Kinetics of Linoleate Oxidation in Model Systems[1,2]

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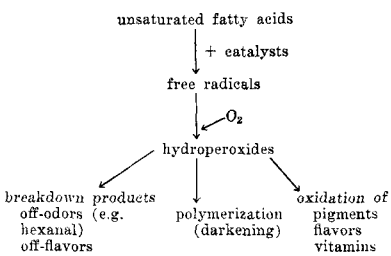
Abstract

Oxidation of methyl linoleate, trilinoleate and linoleic acid has been studied in model systems based on various solid supports. Oxidation was followed by measurement of oxygen absorption, peroxide values and products of oxidation as a function of moisture equilibrium relative humidity. Effects of various metals, histidine and the antioxidants propyl gallate and butylated hydroxytoluene were studied. The results indicate: (a) at 50 C oxidation of protein increases with increasing moisture content and the protein interacts with peroxides changing the overall oxidation rate; (b) increasing moisture content shows the same inhibitory effect on oxidation of trilinoleate as it does on methyl linoleate; (c) the effectiveness of antioxidants is increased with increasing humidity but some chelating agents complexed with metals become catalytic at the higher moisture content; and (d) at moisture contents in the region of capillary condensation, mobility of reactants is enhanced since the rate of oxidation increases significantly.

Introduction

Rancidity resulting from the oxidation of lipids is a primary consideration in the storage stability of many freeze-dehydrated foods. Through a free radical autocatalytic process, molecular oxygen reacts with the unsaturated fatty acids of the lipids. The overall oxidation mechanism is shown below; it results in the typical production of volatile off-odors and flavors.



Lipid oxidation, like most free radical reactions, is very sensitive to catalysts and inhibitors; nonlipid materials such as metals rapidly catalyze the reaction. The oxidation reaction also depends on the physical state of the lipid, i.e., whether it is emulsified with water or deposited on an exposed solid surface. In freeze-dried food the lipid is spread on a large porous matrix, thus oxygen is readily available for reaction and the food oxidizes even at low oxygen partial pressures (1). Under these conditions, however, the presence of small amounts of water markedly inhibits rancidity, as was shown in several food systems stored at different moisture levels (2–4). Salwin (5) proposed that the inhibitory effect of water was related to the water sorption isotherm because the adsorption of the water on the surface of the food excluded oxygen from reacting with the lipids. The water sorption characteristics of foods have been reviewed by Labuza (6) and a typical isotherm relating moisture content to water activity (per cent relative humidity 100) is shown for freeze-dried salmon in Figure 1. The increasing inhibitory effect of water on oxygen absorption in salmon, as water activity increases, can be seen in Figure 2 (7). It was pointed out, however, that above the monolayer value of water adsorption, there occur other reactions such as nonenzymatic browning which also lead to deterioration of foods. The protective effect of water therefore becomes modified.

In the past five years extensive studies (8–10) were undertaken to investigate the mechanisms by which water interacts during lipid oxidation at low moisture content. The studies utilized a model system which was comprised of methyl linoleate, the major oxidizable fatty acid present in foods, deposited on an inert cellulosic support and humidified to different moisture contents. Figures 3 and 4 summarize the results of this work and indicate that: (a) water has an inhibitory effect on up to about 60% relative humidity, at which point capillary condensation of water begins in the porous matrix of the food; (b) water even below the monolayer coverage (at 20% RH for the model system) has an inhibitory effect on oxidation; (c) by hydrating the trace metals, water reduces their catalytic effect on oxidation and demonstrates another of its antioxidant properties;

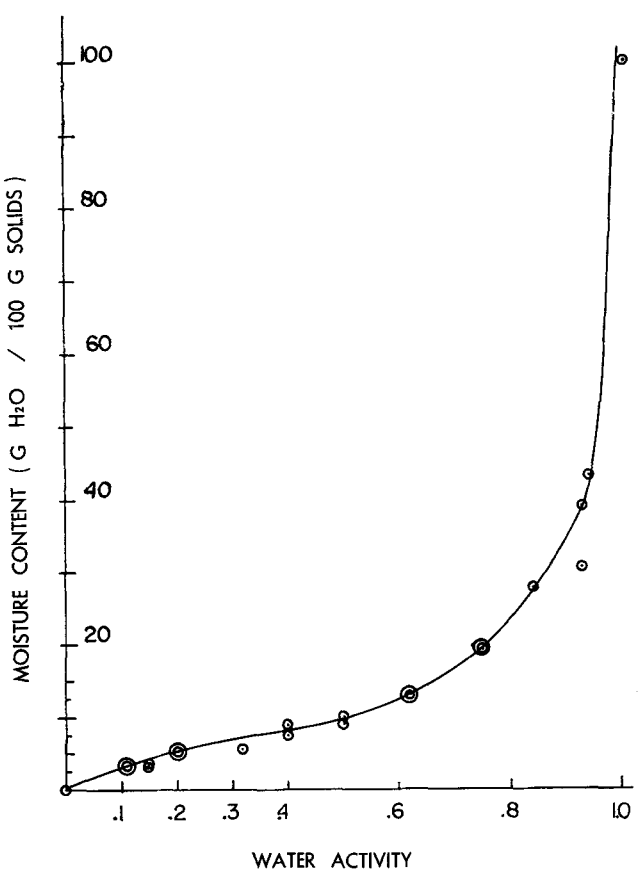


FIG. 1. Moisture adsorbed as a function of equilibrium water activity (per cent RH 100) for freeze-dried salmon at 37 C.

[1] Presented at the AOCS Meeting, New York, October, 1968.
[2] Contribution No. 1396, Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass.

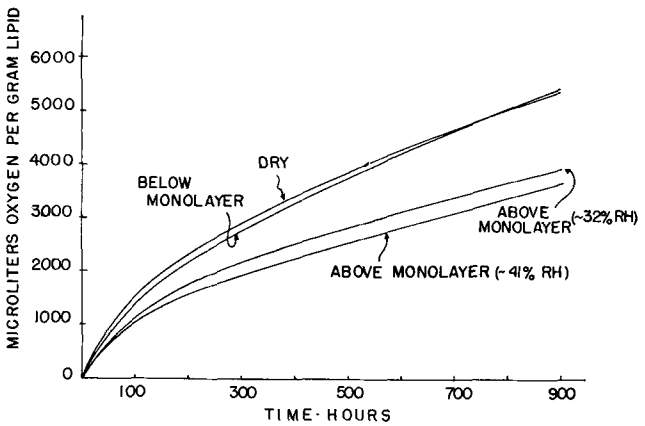


FIG. 2. Oxidation of freeze-dried salmon at 37 C as a function of relative humidity.

(d) the changeover from hydroperoxide monomolecular decomposition kinetics to bimolecular decomposition occurs at higher oxidation levels as the amount of water present increases (10). Based on this observation and through measurements of the kinetics of the reaction, water was shown to inhibit oxidation by hydrogen bonding a portion of the hydroperoxides produced during the propagation step of the reaction. The hydroperoxides are thus removed from further reaction. Once no more hydroperoxides can be removed from the reaction, the rate of oxidation increases rapidly. In addition, the hydration of catalysts reduced the overall rate constants during both periods of oxidation. The overall scheme of water inhibition is shown below:

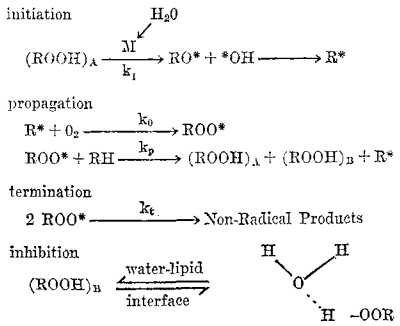


Table I contains a compilation of the measured rate constants for the oxidation of methyl linoleate in the model system. During the initial period of oxidation, initiation is by monomolecular decomposition of hydroperoxides. If the substrate concentration does not change significantly, then the rate of oxidation is:

$$\frac{-d(O_2)}{dt} = \frac{d(ROOH)}{dt} = \frac{k_p}{(2k_t)^{1/2}} k_i^{1/2}[M]^{1/2}[RH_o][ROOH]^{1/2} = K_M[ROOH]^{1/2}$$

K_M represents the overall monomolecular rate constant which can be determined by a plot of the square root of oxygen absorbed (or peroxide content) versus time.

As the amount of hydroperoxide increases, the initiation through ROOH decomposition becomes bimolecular, the decrease in substrate concentration becomes limiting and the rate of oxidation can thus be represented by:

$$\frac{-d(O_2)}{dt} = \frac{d(ROOH)}{dt} = \frac{k_p}{(2k_t)^{1/2}} k_i^{1/2}M^{1/2}[RH][ROOH] = K_B(1-y)\,y$$

where $y = [ROOH] = [O_2]$, and $RH = (RH)_o\,(1-y)$

K_B represents the overall bimolecular rate constant which is determined from a plot of ln (y/1–y)

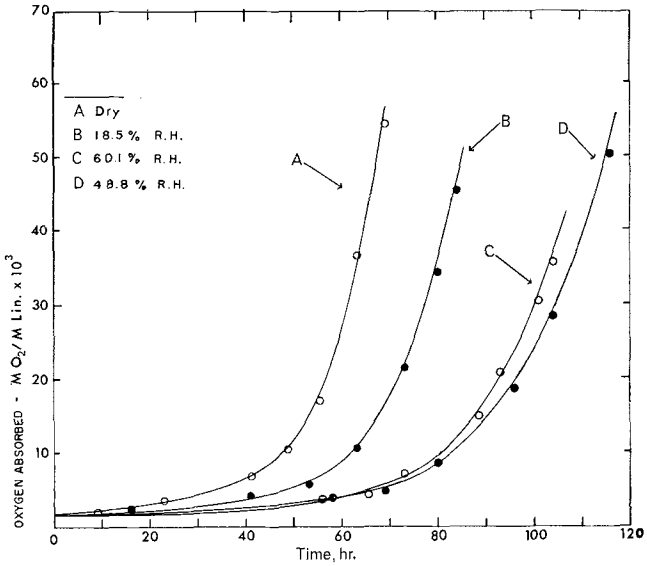


FIG. 3. Effect of moisture content on rate of oxidation of methyl linoleate supported on microcrystalline cellulose model systems 37 C.

(which is the moles oxygen absorbed per mole of substrate corrected for substrate depletion) versus time.

The results shown in Table I support the following conclusions: (a) K_M and K_B decrease in systems humidified to low moisture contents. The induction period (time to reach 500 μl/O_2 absorbed per gram linoleate) increases. (b) Deposition of the linoleate on the solid support lowers K_M but increases K_B as compared to oxidation of linoleate in bulk (11) or in a thin film on glass. This indicates that the support is not completely inert but has some inhibiting properties early in the oxidation reaction. (c) Oxidation of linoleate is more rapid in aqueous emulsion than in dry systems. (d) The oxidation of linoleate present in a food proceeds at a rate 5 to 10 times slower than that in a pure model system; thus, other factors in foods contribute to the inhibition of oxidative rancidity. It was shown (7) that the oxidation mechanism in salmon never entered into the bimolecular period. However, the inhibition by water was similar to the model system results.

Experimental Procedures

Model Systems

Freeze-dehydrated Systems. All systems were prepared according to the method described by Maloney et al. (8). Mixing of the ingredients was conducted at low temperature in a Sorvall Omni mixer. The cellulose system was composed of lipid and microcrystalline cellulose (Avicel American Viscose Corp.) in the ratio of 1:6. The protein systems contained 41 parts lipid to 59 parts protein. In all cases enough water was added prior to mixing to give a smooth continuous gel. Water soluble additives such as cobalt were added as a solution in the mixing water, and lipid soluble additives were combined with the lipid prior to mixing. After preparation the samples were freeze-dried for 48 hr at 100 μ Hg and were then humidified over saturated salt solutions to the desired moisture content. The moisture contents were checked using a gas chromatographic procedure as follows: 20 ml of dry methanol were added to the sample in a 50 ml-stoppered Erlenmeyer flask and shaken for ½ hr. A suitable sample (5 to 10 μl) was withdrawn

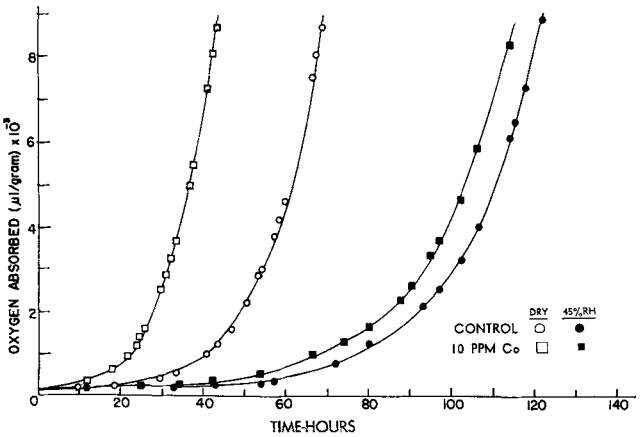


FIG. 4. Effect of humidification on the cobalt catalyzed rate of oxidation of methyl linoleate supported on microcrystalline cellulose model systems 37 C.

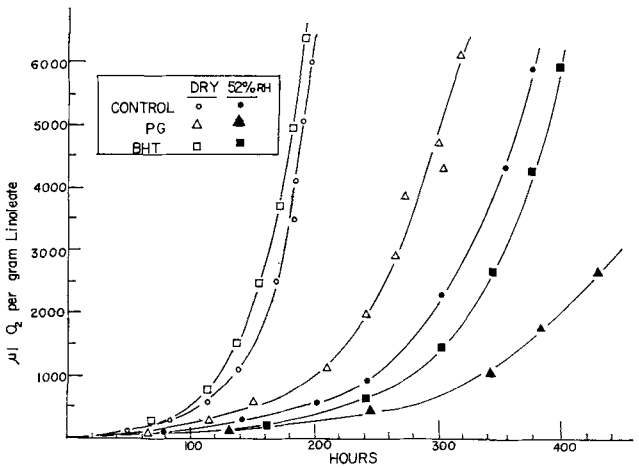


FIG. 5. Effect of humidification on the inhibition of oxidation by propyl gallate (10^{-3} moles PG/mole linoleate) and butylated hydroxytoluene (10^{-3} moles BHT/mole linoleate) in microcrystalline cellulose model systems, 37 C, Run 1.

with a syringe and injected into a Perkin-Elmer Gas Chromatograph equipped with a thermal conductivity detector maintained at 100 C. The column was a 6 ft × ¼ in. copper tube packed with Poropak Q (Waters Associates, Framingham, Mass.) and maintained at 100 C with helium flow at 40 cc/min. A calibration standard was made by adding 1 ml of water to 49 ml of the dry methanol. Standard techniques were used for measurement of peak areas.

Filter Paper System. Ash free filter paper was soaked in a solution containing the desired additives. The paper was then dried in a vacuum for 8 hr at 27 in. Hg and subsequently at 10 μ Hg for 24 hr. The paper was then removed, weighed and dipped into a hexane solution of methyl linoleate for 10 sec, then removed and dried as before. The systems were buffered to the desired pH (8.0) for the data presented in this paper.

Cellulose System. Powdered cellulose (Alphacel, NBCo.) was mixed with purified linoleic acid (Fisher Grade) with a stirring rod in a ratio of 2:1, then the desired amount of water was added by pipette.

Oxygen Absorption

Oxygen absorption was measured directly in Warburg manometers or a Gilson respirometer and the results were analyzed kinetically according to the method of Maloney et al. (8). The cellulose and filter paper systems were oxidized at 37 C whereas the protein-lipid systems were oxidized under accelerated conditions of 50 C after initial results showed the oxidation to be very slow at 37 C.

Peroxide Value

The AOCS method (Cd 8:53) was used on samples of between 2.5 to 5.0 g solids. Peroxide values were converted to oxygen absorbed per gram lipid by using the proper molecular weights and conversion factors.

Hexanal-Heptanal Volatiles Production

The relative amount of volatile decomposition products of lipid oxidation of methyl linoleate, hexanal and heptanal, were determined by injection of a sample from the head space of sealed samples into a F & M 1609 flame ionization gas chromatograph. Conditions were: column: 4 mm × 8 ft, 10% Lac-728 on 60–80 W 600; temperature: 80 C; carrier gas: N_2 60 ml/min; hexanal and heptanal: single peak at 45 sec; sample size: 2 g solids in 25 ml Erlenmeyer Flask.

Protein Solubility Study

To determine the interaction between protein and lipid at the high temperature, a mixture of 1 g methyl linoleate: 5 g gelatin (Difco) was oxidized for 6 days at 50 C and at the desired moisture content. Solubility was determined in ethanol 0.8 M NaCl solutions in several ratios, after several extractions to remove lipid (12). The Folin Ciocalteu method (13) was used to measure the soluble protein. Incorporation of C_{14} from linoleate was determined by the method of Zirlin and Karel (12).

Results and Discussion

Microcrystalline Cellulose Model Systems

Systems similar to those used by Maloney et al. (8) were prepared using different antioxidant mixtures to determine their effectiveness in both the dry and humidified state. Figure 5 shows, for example, the effect of water on the effectiveness of antioxidants BHT (system containing butylated hydroxytoluene) and PG (system containing propyl gallate) at a level of 10^{-3} moles per mole of linoleate. PG is an effective catalyst but BHT is not. BHT may have

TABLE I

Lipid Oxidation
Kinetic Values for Methyl Linoleate Oxidation

System		K_M (moles/mole)$^{1/2}$ hr^{-1}	K_B hr^{-1}	t_1[a] hr
Kern & Dulog (1959)				
Bulk linoleate	40 C	13.0×10^{-3}	6.4×10^{-2}	
	50 C	40.0×10^{-3}	16.6×10^{-2}	
Mabrouk & Dugan (1960)				
Emulsion	40 C	32.3×10^{-3}	8.5×10^{-2}	
	45 C	56.1×10^{-3}	13.2×10^{-2}	
	50 C	95.5×10^{-3}	20.7×10^{-2}	
Present study[b] 37 C				
Thin film[c] (50 cm²/g)		10.9×10^{-3}	6.6×10^{-2}	12
Model system[c] (dry) (10^7 cm²/g)		7.1×10^{-3}	8.2×10^{-2}	20
Model system (dry) 10–20 ppm cobalt		22.5×10^{-3}	65.0×10^{-2}	5–10
Model system (humidified)				
30% RH (3% moisture)		5.3×10^{-3}	5.2×10^{-2}	30
45% RH (4% moisture)		4.6×10^{-3}	4.9×10^{-2}	30
Freeze dried salmon				
Dry		1.8×10^{-3}	.....	40
11% RH		1.2×10^{-3}	.....	50
32% RH		0.8×10^{-3}	.....	70

[a] Time to reach 500 μl/g linoleate.
[b] Methyl linoleate from Mann. Res. Lab, New York.
[c] Average of 15 tests.

TABLE II
Kinetic Constants Methyl Linoleate Oxidation Avicel Model Systems 37 C[a]

Conditions	K_M (moles/mole)$^{1/2}$ hr^{-1}	K_B hr^{-1}	t_i hr
Run 1			
Control			
dry	3.62×10^{-3}	2.88×10^{-2}	100
32% RH	2.07×10^{-3}	1.42×10^{-2}	180
10^{-3}M BHT			
dry	3.16×10^{-3}	2.80×10^{-2}	115
32% RH	2.07×10^{-3}	1.40×10^{-2}	235
10^{-3}M PG			
dry	2.41×10^{-3}	2.30×10^{-2}	140
32% RH	2.00×10^{-3}	0.96×10^{-2}	250
Run 2			
Control	5.1×10^{-3}	4.92×10^{-2}	25
10^{-1}M BHT	0.48×10^{-3}	....	510
10^{-2}M BHT	0.39×10^{-3}	....	800
10^{-3}M BHT	0.38×10^{-3}	....	900

[a] All concentrations based on linoleate content.

TABLE III
Kinetic Values for Trilinoleate Systems 37 C

System	K_M (moles/mole)$^{1/2}$ hr^{-1}	K_B hr^{-1}	t_i[a] hr
Run 1			
Trilinoleate			
dry	1.10×10^{-3}	0.40×10^{-2}	120
11% RH	0.80×10^{-3}	0.25×10^{-2}	160
32% RH	0.54×10^{-3}	0.17×10^{-2}	200
Run 2			
Trilinoleate			
dry	0.50×10^{-3}	0.22×10^{-2}	120
32% RH	0.21×10^{-3}	0.11×10^{-2}	230
Trilinoleate + 1% Linoleic acid			
dry	0.46×10^{-3}	0.23×10^{-2}	170
32% RH	0.25×10^{-3}	0.13×10^{-2}	230

[a] t_i, time to reach 500 μl/g linoleate.

been volatilized during freeze-drying and its final concentration reduced during storage, since it has a high vapor pressure. Run 3 in Figure 6 shows much better results for BHT, perhaps the result of a smaller loss during drying. Kinetic constants for these runs and for other similar tests are shown in Table II for comparison. An examination of the results shows: (a) The overall rate constants for the controls vary between runs. It is also evident that the rate is lower than in the early model system runs reported in Table I. Variations in initial purity of the lipid or in the amounts of trace metals present in the Avicel (less than 2 ppm) or introduced by contamination during preparation may be responsible for this variation. However, within any one run the reproducibility of replicates is good. (b) The effectiveness of propyl gallate increases with humidification, possibly because of better mobility on the surface of the solid matrix, whereas BHT seems to be more effective in the dry state as indicated in Run 3. This run shows that increasing the concentration of BHT beyond a certain limit changes its action to a prooxidant effect since it works as a free radical chain breaker. A test with this type of model system would seem to be better for prediction of antioxidant properties than using the Swift AOM test.

Since most food lipids contain fatty acids as triglycerides, with only small amounts of free fatty acids, it was decided to test both lipid forms in the same type of model system. Trilinoleate was oxidized in a microcrystalline cellulose system at 37 C and oxygen absorption, peroxide value and the production of volatile compounds were examined. The results are shown in Figures 7 and 8 and the kinetic values are tabulated in Table III. The results indicate that: (a) Humidification has the same effect on oxidation of triglycerides as on the methyl ester; the presence of 1% of free fatty acid does not significantly affect the rate of oxidation. It should be noted that the rate constants are about an order of magnitude smaller for trilinoleate than for the methyl ester. In fact, the rates are similar to those for the salmon oxidation (Table I), indicating that the system is more representative of a food. Possibly, this effect may be due to the fact that free radical chains propagate less readily in triglycerides than in the more mobile esters of single fatty acids. It has been shown (18) that the position of the fatty acid on the triglyceride also contributes to the rate of oxidation, with the β position giving the most protection. (b) Peroxide value expressed as oxygen absorbed, as shown by the open circles in Figure 7, is a good index of oxidation up to about 4000 μl O_2/g. This is similar

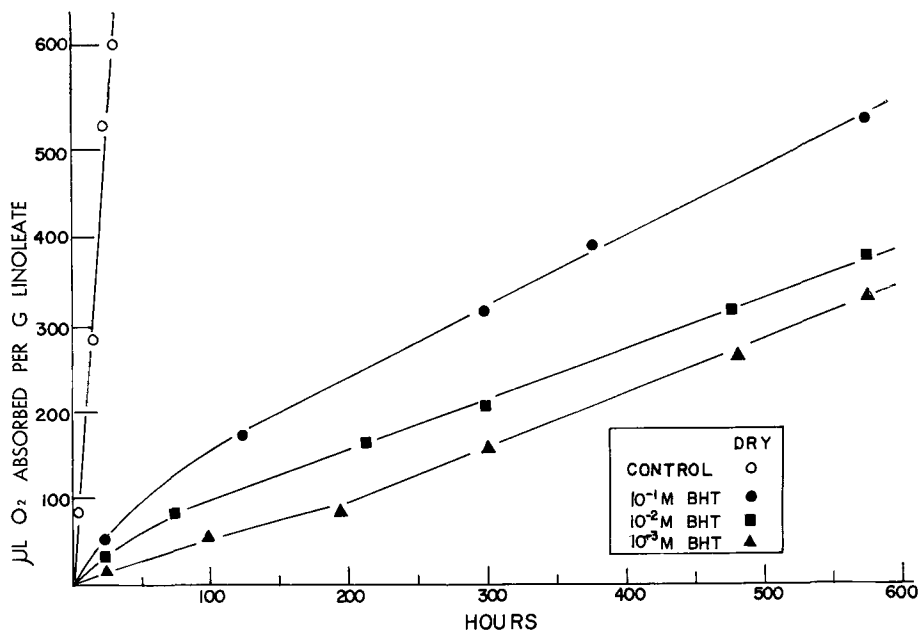


FIG. 6. Effect of BHT at various levels of addition on the oxidation of methyl linoleate supported on microcrystalline cellulose in the dry state at 37 C, Run 2.

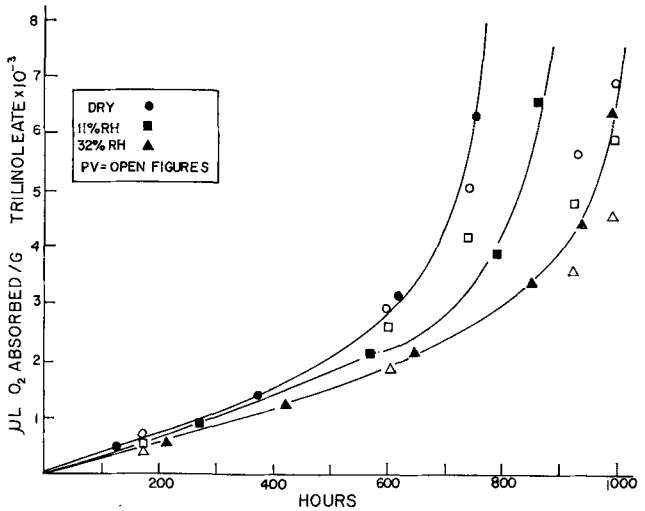


FIG. 7. Effect of humidification on the rate of oxidation of trilinoleate supported on microcrystalline cellulose model systems, 37 C, Run 1.

to the results of Martinez and Labuza (7) for the methyl linoleate system. However, with foods the peroxide value is usually several orders of magnitude smaller than the oxygen absorbed where the peroxide interacts with the other constituents (7,16). (c) Measurement of the production of hexanal is useful as an index of oxidation and is sensitive to the effects of water.

Protein Model Systems

In order to investigate the effect of proteins on the rate of oxidation, a model system was devised utilizing 59% egg albumin (Mann Research Laboratories, N.Y.) as protein and 41% lipid. The lipid used was a mixture of methyl linoleate, methyl oleate (Hormel Institute, Minn.) and a non-oxidizable hydrocarbon (Apiezon B oil MW-360, J. G. Biddle Co., Pa.). Three runs were made under varying conditions (see Table IV). Since preliminary investigations indicated that the rates were too slow to be measured at 37 C, all tests were made at 50 C; however, it was found that the protein also oxidized at this temperature (Fig. 9). Assuming that protein oxidation is independent of the lipid oxidation, the rate of the latter can be separately calculated. Since oleate oxidation occurs at about 12 to 15 times slower than linoleate, its oxidation can be neglected.

Figures 10, 11 and 12 present some data for the total oxygen absorbed per gram of sample, which accounts for both protein and linoleate oxidation. At

TABLE IV

Protein Model System Parameters[a]

System	Run 1	Run 2	Run 3
Component			
Protein (g/100 g dry system) (spray dried egg albumin)	59	59	59
Lipid (g/100 g dry system)	41	41	41
(distribution %)			
Methyl linoleate	10%	30%	40%
Methyl oleate	50%	30%	20%
Apiezon B	40%	40%	40%
Additives			
Myoglobin (mg/100 g system)	20	....	....
Cobalt (g/g linoleate)	....	10	10[b]
Propyl gallate[c] (ppm on solids basis)	50	150	150
Butylated hydroxytoluene[c] (BHT) (ppm on solids basis)	50	150	150
Histidine[c] (moles/mole linoleate)	....	....	10^{-3}

[a] Monolayer equilibrium humidity, 31% RH.
[b] Added only to catalyzed sample.
[c] Eastman Kodak (added only to specific samples).

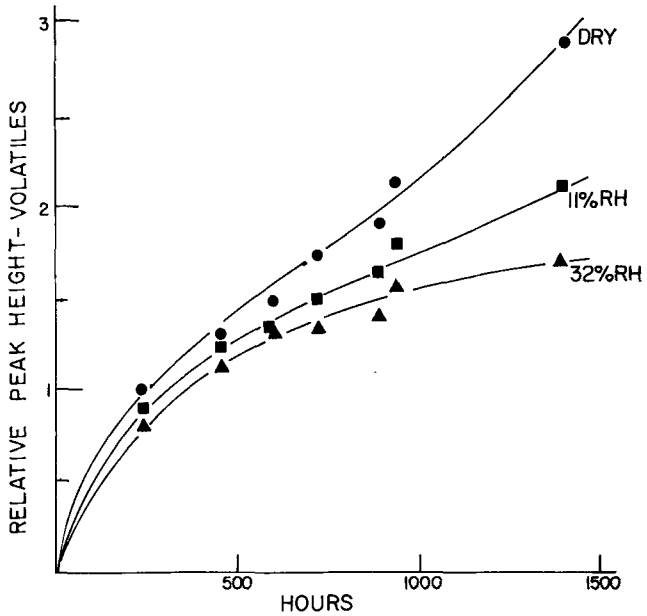


FIG. 8. Effect of humidification on the production of hexanal and heptanal during oxidation of trilinoleate supported on microcrystalline cellulose model systems, 37 C, Run 1.

low linoleate concentration (10% of the lipid), oxidation is very slow although myoglobin (Mann Research Labs., N.Y.) was present as a catalyst for all three systems of Run 1. With increased linoleate concentration, the total oxygen absorbed increases proportionately. Normally with a fatty food such as ground beef, linoleate would comprise less than 2% of the fatty acids with oleic and stearic acid predominating. This low value means a low rate of oxidation for foods, which is difficult to measure manometrically as was the case for Run 1 of this system.

An examination of Table V, which represents the kinetic values for the three runs at all conditions tested, indicates: (a) In almost all cases humidification to 31% RH (the monolayer equilibrium relative humidity) decreased the rate constants and increased the measured induction time. This would be expected from the previous results of the cellulose system and with foods. Above 40% RH (the point at which condensation of water in the solid matrix capillaries takes place to produce water of a more liquid-like structure) a catalytic effect on lipid oxidation was shown in some cases. It is possible that this catalysis

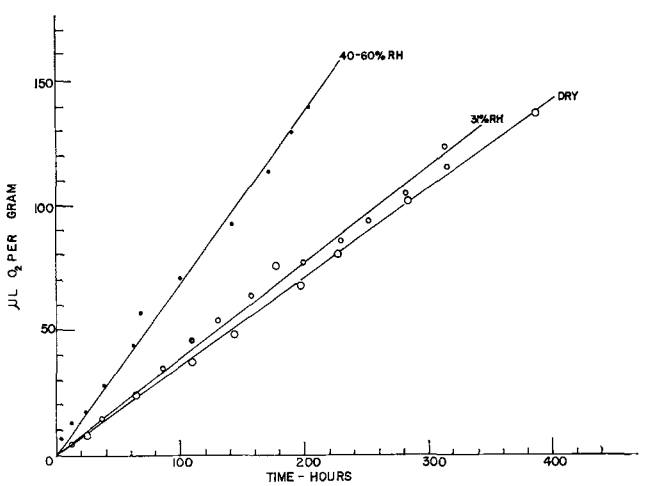


FIG. 9. Rate of oxidation of egg albumin as a function of relative humidity at 50 C.

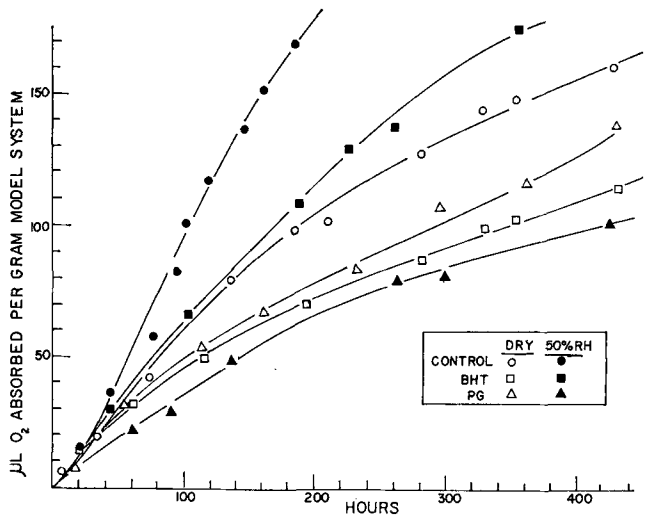


FIG. 10. Effect of moisture content and antioxidants on the total oxygen adsorbed per gram sample of protein-linoleate model systems at 10% linoleate, 50 C, Run 1.

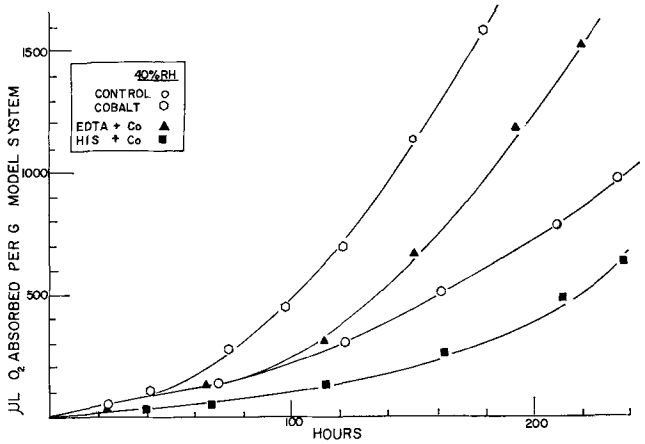


FIG. 12. Effect of the chelating agents EDTA and histidine on the cobalt catalyzed oxidation of protein-linoleate model systems at 40% linoleate and 40% RH, 50 C, Run 3.

results from products produced from protein oxidation, which is accelerated at the higher humidities. (b) The antioxidants PG and BHT were effective at 31% RH and 50% RH when linoleate concentration was low compared to protein. At increased linoleate (Run 2) both were effective at 31% RH but at 60% RH the rate in the monomolecular decomposition period was increased. (c) In Run 3 with 40% linoleate, oxidation proceeded rapidly in the humidified state. Histidine did not have as good antioxidant properties as on cellulose but in combination with cobalt on the protein matrix it became a very effective antioxidant. EDTA (system containing sodium diethyl-diaminetetra-acetic acid) showed catalytic activity at 40% RH. In combination with cobalt it decreased only slightly the catalytic activity of cobalt even though the EDTA-cobalt molar ratio was 10:1. (d) The overall magnitudes of the rate constants are lower than the average values presented in Table I for methyl linoleate but similar to those for trilinoleate. This indicates that the protein may have a modifying effect on the lipid oxidation mechanism.

Interactions between lipid, protein and water were also demonstrated in samples of gelatin-methyl linoleate oxidized for 6 days at 50 C (Fig. 13). It can be seen that at the low humidity where oxidation would occur most rapidly (approximately the same protein-lipid ratio as in Run 3), the protein is apparently degraded into fragments of lower molecular weight. This results in an increased solubility of the protein in mixtures of 0.8 M NaCl with ethanol. At 60% RH however, where oxidation of protein is enhanced and lipid oxidation is reduced, aggregation occurs, decreasing protein solubility. El-Gharbawi and Dugan (19) also found reduced solubility of soluble protein nitrogen for dried beef (3% moisture) stored for 24 weeks at 80 F and 2% oxygen. These conditions were optimum for oxidation of the lipid followed by interaction with the protein fraction and gave reduced solubility. With the long storage period any scission of protein would be masked by the other interactions that occur and thus show

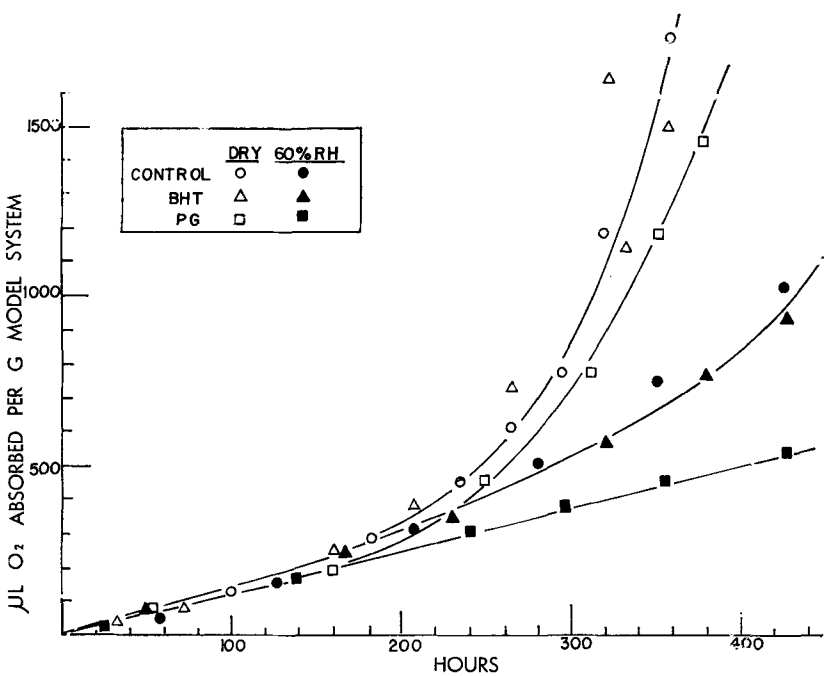


FIG. 11. Effect of moisture content and antioxidants on the total oxygen adsorbed per gram sample of protein-linoleate model systems at 30% linoleate, 50 C, Run 2.

TABLE V

Kinetic Constants Protein Model Systems 55 C

Conditions	K_M (moles/mole)$^{1/2}$ hr^{-1}	K_B hr^{-1}	Induction time hr
Run 1			
Control			
dry	1.74×10^{-3}	0.82×10^{-2}	75
31%	1.25×10^{-3}	0.74×10^{-2}	140
50%	1.82×10^{-3}	0.58×10^{-2}	55
BHT			
dry	1.39×10^{-3}	0.69×10^{-2}	50
31%	1.49×10^{-3}	0.31×10^{-2}	85
50%	0.87×10^{-3}	0.87×10^{-2}	160
PG			
dry	1.0×10^{-3}	0.64×10^{-2}	75
31%	0.79×10^{-3}	0.78×10^{-2}	180
50%	0	0	<400
Run 2			
Control			
dry	1.59×10^{-3}	0.99×10^{-2}	60
31%	1.30×10^{-3}	0.64×10^{-2}	55
60%	1.14×10^{-3}	0.81×10^{-2}	70
BHT			
dry	0.89×10^{-3}	1.07×10^{-2}	70
31%	0.83×10^{-3}	0.30×10^{-2}	100
60%	1.74×10^{-3}	0.20×10^{-2}	50
Run 3			
Control			
31% RH	2.47×10^{-3}	0.79×10^{-2}	85
40% RH	1.75×10^{-3}	0.82×10^{-2}	65
Cobalt			
31% RH	1.95×10^{-3}	1.21×10^{-2}	85
40% RH	3.50×10^{-3}	1.18×10^{-2}	40
Histidine			
31% RH	1.75×10^{-3}	0.74×10^{-2}	100
40% RH	1.45×10^{-3}	1.26×10^{-2}	85
EDTA			
31% RH	1.29×10^{-3}	0.74×10^{-2}	100
40% RH	1.59×10^{-3}	1.53×10^{-2}	50
Co + EDTA			
31% RH	2.13×10^{-3}	1.02×10^{-2}	70
40% RH	2.02×10^{-3}	1.12×10^{-2}	70
Co + HIS			
31% RH	1.39×10^{-3}	0.77×10^{-2}	110
40% RH	1.16×10^{-3}	0.84×10^{-2}	125

solubility losses. From Table VI it can be seen that a direct reaction occurs between linoleate and protein, as evidenced by incorporation of ^{14}C-labeled linoleate into the protein (12) which is enhanced at the higher

TABLE VI

Incorporation of ^{14}C-labeled Linoleate in Gelatin at 50 C

Days	Per cent ^{14}C incorporated into gelatin 0.1% RH	60% RH
0	0.90	0.90
3	1.30	1.30
6	2.12	4.75

humidity. This could account in part for the reduced solubility.

In addition to the effect of linoleate on protein solubility, the protein interacts with the lipid oxidation mechanism as evidenced by the peroxide values. In Table VII, the peroxide value converted to oxygen absorbed per gram linoleate is compared to the actual monometric determinations corrected for protein oxidation. It can be seen that although the peroxide determination compares favorably in the first 24 hr, after that time it becomes 10 to 20 times less than the actual value. This has been observed in salmon (7) as well as in beef (16). It is possible that the peroxides react with the protein through a free radical mechanism and become tied up to the protein as indicated by the above results. This also indicates that peroxide determination is not a useful technique for measurement of oxidation in foods containing proteins, especially if stored under accelerated conditions such as at 50 C.

High Humidity Systems

It had been observed in the microcrystalline cellulose systems that as the water content was increased above the equilibrium humidity for the beginning of capillary condensation, the effect of water as an oxidation inhibitor was modified. Figure 14 indicates the effect of high moisture levels on oxidation of linoleic acid supported on powdered cellulose (about

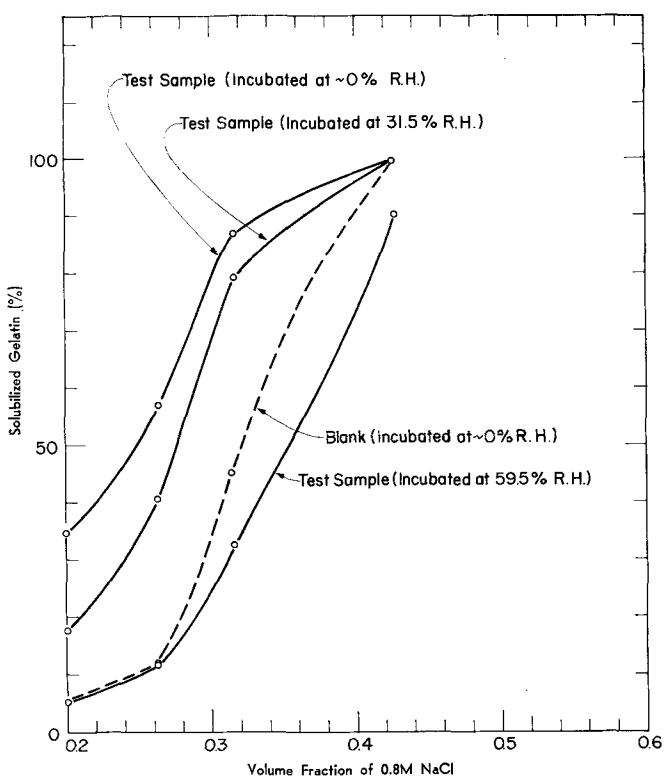


FIG. 13. Solubility of gelatin cooxidized with methyl linoleate for 6 days at 50 C. Volume fraction of 0.8 M NaCl in ethanol.

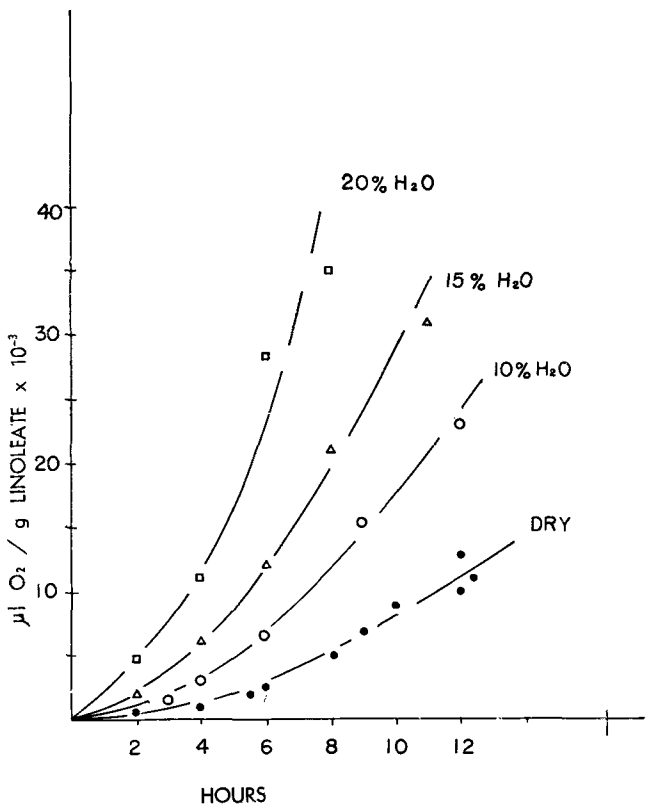


FIG. 14. Effect of high moisture content on the oxidation of linoleic acid (Fisher Purified) supported on powdered cellulose at 37 C.

TABLE VII
Comparison of Manometric Oxygen Adsorbed With Peroxide Value
Protein Model System (Run 2 Control)

Time (hr)	Relative humidity <0.01%		Relative humidity 31%		Relative humidity 60%	
	Oxygen adsorbed[a]	PV[b]	Oxygen adsorbed	PV	Oxygen adsorbed	PV
24	284	115	240	72	230	127
72	700	122	650	138	750	129
232	3650	144	2070	261	2450	100
337	11350	295	3900	173	4600	165

[a] Microliter O_2/g linoleate (manometric data).
[b] Microliter O_2/g linoleate = (PV meq/K_g) × 11.2.

40 ppm trace metal content). The rate increased with humidity, as indicated by the rate constants in Table VIII. These moisture levels fall within the capillary range where mobility of water is enhanced. Figure 15 shows the bimolecular oxidation region, in which a similar effect occurs with the system comprised of methyl linoleate deposited on filter paper and buffered to pH 8. At very high humidity (98% RH) the rate of oxidation is increased. Table IX contains the rate constants for this experiment (Run 22) as well as for Run 20 at lower humidity. At 52% RH the rate of oxidation was decreased for the control and a strong antioxidant effect for cobalt-histidine complex similar to that which occurred in the protein system was observed. The large increase in rate at 98% RH probably can be attributed to increased mobility of the catalysts which could react to a larger extent than at lower water concentration. It is possible that their catalytic activity is less, but that the

TABLE VIII
Kinetic Values: Linoleic Acid Cellulose System
37 C

Conditions	K_B	t_i
	hr^{-1}	hr
Dry (0.1% RH)	39×10^{-2}	2
10% H_2O (45% RH)	40×10^{-2}	1.8
15% H_2O (75% RH)	48×10^{-2}	1.2
20% H_2O (85% RH)	58×10^{-2}	0.4

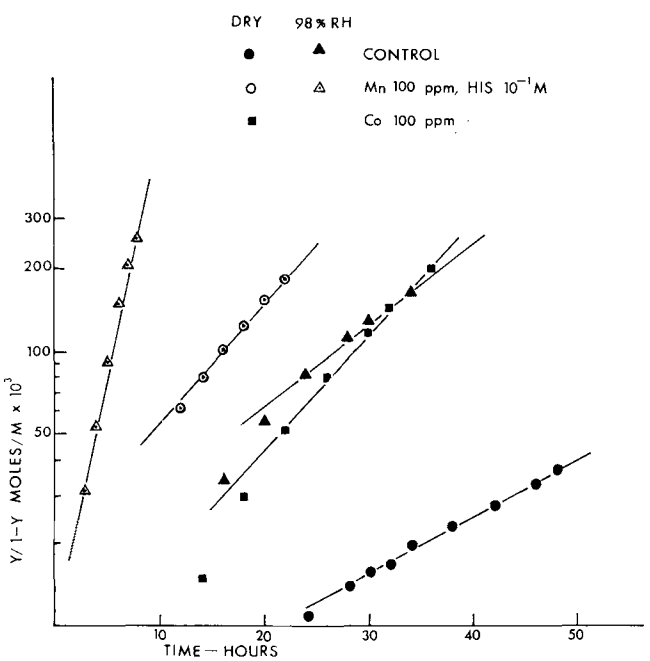


FIG. 15. Bimolecular rate plot showing effect of high moisture content (98% RH) on the catalyzed rate of oxidation of methyl linoleate supported on filter paper, 37 C, Run 22.

TABLE IX
Kinetic Values for the System: Methyl Linoleate Filter Paper
37 C

Conditions	K_M	K_B	t_i
	(moles/mole)$^{1/2}$ hr^{-1}	hr^{-1}	hr
Run 20			
Control			
dry	16.5×10^{-3}	7.8×10^{-2}	15.5
52% RH	13.0×10^{-3}	7.1×10^{-2}	18.0
100 ppm cobalt			
dry	24.3×10^{-3}	7.6×10^{-2}	7.4
52% RH	26.8×10^{-3}	9.7×10^{-2}	0.2
100 ppm cobalt + histidine (10^{-1}M)			
dry	15.5×10^{-3}	9.2×10^{-2}	12.0
52% RH	6.6×10^{-3}	6.6×10^{-2}	18.0
Run 22			
Control			
dry	7.0×10^{-3}	4.6×10^{-2}	17
98% RH	20.2×10^{-3}	6.8×10^{-2}	8.0
Mn 100 ppm + 10^{-1}M histidine			
dry	31.0×10^{-3}	10.3×10^{-2}	2.6
98% RH	92.0×10^{-3}	38.5×10^{-2}	0.6
Cobalt 100 ppm			
dry	12.5×10^{-3}	9.1×10^{-2}	2.0

increased mobility enhances the overall rate, especially for the histidine-manganese complex. The details of the histidine interactions with metals will be presented elsewhere. It is also interesting to note that the rate constants compare favorably with the values found for oxidation of linoleate in emulsions in Table I. These results lead one to question the extent of oxidation occurring in intermediate moisture foods which have an equilibrium humidity of about 75–80% RH. Research on these systems is now in progress in our laboratories.

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