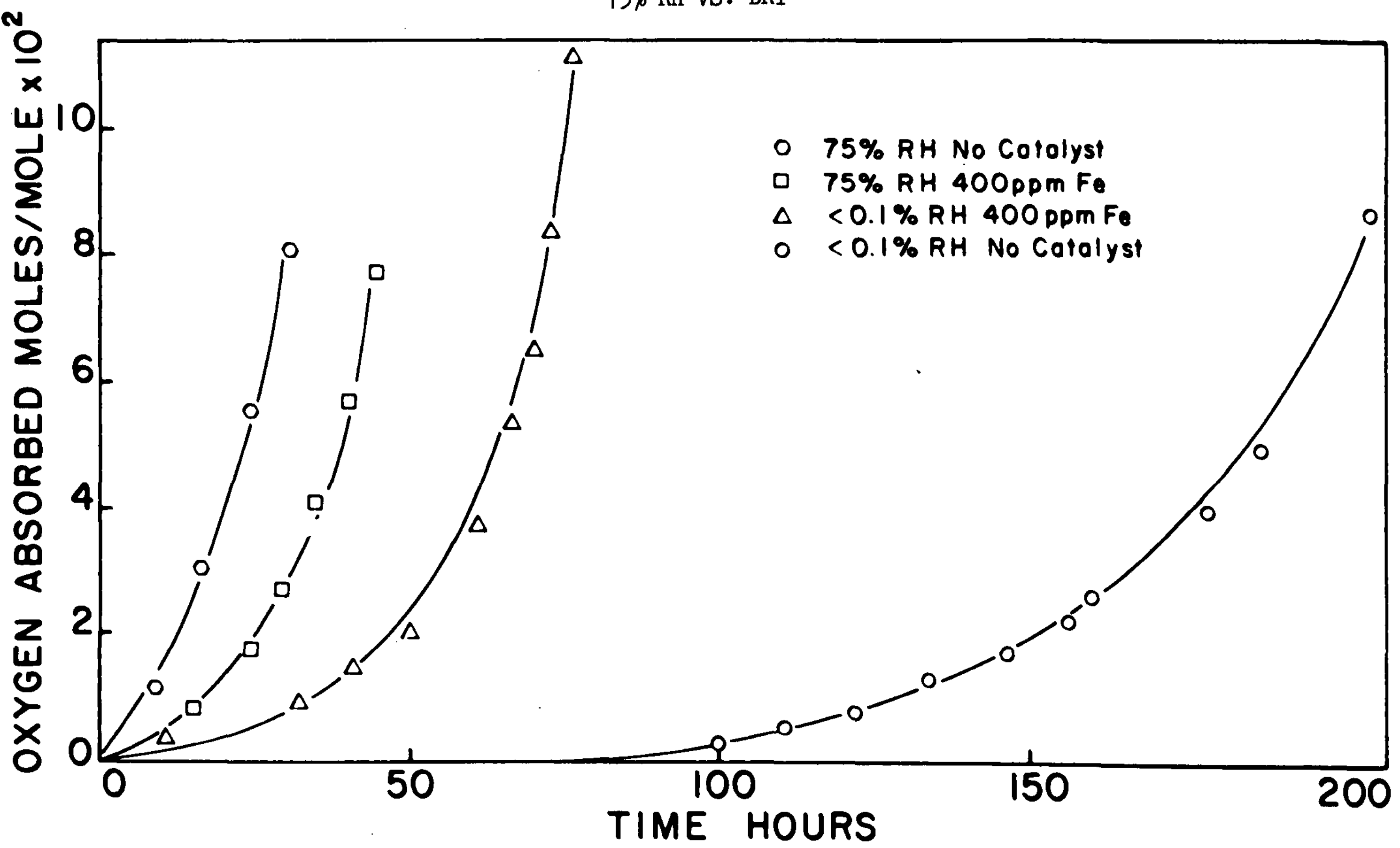


FIGURE 16, RUN #2 - OXIDATION OF CELLULOSE/ 30% GLYCEROL MODEL SYSTEM EFFECT OF 75% RH VS. DRY.

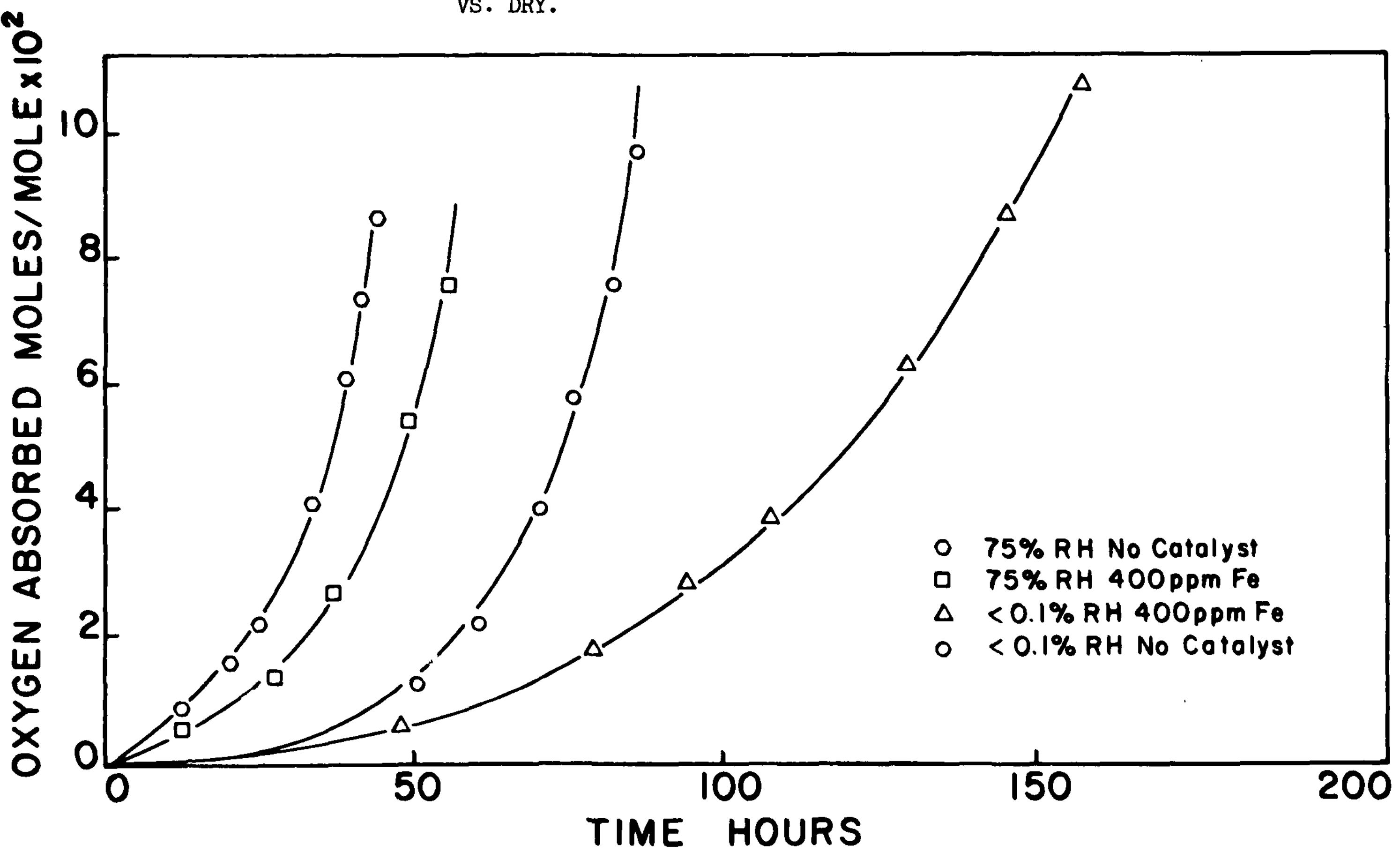
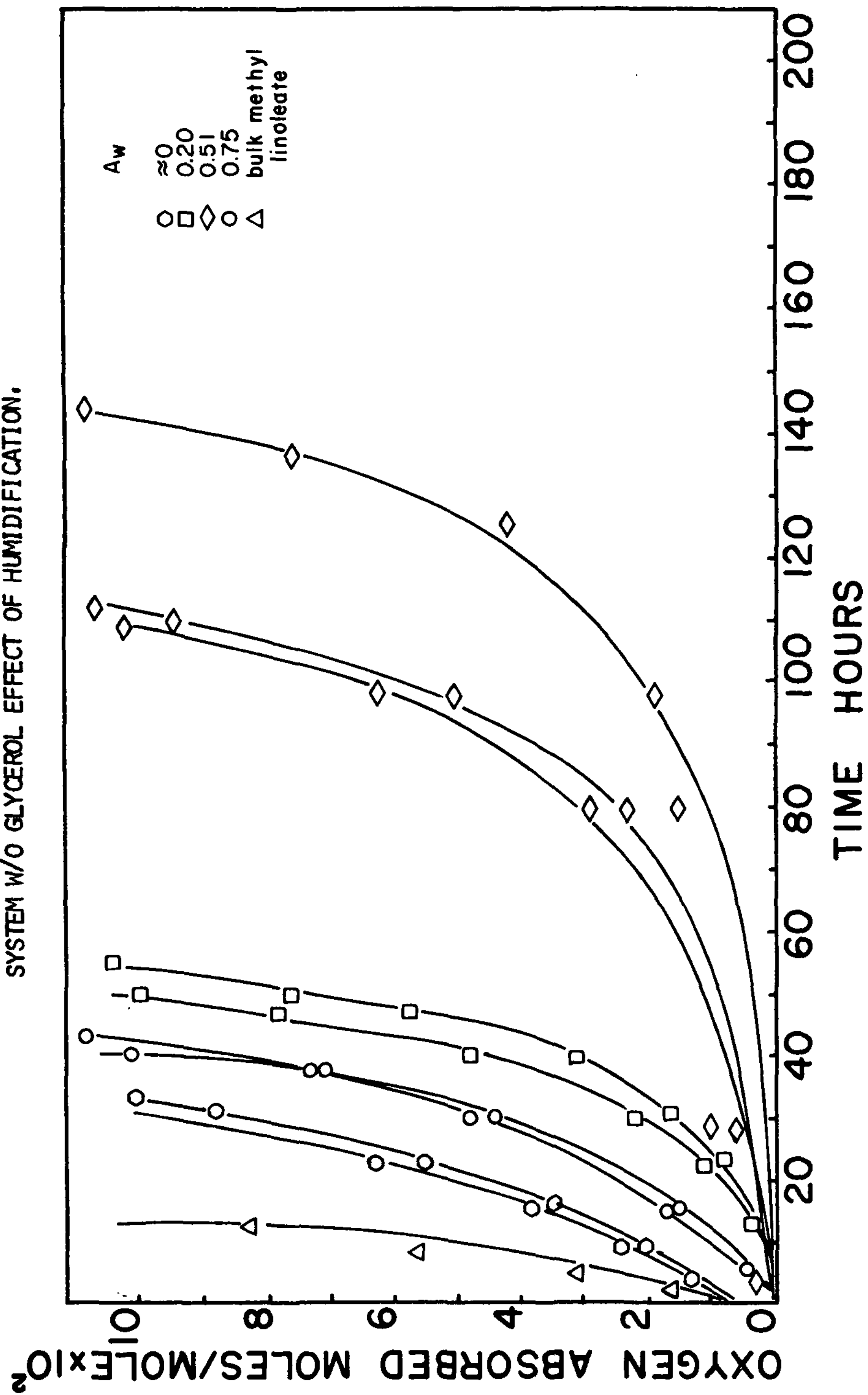


FIGURE 17. RUN #6 - OXIDATION OF CELLULOSE MODEL SYSTEM W/O GLYCEROL EFFECT OF HUMIDIFICATION.



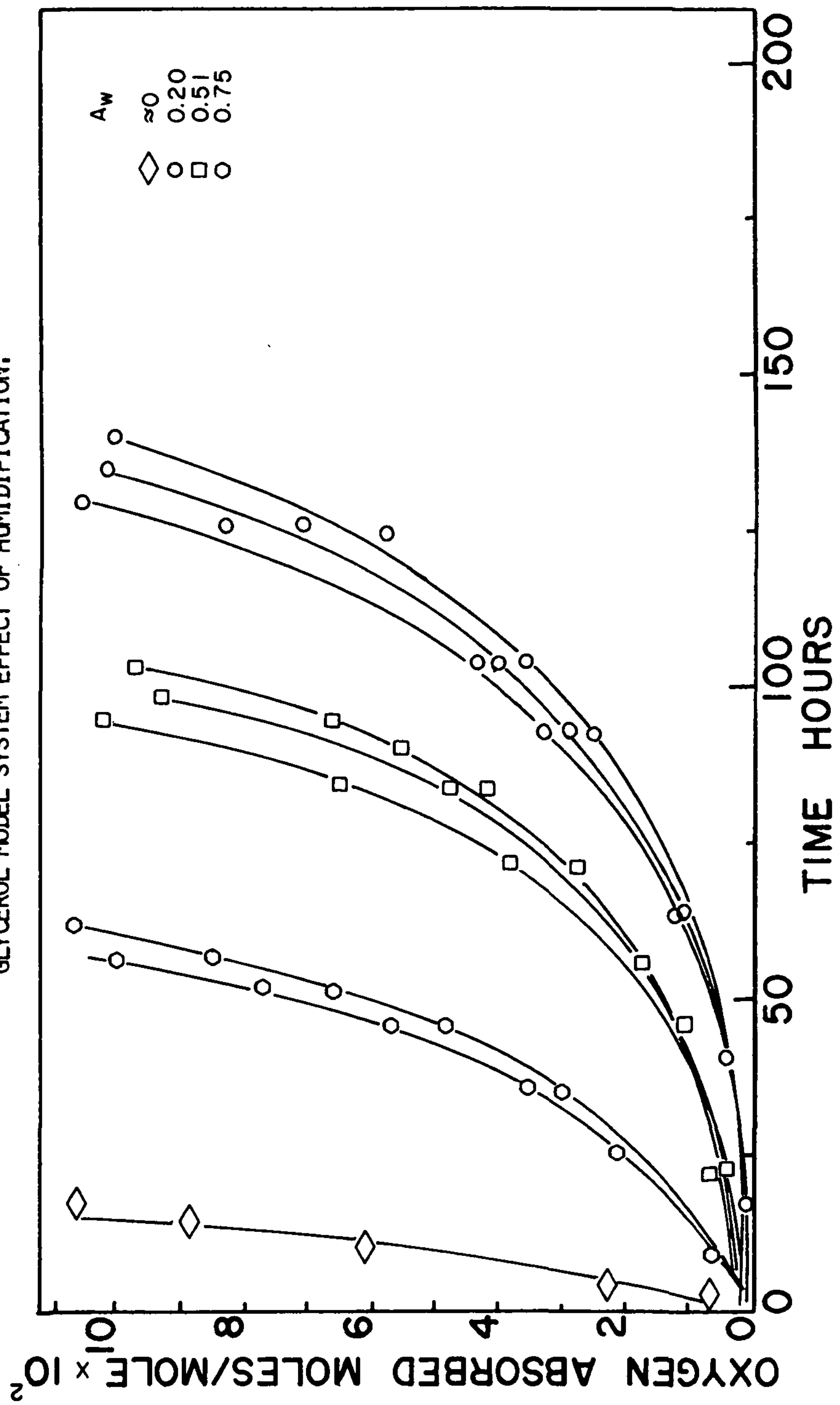


FIGURE 18. RUN #6 - OXIDATION OF CELLULOSE/30% GLYCEROL MODEL SYSTEM EFFECT OF HUMIDIFICATION.

Figure 19. Run #7 - Effect of humidification on oxidation of cellulose model system w/o glycerol.

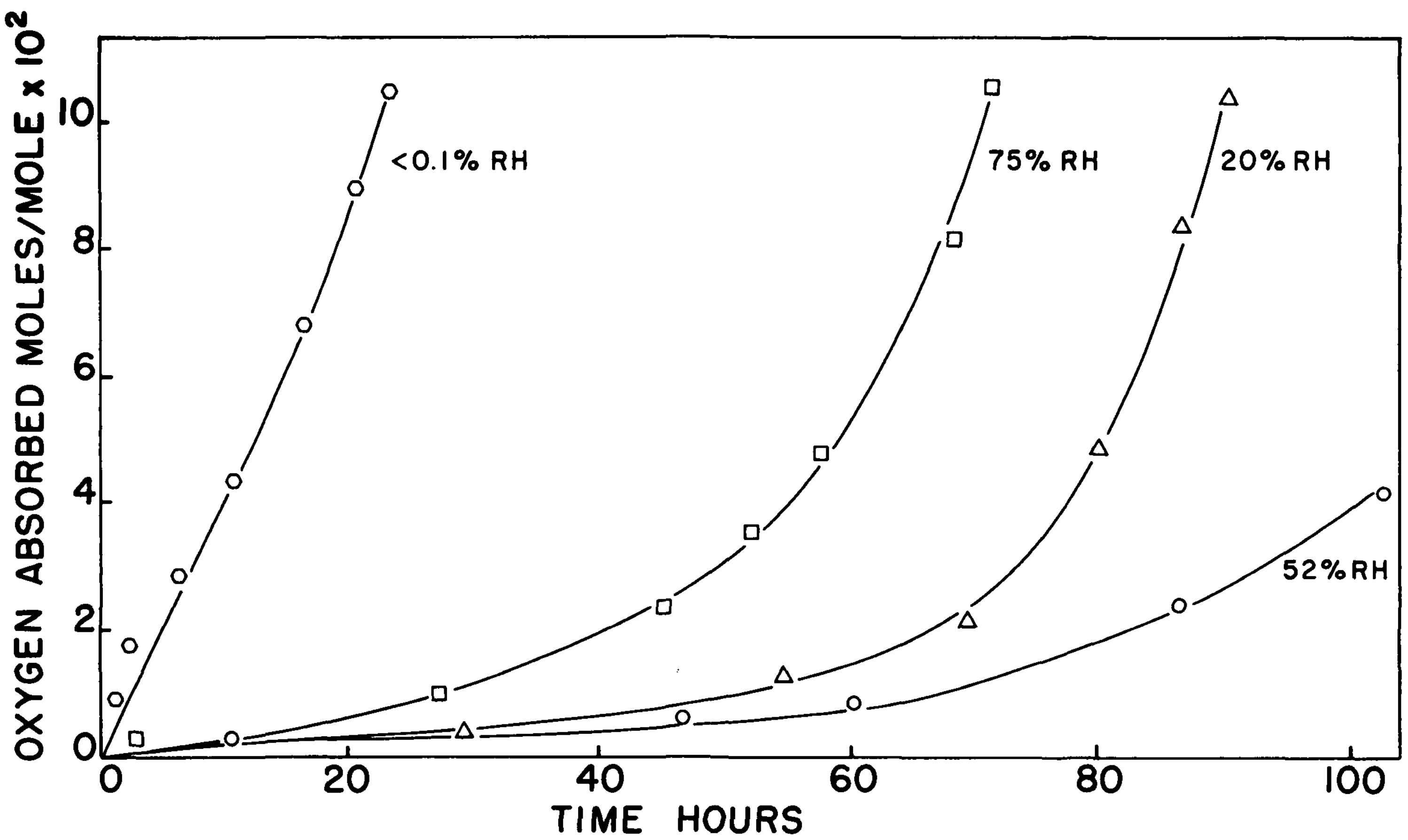


FIGURE 20. RUN #7 - EFFECT OF HUMIDIFICATION ON OXIDATION OF CELLULOSE/30% GLYCEROL MODEL SYSTEM.

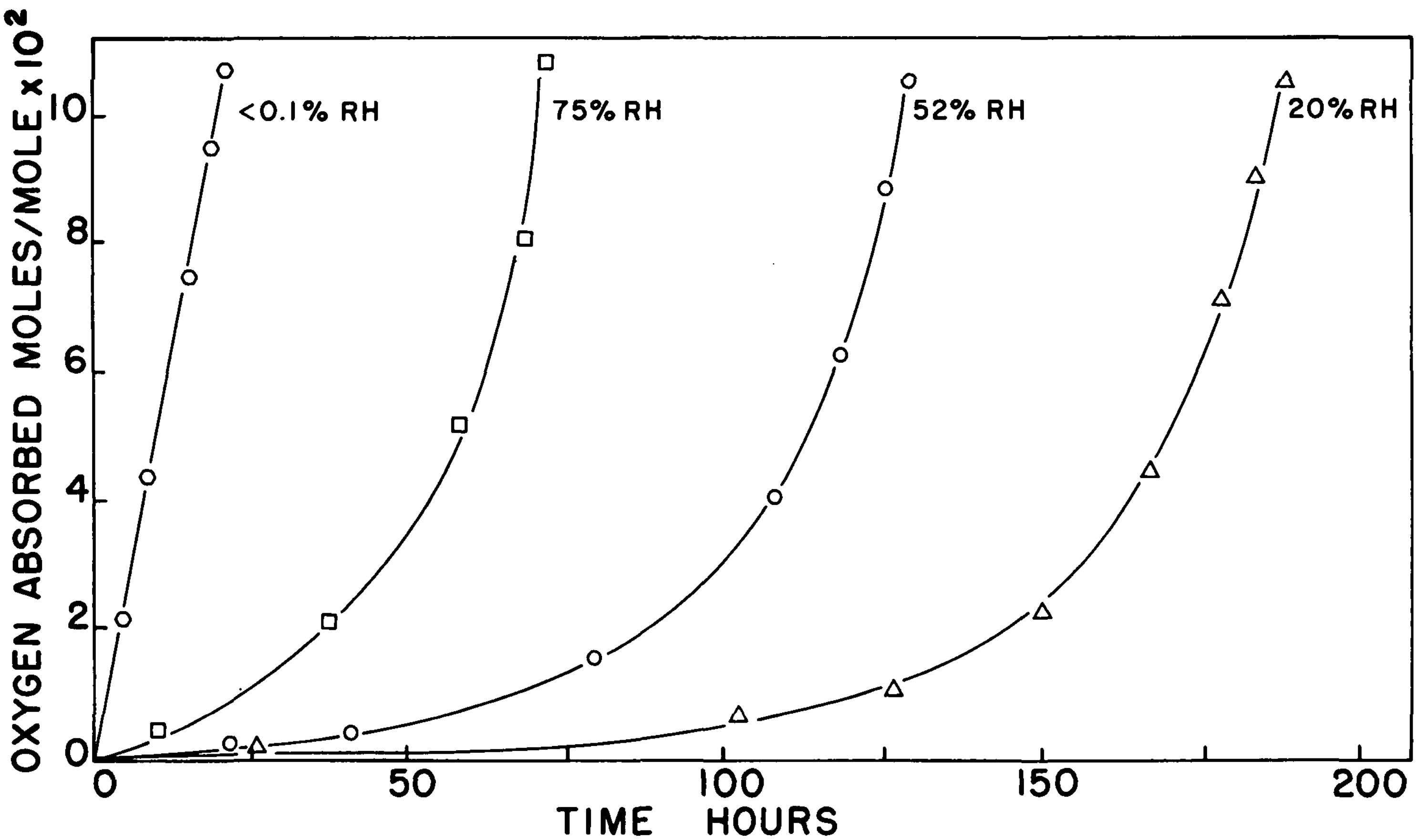


Figure 21. Run #14 - Effect of humidification on oxidation of 20% glycerol/protein A system.

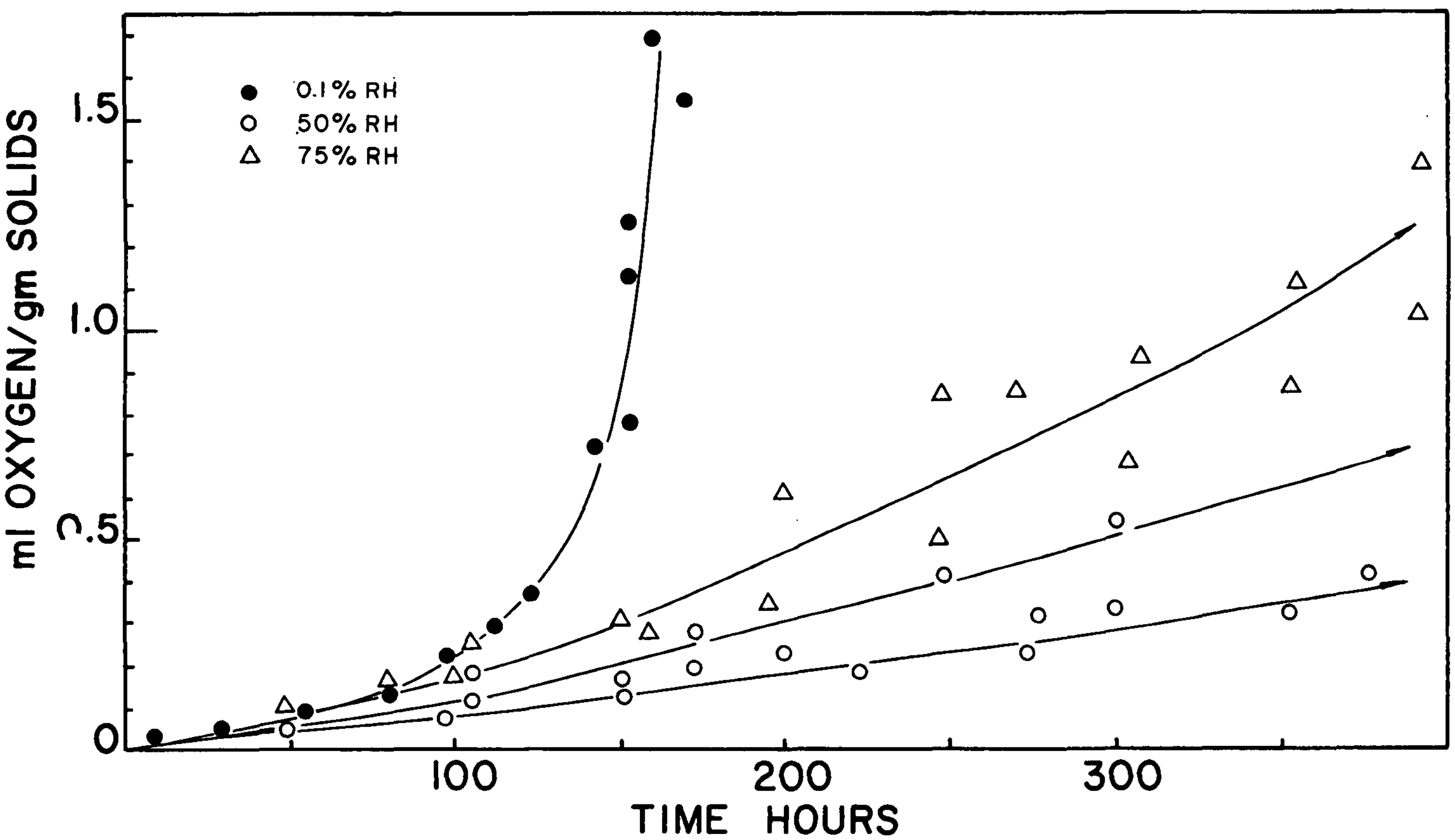


Figure 22. Run #14 - Effect of humidification on oxidation of 20% glycerol/protein B system.

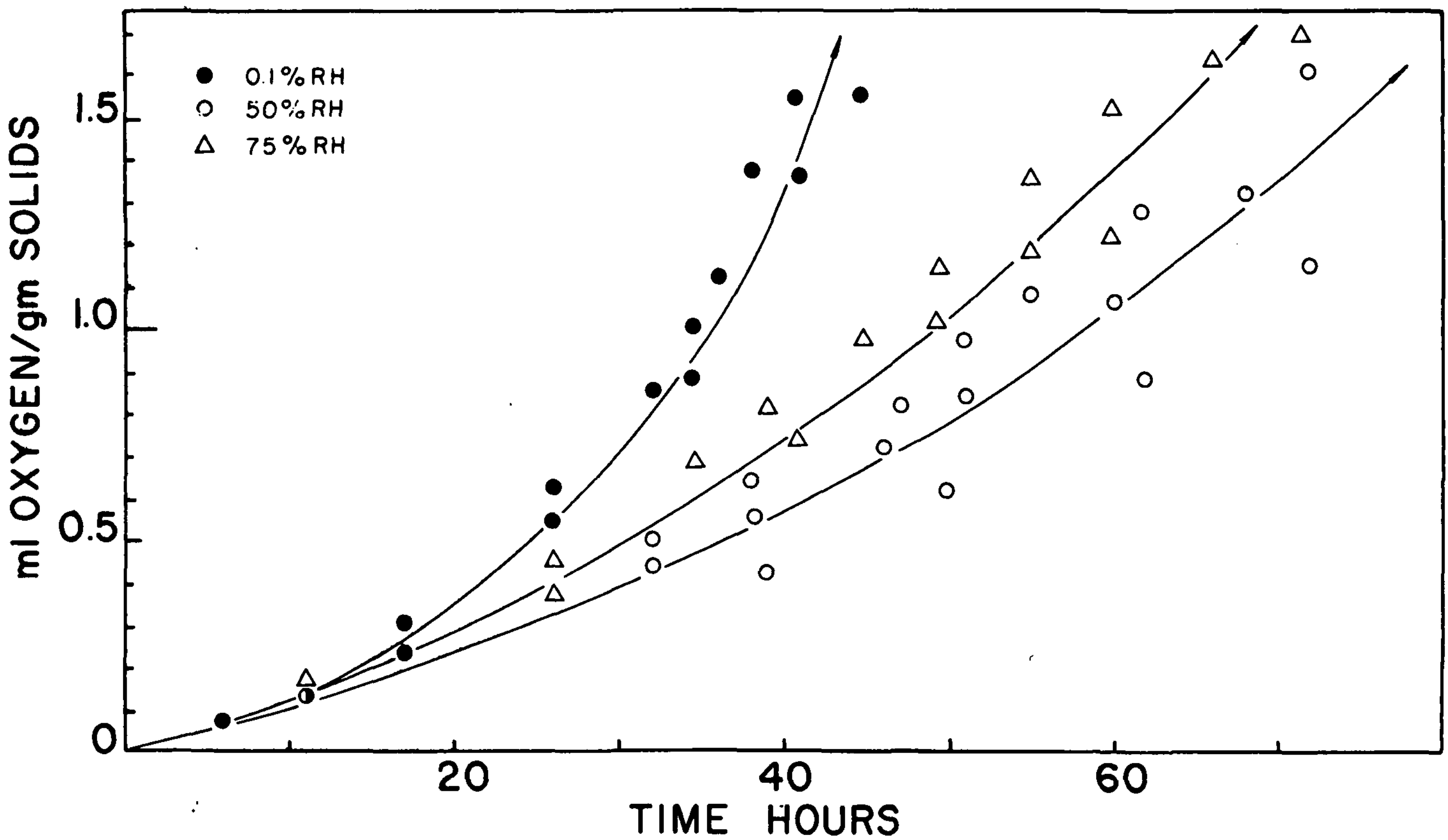


Figure 23. Run #15 - Effect of humidification on oxidation of 20% glycerol/protein A system.

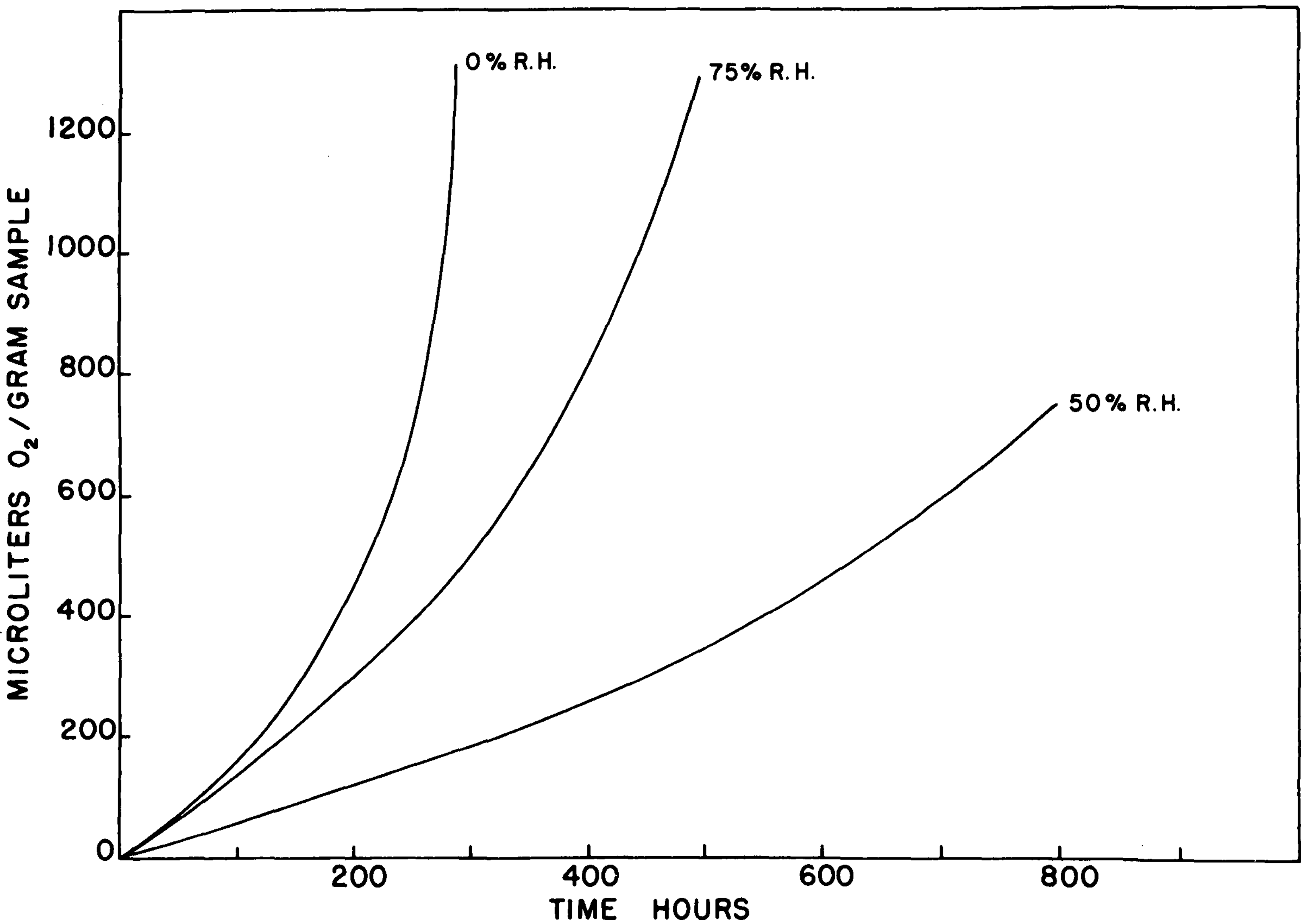


FIGURE 24. RUN #15 - EFFECT OF HUMIDIFICATION ON OXIDATION OF 20% GLYCEROL/ PROTEIN B SYSTM.

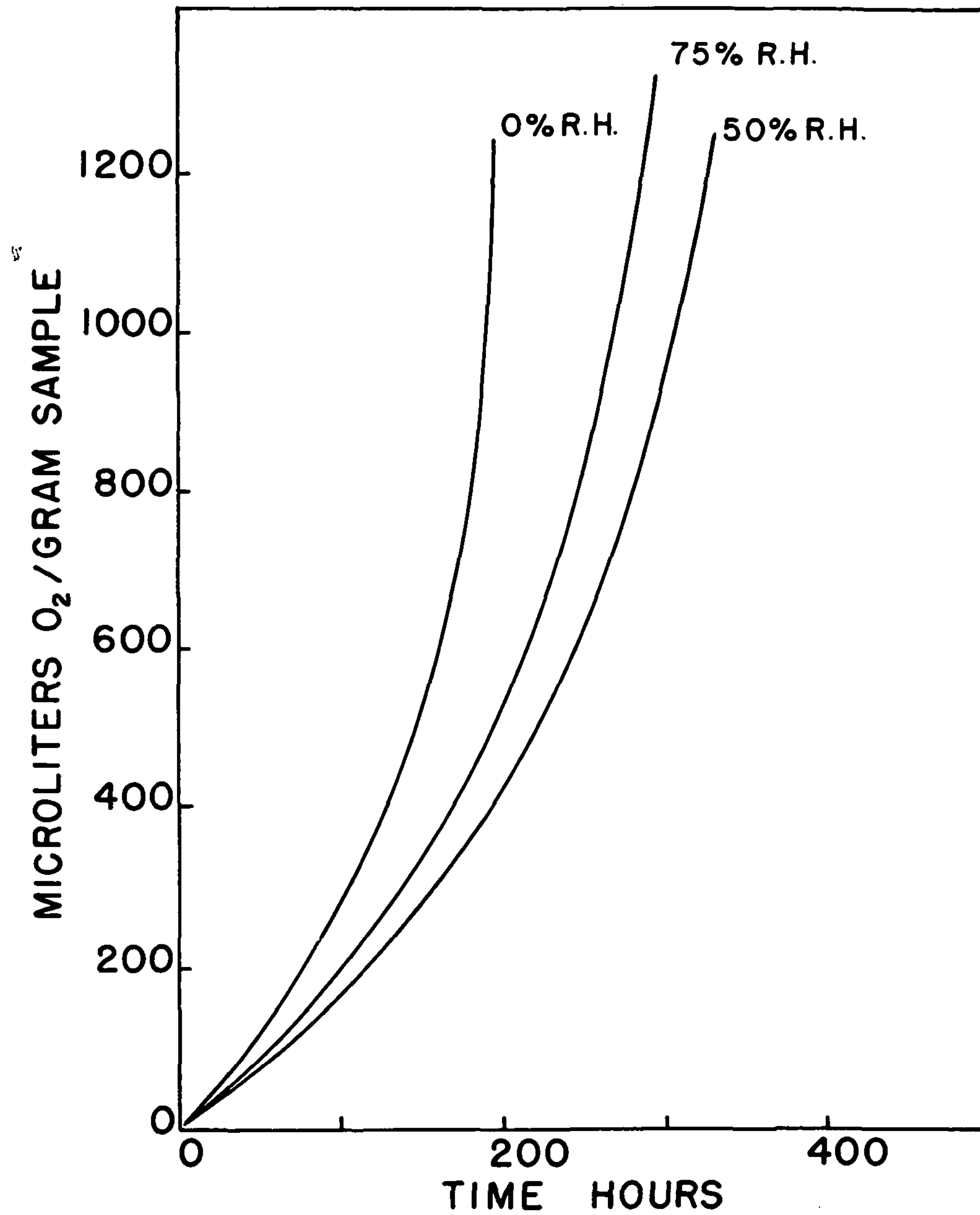


FIGURE 25. RUN #16 - EFFECTIVENESS OF PHENOLIC ANTIOXIDANTS IN CELLULOSE/30% GLYCEROL MODEL SYSTEM, 20% RH.

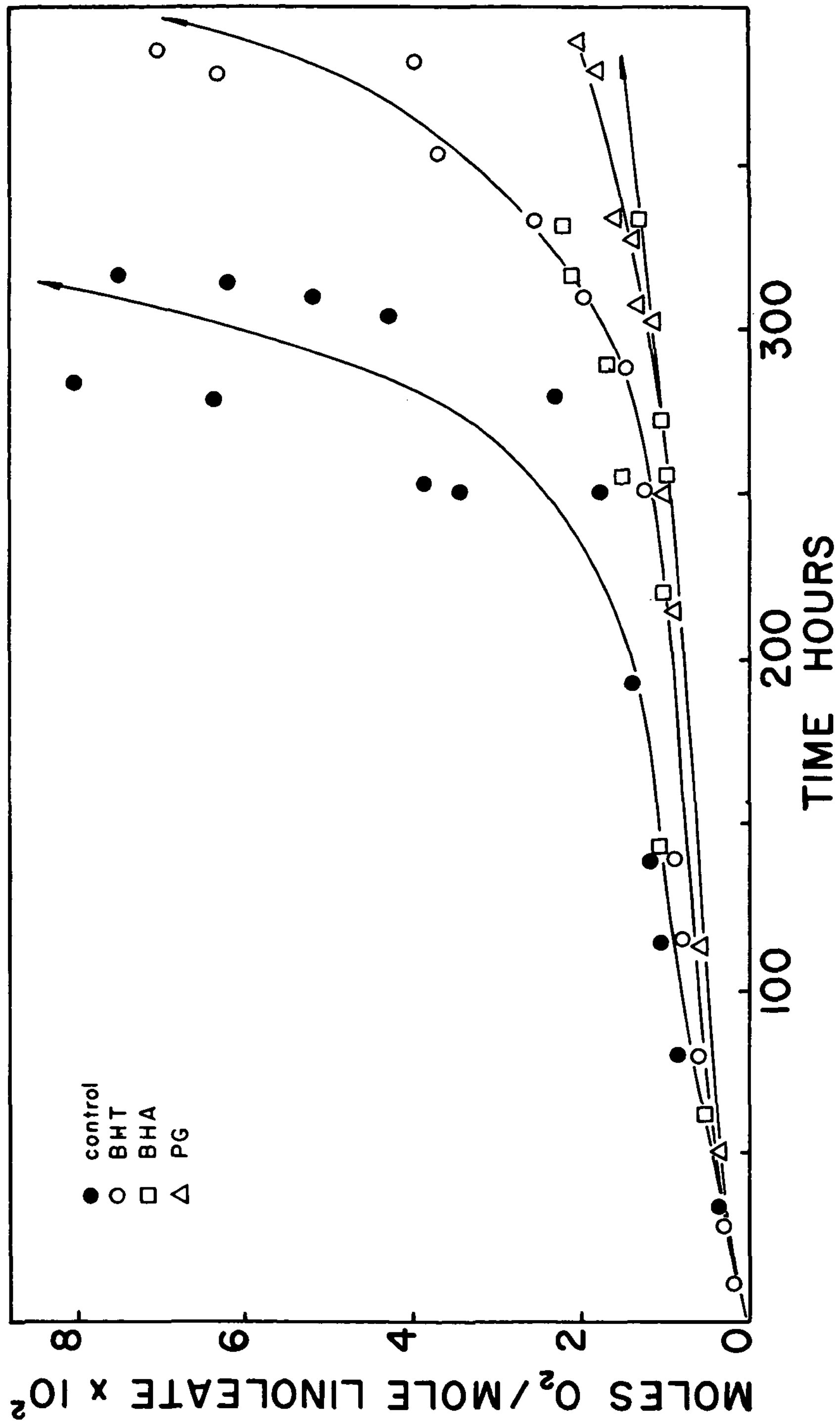
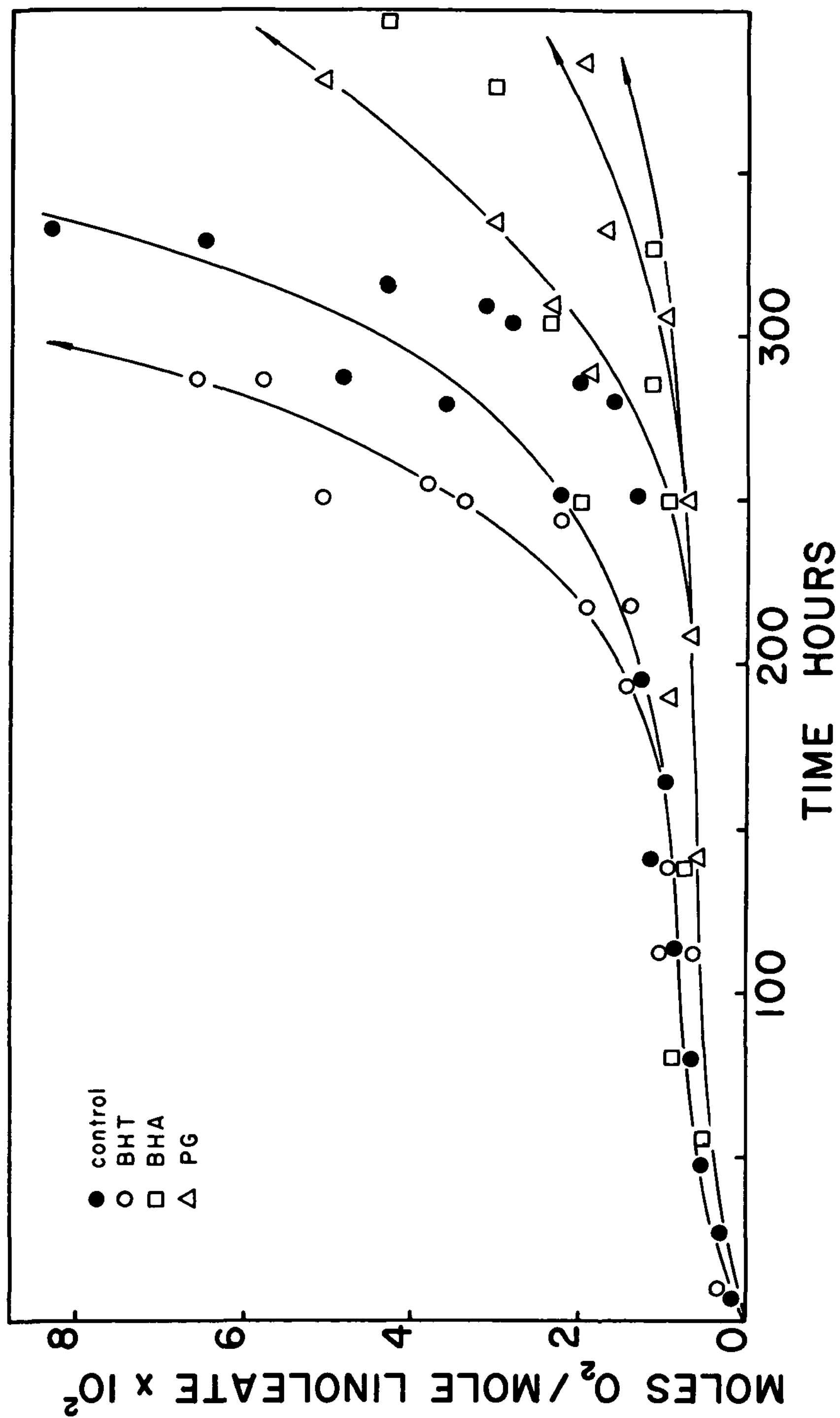


FIGURE 26. RUN #16 - EFFECTIVENESS OF PHENOLIC ANTIOXIDANTS IN CELLULOSE/30% GLYCEROL MODEL SYSTEM, 51% RH.



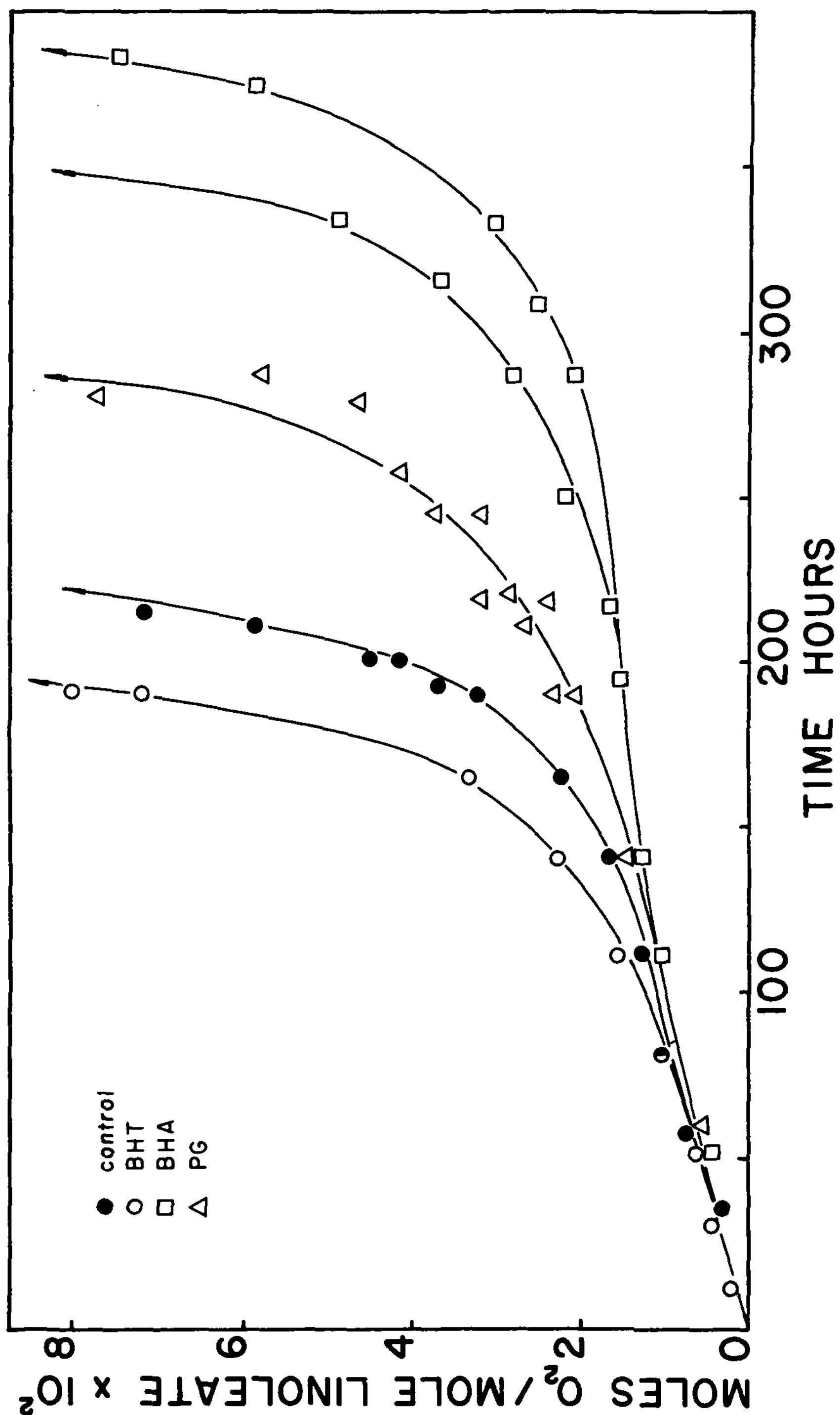


FIGURE 27. RUN #16 - EFFECTIVENESS OF PHENOLIC ANTIOXIDANTS IN CELLULOSE/30% GLYCEROL MODEL SYSTEM, 75% RH.

FIGURE 28. RUN #17 - EFFECTIVENESS OF ANTIOXIDANTS IN A CELLULOSE/30% GLYCEROL MODEL SYSTEM, 20% RH

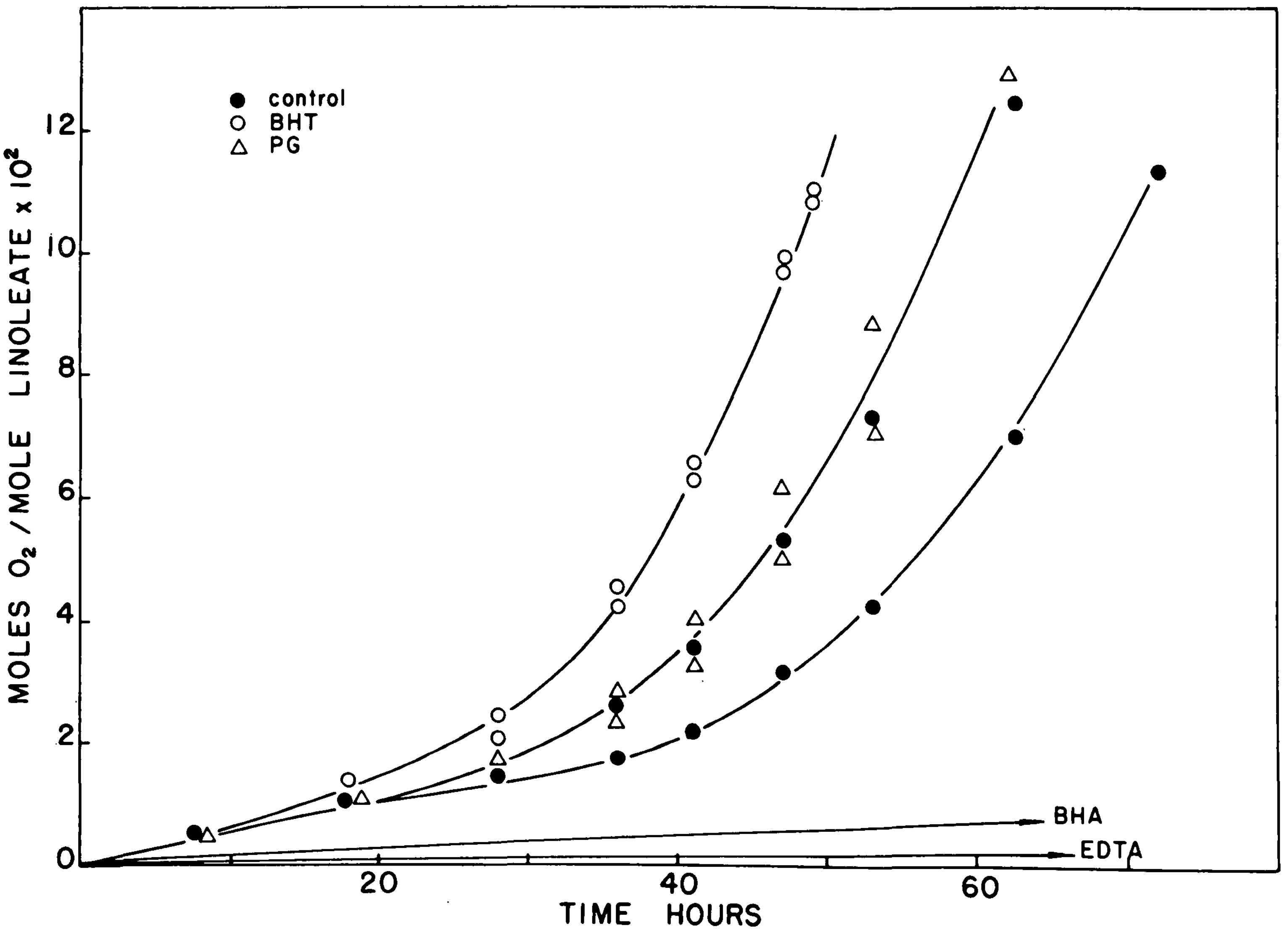


FIGURE 29. RUN #17 - EFFECTIVENESS OF ANTIOXIDANTS IN A CELLULOSE/30% GLYCEROL MODEL SYSTEM, 75% RH.

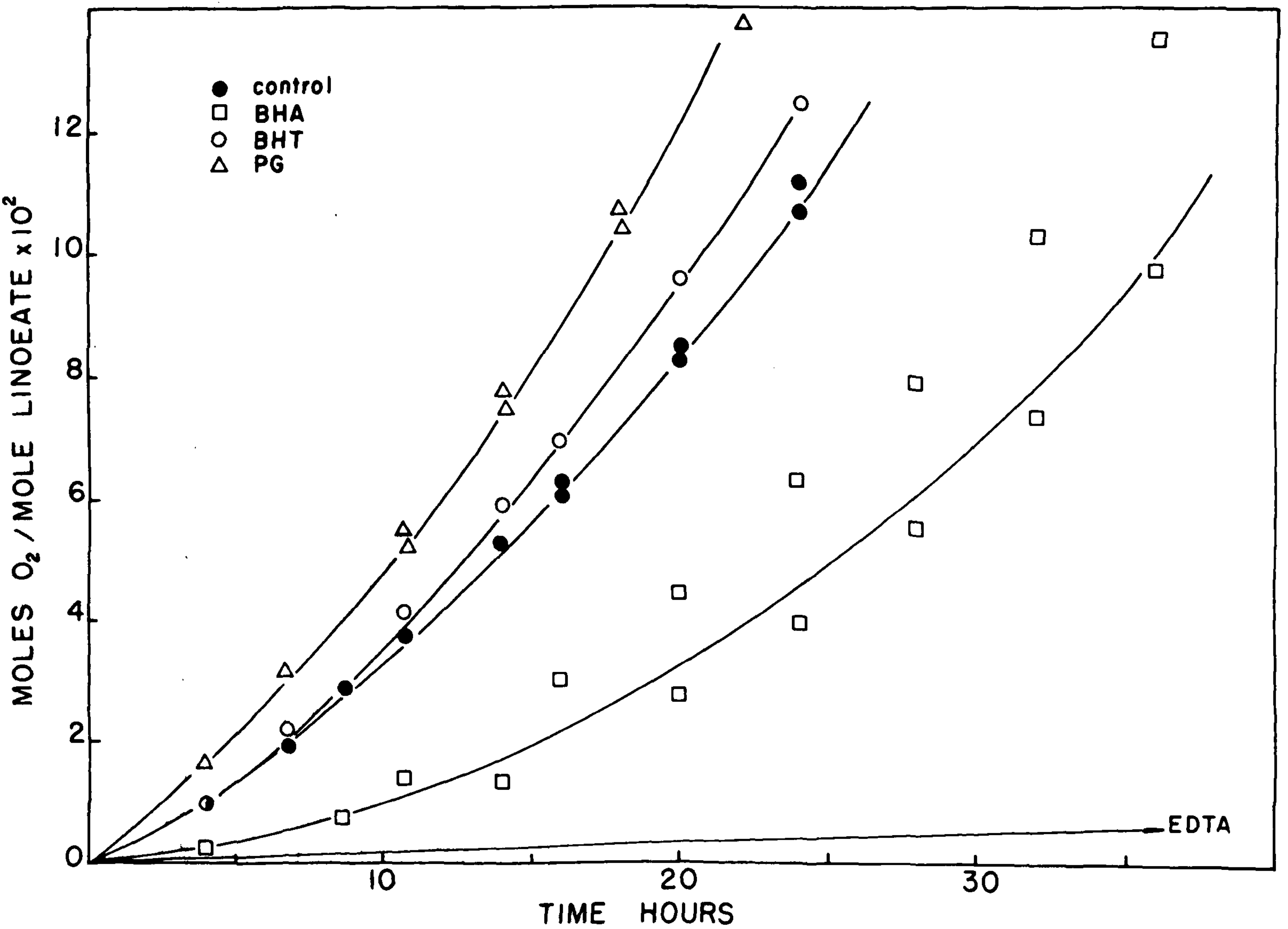


FIGURE 30. RUN #17 - EXTENTION OF OXIDATION CURVES FOR BHA AND EDTA IN CELLULOSE/30% GLYCEROL SYSTEM.

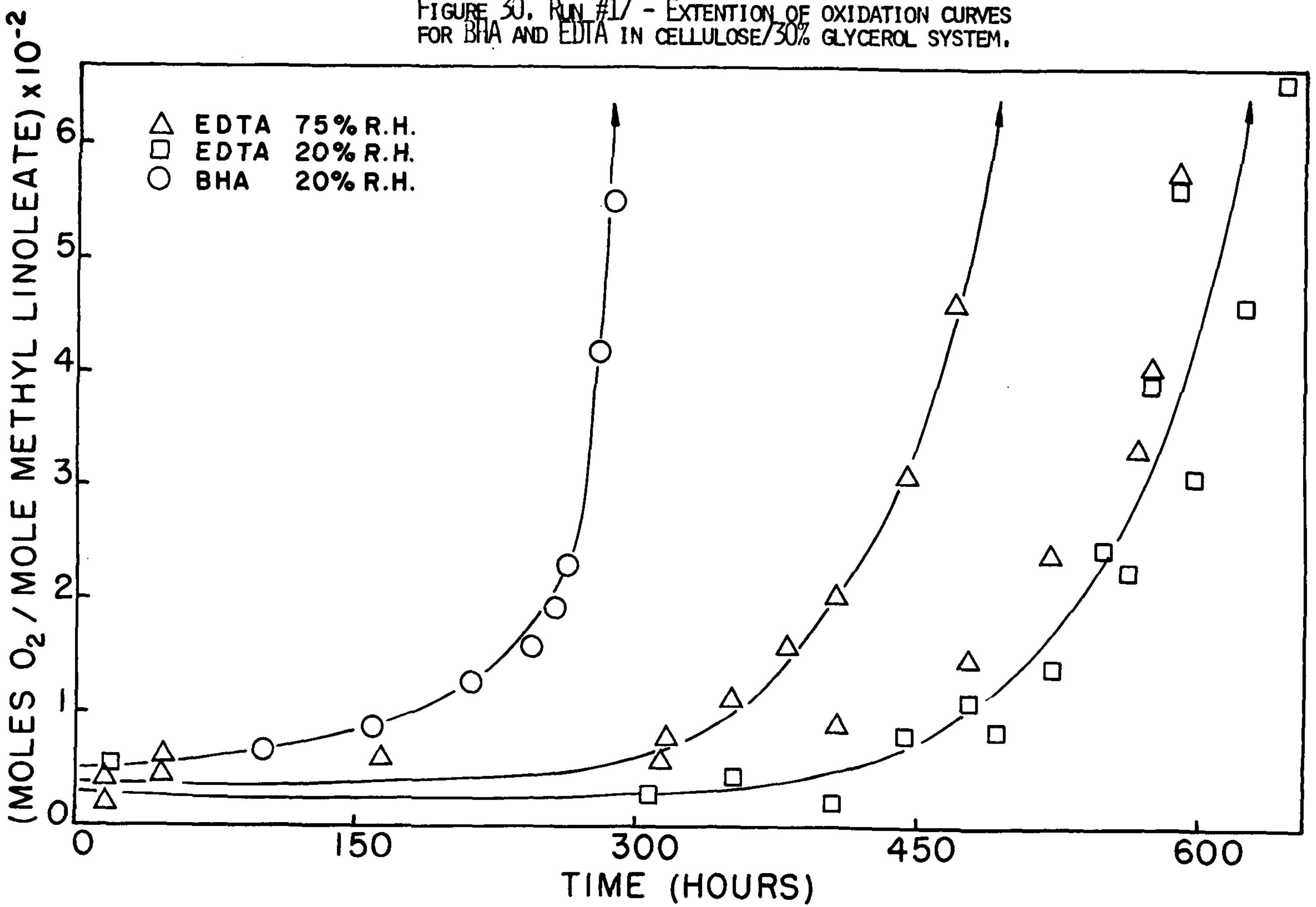


FIGURE 31. RUN #18 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A CELLULOSE/30% GLYCEROL MODEL SYSTEM, 61% RH.

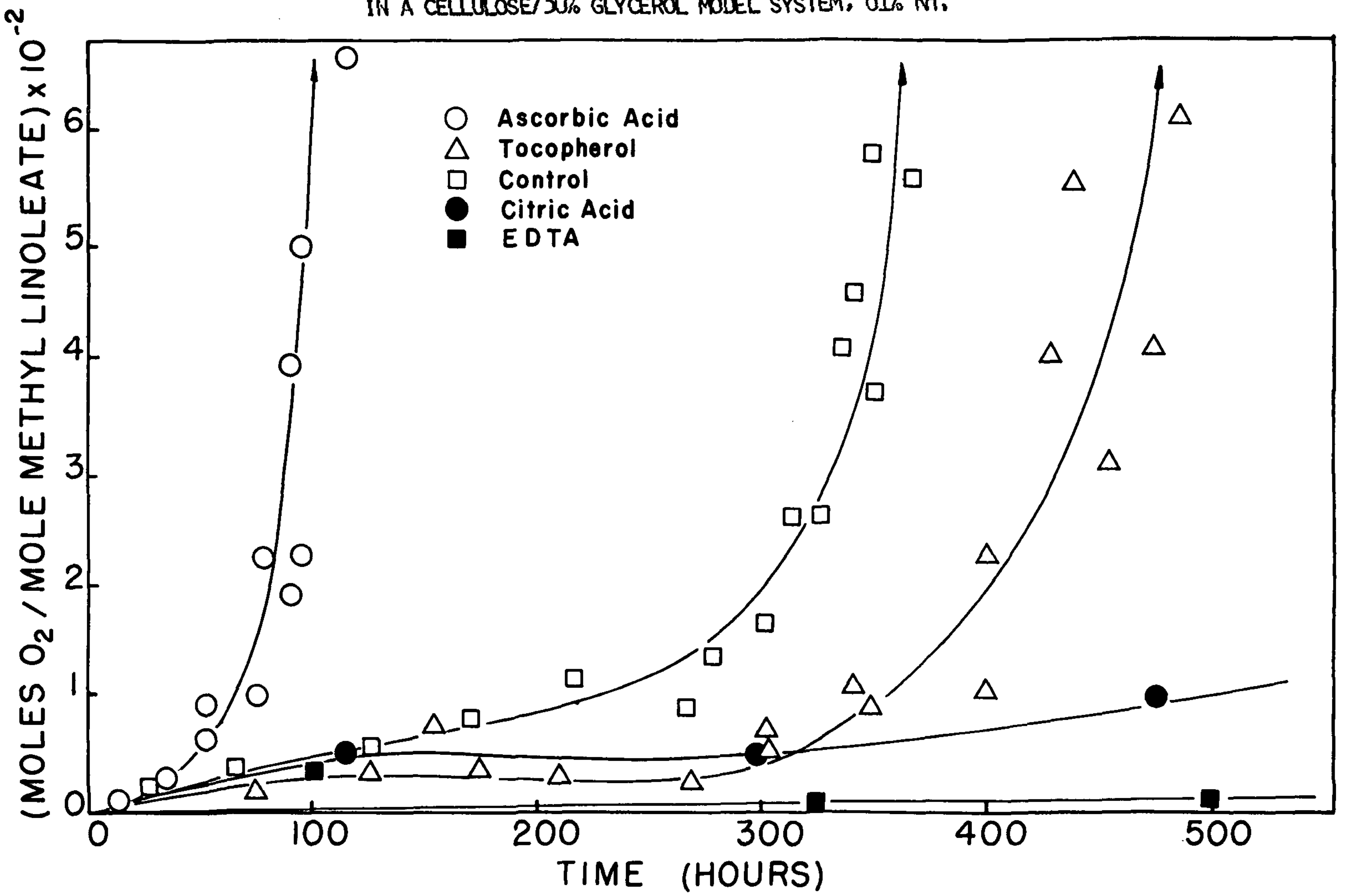


Figure 32. Run #18 - Effectiveness of various antioxidants in a cellulose/30% glycerol model system, 61% RH.

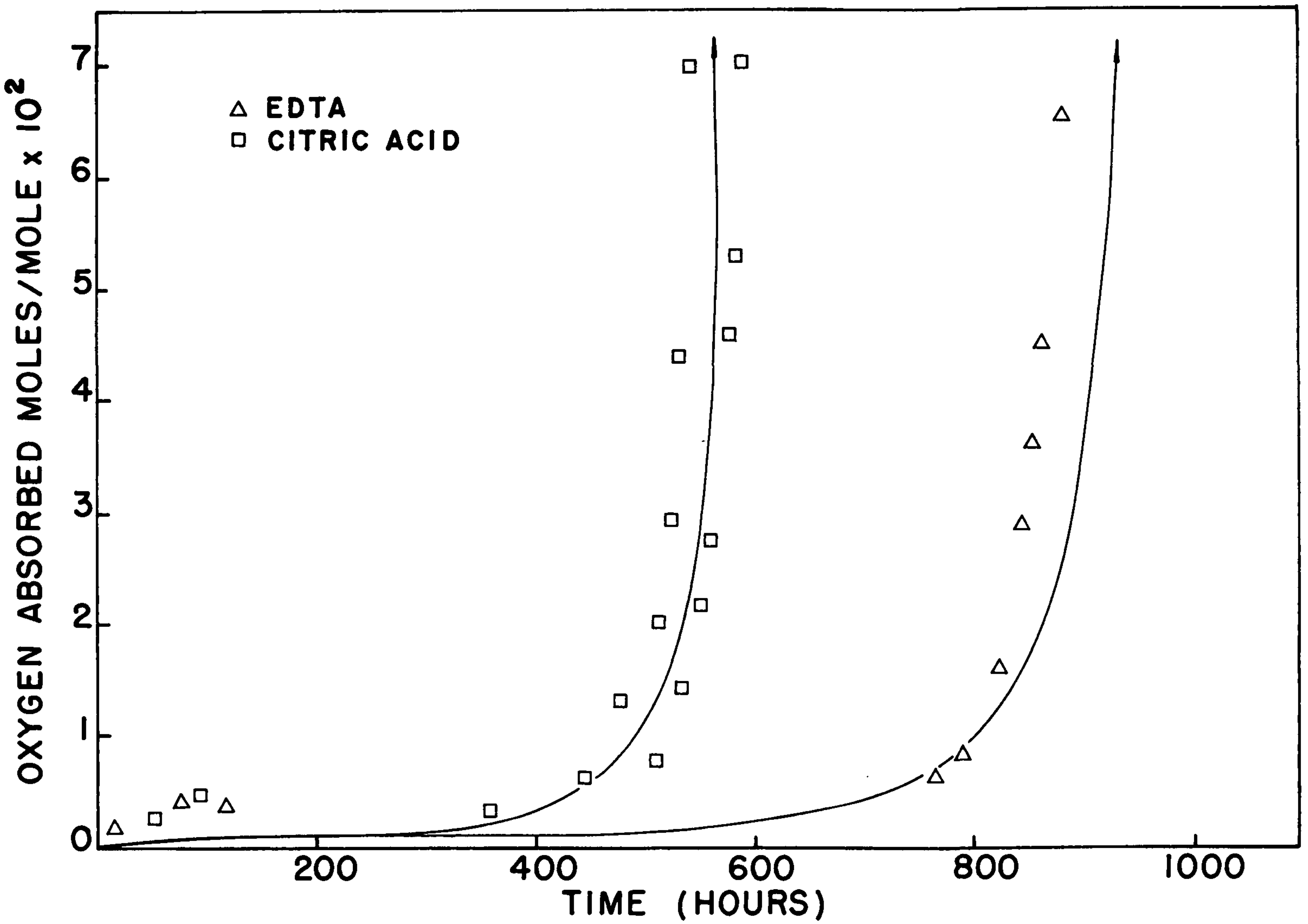


FIGURE 33. RUN #18 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A CELLULOSE/30% GLYCEROL MODEL SYSTEM, 75% RH.

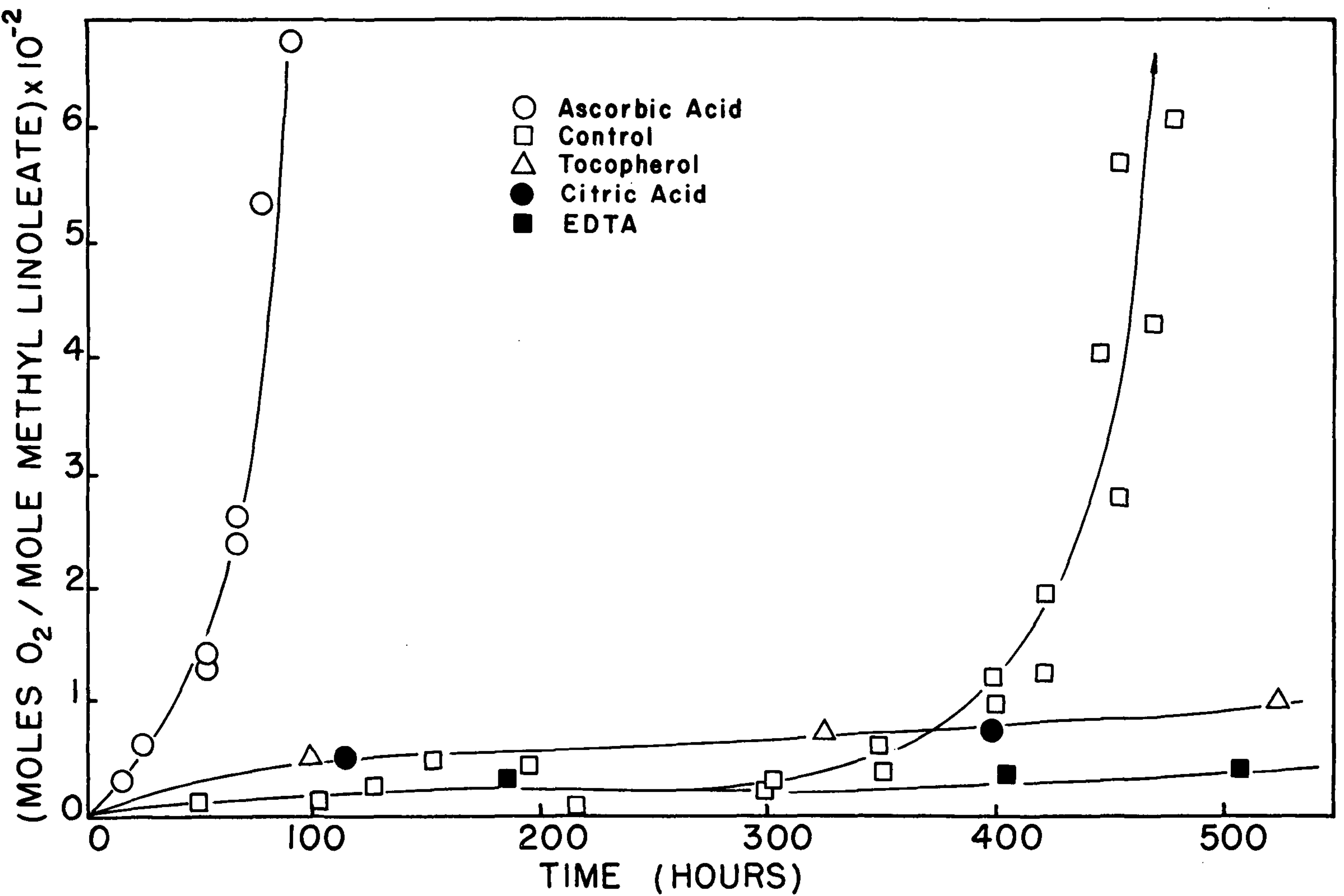


FIGURE 34. RUN #18 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A CELLULOSE/30% GLYCEROL MODEL SYSTEM, 75% RH.

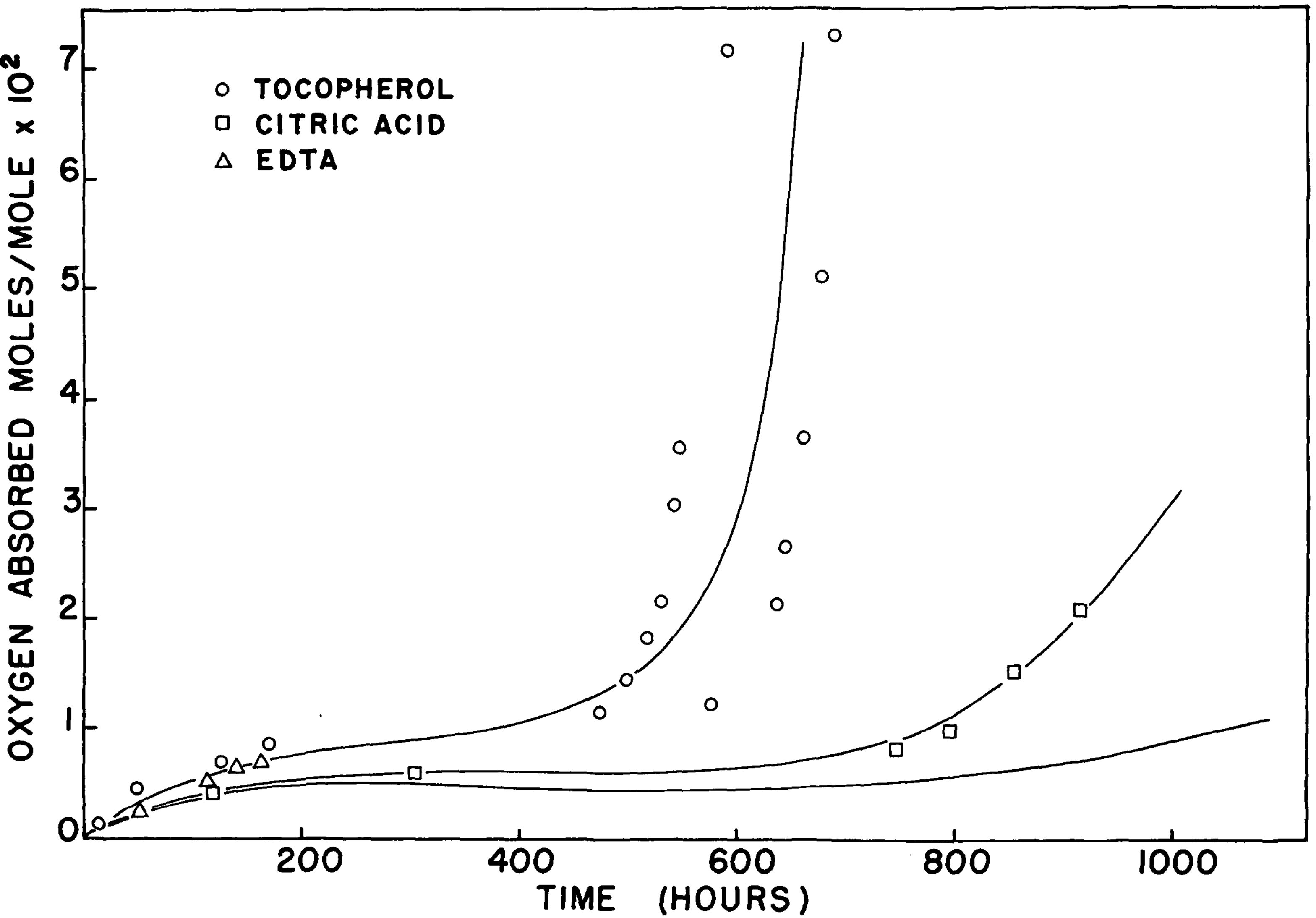


FIGURE 35, RUN #19 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A PROTEIN A/20% GLYCEROL MODEL SYSTEM, 61% RH.

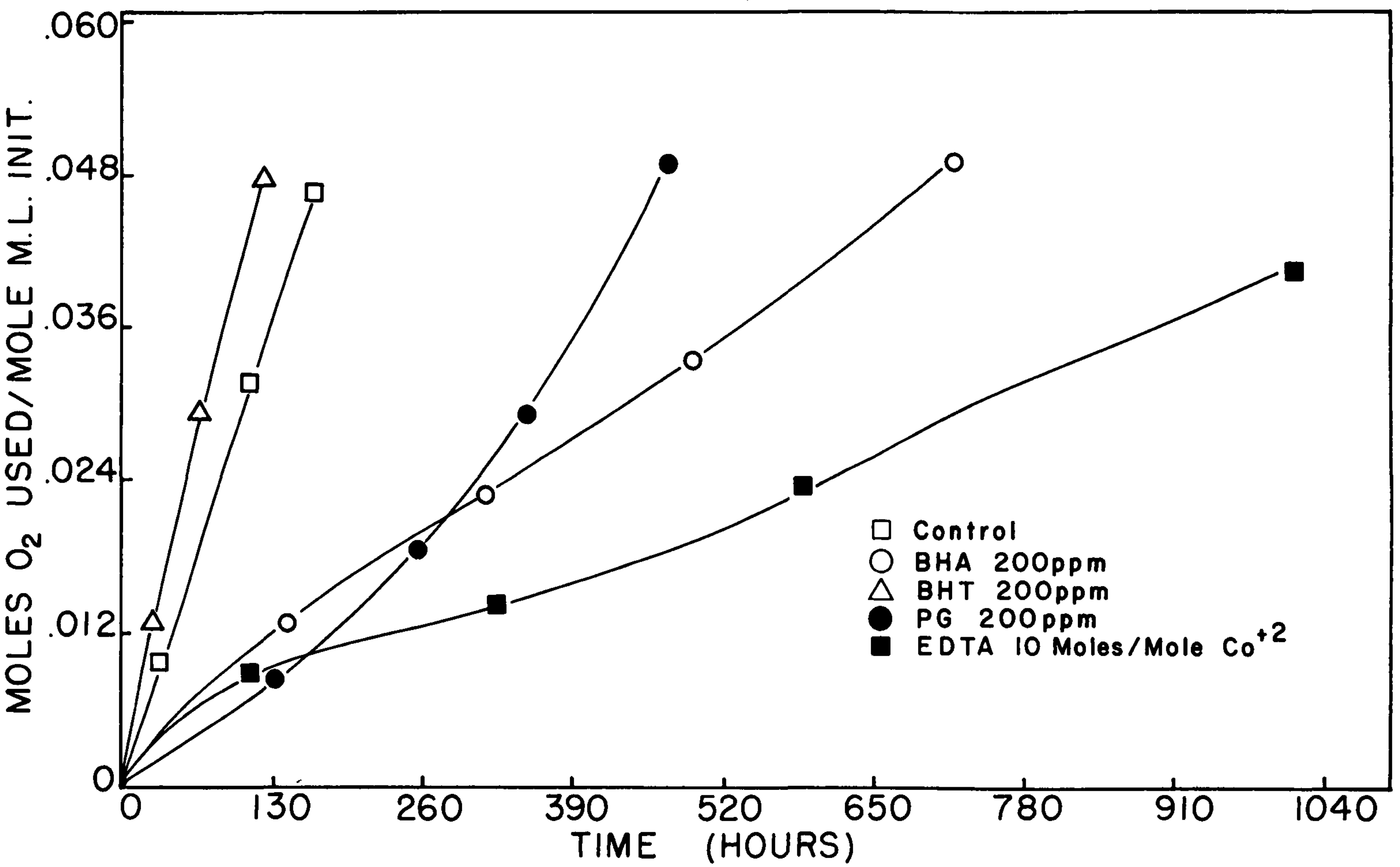


FIGURE 36, RUN #19 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A PROTEIN A/20% GLYCEROL MODEL SYSTEM, 75% RH.

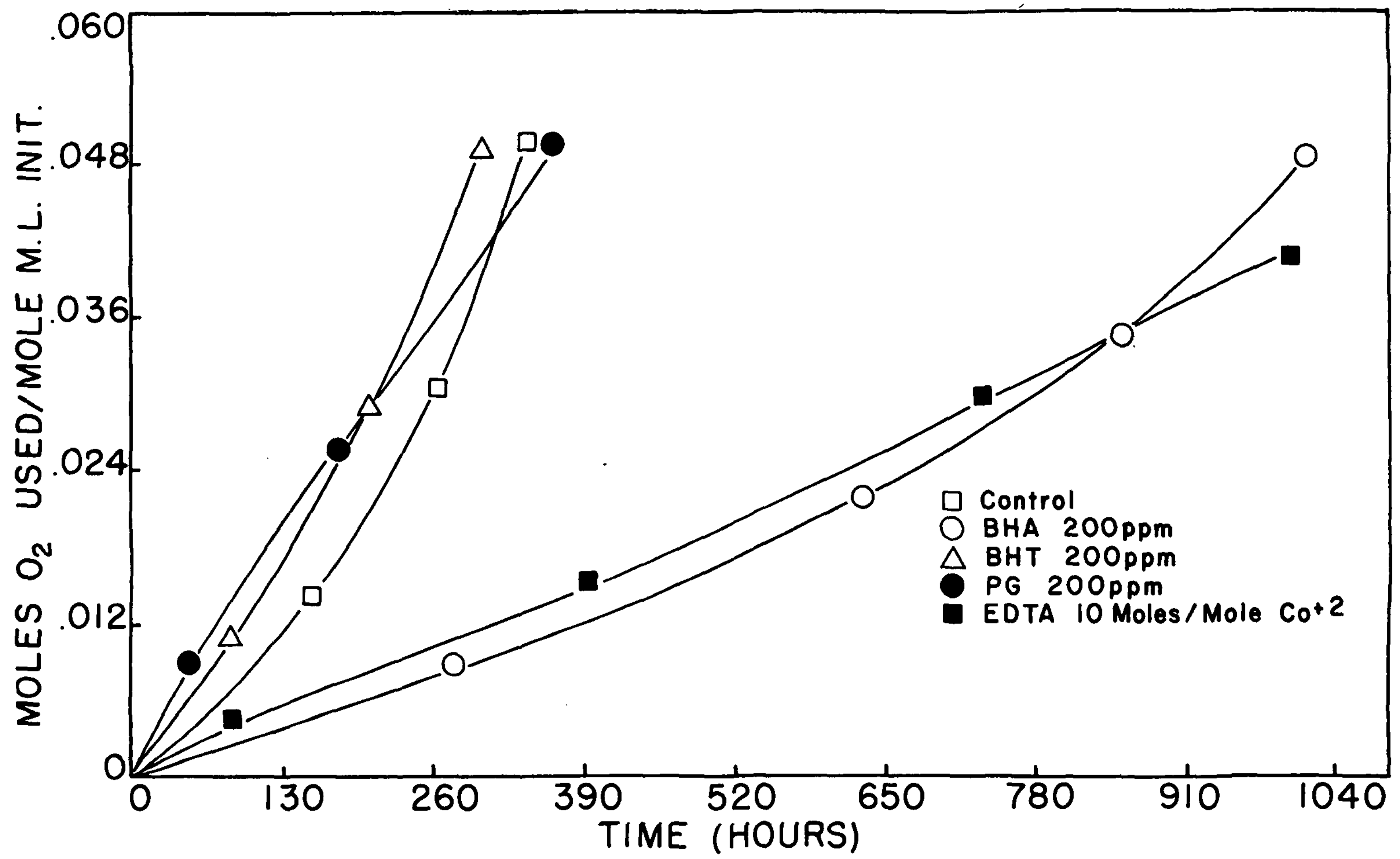


FIGURE 37, RUN #20 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A PROTEIN A/20% GLYCEROL MODEL SYSTEM, 61% RH.

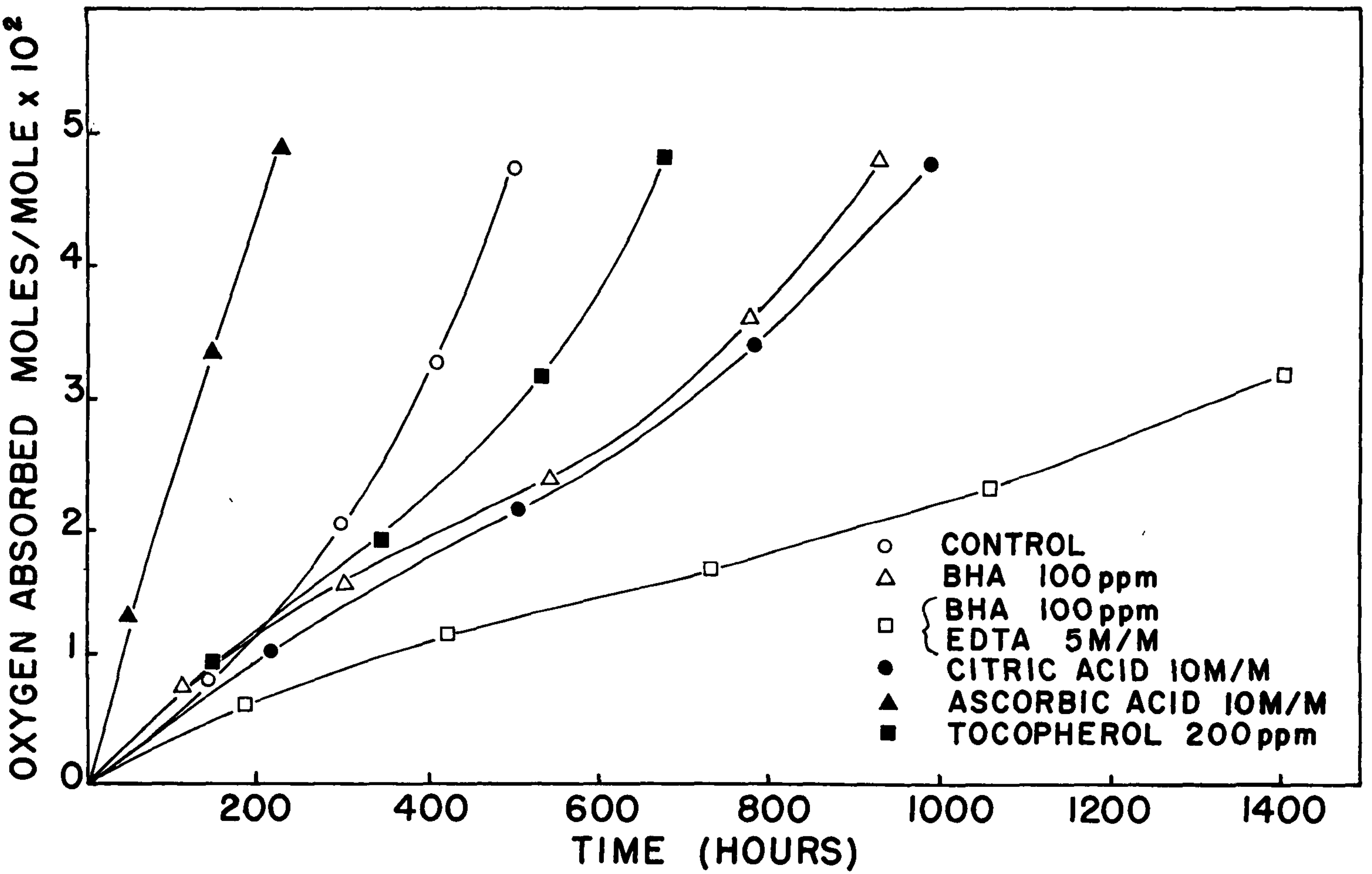


FIGURE 38, RUN #20 - EFFECTIVENESS OF VARIOUS ANTIOXIDANTS IN A PROTEIN A/20% GLYCEROL MODEL SYSTEM, 75% RH.

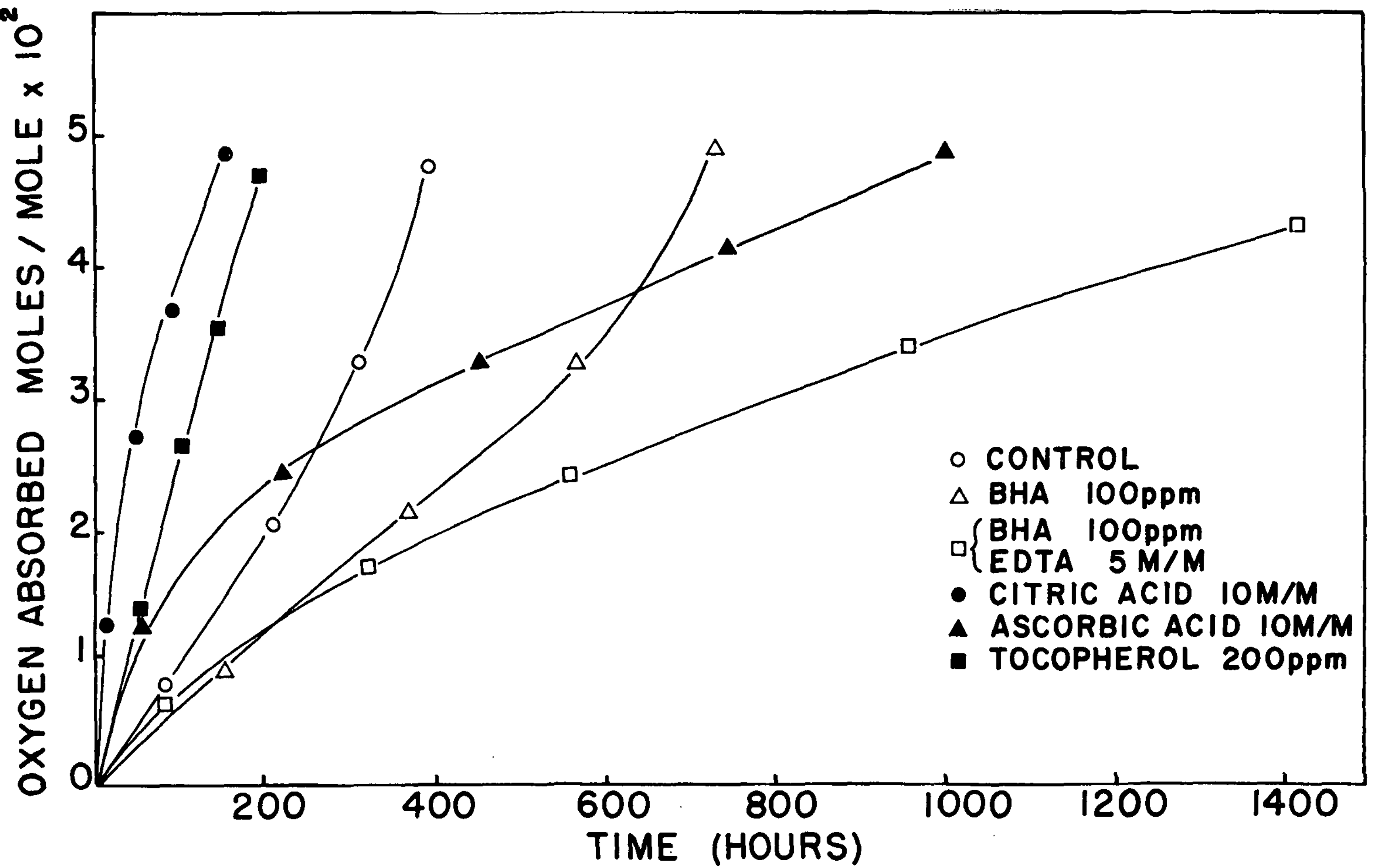
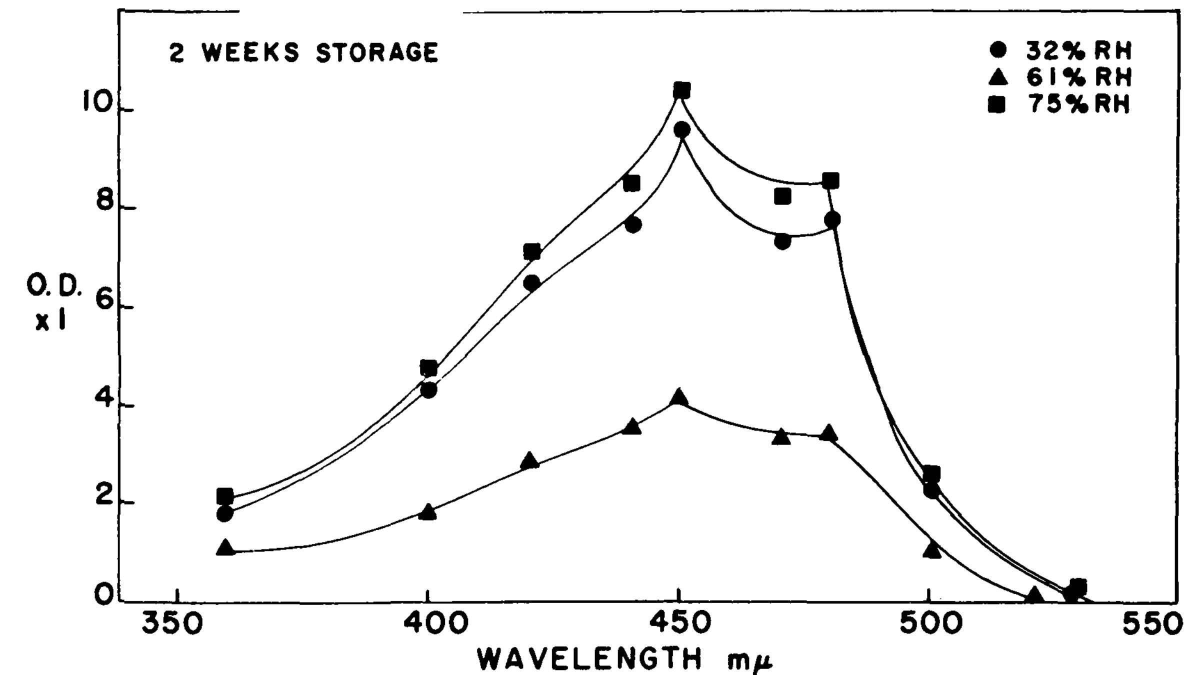


FIGURE 39, RUN #22 - CAROTENOID PIGMENT ABSORPTION CURVE FOR CARROT INTERMEDIATE MOISTURE FOOD SYSTEM AT 2 AND 6 WEEKS STORAGE.



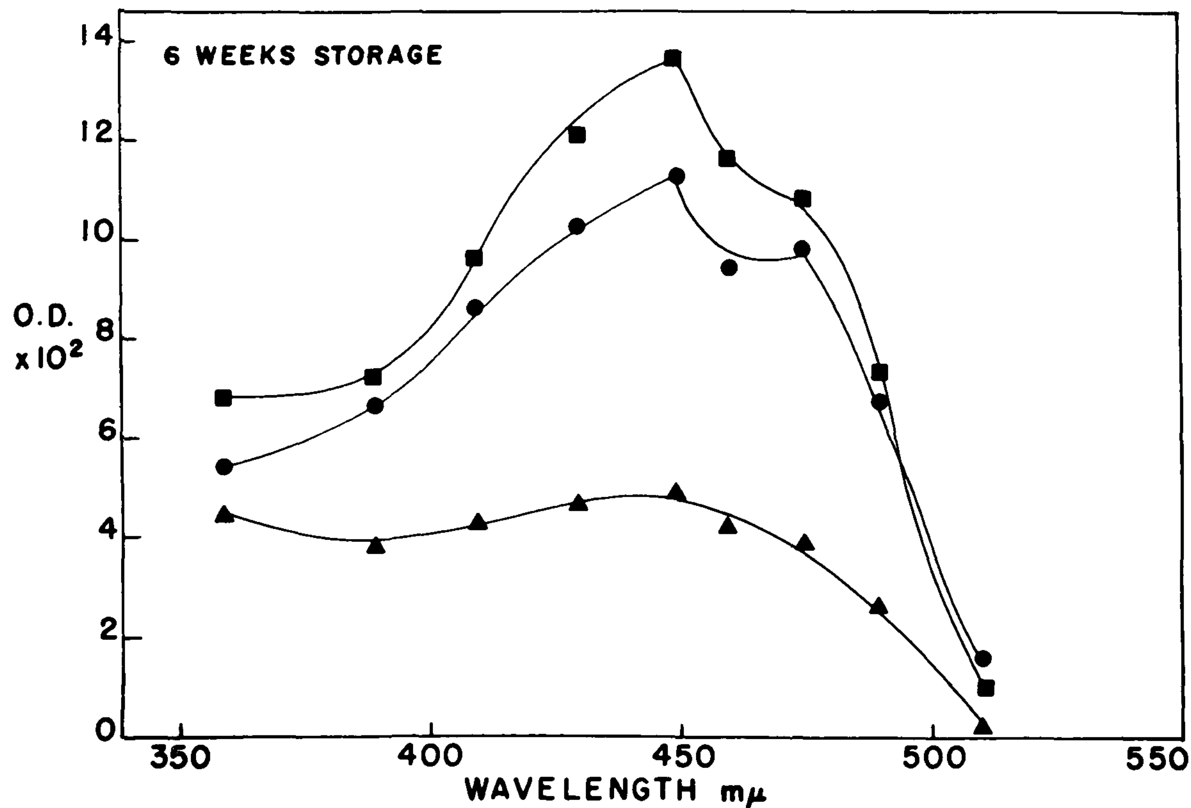


FIGURE 40, RUN #22 - LOSS IN CAROTENOID INDEX AS A FUNCTION OF RELATIVE HUMIDITY IN CARROT INTERMEDIATE MOISTURE FOOD SYSTEMS.

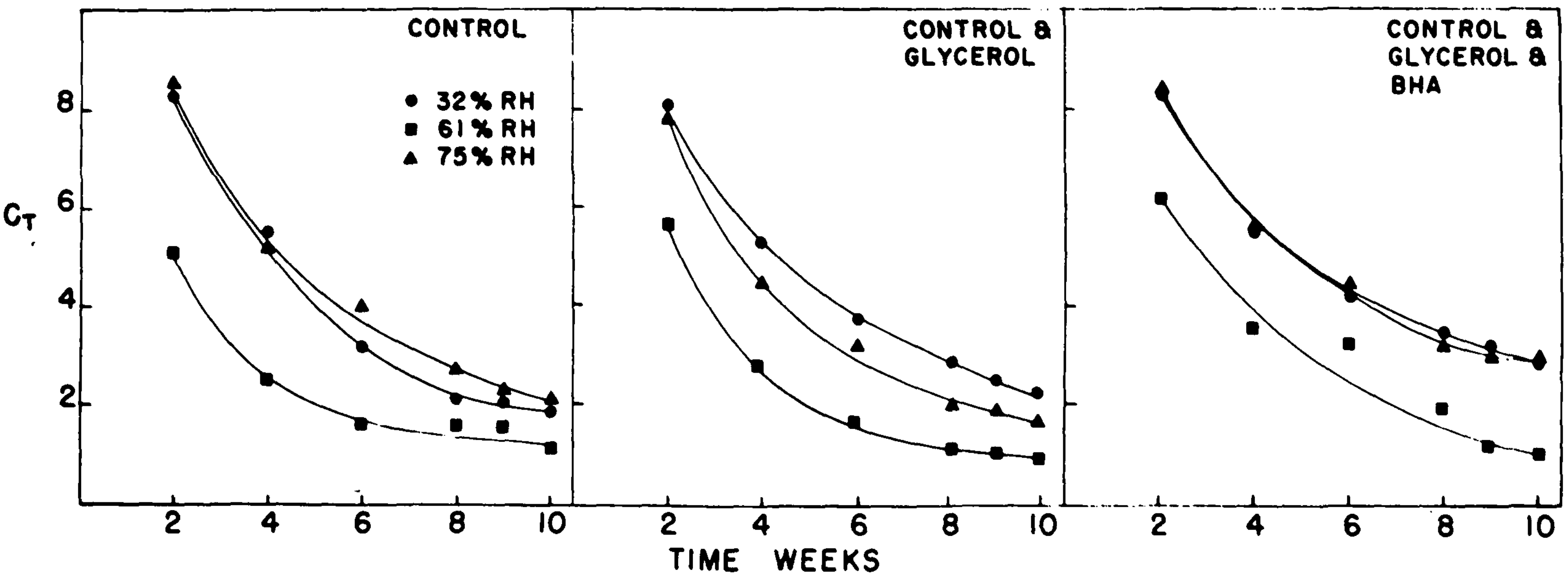


FIGURE 41, RUN #23 - PRODUCTION OF PEROXIDES FOR CHICKEN/CELLULOSE/GLYCEROL INTERMEDIATE MOISTURE FOODS AT THREE TEMPERATURES.

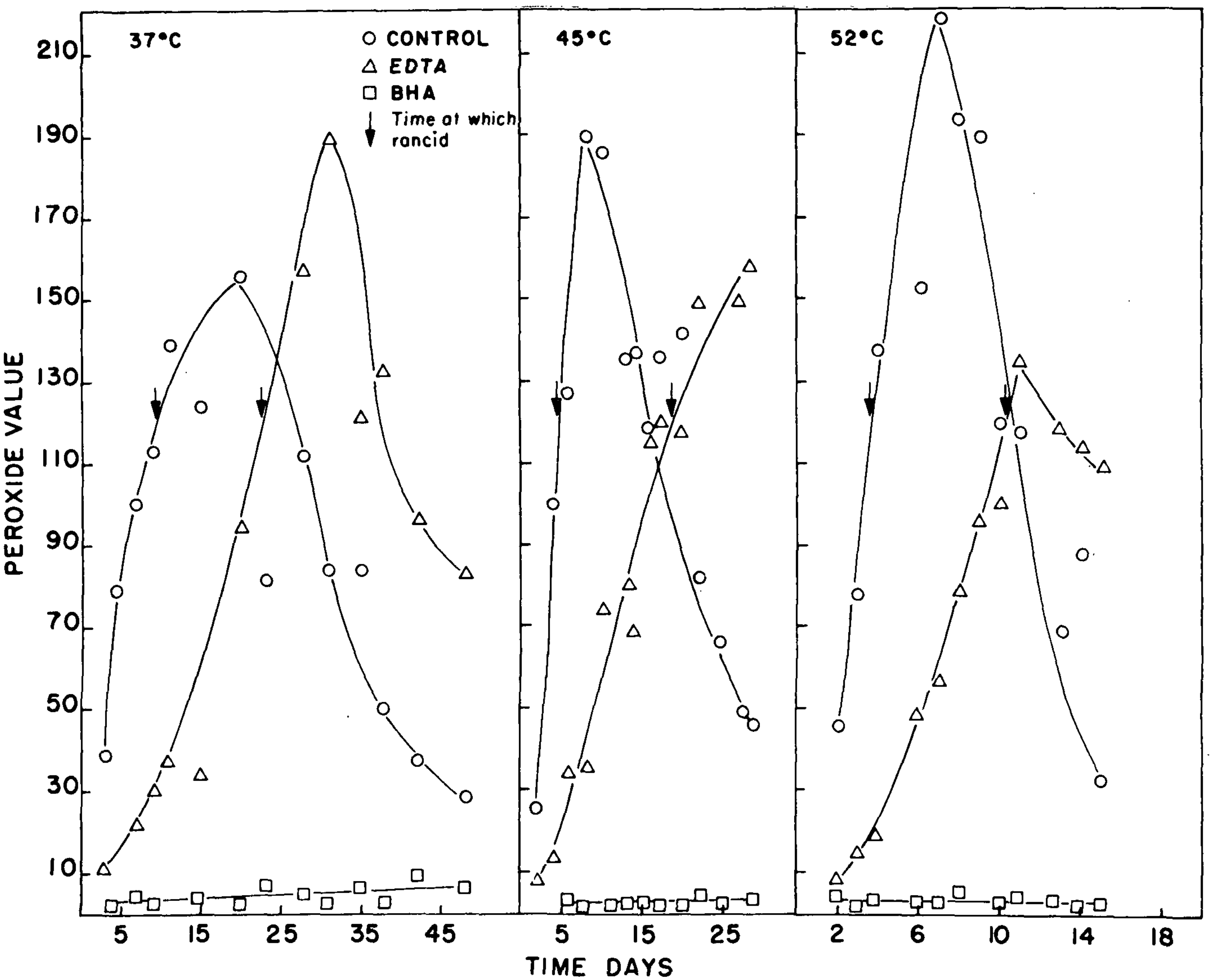
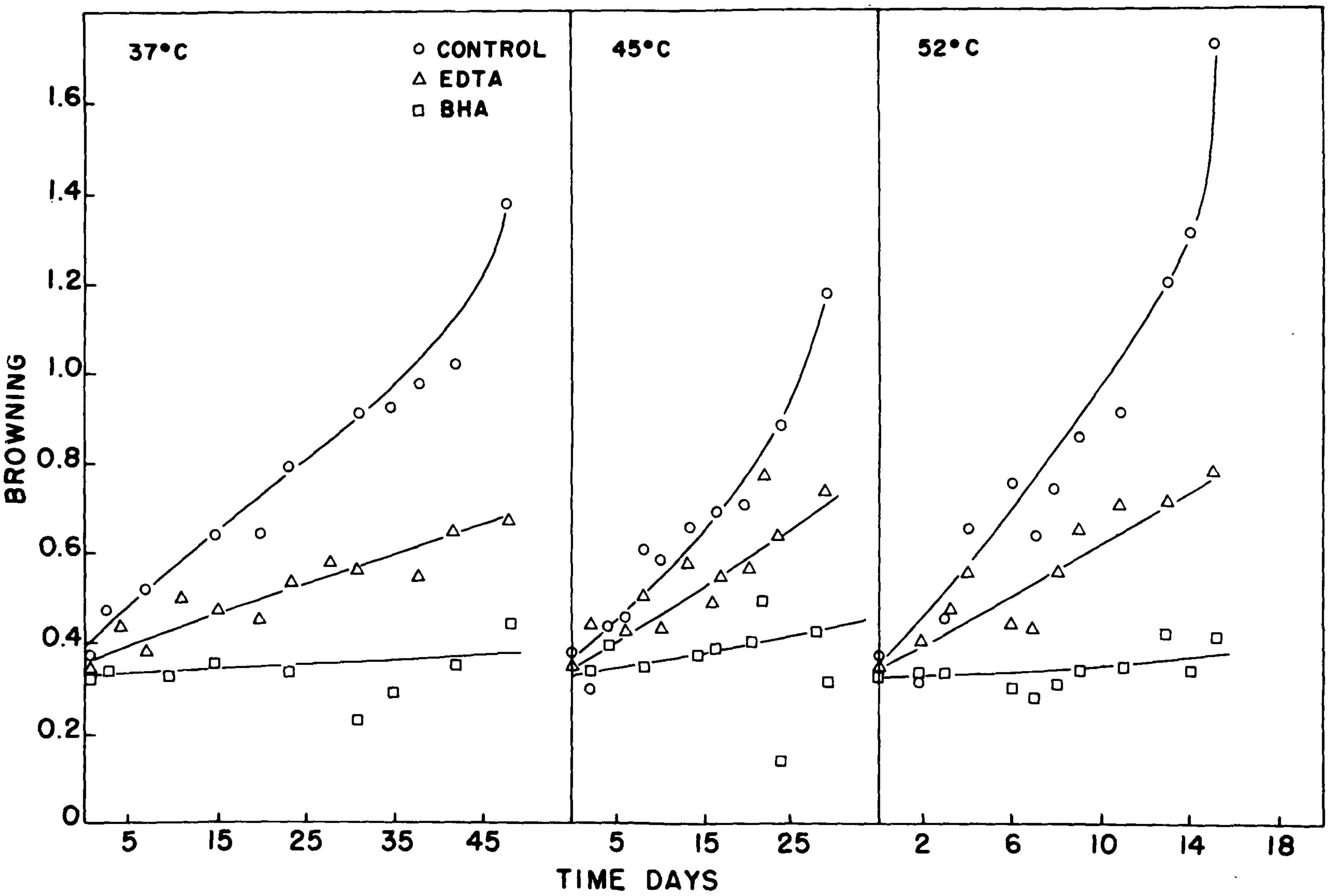


FIGURE 42, RUN #24- BROWNING DEVELOPMENT FOR CHICKEN/CELLULOSE/GLYCEROL INTERMEDIATE MOISTURE FOODS AT THREE TEMPERATURES.



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