Effects of Model System Composition on Autoxidation of Methyl Linoleate

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Autoxidation of methyl linoleate was studied in model systems containing added cobalt nitrate as well as water-binding agents including cellulose, dextran, and glycerol. In all systems, addition of water is antioxidant up to a critical water activity, above which further increases in water content promote oxidation. It was found that the critical water content and the critical water activity, corresponding to the inversion of the effect of water, depended on the composition of the model system. Addition of cobalt and of glycerol decreased the numerical value of the critical water content and of water activity. Dextran addition resulted in an increase in the critical water content. The results were explained as follows: a small amount of water is bound to polysaccharides and does not affect oxidation. Additional amounts are antioxidant due to hydration of catalysts and of hydroperoxides. At high water contents, however, these antioxidant effects are overshadowed by the ability of water to solubilize and thus mobilize metallic pro-oxidants.

Relatively small increases in the water content of dehydrated foods often retard oxidative deterioration. When foods or model systems contain significant amounts of metal catalysts, small amounts of water can be antioxidant by hydrating the catalysts and thus reducing their activity. The existence of such hydration complexes has been demonstrated by Dean and Skirrow (1958); their formation prolongs the induction period and decreases the rate of monomolecular decomposition of hydroperoxides. Addition of water may also retard autoxidation by hydrogen-bonding of hydroperoxides. This latter phenomenon is evidenced by an upward shift in the extent of oxidation at which monomolecular decomposition of hydroperoxides changes to the more rapid bimolecular decomposition. These mechanisms have been described in studies by Maloney *et al.* (1966), Labuza *et al.* (1966, 1969), and Karel *et al.* (1966, 1967). In addition, Rockland (1969) has suggested that water may retard autoxidation by direct effects on free radicals.

There are, however, other reports in the literature indicating that water may promote oxidation. These observations refer especially to situations in which the water content is high. Kamiya and Ingold (1964) showed a pro-oxidant effect of 10% water added to a metal-catalyzed model. Likewise, when 12% water was added to lard, a pro-oxidant effect was noted (Lips, 1949). Phillips and Williams (1952) studied rendered chicken fat and showed that the addition of 67% water was pro-oxidant. Rockland (1957, 1969) has shown that walnuts and beans tend to oxidize faster at very low and very high moisture contents compared to moisture levels between these extremes.

Karel (1960) has suggested that increases in water content to high levels may increase the mobility of various pro-oxidants that are present in the system but relatively inaccessible to reactants until the water content reaches a high level. The increased mobility could occur either directly by solubilization of catalysts or indirectly by plasticization of polymeric crystallites in the system.

Little systematic work has been reported on the interactions between water content and system composition for lipid oxidation over the entire range of water activities. Water activity is defined as the ratio of partial pressure of water in food to the vapor pressure of pure water at the given temperature. It is generally accepted that water activity is related to availability of water for various deteriorative reactions in foods. Comprehensive reviews of the role of water activity in food stability have been published recently (Labuza, 1968; Labuza *et al.*, 1970). Such information is needed if optimum water contents are to be selected to retard lipid oxidation in foods. This is especially true for intermediate-moisture foods, which may have a water content in the range in which water changes from a predominantly antioxidant component to a pro-oxidant. The studies reported here were performed to determine the effect of system composition on oxidation at various water activities, especially those associated with intermediate-moisture foods.

MATERIALS AND PROCEDURES

Purified model systems were designed to represent features of the water and lipid phases found in foods. The basic system contained two components: redistilled methyl linoleate that had less than 1% diene conjugation as measured by ultraviolet absorbance at 233 mμ (Privett and Blank, 1962), and microcrystalline cellulose (Avicel PH-101, FMC Corp.). The ratio of these ingredients was 1 to 6 by weight. Effects of other components on oxidation were determined by adding them to the basic system. The added components were: glycerol (three parts, based on the total weight of the system); Dextran-10, three parts (a polysaccharide of 10,000 mean molecular weight, Pharmacia, Uppsala, Sweden); cobalt as $Co(NO_3)_2$ at 100 ppm of cobalt in methyl linoleate (equivalent to 5μmoles of cobalt per mole of methyl linoleate), and citric acid buffer containing 0.27 mole of citric acid and 1.25 moles of Na_2HPO_4 per mole of methyl linoleate (pH 6.4).

The ingredients were premixed by manual stirring in a 400-ml stainless steel Sorvall Omnimixer cup modified with a 1/4 in. Swagelock extrusion port in the bottom and were then blended at approximately 10,000 rpm for 20 min while the cup was held in melting ice. The extrusion port was then opened, and samples weighing about 4 g were extruded directly into glass reaction vessels. These samples were immediately frozen in liquid nitrogen and freeze-dried for 48 hr at room temperature at less than 100 μ total pressure. After drying, the samples were humidified to the desired water activity over saturated salt solutions in an evacuated desiccator.

Oxygen absorption was followed in triplicate samples at each water activity at 37° C with standard Warburg manometric techniques. The initial runs with no additives were

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performed at 40° C as part of another study. Peroxide values were measured on lipid extracted from the samples, using methods suggested by Lea (1952). The peroxide values were determined on samples prepared and incubated under conditions identical to those for samples used to measure oxygen absorption.

Water analysis was performed on representative samples by extraction with 20 ml of dry methanol during 30 min of shaking. Five μl of the extract were injected into a Perkin Elmer Model 154 vapor fractometer fitted with a 72 × 1/4 in. copper column packed with Porapak Q. Helium carrier gas flowed at the rate of 50 ml per minute. The temperature of the column, port, and thermal conductivity detector was 130° C. Detector response was recorded on a strip chart and compared to the area of a standard solution containing 1 g of water per 20 g of methanol.

RESULTS

Figure 1 shows oxygen absorption by the methyl linoleate-cellulose system with no additives. Addition of water to this system is seen to be increasingly antioxidant when water activity is increased from near zero to the highest activity studied, which was 0.75. Figure 2 shows that when 100 ppm of cobalt are added to the system, the addition of water is increasingly antioxidant up to a water activity of 0.51, but above this level oxidation rates increase again. When the cobalt catalyst is present, there is a water activity at which the effect of added water on the rate of oxidation is reversed. This water activity is referred to as the system's critical water activity.

Figure 3 shows the same system but containing, in addition to the cobalt, 30% dextran by weight. The patterns of relative rates of oxidation as a function of water activity are essentially the same in Figures 2 and 3. The main effect of dextran is an increase in the water content at a given water activity. The magnitude of this increase is seen in Table I. The critical water activity also shifts as a result of dextran addition from the 0.51 level to about midway between the 0.51 and 0.20 levels.

When glycerol is added to the system, with cobalt present, the main effect is a shift of the critical water activity from the 0.51 level to a level well below 0.20 (Figure 4). Additional experiments, not reported here (Heidelbaugh, 1969), indicated

Table I. Ratio of Water to Lipid in Model Systems Studied

	Model System		
Water Activity	Methyl Linoleate (1) Cellulose (6)	Methyl Linoleate (1)[a] Dextran (3) Cellulose (6)	Methyl Linoleate (1) Glycerol (3) Cellulose (6)
0.11	0.13	0.26	0.15[b]
0.20	0.19	0.36	0.30
0.32	0.24	0.50[b]	0.47
0.43	0.27	0.62	0.70
0.51	0.33[b]	0.67	0.84
0.61	0.41	0.86	1.37
0.75	0.53	1.11	2.28
0.80	0.59	...	2.68
0.84	0.62	1.25	3.78
0.91	0.71	...	6.22
0.96	...	2.29	...

[a] Proportions on basis of weight. [b] Near the critical water activity level. Critical activity levels apply to systems containing 100 ppm of cobalt. Water-to-lipid ratios apply to systems with as well as without the added catalyst.

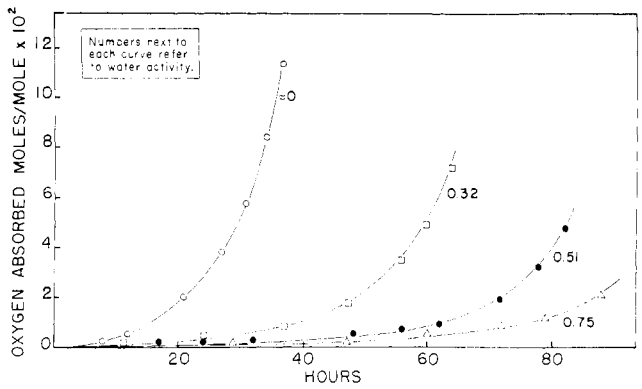

Figure 1. Oxygen absorption at 40° C by methyl linoleate-cellulose system

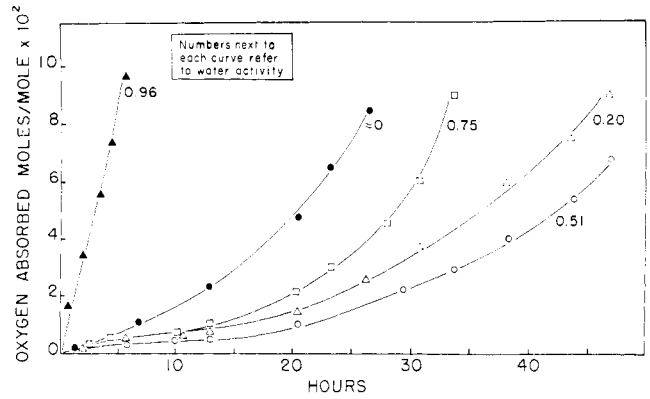

Figure 2. Oxygen absorption at 37° C by methyl linoleate-cellulose system containing 100 ppm cobalt as $Co(NO_3)_2$

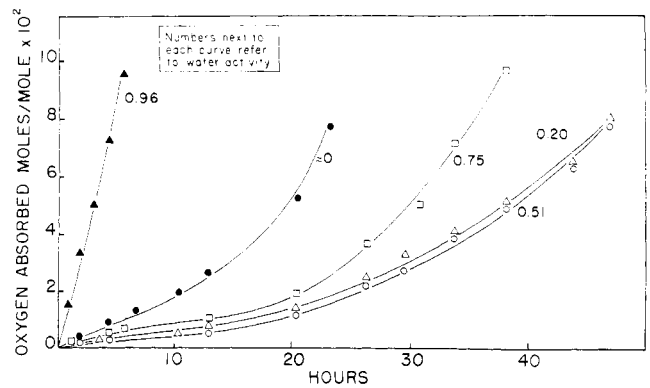

Figure 3. Oxygen absorption at 37° C by methyl linoleate-dextran-cellulose system containing 100 ppm cobalt as $Co(NO_3)_2$

that with 30% glycerol and 100 ppm cobalt in the system, the critical water activity occurs at an activity level between zero and 0.11.

When buffer is added to the system in the presence of glycerol and cobalt, the general pattern is an increasing prooxidant effect with increasing water activity. With the buffer present, however, the effects of water were poorly reproducible. This poor reproducibility is attributed to the presence of trace metal contaminants in the buffer salts and to the concentration of salts at lipid-water interfaces, which increased variability in the rates of oxidation.

In all of the runs, the peroxide values gave good agreement with the oxygen absorption data up to at least the 20% level of oxidation (calculated on the basis of moles of oxygen per mole of methyl linoleate).

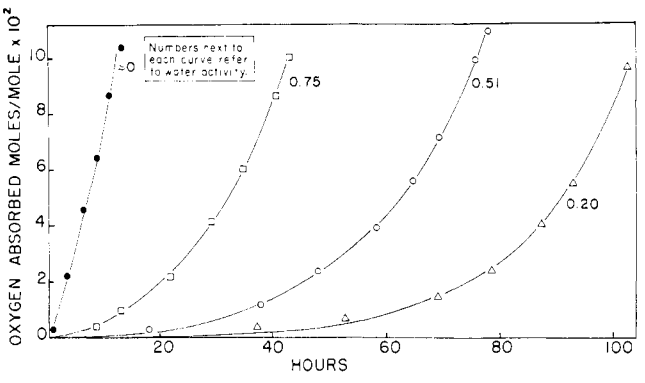

Figure 4. Oxygen absorption at 37° C by methyl linoleate-glycerol-cellulose system containing 100 ppm cobalt as $Co(NO_3)_2$

DISCUSSION

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

As water activity increases, 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. This explains the existence of a critical water activity up to which the continued increase of water activity is increasingly antioxidant, but above which any further rise in water activity is increasingly pro-oxidant. Knowledge of a system's critical water activity is therefore essential for determining the predominant effect of water on rates of oxidation.

The critical water activity and the critical water content depend strongly on the composition of a food. The following changes in composition with respect to the basic cellulose-methyl linoleate system were studied.

Addition of Dextran. This inert, polymeric component binds some of the water and thus prevents a portion of the total water from participating in the mechanisms affecting the rate of oxidation. As a consequence, the critical water content is shifted from 0.33 to 0.5 g of water per g of linoleate.

Addition of the water-soluble metallic catalyst, cobalt, shifts the critical activity of water downward. Solubilization of the cobalt by water has a highly pro-oxidant effect and counteracts the antioxidant effects when water content reaches even a moderate level.

Addition of glycerol shifts the critical water activity to a very low level. Glycerol displaces water from cellulose surfaces and therefore increases the total amount of "free" water, that is, water not bound to cellulose. Of course, some of that "free" water is strongly bound to glycerol itself. It is apparent, however, that water bound to glycerol is capable of solubilizing catalysts and therefore accelerating oxidation. This is in contrast to water bound to dextran or cellulose, which is not capable of such activity.

Solubility tests show that "dry" glycerol is also incapable of solubilizing salts of cobalt. There is, however, some direct pro-oxidant effect of the glycerol at a water activity of zero, and it is not known whether this is due to some ability of the glycerol to mobilize catalyst in the system. In any case the solubilizing of pro-oxidants appears greatly enhanced at high water activities. This behavior is reminiscent of the known ability of hydrated glycerol to plasticize polymeric films. The optimum water activity for glycerol-containing systems coincides with a water content adequate for formation of glycerol-monohydrate. It is not known, however, whether this form of glycerol is particularly effective in inactivating catalysts, or if the coincidence is fortuitous and the water activity effects fully accounted for by the factors mentioned. Experiments with a wider range of compositions are planned to explore further system interactions.

The literature contains apparently conflicting reports regarding the effects of water content on the stability of lipids in foods and model systems. Some studies report that addition of water retards oxidation and others report the opposite. A critical review of the reports, prompted by the findings of our research, showed in general that reports indicating that water retarded oxidation were dealing either with systems having low water-binding capability (*e.g.*, adding water to bulk oil) or with relatively low water activities. On the other hand, reports showing a pro-oxidative effect of increasing water dealt generally with high water activities.

Predictions of the effects of change in water activity or water content cannot be made without knowledge of a system's critical water activity, which, in turn, depends on composition. Therefore, additives can have different effects on lipid oxidation depending upon their interaction with other system components. Judging from the water activities and system composition at which these effects were exhibited in the model systems studied here, it is apparent that these considerations are very important to the design of intermediate-moisture foods.

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