Metal-Catalyzed Oxidation in the Presence of Water in Foods[1]

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ABSTRACT

Methyl linoleate was oxidized in model systems consisting of either cellulose or casein with which the lipid was dispersed with water containing cobalt salts. The dispersion was extruded into Warburg flasks, frozen and freeze-dried at 100 μ Hg and with platen temperatures of 80 F. The samples were then humidified over saturated salt solutions to give moisture contents from less than 1 g H_2O/100 g solids up to 30 g H_2O/100 g solids. The higher moisture contents were obtained by addition of glycerol to the model system during preparation and humidification at 60-75% RH. Chelating agents including EDTA and citric acid in concentrations of 1 to 10 moles per mole of cobalt ion were used in some experiments. Oxidation was followed manometrically and by peroxide analysis. At low water contents, water acts as an antioxidant through hydration of metallic catalysts. As the moisture content increases, water promotes oxidation through its solvent activity. In the region of capillary condensation, antioxidant effect of metal hydration is overshadowed by the prooxidant effect of metal solubilization. The water soluble chelating agents such as EDTA act on metals in aqueous solution and their activity is promoted by increased moisture content.

INTRODUCTION

Storage of processed foods, whether dehydrated, intermediate moisture, or in their natural state, is limited by many deteriorative reactions. The basic premise of food processing is the elimination of the condition under which microbiological deterioration can take place. This, for example, is accomplished in canning by heat destruction of the microbes and in drying by removal of water which is needed for growth. Under these conditions then, microbiological decay no longer occurs but rather some chemical reaction now limits storage stability.

Of primary concern for many foods is the loss of stability and good quality through the oxidation of lipids, primarily those containing unsaturated double bonds. The basic reaction proceeds by a free radical mechanism which is catalyzed by the various trace metals contained in foods. These include the iron contained in the myoglobin of meat, the hemoglobin of blood and the manganese of chlorophyll in plants. Overall the oxidation of lipids leads to production of off-flavors and odors, crosslinking reactions with proteins reducing their solubility and biological availability, and cooxidation of various pigments, flavors and vitamins. Schultz and Day (1) have thoroughly reviewed the deteriorative aspects of lipid oxidation of foods.

In terms of water content, it has been known for many years that, especially for dehydrated foods, overdrying or overbaking causes an increased rate of lipid oxidation (2-6). In our laboratories a major study was made of oxidation at low moisture content to determine the mechanism by which water interacted in the lipid oxidation process (7-8). The studies were confined to the low moisture range of water adsorption where the equilibrium relative humidity is approximately 0% to 50% RH (region A and B of Fig. 1). This paper is a review of the results of these studies and of further work which was done at higher moisture levels.

The effect of water on metal-catalyzed lipid oxidation can be understood in terms of the various properties of water which include: dissolution of solutes, mobilization of molecules, solvent for reactants, and reaction with other molecular species. The analysis of these properties in terms of overall food quality has been discussed in detail by Labuza et al. (10) and Labuza (11). This paper will deal specifically with the lipid oxidation mechanism.

METHODS

Model Systems

All systems were prepared according to the method described by Maloney et al. (7). The freeze-dried systems consisted of either the cellulose system, cellulose (6 pt) to methyl linoleate (1 pt), or the protein system, casein (4 pt) to methyl linoleate (1 pt). These were mixed with sufficient water to make a paste, extruded into reaction vessels, freeze dried at 80 F and 100 μ Hg for 48 hr, and then were rehydrated by humidification over various saturated salt solutions to reach the desired moisture level. Cobalt as a catalyst was added as a salt of nitrate to the mixing water at 100 ppm on a linoleate basis unless otherwise stated. The various chelating agents and antioxidants were also added in the mixing water. Chelating agents such as EDTA, ascorbic acid and citric acid were added at 10 moles per mole of added cobalt. The antioxidants BHA, BHT, tocopherol and propyl gallate (PG) were added at 200 ppm on a linoleate basis. For the systems which were representative of intermediate moisture foods, glycerol was added as a humectant as de-

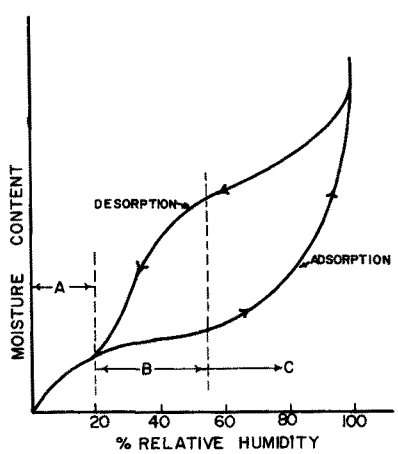

FIG. 1. Typical moisture sorption isotherm for food or model food system.

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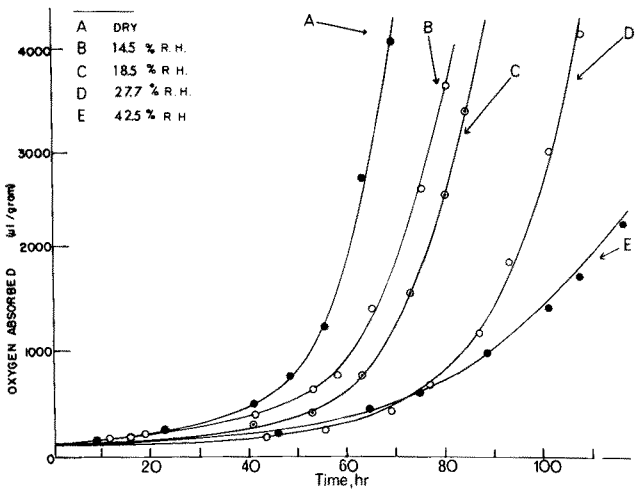

FIG. 2. Oxygen absorption of cellulose-linoleate model system as a function of relative humidity at 37 C.

scribed by Labuza et al. (12). For the cellulose system, 1 pt glycerol to 1 pt linoleate was used, while 1.25 pt glycerol to 1 pt linoleate was used for the casein system. These gave moisture contents of 25-30 g H_2O/100 g solids in the intermediate moisture range. One intermediate moisture food system was also studied consisting of 27.4% Gerbers Strained Baby Food Chicken, 53.4% microcrystalline cellulose powder and 19.2% glycerol which were mixed together to give an equilibrium humidity of 75% at a moisture content of 34 g H_2O/100 g solids.

Oxygen Absorption

Oxygen absorption at 37 C was measured directly in Warburg manometers and the results were analyzed kinetically according to the methods described by Labuza et al. (9). Specifically, the data were compared for oxygen absorbed per gram of linoleate, for time to reach 1% oxidized (moles/mole oxidizable linoleate), which is a measure of the induction period and often corresponds to a level of oxidation at which foods would be just becoming rancid; and for the rate constant K_m which corresponds to the overall reaction rate during the initial stages of oxidation. Duplicate or triplicate samples were prepared for all runs and the average is shown for the kinetic data. Duplicate samples usually showed less than ± 5% difference. Where variations were larger, all data are shown. It must be noted that due to the initial variation in the linoleate (always greater than 99.9% pure) and unintentional introduction of extraneous trace metals

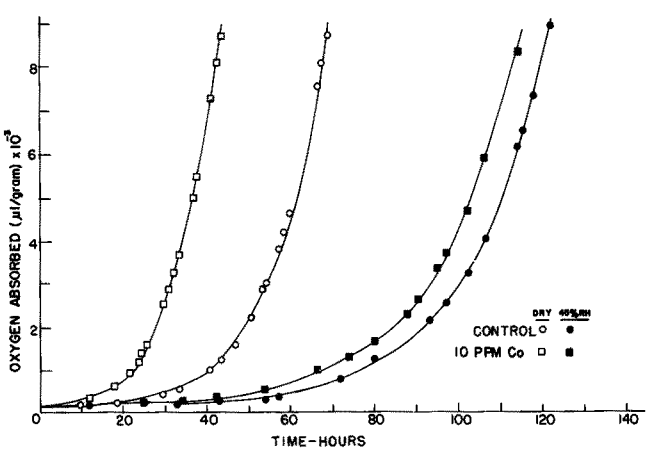

FIG. 3. Effect of humidification to 45% RH on a cobalt nitrate catalyzed (10 ppm cobalt) cellulose-linoleate model system.

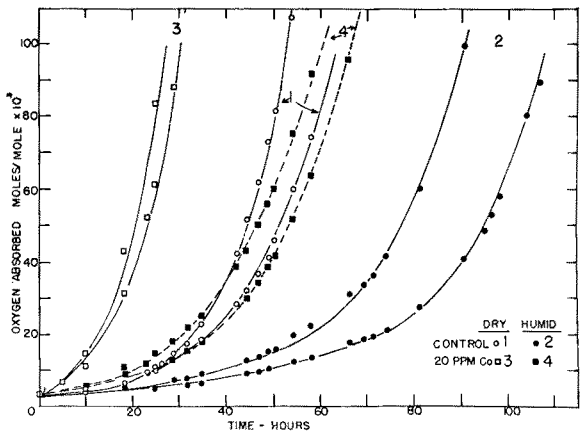

FIG. 4. Effect of humidification to 59% RH on a cobalt acetate (20 ppm cobalt) catalyzed cellulose-linoleate model system.

during mixing, absolute values between runs cannot be compared but values within a run are comparable. The trends of the various treatments can be compared between runs, however.

Peroxide Values

The AOCS method (Cd 8:52) was used on samples sized to give between 0.2 to 0.3 g linoleate in 30 ml Erlenmeyer flasks which were covered with rubber serum caps and held at 37 C until analyzed.

RESULTS AND DISCUSSION

Water-Inhibition Theory for Low Moisture Content

In general, with foods in the dehydrated state, stability to lipid rancidity development as caused by oxidation of unsaturated fatty acids increases as the moisture content increases. Maloney et al. (7) and Labuza et al. (8) studied this in detail by using the cellulose-linoleate model systems. Figure 2 summarizes their results in uncatalyzed systems and shows that the extent of oxidation as well as the rate of oxidation decreased as the equilibrium relative humidity (Fig. 1) or water activity (a_w = % Relative Humidity ÷ 100) increased. Previous work by others in foods attributed this result to either reduction in oxygen diffusion rate by water (13), lowering of catalyst effectiveness by chelation or hydroxide formation (14), or exclusion of oxygen from adsorption on specific sites by water (6), as well as other mechanisms. In studies of catalyzed systems (8), as shown in Figures 3 and 4, it can be seen that in the humidified state the catalyst activity decreases significantly. The reduction in rate depends both on the type of catalyst salt used, due to its hydration state, as well as on the level of humidification. Based on the results of both catalyzed and uncatalyzed systems, a theory of oxidation inhibition was proposed (7-9) in terms of the reactant properties of water. The overall scheme of water inhibition is as follows:

Initiation:

(a) monomolecular $[ROOH]_A \xrightarrow[H_2O]{Metals} RO^* + {}^*OH \rightarrow R^*$

(b) bimolecular $2[ROOH]_A \xrightarrow[H_2O]{Metals} RO^* + ROO^* + H_2O \rightarrow R^*$

Propagation:

$R^* + O_2 \longrightarrow ROO^*$

$ROO^* + RH \longrightarrow [ROOH]_A + [ROOH]_B + R^*$

TABLE I

Effect of Humidification on Rate Constants

	Time to reach 1% oxidized, hr	Monomolecular rate constant of test sample/ rate constant of dry control
Control		
Dry	25	---
32% RH	35	0.89
45% RH	55	0.77
10 ppm Cobalt		
Dry	16	2.64
32% RH	28	0.90
45% RH	50	0.75
10 ppm Cobalt + EDTA		
Dry	27	1.30
32% RH	40	0.85
45% RH	101	0.46

Termination:

$$2ROO^{*} \longrightarrow \text{nonradical products}$$

Inhibition:

$$[ROOH]_B \underset{}{\overset{\text{water-lipid interface}}{\rightleftharpoons}} \overset{H}{O}\!-\!\overset{H}{H} - OOR$$

According to this theory, water shows essentially two antioxidant mechanisms at low moisture content. First, and probably most important, the water hydrates trace metal catalysts present, thus lowering their catalytic effectiveness, and also in some cases forms insoluble hydroxides with metals, thereby taking them out of the reaction completely (15). Thus the overall rates of oxidation, in the monomolecular rate period as well as in the faster bimolecular rate period, are decreased. However, the total reduction in rate cannot be completely attributed to catalyst inhibition, since it was found that there was a higher extent of oxidation when the changeover to the bimolecular oxidation period occurred (end of induction period). This has been attributed to a hydrogen-bonding of the amphipolar hydroperoxides formed after migration to the lipid water interface. In effect this lowers the total effective hydroperoxide content (16). In addition some product inhibition by water in the bimolecular initiation reaction may also occur, thus lowering the overall reaction rate and extent.

Chelating Agents in Low Moisture Systems

Further work in these systems was carried out with various antioxidants and chelating agents and has been summarized by Labuza et al. (9). Table I shows the effectiveness of the chelating agent EDTA in combination with increasing moisture contents. At 45% RH the induction time is nearly double that of the controls and was much better than the effectiveness shown with phenolic antioxidants in humidified samples (9). This increasing effectiveness can be attributed to the solubilization and mobilization properties of water. As water content is increased from the dry state to above the level where one layer of water covers the surface, diffusion of solutes as well as solubilization of previously undissolved species becomes significant (17). Thus it is felt that the higher humidity allows better mobilization of chelating agents to contact trace metals and lower catalytic activity. In systems which contain a high protein content, however, EDTA was not as effective, probably because it became chelated to protein bound metals which were exposed as the amount of water increased (9).

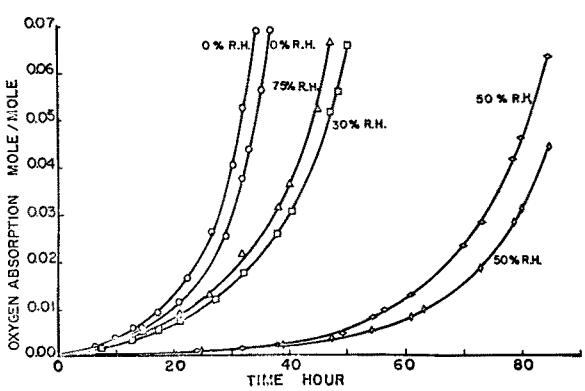

FIG. 5. Effect of humidification in the intermediate moisture range on oxidation rate as compared to lower relative humidities for a cellulose-linoleate-glycerol model system. 100 ppm cobalt added in all systems.

Oxidation in Intermediate Moisture Systems

In several studies reviewed previously (9) it was found that at water activities above 0.5, and especially in aqueous systems, oxidation proceeded very rapidly. This result was important from the standpoint of intermediate moisture foods which range in water activity from a_w=0.6 to 0.85 and have moisture contents of 25 to 45 g H_2)/100 g solids. To achieve these conditions usually some humectant such as sugars or glycols are added to the food. Based on work at low humidities, it was initially felt that the antioxidant effects of water would predominate. Subsequent work showed, however, that in region C of the moisture isotherm

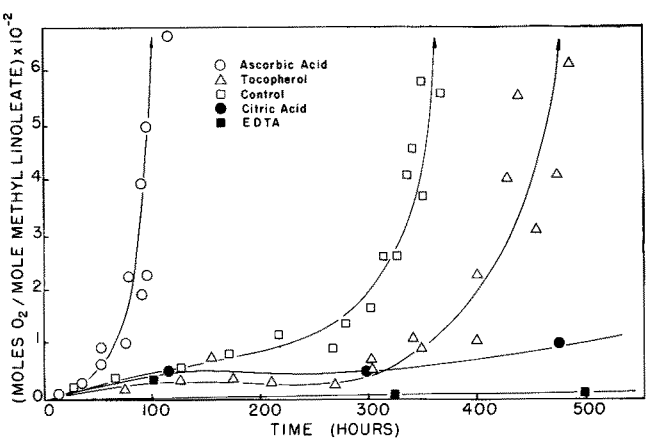

FIG. 6. Effectiveness of various antioxidants on oxidation in a cellulose-linoleate-glycerol model system at 61% RH. All systems 100 ppm cobalt; tocopherol at 200 ppm linoleate basis; chelating agents-ascorbic acid, citric acid and EDTA at 10 moles/mole cobalt added.

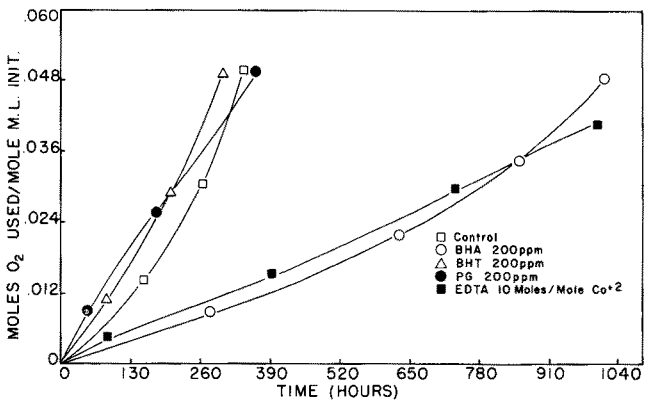

FIG. 7. Effectiveness of various antioxidants on oxidation in a protein-linoleate-glycerol model system at 75% RH. All systems 100 ppm cobalt.

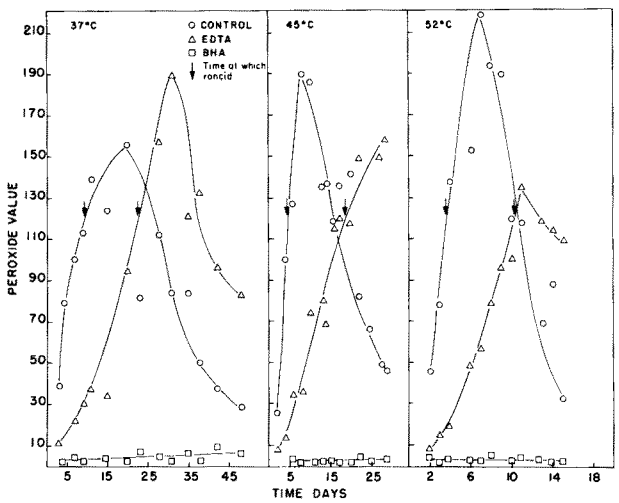

FIG. 8. Production of peroxides in a chicken-cellulose-glycerol intermediate moisture food at three temperatures. EDTA added at 400 ppm on a solids basis. BHA added at 200 ppm on a total fat basis.

TABLE II

Moisture Contents of Model Systems, g H_2O/100 g solids

Per cent RH	Control system	Control + glycerol[a] system
< 0.1	0.01	0.7
32	5.5	5.4
51	7.6	8.7
61	9.5	13.3
75	11.1	18.6
84	14.2	34.5

[a]EDTA addition had no effect on moisture level.

TABLE III

Chicken-Cellulose-Glycerol System, Oxidation Rates of $\mu/O_2/g/day$

	<0.1% RH	32% RH	75% RH
Control	0.59	0.37	1.00
+ 100 ppm citric acid (solids basis)	0.40	0.29	0.74
+ 200 ppm BHA (lipid basis)	0.17	0.10	0.23

(Fig. 1) the solvent and mobility properties of water predominate. Thus, as seen in Figure 5 for a cellulose-linoleate-glycerol system, the oxidation extent and rate in the intermediate moisture range tends toward the rate of the dry condition. Heidelbaugh and Karel (submitted for publication) have shown this to be due primarily to mobilization of trace metals, including those which had not been previously exposed at the lower humidities. The mobility and solubilization of these catalysts thus overpowers the antioxidant effect of water on these metals. The use of a water soluble liquid humectant such as glycerol caused this to occur at lower humidities than without glycerol present.

Metal Chelators in the Intermediate Moisture Range

Labuza et al. (12) have studied the effects of various chelating agents as compared to antioxidants in the intermediate moisture range for both protein and cellulose based systems. As was expected, both EDTA and citric acid are very effective in a cellulose system, increasing the induction time by three to four times, as compared to only a 50% increase for phenolic antioxidants. Figure 6 shows an example of the protection afforded. In protein systems, however, possible binding of EDTA to protein-bound trace metals caused it to have less effectiveness and in fact, as shown in Figure 7, it was not better than BHA, a phenolic antioxidant.

More recent studies in the intermediate moisture range have further elucidated the interaction of metals, water and oxidizing lipids. As shown in Figure 8 for a protein-linoleate-glycerol system containing 100 ppm cobalt as the catalyst, the oxidation rate decreases as moisture content (or water activity) increases from the dry state. Glycerol itself seems to afford some chelating activity as compared with EDTA and, in combination, a synergistic effect exists. However, for the control which does not contain glycerol, as well as the control with EDTA and a glycerol-containing control, the oxidation rate peaks at 61% RH and then decreases again. It had been previously observed that oxidation was more rapid at 61% RH as compared to 75% RH (12) for both cellulose- and protein-based systems.

One explanation proposed may be the fact that for most of these systems the moisture content increase in the range of water activity 0.5 to 0.8 (Table II) may be enough to dilute the reacting metal catalysts. Thus by mass action the rate would be expected to decrease. However, the mixture of EDTA with glycerol did not show this effect but rather showed a slow increase in rate after the initial decrease. No answers have yet been found to elucidate these unusual results. However, in previous experiments with EDTA at the same concentration the protein system showed slightly faster oxidation at 61% RH as compared to 75% RH. It is possible that in the present experiment the initial peroxide levels were very low so that the combination effect of chelation caused the reaction to be very slow and enhanced the antioxidant properties of water. Thus the rate did not begin to increase until it reached 75% RH. This in fact is similar to the results of cellulose systems to which no antioxidants have been added (Fig. 5). A second explanation is that it is possible that at 50-60% RH, the moisture present allows swelling of the protein, exposing new trace metals which catalyze the reaction. Thus, by either increasing the water content to dilute their concentration or by adding a chelating agent with a mobilizing agent such as glycerol, their activity is decreased.

The above mechanisms have been further elucidated in various food systems (18) and are illustrated in Figure 8 and Table III for a chicken system at 75% RH. It is obvious that the phenolic antioxidant gives the best protection as compared to either EDTA or citric acid. This is as would be expected on the basis of previous model system results. Also, as with the model systems, the rate of oxidation was

accelerated at 75% RH, as compared to lower relative humidities. It is interesting to note that by plotting the log of the initial rates of peroxide formation as a function of the reciprocal absolute temperature an activation energy of 10 Kcal was found for the control and an activation energy of 17 Kcal/g mole for the samples containing EDTA. This behavior would be expected if catalytic activity of metals is completely eliminated.

ACKNOWLEDGMENTS

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