Effect of Water-binding Agents on the Catalyzed Oxidation of Methyl Linoleate

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Abstract

Autoxidation of methyl linoleate was studied in model systems containing added metals and different water-binding agents including cellulose, dextran and glycerol. In all systems, addition of water was increasingly antioxidant up to a critical level of water activity, above which further additions promote oxidation. The specific level of water activity at which oxidation rates are minimal and the water content at this critical activity depend on the composition of the system. Results of the present study and much of the conflicting data in the literature can be explained as follows. A small amount of water is tightly bound to polysaccharides and does not affect lipid oxidation. Additional amounts of water are antioxidant because of their ability to hydrate metallic catalysts and to form hydrogen bonds with hydroperoxides. At high water contents the water's solvent action mobilizes catalysts, thereby overcoming the antioxidant effects. The present study indicates that water bound to glycerol is still capable of mobilizing catalysts. Depression of water activity by addition of agents such as glycerol has different effects on oxidation than does depression of water activity by other means. Interaction between the effects of system composition and water activity must be considered when either is varied to maximize storage stability of foods.

Introduction

The literature contains many apparently conflicting reports concerning the effects of water content on the rate of lipid oxidation in foods. Some reports show that addition of water is antioxidant, while others indicate the opposite effect. A critical review of this literature suggests that water usually appears to be antioxidant in systems having water activities characteristic of "dry" foods. In contrast, studies suggesting a pro-oxidant effect usually concern systems having high water activities, typical of fresh foods. However, the effect of water on lipid oxidation over the entire range of possible water activities in foods has not been systematically studied.

Some examples of studies suggesting an antioxidant effect of added water are those performed on cereals, baked goods and dehydrated foods (1–6). In contrast, typical studies indicating that water can have pro-oxidant effects are those reported by Lips (7), Loncin et al. (8), Phillips and Williams (9), and Rockland (10).

Mechanisms suggested for the antioxidant effect of water include the protection of labile groups by water, hydration of metal catalysts, acceleration of production of antioxidant compounds, and a decrease in oxygen diffusivity. Pro-oxidant effects of water have been attributed to increased mobilization of reactants by solvation.

Members of our laboratory have investigated extensively the effects of increasing water activity (a_w) from the dry state up to a level of around 0.5 in methyl linoleate-cellulose model systems (11–15). Water activity is defined as the ratio of the partial pressure exerted by the water in the food to the vapor pressure of pure water at the same temperature. A recent review of this subject was published by Labuza, 1968 (16).

These studies showed that water added in limited quantities was antioxidant. The effect was attributed primarily to two mechanisms: (a) the reduction in activity of trace metals by water, and (b) hydrogen bonding of hydroperoxides at the oil-water interface.

The objective of the present study was to evaluate, in previously developed model systems, the effects of addition of water-binding agents on lipid peroxidation. The results of oxidation studies in model systems were also to be compared with those in one food item.

Experimental Procedures

Model System

Methyl Linoleate. The methyl linoleate used in this study was procured from The Hormel Institute, University of Minnesota, Austin, Minnesota. Hormel estimated the product to be 99% pure by gas liquid and thin layer chromatographic analysis. Upon receipt, each lot was stored at −40 C. Prior to use, each lot was redistilled at less than 50 μ Hg. The redistilled samples were stored in ground-glass-stoppered receiving flasks under nitrogen at −40 C until used.

Glycerol. Glycerol was selected as a model component because it gave the system the water adsorption isotherm characteristic of intermediate-moisture foods. Glycerol of spectrographic quality was used without further purification; it was analyzed for water content as described below. The weight of glycerol and water added to the model system during preparation was adjusted for the water content of the glycerol when the system components were mixed.

Dextran. Dextran-10 (Pharmacia, Uppsala, Sweden) was selected to replace glycerol as water-binding agent in the second model system. Dextran-10 is a polysaccharide having an average molecular weight of 10,000.

Cellulose. Various solid supports have been used by workers studying the oxidation of lipids dispersed on them. Labuza et al. (13) and Maloney et al. (15), after reviewing numerous solid supports used by others, selected microcrystalline cellulose (Avicel FMC Corporation); this material was also used in the present study.

Water. The water used in preparing the model systems was distilled and further purified by passage through a mixed-bed ion-exchanged column; afterward it was redistilled in an all-glass still. The deionized, redistilled water was held in polyethylene containers until used.

Food System: Selection of Ingredients

Pork. Pork was selected for comparison with the

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methyl linoleate-cellulose models (Gerber brand strained pork and pork broth).

Model System and Food Composition: Analysis

Moisture analysis was conducted by extraction of samples with anhydrous methanol and gas chromatographic analysis of the methanol using a Perkin Elmer Model 154 vapor fractometer and a Poropak Q column. Details of the method are described elsewhere (17).

Glycerol Analysis by Gas Chromatography. An F & M 240 programmed-temperature gas chromatograph fitted with an F & M Model 1609 flame ionization detector was used for analysis of the glycerol content of the model systems.

Samples for analysis were prepared by extracting approximately 1 g of dry sample with 20 ml of methanol. Extraction was carried out by shaking at room temperature, at speed setting 7, on a New Brunswick Model G-33 shaker for 30 min.

A 5 μl portion of the extract was withdrawn using a Hamilton 701-NCH microliter syringe. This portion was injected into the flame ionization detector, at an injection port temperature of 345 C, detector temperature of 280 C, and column temperature of 227 C. The column used was 36 × .25 in. copper tubing packed with 80/100 mesh Poropak Q. Carrier gas was nitrogen flowing at 60 ml/min; detector hydrogen flowed at 50 ml/min, and detector air flowed at 375 ml/min.

To compute the glycerol content of the sample, peak areas of duplicate portions were compared to peak areas of a standard solution of glycerol in methyl alcohol injected under the same conditions as the samples.

Methyl Linoleate Analysis by Gas Chromatography. The same equipment and sample preparation were employed for methyl linoleate analysis as for the glycerol analysis by gas chromatography, with the following variations. The column used was stainless steel, 36 × .25 in., packed with 5% FFAP on Chromosorb W. Injection port temperature was 340 C, detector temperature, 260 C, and column temperature, 230 C. Carrier gas was nitrogen flowing at 70 ml/min; detector hydrogen flowed at 50 ml/min and detector air at 375 ml/min. The quantity of methyl linoleate was determined by comparison of peak areas of samples with those of a standard prepared by addition of weighed methyl linoleate (approximately 1 g) to methanol brought to 50 ml in a volumetric flask.

Model System and Food: Preparation

Formula Selection. A series of methyl linoleate-cellulose model systems containing different percentages of glycerol was employed to determine the effect of glycerol on the water adsorption isotherm of the system. The composition of the series was based on a constant gravimetric ratio of methyl linoleate to cellulose (1:6), with glycerol added to give 0%, 10%, 20%, 30% and 40% glycerol contents on the basis of the total weight of all ingredients including glycerol but excluding water. The water content of the system after equilibration at each desired relative humidity was expressed as weight per total dry weight (i.e., all components other than water).

Because the sorption isotherm of the 30% glycerol formulation most resembled that of intermediate-moisture foods, it was chosen for the oxidation study. For comparison with the glycerol-containing system, a dextran model was used that contained the gravimetric ratio of methyl linoleate-dextran-cellulose, 1:3:6. For the food system, 27.5% glycerol was added to pork obtained directly from the commercial package.

In all of the model systems (other than the food) 100 ppm of cobalt as cobalt nitrate were added so that autoxidation could be studied in the metal-catalyzed state and so that variations in trace quantities of metals in the system components would be negligible in relation to the quantity of cobalt. The amount of cobalt was calculated on the basis of weight of cobalt ion to weight of methyl linoleate in the system.

Mixing, Freeze-Drying and Humidification. The weighed microcrystalline cellulose was added to a 400 ml Sorvall omnimixer cup modified at the bottom with a .25 in. Swagelock fitting. The methyl linoleate was added to the cellulose and stirred to make a paste. Water solutions of cobalt nitrate and water-binding agent were added and mechanically blended for 30 min while the cup was submerged in melting ice. After mixing, a 4 in. stainless steel tube was attached to the Swagelock fitting on the bottom of the cup and about 4 g of material were extruded into a specially designed Warburg flask without a center well or side arm. The samples were immediately frozen in liquid nitrogen and freeze-dried for 48 hr at room temperature and a pressure of less than 100 μ. After drying, the samples were held for 12 hr in evacuated desiccators over saturated salt solutions for equilibration to the desired relative humidity.

Oxidation Studies. After equilibration with the desired relative humidity, the samples were placed on Warburg manometers. Oxygen absorption was followed by the standard Warburg technique at 37 C.

Results

Water sorption by the different model systems is shown in Table I. As expected, the addition of water-binding agents such as glycerol and dextran increased the amount of water held by the systems at high humidities. At low humidities, however, glycerol actually decreased the amount of water held. This phenomenon, which we have observed consistently at humidities corresponding to less than monolayer coverage (18), we attribute to the fact that glycerol displaces some water from sites on cellulose.

Theoretical isotherms were also calculated for the glycerol-containing system by summing the water contents that would be sorbed by each component at the given water activity. The theoretical isotherms always fell above the observed isotherms, giving fur-

TABLE I

Moisture Contents in Model Systems and in Pork Containing Added Water-binding Agents, After Equilibration Over Salt Solutions Maintaining Specified Relative Humidities at 37 C
Moisture content, g water/100 g dry matter

RH%	Model system[a]			Pork	
	No additional water-binding agents	Glycerol 30%	Dextran 30%	No glycerol	Glycerol 27.5%
[b]	0.90	0.86	.....	.....	.....
11	1.8	1.5	2.55	2.4	1.7
20	2.7	3.0	3.6	3.6	3.8
32	3.4	4.7	4.95	5.05	6.1
43	3.8	7.0	6.2	6.6	9.3
51	4.7	8.4	6.7	8.0	11.8
61	5.8	13.7	8.6	12.0	18.3
75	7.6	22.8	11.1	19.3	31.7
80	8.4	26.8	.....	.....	.....
84	8.9	37.8	12.5		
91	10.1	62.2	.....		
96	....	.....	22.9	78.1	117.1

[a] Model system: methyl linoleate plus microcrystalline cellulose (1:6 w/w).
[b] Equilibrated over silica gel at a relative humidity greater than zero but less than 11%.

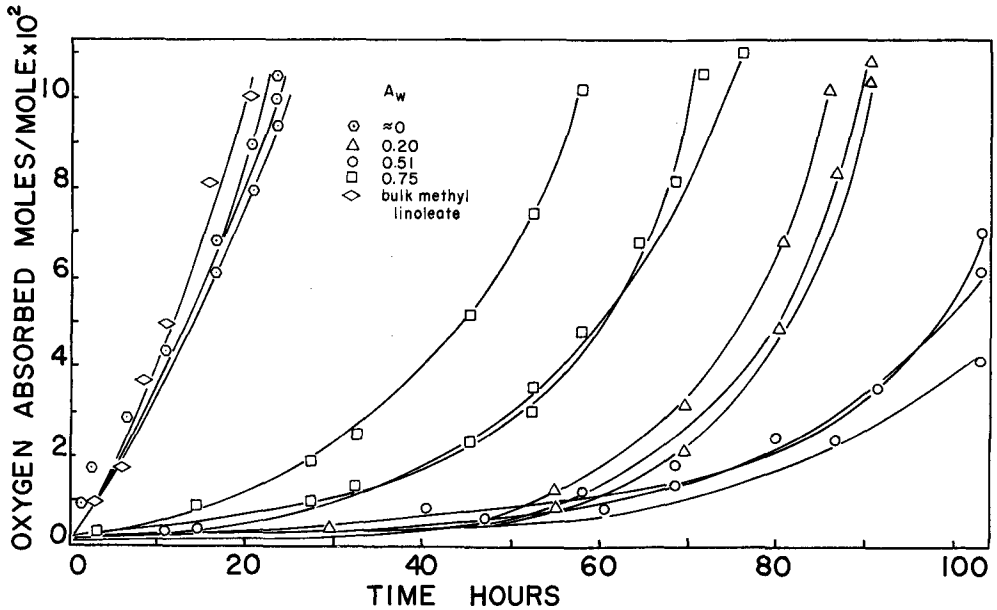

FIG. 1. Oxygen uptake at 37 C by methyl linoleate-cellulose system containing 100 ppm cobalt as $Co(NO_3)_2$ (Run 10).

ther indication of the interaction of components within the system.

Runs in which we studied oxidation in the absence of added water-binding agents showed that the rate of oxidation decreased as water content and water activity increased, up to a water activity level of 0.51. A typical result is shown in Figure 1. This finding is identical to those of Labuza (19), Maloney (20), and Yeh (21). Further increase in water activity above the 0.51 level to 0.75 resulted in an increased rate of oxygen uptake. Yeh (21) also reported increased oxidation when 100 ppm of cobalt were present in his system without glycerol. In the batches containing glycerol (Fig. 2), the rate of oxidation increased as water activity was increased, with the exception that in all cases the fastest oxygen uptake was at conditions of water activity near zero.

The effect on rate of oxygen uptake at high water activity (0.96) is also seen in Figures 3 and 4. Oxidation at a water activity of 0.96 was significantly faster, with or without dextran, compared to all other water activities studied.

Oxidation of pork alone is shown in Figure 5, and pork with glycerol in Figure 6. These results are typical of runs designed so that oxidation of the pork obtained from the commercial package could be compared to the same product containing 27.5% of glycerol on a dry weight basis. These Figures show that addition of glycerol to the pork resulted in a decreased rate of oxidation at near zero water activity but increased rates at 0.20 and 0.52. Glycerol addition had no significant effect on oxidation at an a_w of 0.75.

Oxygen uptake by methanol-chloroform (3:1)-extracted pork was not significant at water activities of 0, 0.20, or 0.51 at 37 C over the time interval (400 hr) in which the nonextracted pork system was observed. At an a_w of 0.75, the extracted pork consumed about 40 μl of oxygen per gram of dry food during this time interval. This uptake represents about 10% of the total oxygen uptake by the slowest oxygen-consuming pork system.

Peroxide values were determined periodically by Lea's method (22), and browning (absorbance measured at 240 mμ) was also followed during the course

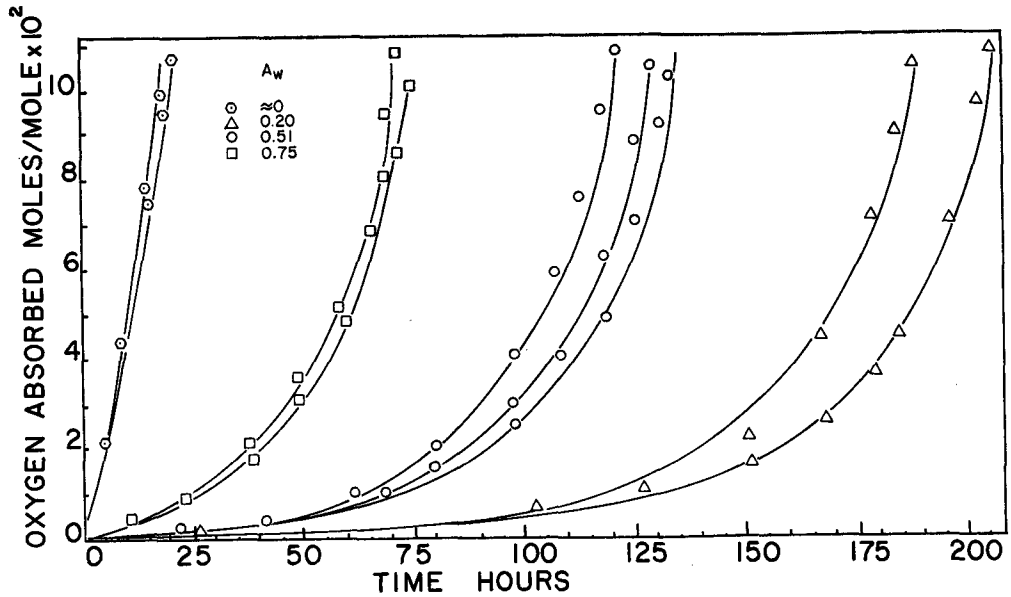

FIG. 2. Oxygen uptake at 37 C by methyl linoleate-glycerol-cellulose system containing 100 ppm cobalt as $Co(NO_3)_2$ (Run 10).

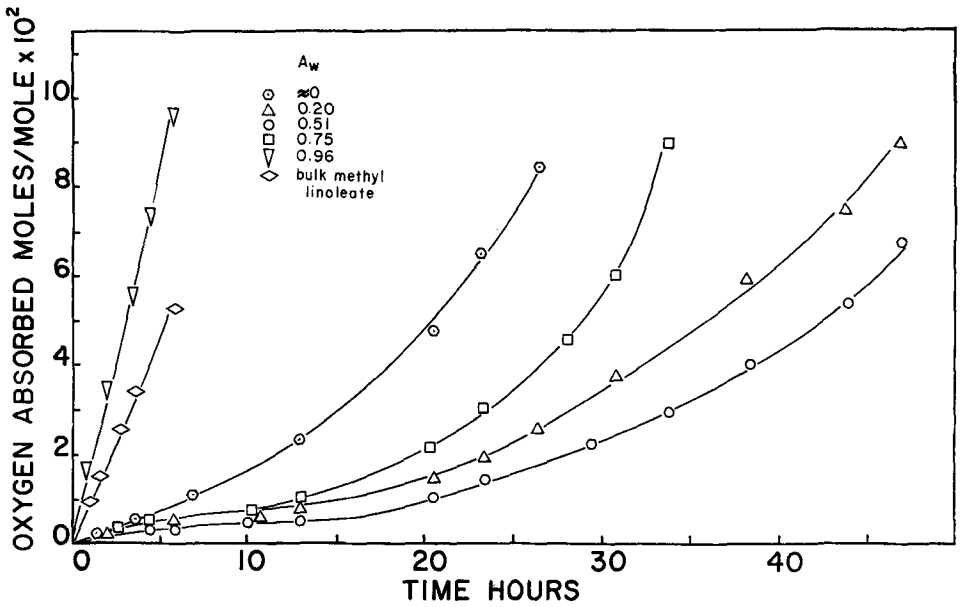

FIG. 3. Oxygen uptake at 37 C by methyl linoleate-cellulose system with 100 ppm cobalt as $Co(NO_3)_2$ (Run 19).

of oxidation of the pork foods. No significant browning was observed in this system during 14 days at 37 C, which was a longer period than for the oxidation study. This lack of development of browning is probably due to the relatively short period of the study. Likewise, no significant peroxide value was recorded for the pork during the time that oxidation was measured; the lack is attributed to the breakdown of hydroperoxides as rapidly as they formed. The head space over pork samples was monitored for carbon dioxide content at 37 C for 14 days; no significant change was noted. The method used for CO_2 analysis has been reported previously (23).

Discussion

Effects of Water Activity on Determining the Predominant Effect of Water

Neither changes in water activity nor in water content, per se, adequately predict changes in rates of oxidation. An explanation can be formulated in terms of the predominant effect of water at any given water activity.

Water affects the rate of lipid oxidation in at least the following three important ways: (a) An antioxidant effect due to bonding of hydroperoxides, which decreases their reactivity. (b) An antioxidant effect due to hydration of metal catalysts, which reduces their catalytic action. (c) A pro-oxidant effect due to an increase in the mobility of reactants.

As water activity is increased, the two antioxidant effects are generally seen before the pro-oxidant effect becomes significant. With continued increase in water activity, the pro-oxidant effect tends to predominate. Thus there exists a critical water activity below which continued increase of water activity is increasingly antioxidant, but above which continued increase of water activity is increasingly pro-oxidant. It follows that knowledge of a system's critical water activity is essential for determining the predominant effect of water on rates of oxidation.

For the methyl linoleate-cellulose system studied here, this critical water activity is near a water activity of 0.51. For the methyl linoleate-glycerol-cellulose system, the critical activity is between zero and 0.11.

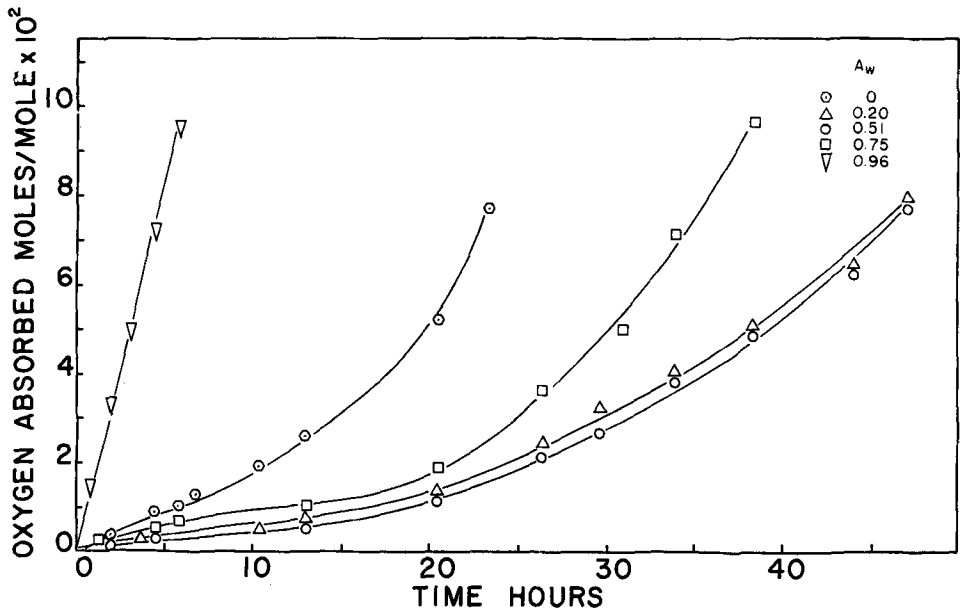

FIG. 4. Oxygen uptake at 37 C by methyl linoleate-dextran-cellulose system with 100 ppm cobalt as $Co(NO_3)_2$ (Run 19).

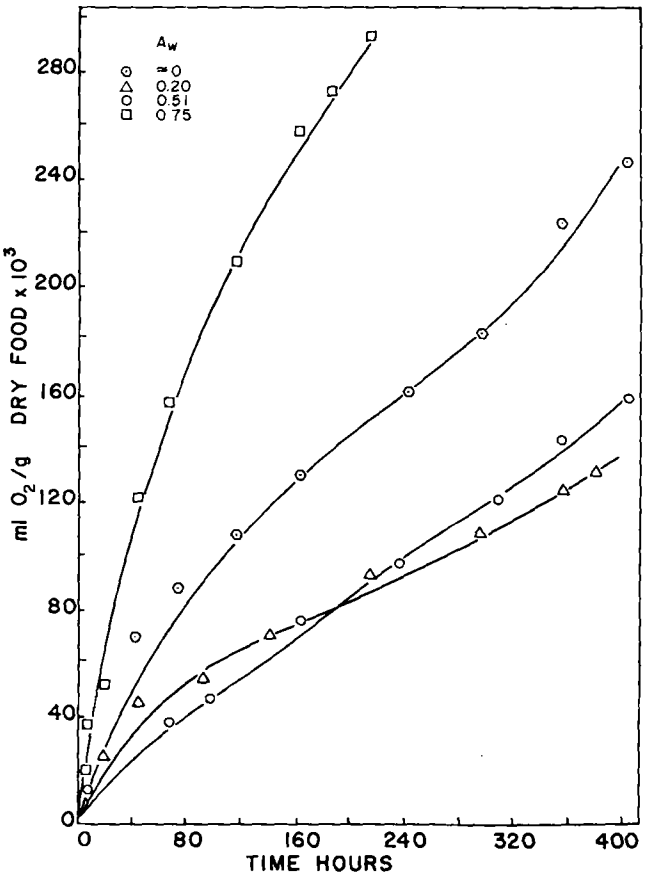

FIG. 5. Oxygen uptake at 37 C by pork.

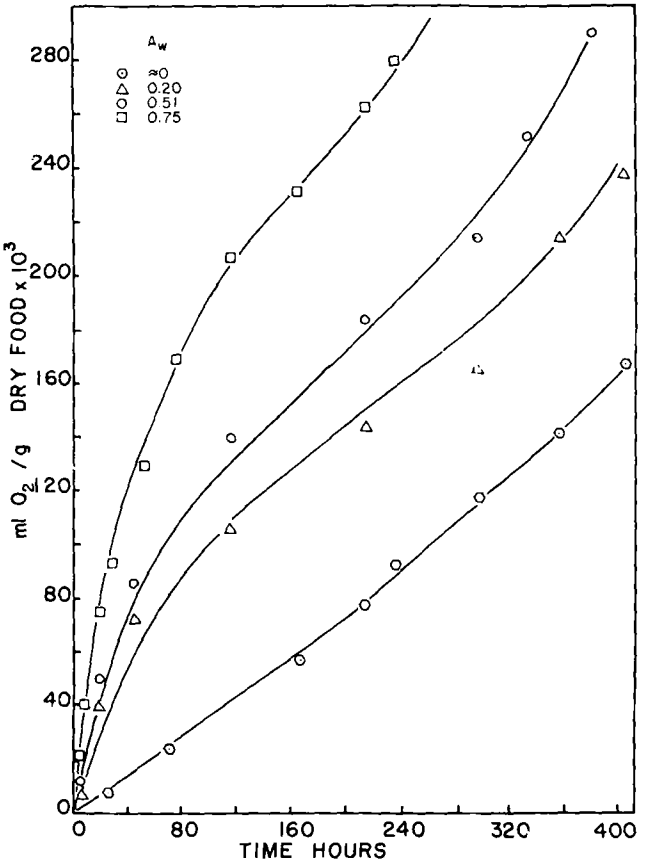

FIG. 6. Oxygen uptake at 37 C by pork containing glycerol.

Effect of System Composition on Determining the Predominant Effect of Water

We have considered above the case of increasing water activity by addition of water in a system with a constant chemical composition of either methyl linoleate-cellulose (1:6) or methyl linoleate-glycerol-cellulose (1:3:6 w/w). Let us now consider the case in which there are changes in system composition such that the change in components results in a change of the system's critical water activity. We may consider three types of component changes.

Addition of Pro-oxidants in Concentrations That Do Not Themselves Affect Water-binding Properties. At high water activities such additives would tend to shift the effect of increased water content to favor the pro-oxidant effect attributable to increased mobility of reactants. Other factors being equal, the critical a_w would be lowered by such addition of a pro-oxidant. The effect was seen by Yeh (21), who added cobalt, by Karel (24) using unpurified linoleic acid, and by Heidelbaugh (18) when large amounts of buffer salts were added to the model system described here.

Addition of Water-binding Agents That Have Little Effect on Other Physicochemical Relationships. The expected results of such addition would be that more water would be subtracted from the total as bound water, giving essentially the same net unbound water at essentially the same critical water activity with and without the additive. That is, at the critical water activity we would find more water per unit of oxidizable substrate. In the present study the critical activity with dextran present occurred with about 8.7 moles of water per mole of methyl linoleate (based on an estimated critical a_w of 0.35). Without dextran the ratio was 5.4 moles/mole (based on an estimated critical a_w of 0.51).

The downward shift of critical a_w by the dextran was probably due to pro-oxidants added with the dextran or to the solubility of dextran, allowing the water bound to it to engage to some degree in the oxidative mechanisms.

Addition of Water-binding Agents That Displace Water From Other System Components. This case is exemplified by the addition of glycerol, which had the net effect of shifting the critical activity downward. There were less than 1.7 moles of water per mole of methyl linoleate at the critical a_w in the system containing glycerol, compared to 5.4 moles/mole at the critical a_w for the system without glycerol.

Addition of glycerol to the pork had qualitatively the same effect on water content and location as it did in the cellulose. The effect on oxidation of the addition of glycerol to pork was likewise qualitatively similar to its effect on oxidation in the cellulose system. In both cases, addition of glycerol resulted in a lowered critical water activity. Quantitatively, the effect in pork was a decrease in critical activity from near 0.40 to near zero.

Effects of Glycerol on the Predominant Effect of Water

The participation of glycerol-bound water in physicochemical phenomena is well known, for example, in plasticizing polymers. This study demonstrates that glycerol displaces water from other system components. The displaced water becomes bound to the glycerol. The glycerol-bound water is able to participate in oxidation reactions so that the pro-oxidant effects of water predominate at all water activities excepting the very lowest (above zero but below 0.11). Such an effect on oxidation can be explained if we assume that water bound to glycerol is reduced in its antioxidant effect but continues to act

as a pro-oxidant by retaining its ability to mobilize reactants.

In pork, glycerol had essentially the same effect on oxidation as in the cellulose-based model system. This indicates that the same mechanisms operate in both systems.

Conflicts in the literature concerning interpretation of results of oxidation experiments using various systems with different water contents may be attributed, to a large degree, to the different effects of water at various water activities and to factors that shift the predominating effect.

The depression of water activity by addition of water-binding agents such as glycerol has different effects on oxidation than does depression by other means. Interactions of system components must be thoroughly understood on a theoretical ground or from empirical determinations if water activity is to be varied in a food to maximize storage stability.

ACKNOWLEDGMENTS

Work supported in part by U.S. Public Health Service Research Grant FD-00050 (formerly UI-00125), National Aeronautics and Space Administration Contract NAS 9-9426, and the Air Force Institute of Technology, United States Air Force. Contribution No. 1663 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

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[Received July 15, 1970]