Effects of Humidification on Activity of Catalysts and Antioxidants in Model Systems[1]

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Abstract

Studies on freeze-dried model systems containing methyl linoleate and various additives have shown that water content plays an important role in controlling the activity of antioxidants and catalysts; water can counteract the catalytic activity of certain metals, and can enhance the antioxidant effect of some chelating agents. Different additives, however, respond differently to the effects of humidification, especially at high water activities. Data are presented which show that complexes of manganese and histidine show enhanced prooxidant activity at high water contents, while those of cobalt and histidine become more antioxidant. The effectiveness of EDTA as a chelating agent is also enhanced as the moisture content is increased. However, the effects of humidification are dependent on other components of the system, including the nature of the hydrophilic support.

Introduction

In a previous paper (Tjhio and Karel, unpublished data) we reported on the oxidation of methyl linoleate in a freeze-dehydrated model system based on filter paper. In these studies, which were conducted in the absence of water, we observed that histidine was antioxidant in early stages of the oxidation and became slightly prooxidant in the later stages. We also found that the prooxidant activity of cobalt and of manganese salts increased with increasing pH. At pH 9.0 histidine increased the catalytic activity of manganese. Conditions resulting in an increased prooxidant effect of the manganese-histidine complex also resulted in the formation during oxidation of decomposition products of histidine. In contrast, at pH 8.0 histidine counteracted the catalytic activity of cobalt, and the cobalt-histidine complex acted as a neutral additive. This paper reports the effects of humidification on model systems containing the additives studied previously in the absence of water.

Materials and Methods

Materials, preparation, buffering and oxidation of the model systems were described in detail by Tjhio and Karel (unpublished data) and by Maloney et al. (1). In summary, the procedure was as follows.

Solid Supports

Ash-free filter paper was chosen as the solid support to simulate the hydrophilic porous structure of freeze-dried foods (2). The filter papers were 4.25 cm in diameter and weighed about 1.00 g each. The amount of lipid dispersed on the filter paper was in the range of 80–100 mg/filter paper.

Microcrystalline cellulose (Avicel, American Viscose Corp., Marcus Hook, Pa.) was mixed with lipid in the ratio of 6:1. This mixture was beaten into a gel, using 30 parts water containing the additives. This was then freeze-dried in the desired reaction flasks for 48 hr at 100 μ Hg. This is the system used by Maloney et al. (1).

Methyl Linoleate

Methyl linoleate of the highest purity commercially available (The Hormel Institute, Minneapolis, Minn.) was further purified using two vacuum distillation processes, a technique similar to that used by Wallace (3).

Amino Acid

l-Histidine was of the highest grade commercially available (Cal. Biochem., Los Angeles, Calif.). For each run, a solution of the amino acid (in base form) was freshly made up in twice-distilled deionized water at the desired concentration (moles histidine per mole linoleate).

Metal Catalysts

Manganese sulfate monohydrate and cobalt chloride hexahydrate were of reagent grade (Malinckrodt Chemical Works, St. Louis, Mo.). Solutions of the metal salts were made up in twice-distilled deionized water, and the concentrations were based on the amount of solution absorbed by each filter paper. The concentration of the metal salts is expressed as ppm with respect to the weight of linoleate.

Buffer Solutions

Ten milliliters of additive solution were adjusted to the desired pH by adding a few drops of 10 N sodium hydroxide solution. To this solution of 0.5 ml (5%) of a buffer solution was added. The solution was then ready for application. The buffers used were as follows: for pH 4.0, 12.29 parts of 0.1 M citric acid and 7.71 parts of 0.2 M disodium phosphate; for pH 8.0, 0.55 parts of 0.1 M citric acid and 19.45 parts of 0.2 M disodium phosphate; for pH 9.0, 50 ml of 0.025 M borax and 4.6 ml of 0.1 M hydrochloric acid. The pH was maintained at the highest feasible level, namely pH 8.0 for systems containing manganese sulfate. These upper pH limits were set because above them the metals precipitated as hydroxides. The rate of oxidation of control samples was the same at pH 8.0 and at pH 9.0, and the controls were adjusted to either pH. The pH of each solution was checked both before application to the filter paper and in a methanolic solution after extraction from the filter paper. Of course, the pH of the dry system in the absence of water could not be defined or measured.

Preparation of the Filter Paper Model Systems

The filter paper was dipped into a buffered aqueous solution containing water-soluble additives. It was then dried in air on a stainless-steel wire tray for 10 min and placed in a beaker in a vacuum desiccator. The desiccator was evacuated by pumping with an aspirator for 8 hr. It was then connected to a vacuum pump through a liquid nitrogen trap, and the filter paper further dried for 24 hr at a pressure of 5–10

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μ Hg. The dehydrated filter paper containing water-soluble additives was then weighed, dipped into the methyl linoleate solution in *n*-hexane (50–60%) for 10 sec, and dried on a stainless-steel wire rack at room temperature for 5 min. The vacuum dehydration procedure was then repeated.

Humidification of the Model System

After samples were dried, they were humidified in vacuum desiccators over saturated salt solutions adjusted to specific water activities (4). This was done by evacuating the desiccator and allowing the sample to equilibrate for 4 hr at 37 C. Control samples were maintained in the dry state but were treated in the same manner and were held over magnesium perchlorate. After equilibration, the vacuum was broken with air which had been previously adjusted to the proper humidity by bubbling through saturated salt solutions.

Oxidation

Reaction flasks containing the model systems were attached to the manometers of a Gilson differential respirometer (Rainin Instrument Co., Boston, Mass.), and the oxidation conducted under air at 37 C in a water bath. To eliminate the possibility of oxygen depletion, the reaction flasks were flushed at suitable intervals with dry air or air humidified to the desired relative humidity. Oxidation was followed, using similarly treated samples stored in 50 ml Erlenmeyer flasks, by recording the increase in conjugated hydroperoxides. The conjugated hydroperoxides were measured by UV absorption at 233 mμ (5). Dry methanol was used for extraction of the sample.

Moisture Analysis

The moisture content of the samples was determined by gas chromatographic analysis after extracting the model system with anhydrous methanol. The gas chromatograph (Perkin-Elmer model 154, Perkin-Elmer Co., Norwich, Conn.) was operated under the following conditions: column, 6 ft × ¼ in. copper; column packing, Porapak Q; column temperature, 100 C; flow rate carrier gas (helium), 40 ml/min; filament, 8 volts; sample size, 5 μl; attenuation, 1 − 32 ×; solvent, reagent-grade dry methanol. The moisture contents at the relative humidities employed in this study are given in Table I.

Oxidation Rate Constants

The following terms were used as the oxidation rate constants:

TABLE I

Moisture Contents of Model Systems After Equilibration at Several Humidities

Humidification	g H_2O / 100 g Solids	g H_2O / 100 g Cellulose
Microcrystalline cellulose		
Dry[a]	0.90	1.05
11% RH	1.8	2.1
32% RH	3.3	3.9
45% RH	4.2	4.8
52% RH	4.8	5.6
75% RH	7.5	8.8
98% RH		
Filter paper		
Dry[a]	0.94	1.65
11% RH		
32% RH		
45% RH		
52% RH	4.75	8.17
75% RH	7.5	13.3
98% RH	8.1	14.4

[a] <0.1%.

t_i (hr) = the induction period equivalent to the time required to reach an oxygen absorption of 5 mmoles O_2/mole linoleate.

K_M ($[\frac{\text{moles } O_2}{\text{mole linoleate}}]^{1/2}$ hr^{-1}) = the oxidation rate constant during the initial monomolecular period.

K_B (hr^{-1}) = the oxidation rate constant during the subsequent hydroperoxide bimolecular decomposition period.

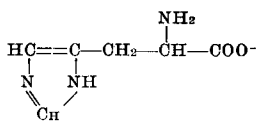
The derivation of these terms is described by Maloney et al. (1) and Labuza et al. (6). The rate constants of samples treated by humidification or by the introduction of additives (subscript t) are divided by the rate constant for the dry control (no additives, subscript c). The effect is antioxidant if the value falls below 1 and prooxidant if it is above 1.

Results and Discussion

In previous studies (1,7, Tjhio and Karel, unpublished data) we found that histidine in the dry state had an antioxidant effect in low concentrations (10^{-3} M) and only in the very early stages of oxidation. At high concentrations (10^{-1} M) histidine was slightly prooxidant, especially during advanced stages of oxidation. Humidification of the filter paper system to high humidity increased this prooxidant effect to quite significant levels.

Table II shows the effect of humidification on the extent of oxidation of methyl linoleate dispersed on filter paper. The control shows the expected decrease in rate at 75% RH, as found by Maloney et al. (1) for microcrystalline cellulose model systems, but the extent of oxidation at 98% RH exceeds that of the dry control. In Table II the average of the kinetic data measured from manometric oxygen absorption for five runs on the same system containing only histidine shows a similar prooxidant effect at 98% RH but no real effects at lower humidities.

At pH 9.0 histidine is present in the following form:



The solubility of histidine in water is high because of the carboxylate group. The negatively charged histidine molecule should move freely in the aqueous medium to react with water-soluble radicals or oxidation products (e.g., hydroperoxides), which would be more soluble in water than the original substrate. The higher degree of mobility of histidine in aqueous medium may then account for its prooxidative action,

TABLE II

Effect of Humidification on the Antioxidant-Prooxidant Activity of Histidine During Methyl Linoleate Oxidation in Filter Paper Model Systems

Humidification	Induction time (hr)	Oxygen absorbed (mmole O_2/mole)[a]	$(K_M)_t/(K_M)_c$	$(K_B)_t/(K_B)_c$
Control				
Dry[b]	15	6.5		
52% RH				
75% RH		3.5		
98% RH	14	8.9		
10^{-1} M Histidine				
Dry[b]	15.5	8.0	1.0	1.2
52% RH	15.0		1.0	0.9
75% RH		21.6		
98% RH	4.0	36.8	4.5	3.3

[a] At 24 hr based on UV diene conjugation.
[b] <0.1%.

TABLE III

Effect of Humidification on the Catalytic Activity of Metal Salts During Linoleate Oxidation in the Filter Paper Model System

Humidification	Extent of Oxidation (mmoles O_2/mole)[a]			$(K_M)t/(K_M)c$	$(K_B)t/(K_B)c$
	12 hr	39 hr	47 hr		
Control					
Dry[b]	5.1	8.2	8.4		
52% RH					
98% RH	8.1	13.9	48.4		
100 ppm $CoCl_2$					
Dry[b]	7.2	28.3	38.3	3.2	1.5
52% RH				2.2	1.3
98% RH	2.9	14.8	28.3	1.0	1.0
100 ppm $MnSO_4$					
Dry[b]	5.4	40.6	48.6	4.7	2.0
52% RH				2.0	1.5
98% RH	4.9	68.4	134.0	3.9	2.7

[a] Based on diene conjugation measurement.
[b] <0.1%.

which is higher in aqueous medium than in dry or almost dry systems.

The effects of addition of the salts of cobalt and of manganese at different humidities are compared in Table III for the filter paper system. Based on the ratios of the rate constants, the effects of salts of cobalt and of manganese are similar at lower humidities. Humidification to 52% RH tends to decrease the values of K_M and K_B from those in the dry system. However, catalysis is not significantly lower than catalysis in the dry state because of the simultaneous decrease in induction times.

Humidification to 98% RH appears to exert different effects on the two metals. Cobalt showed a lower catalytic activity than in the dry system or at 52% RH while manganese showed higher prooxidant activity than at 52% RH. Apparently, humidification to 98% RH resulted in a moisture content high enough to cause different effects on the behavior of cobalt and manganese. At this moisture content, it may be assumed that cobalt and manganese ions were hydrated. The water in excess of hydration may exert the following effects in systems containing cobalt or manganese. (a) It may aid in the dispersion of the fat in a monomolecular layer in cellulosic materials such as the filter paper used in this work, and thus accelerate the oxidation by increased surface catalysis. Similar surface effects were noted by Honn et al. (8) in the oxidation of soybean oil. (b) It may increase the internal surface area of the cellulosic filter paper, thereby increasing the total area on which oxidation of the dispersed lipid occurs (2). (c) It might result in a continuous network of tiny water channels throughout the cellulose material. This could result in bulk transport of the catalyst and a subsequently higher rate of oxidation.

If only the preceding hypotheses are accepted, identical results in cobalt- and manganese-catalyzed systems humidified to 98% RH would be expected. However, this is not the case. The difference in the behavior of cobalt and manganese may be based on the difference in the magnetic moments of cobalt and manganese complexes (Tjhio and Karel, unpublished data). Its relatively stronger magnetic properties make it very likely that manganese, although surrounded by water molecules, still has the ability to attract the polar hydroperoxides present in aqueous medium, hold them long enough to allow electron transfer to occur, and then initiate the free radical chain reaction.

Relative humidities of 52% and 98% were used in studies of the effect of humidity on the catalytic effect of histidine in the presence of salts of either

TABLE IV

Effects of Humidification on the Activity of Histidine-Metal Complexes During the Oxidation of Methyl Linoleate in Filter Paper Model Systems[a]

Humidification	Induction time (hr)	$(K_M)t/(K_M)c$	$(K_B)t/(K_B)c$
10^{-1} M Histidine + 100 ppm $CoCl_2$			
Dry[b]	14	1.0	1.0
52% RH	17	0.7	0.9
98% RH	20	0.9	0.9
10^{-1} M Histidine + 100 ppm $MnSO_4$			
Dry[b]	< 1	4.4	2.0
52% RH	< 1	4.8	3.4
98% RH	< 1	22.0	6.7

[a] Average of five runs.
[b] <0.1%.

cobalt or manganese. The ratios of the parameters of filter paper model systems containing cobalt and histidine and manganese and histidine are summarized in Table IV.

As reported previously (Tjhio and Karel, unpublished data), histidine in the dry state completely eliminates the prooxidant activity of cobalt chloride but actually enhances the catalytic activity of manganese sulfate. Humidification of the model system to 52% further accentuated the differences between the behavior of manganese and that of cobalt; at 98% RH these differences are striking. At 98% RH the combination of manganese and histidine has a particularly powerful prooxidant effect, whereas the combination of cobalt and histidine has a slight antioxidant activity.

Our findings with respect to the effect of humidity on the catalytic activity of manganese combined with histidine were similar to observations made by Karel (2). He found that the rate of oxidation of linoleic acid (which was dispersed on filter paper), with or without copper added, was increased by the addition of water to the system. However, Maloney et al. (1) and Labuza et al. (6), studying the oxidation of methyl linoleate dispersed on microcrystalline cellulose, found that water decreased the oxidation rate of methyl linoleate in the cobalt-catalyzed, as well as in the uncatalyzed, system. It is possible that the site of water adsorption is not identical on Avicel and on filter paper. Filter paper probably has a more continuous structure than microcrystalline cellulose. In filter paper the water absorbed forms very small channels through which the catalyst can pass freely and move to the oil-water interface, hence having a greater facility for interaction with the substrate.

However, at low concentrations the presence of water modifies the prooxidant effect of the cobalt-histidine chelate in both systems. This is similar in the case of EDTA, which is the common chelating

TABLE V

Effects of Humidification on the Activity of EDTA and Cobalt Nitrate During the Oxidation of Methyl Linoleate in Microcrystalline Cellulose Model Systems

Humidification	Induction time (hr)	$(K_M)t/(K_M)c$	$(K_B)t/(K_B)c$
Control			
Dry[a]	25		
45% RH	55	0.77	0.60
10 ppm Cobalt			
Dry[a]	16	2.64	1.32
32% RH	28	0.90	0.60
45% RH	50	0.75	0.57
10 ppm Cobalt + EDTA[b]			
Dry[a]	27	1.30	1.02
32% RH	40	0.85	0.80
45% RH	101	0.46	0.56

[a] <0.1%.
[b] At 10 moles per mole cobalt.

agent added to most oxygen-susceptible systems. Table V contains the kinetic constants calculated from manometric oxygen absorption for microcrystalline cellulose systems. In the dry state EDTA reduces the catalytic activity of the added cobalt to almost that of a system with no catalyst. In addition, however, an additional antioxidant effect is observed for this complex at increased humidities. This may be due to induction of hydroperoxide breakdown of the complex into nonradical forms.

Karel and Labuza (9) used freeze-dried food systems in their studies on the rate of oxidation of a mixture of methyl linoleate and methyl oleate in the presence of proteins. They found that histidine in combination with cobalt was effective in reducing the rate of oxidation at 31% RH (at the monolayer value of water). At 40% RH (above the monolayer value) cobalt plus histidine also had some antioxidant activity. Our observations of the antioxidant activity of cobalt combined with histidine in the filter paper system agree with these findings.

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