Oxidation at Intermediate Moisture Contents [1,2]

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ABSTRACT

In the study of the oxidation rate of methyl linoleate in protein and cellulose systems, a pro-oxidant effect was found at intermediate moisture contents. At low water content, water hydrates metals and hydrogen bonds with peroxides, and an overall decrease in the rate of lipid oxidation results. With an increase in the water content to the region with a water activity of 0.6 to 0.7, the water predominantly acts as a solvent to dissolve and mobilize previously unavailable trace metals with the result of increased oxidation rates. Use of chelating agents such as ethylenediaminetetraacetic acid and citric acid reduced oxidation significantly although some anti-oxidant activity was also observed for butylated hydroxyanisole. These results have important implications in the preparation of intermediate moisture foods.

INTRODUCTION

It has been well documented for both dehydrated foods and model systems that by increasing the moisture content (or equilibrium relative humidity of a food), rancidity could be slowed or prevented for long periods. The work of Maloney et al. (1) and Labuza et al. (2) elaborated the mechanism by which water exerted its protective behavior. This antioxidant action is attributed to hydrogen bonding of peroxides and hydration of metal catalysts. The overall effect is to lower the reaction rate constants.

Recently, Labuza (3) reviewed the kinetics of lipid oxidation in relation to the action of water. Several discrepancies were observed at the relative humidities where water was able to condense in capillaries of the porous matrix. For example, the oxidation rate of linoleic acid increased in this region. Tjhio et al. (4) also showed that rates of oxidation were higher at 98% RH for metal catalyzed linoleate deposited on filter paper than at lower relative humidities.

Acker (5) has shown increased enzymatic activity in the capillary condensation region of the moisture-relative humidity isotherm for model systems containing hydrolytic enzymes. The conclusions drawn were that mobility of reactants increased in this region and more water was available for hydrolysis. Rockland (6) also demonstrated that decrease in free radical content and in phosphorescent decay are greatly accelerated in the region of intermediate moisture content. This region is characterized by a moisture content of 20 to 40 g H_2O/100 g solids and a high relative humidity (60% to 80% RH).

The above results bring into question the stability to oxidation of foods held at these moisture contents. The importance can be seen in the rapid development of the wet moist pet foods which are intermediate moisture foods and accounted for a $100 million dollar market in 1969. These foods contain an antimycotic to eliminate any microbial deterioration which can occur at the high relative humidity and they are usually prepared from cooked food components to eliminate enzymatic activity. Thus, lipid oxidation (fat contents are usually greater than 10% to 15%) and nonenzymatic browning are probably the primary

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TABLE I

Model System Composition[a]

System	g/100 g solids
Cellulose	
Microcrystalline cellulose	60
Methyl linoleate	10
Glycerol	30
Water (per isotherm)	
Protein	
Casein	64
Methyl linoleate	16
Glycerol	20
Water (per isotherm)	

[a]Additives: Cobalt, 50 ppm (on linoleate basis) in cellulose systems; Cobalt, 100 ppm (on linoleate basis) in protein systems; BHA, butylated hydroxyanisole, 200 ppm linoleate basis; BHT, butylated hydroxytoluene, 200 ppm linoleate basis; PG, propyl gallate, 200 ppm linoleate basis; *α-dl*-tocopherol, 200 ppm linoleate basis; EDTA, ethylenediaminetetraacetic acid, 10 moles/mole cobalt ion; ascorbic acid, 10 moles/mole cobalt ion; citric acid, 10 moles/mole cobalt ion.

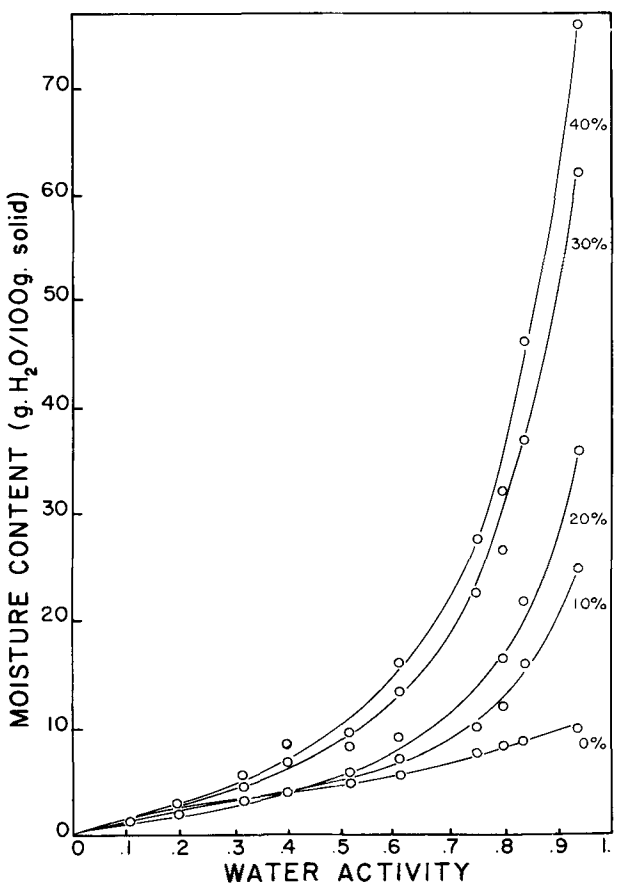

FIG. 1. Effect of glycerol content (per cent of total solids) on moisture sorption isotherm of cellulose systems.

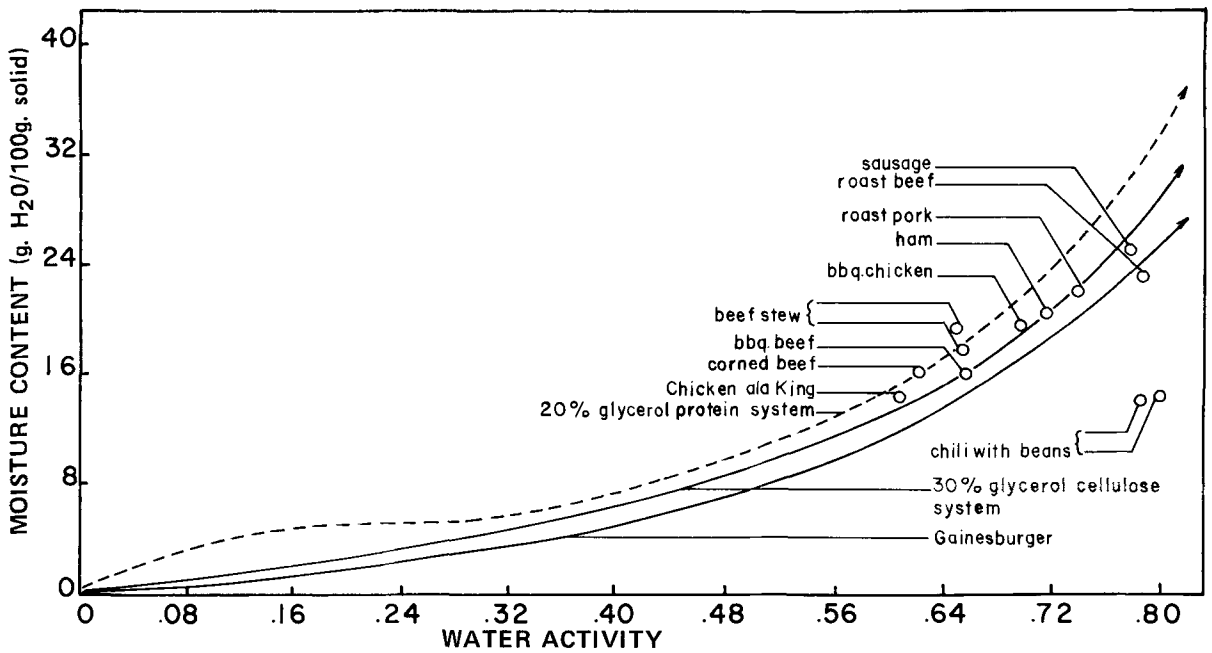

FIG. 2. Comparison of isotherms of intermediate moisture model systems to actual foods.

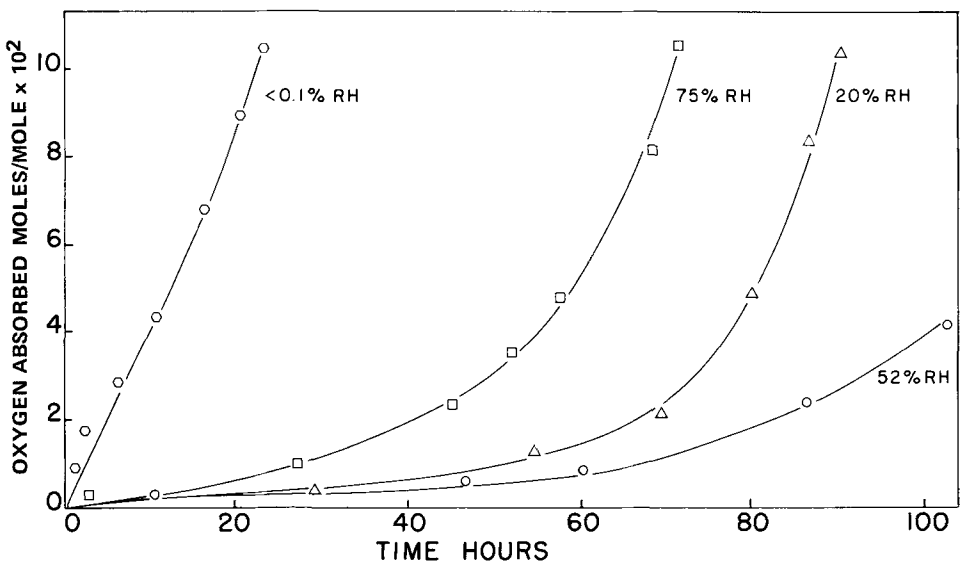

FIG. 3. Uptake of oxygen by cellulose model system without glycerol: effect of relative humidity.

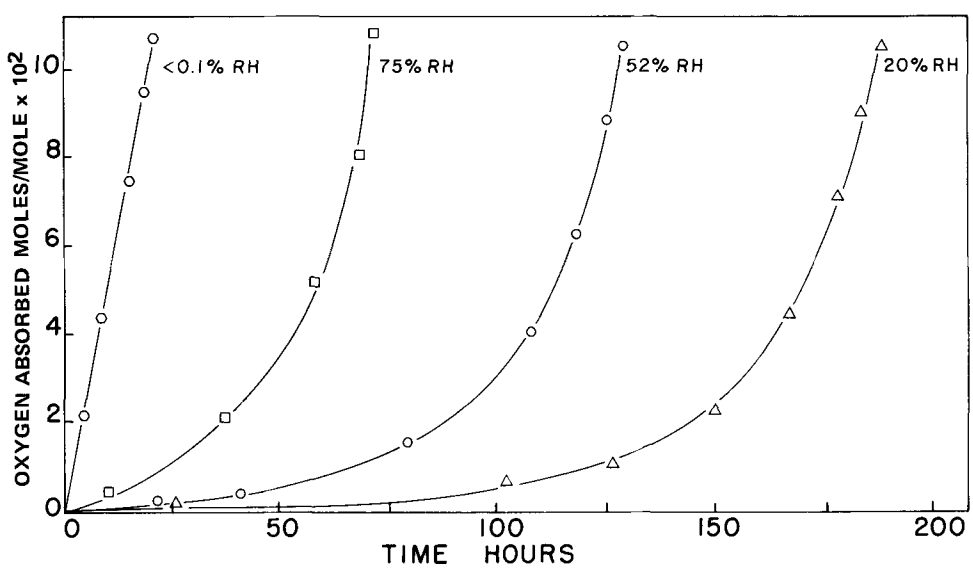

FIG. 4. Uptake of oxygen by cellulose model system with 30% glycerol: effect of relative humidity.

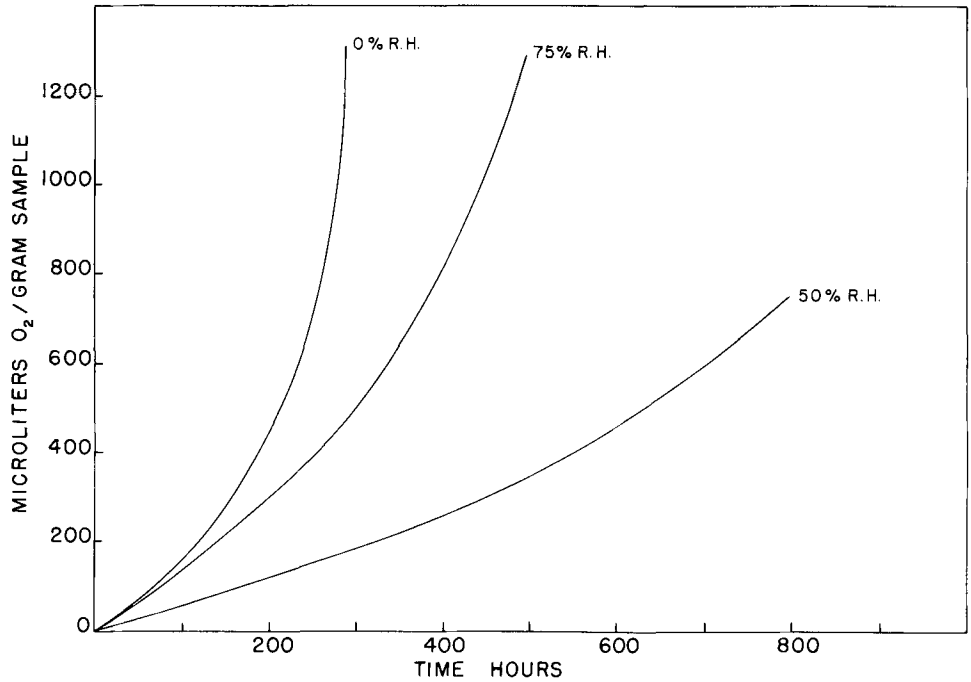

FIG. 5. Uptake of oxygen by protein model system with 20% glycerol: effect of relative humidity.

causes of deterioration. Our study was undertaken to determine, at intermediate moisture levels, the extent of lipid oxidation in model food systems and the effectiveness of the various food grade antioxidants in preventing the reaction. It was hoped that by undertaking this study, the mode of interaction of water in oxidizing systems could be further elucidated.

EXPERIMENTAL PROCEDURES

Model System Preparation

All systems were prepared according to the method of Maloney et al. (1). Briefly, the system components were mixed together at high speed and extruded into Warburg flasks. The flasks were frozen in LN_2 and freeze dried for 48 hr at 100 μ Hg and room temperature. The samples were then humidified for 24 hr over saturated salt solutions at the desired relative humidity and placed on manometers to measure oxygen uptake. Water soluble additives were added as a solution in the mixing water and lipid soluble additives were combined with a minimum amount of methanol. Table I shows the composition of the systems after humidification. The amount of water in the final system depends on the relative humidity the system was exposed to and the humectant content (i.e., glycerol). To obtain the desired moisture range, 20 to 30 g H_2O/100 g solids at 60% to 75% RH, a carbohydrate such as sucrose, glucose, glycerol, or propylene glycol is usually added; this is especially true for the wet moist pet foods. Glycerol was used extensively in this study because if intermediate moisture foods are to be used for long-range space flights, glycerol would be easily obtained as the product from conversion of expelled carbon dioxide. Also, glycerol absorbs more water per unit of weight. To determine the glycerol content needed to raise the moisture content to a level that correlated with a satisfactory texture and palatability, isotherms were prepared at four glycerol contents (see cellulose system in Fig. 1). These isotherms, the isotherm for "Gainesburger," and the moisture content-water activity point of 11 intermediate moisture

TABLE II

Kinetic Oxidation Constants: Cellulose-Glycerol-Linoleate Systems

	Time to reach 1% oxidized, hr			Time to reach 3% oxidized, hr		
System	20% RH	51% RH	75% RH	20% RH	51% RH	75% RH
Run 10						
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 11						
Control	21	---	4	42	---	9
BHA	170	---	10	270	---	19
EDTA	480	---	380	570	---	510
Run 12		61% RH			61% 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

TABLE III

Kinetic Oxidation Constants: Protein-Glycerol-Linoleate Systems

System	Time to reach 1% oxidized, hr		Time to reach 3% oxidized, hr	
	61% RH	75% RH	61% RH	75% RH
Run 1				
Control	23	120	98	300
BHA	90	318	425	860
BHT	16	76	63	213
PG	144	64	288	220
EDTA	161	277	710	808
Run 2				
Control	150	193	281	449
BHA (100 ppm)	132	187	449	684
BHA (100 ppm)/EDTA (5 m/m)	154	256	768	1154
Citric acid	15	200	76	674
Ascorbic acid	37	45	313	152
α-*dl*-Tocopherol	49	220	145	550

foods, which was prepared according to the methods of Hollis et al. (7), are compared in Figure 2. The isotherm for the cellulose system containing 30% glycerol and the 20% glycerol system for the protein system were found to give the moisture content desired to be employed as models for the intermediate moisture range.

Extent of Oxidation

Oxygen absorption was measured in Warburg manometers at 37 C. The data were collected as moles of oxygen per mole linoleate or milliliter of O_2 per total dry weight of sample. To simplify data presentation, the average time of duplicate or triplicate samples to reach 1% oxidized (moles O_2 per mole linoleate) were calculated. At 1% oxidation, the induction period for oxidation has just ended and for a food rancidity would be just detectable. At 3% oxidation, the samples would be entering the rapid bimolecular oxidation period. As with previous studies (1-3), all variables have to be tested in any one run because of the variability in initial linoleate levels. Comparison of numbers between runs is meaningless, but comparison of the trends between runs can be made. In all cases the average of three Warburg samples is presented. These did not vary by more than ±5%.

RESULTS AND DISCUSSION

Effect of Water and Glycerol

Cellulose model systems with or without 30% glycerol were prepared and oxidized at 37 C in air. The results of Run 7, which is representative of the data, are shown in Figures 3 and 4 for the respective systems. The results of the system without glycerol (Fig. 3), show that increasing the moisture content up to 50% RH significantly decreases the rate of oxidation. This substantiated the previous findings of Maloney et al. (1) and Labuza et al. (2) for the same system. The system oxidized, however, at a faster rate at 75% RH than did the samples at 20% RH or 50% RH. The 20% RH, 50% RH, and 75% RH samples contain 5, 10, and 28 g H_2O/100 g solids respectively or almost three times as much water at 75% RH than at 50% RH. These results correspond to those found for linoleic acid at high water content and they are indicative of the much larger rates found in oxidation of linoleate in emulsions (3). With the addition of glycerol (Fig. 4), the transition to faster oxidation rates occurs at 50% RH rather than at the higher RH of 75%, whereas the 20% RH oxidizes at the slowest rate. These results were consistent in all 12 runs performed with different metal catalyst concentrations.

Similar results were found for the glycerol-linoleate-protein model system (Fig. 5). The addition of water alone or water and glycerol caused more rapid oxidation at 75% RH, as was found for the cellulose systems. At first glance, it would appear that glycerol is acting as a catalyst; however, glycerol alone or in combination with nonlipid components did not absorb oxygen at 37 C or increase the rate of oxidation in combination with bulk linoleate. The major factor, therefore, is increased mobility of the catalysts in the aqueous phase, as was concluded by Heidelbaugh (8). Increased aqueous mobility in the intermediate moisture range has also been found by Acker (5) for enzyme activity, by Duckworth and Smith (9) for ionic mobility, and by Rockland (6) for other reactions. Since glycerol is soluble in the aqueous phase and is very immiscible with the lipid, it tends to increase the amount of available mobile phase present. Thus, when glycerol is in the system, the transition relative humidity below which the antioxidant effects of water predominate is lowered significantly and the prooxidant effect of catalyst mobility becomes greater.

Antioxidant Effectiveness at Intermediate Moisture Values

Because of the high moisture content at intermediate moisture values, the effectiveness of various food grade antioxidants may differ due to their mode of action. Table II shows the kinetic data for three runs in which the typical phenolic type antioxidants that act as free radical chain breakers (added at 200 ppm) and various chelating agents were tested in the cellulosic system. In Runs 10 and 11, the time to reach 1% oxidation is much more rapid for the control system held at 75% RH, showing the accelerating effect of water. For all systems in Run 12 except ascorbic acid and for the protein system data presented in Table III, the samples at 61% RH oxidize much faster than at 75% RH. This is a reversal of the previously stated hypothesis but it can be explained by the same factors that affect nonenzymatic browning which shows a rate maximum in the intermediate moisture range (10). All systems at 75% RH have nearly two to three times the moisture content of those at 61% RH. It appears that the solubilization and mobility effect already occurs at 61% RH and the addition of more water only tends to dilute the reactants and catalysts, and thus, due to mass action, the rate decreases. The reversal found with ascorbic acid may be due to a pH effect which is not yet understood.

In the cellulose systems, butylated hydroxyanisole (BHA) appears to be the best phenolic antioxidant, although PG is more water soluble and would be expected to work better. Tocopherol is also quite good, but it is prohibitively expensive to use for foods. The metal

chelating agents appear to give the best protection; ethylenediaminetetraacetic acid (EDTA) is superior, followed by citric acid, and then by the phenolic antioxidants. Ascorbic acid, which has been used as a chelating agent in dry systems, acts as a strong prooxidant probably due to oxidation of the ascorbic acid itself (11) or to the fact that, at high moisture levels, the chelated metal-ascorbic acid complex may act as a better catalyst (12).

Table III shows similar results in the protein systems for these antioxidants. It appears, however, that BHA may be better in this case than EDTA. The EDTA may be complexed with the metals contained in the protein matrix and thus may be unable to react with the free trace metals. Thus, EDTA may not work well in protein-type foods such as meats that contain a high, bound-metal concentration. The EDTA/BHA combination would appear to give the best protection. Also, in the protein system at 61% RH, citric acid acted as a catalyst possibly due to a pH effect which was diluted at 75% RH where oxidation was similar to the control.

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