

Effect of Synthetic Chelates on the Autoxidation of Unsaturated Fatty Acid Esters

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Metals of the first transition series were chelated with Schiff bases obtained from salicylaldehyde and ethylenediamine (I) and from salicylaldehyde and 1,3-propylene diamines substituted in position 2 with various fatty chains (II). Both types of chelates catalyze the autoxidation of unsaturated fatty acid esters to varying degrees according to the metal constituent. Chelates of type I are sparingly soluble in fats and necessitate studies on heterogeneous systems. Chelates of type II, which are highly fat-soluble, are about 10 times as active as type I chelates, and the activity of type II chelates differs with substituted fatty chains as well as with metal constituents. Among the chelates studied, those with oleic chains were the most active; Zn^{II} chelates were not autocatalytic.

THE PRESENT INVESTIGATION was aimed at catalyzing and utilizing autoxidation as a means of obtaining polyfunctional derivatives of long-chain fatty acids from an unsaturated oil (3). Various functionalities can be introduced in a fatty acid chain via the hydroperoxide group in a known and specific position. Some possible chemical modifications include the introduction of a second double bond to convert oleic acid into a conjugated dienoic drying oil, preparation of ω -unsaturated long-chain aldehydes, and cleavage of hydroperoxides into ω -unsaturated short-chain fatty acids.

Procedure

Because this study was concerned primarily with catalytic effects, other reaction variables were chosen arbitrarily as follows: Oxidation was with dried air at a flow rate of 50 liters per hour per 30 grams of substrate at 30° C. in glass apparatus. A series of oxidations was studied also with oxygen in a Warburg respirometer. In nearly all cases, the substrate was methyl oleate, apparently free of trans isomer, of known concentration in the range of 93 to 99% pure as determined by gas-liquid chromatography (GLC). In a few cases, the substrate was a mixture of methyl linoleate and methyl linolenate.

Choice of Catalyst

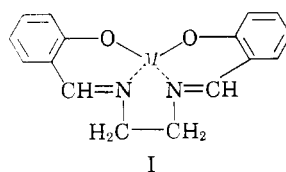
Chalk and Smith (2) demonstrated that chelation may suppress, intensify, or have no effect on the catalytic activity of metals. The present study showed that catalytic activity depends on both the chelating agent and the metal. Chelate-metal ion-hydroperoxide complexes may be formed which have different catalytic activities and which may be the primary centers of oxidation-reduction reactions.

In any case, chelation seems to modify the oxidation potential.

In heavy metal salts or soaps, the bond between carboxyl group and metal varies with environment. In nonpolar solvents, soaps are combined in micelles of aggregation, in which ionic character increases with increasing polarity of the metal-carboxyl bond and decreases with increasing chain length of the fatty acid. Micelle formation probably is responsible for the formation of active centers, in which case the primary autoxidative processes occur in micelles. That various heavy metal soaps would be expected to give similar catalytic behavior on a given substrate is illustrated in Figure 1.

Calvin Chelates

Chelates formed by metals of the first transition series with the Schiff bases obtained from salicylaldehyde and ethylenediamine (I) were selected. Such chelates were prepared first by Pfeiffer (5), then by Tsumaki (6). Later,



they were studied thoroughly by Calvin and coworkers (7, 4). As suggested by Calvin, these compounds will be indicated as SaEn chelates.

SaEn chelates appeared promising for these studies because they can be purified, they are stable, their structure has been studied, the relation between composition and oxygen transport has been investigated, and information is available on their polarographic reduction potentials.

SaEn chelates of different metals vary in bonding from ionic to covalent. For

example, the oxygen-carrying action of the cobalt chelate can be explained by a reversible mechanism in which the metal maintains its valence during oxygen transport. This situation does not change, within certain limits, whether the cobalt chelate is used in the solid state or in solution. Certain crystalline forms may occlude and release oxygen in a manner that is almost perfectly reversible. Calvin chelates of this type, however, are sparingly soluble in oil and fatty acid methyl esters and, therefore, necessitate operation in a nonhomogeneous system.

The differing catalytic activities of various metals in a series of SaEn chelates are shown in Figure 2. Fe^{II} has definite catalytic activity without an induction period in the initial stages of oxidation; Ni^{II} , Cu^{II} , and Zn^{II} have varying induction periods; and Co^{II} has no catalytic effect. The peculiar noncatalytic behavior of the $CoSaEn$ chelate might result because the required reversible oxygenation of $CoSaEn$ chelate is blocked by the formation of Co^{+3} ions that are

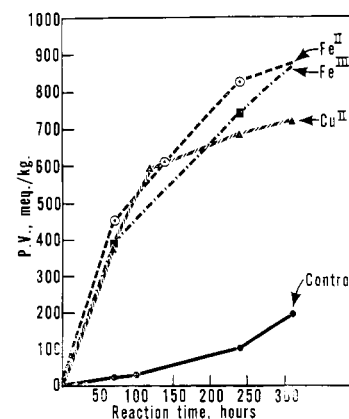


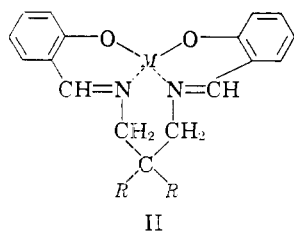
Figure 1. Autoxidation of methyl oleate catalyzed by oleic acid soaps

necessary for charge transfer. Nickel and copper approach this behavior in the initial phases of autoxidation, but subsequently show definite catalytic action. The metal-ligand bonds of the Fe^{II} chelate are ionic, and its behavior is similar to that of the metal soaps.

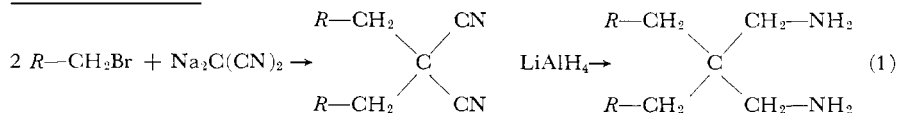
Differentiated catalytic behavior also was observed in a mixture of methyl linoleate and methyl linolenate. The autoxidation curves in Figure 3 again show the existence of three different types of behavior: Fe^{II} and Fe^{III} have no induction period; Co^{II}, Cu^{II}, and Ni^{II} have an induction period in initial phases of oxidation; and Zn^{II} has a prolonged induction period. The similarity of Co^{II}, Ni^{II}, and Cu^{II} may be more apparent than real. During the early part of the oxidation, Co^{II} produces significantly faster decreases in iodine value (I.V.) than Ni^{II} and Cu^{II}. For example, after 75 hours, I.V. data were as follows: Co^{II}, 100; Cu^{II}, 181; and Ni^{II}, 176. Thus, it appears that under the aspect of an induction period, during which all three metals cause little increase in peroxide value (P.V.), a difference in mechanisms is concealed.

"Soluble" Chelates

Because of the low solubility of Calvin chelates in the substrates, the systems studied were heterogeneous. Therefore, a series of new chelates that were more soluble in fats and yet maintained the unique catalytic behavior of the Calvin compounds was prepared. The new chelates (II) are designated "soluble" chelates. Substituent *R* was (a) C₁₂H₂₅, (b) C₁₆H₃₃, (c) C₁₉H₃₇, or (d)



(d) C₁₇H₃₃. The intermediate 1,2-dialkylpropylenediamines were synthesized through the corresponding malonic dinitriles (Equation 1). Substituent *R* was (a) C₁₁H₂₃, (b) C₁₅H₃₁, (c) C₁₇H₃₅, or



(d) C₁₇H₃₃. The different lengths of substituent *R* imparted modifications in chelate stability, and the different degrees of unsaturation permitted the formation of different chelate-peroxide complexes.

Chelates were purified by silicic acid column chromatography, and the purity of each fraction was verified by thin-layer chromatography (TLC). When a column fraction gave only one spot by

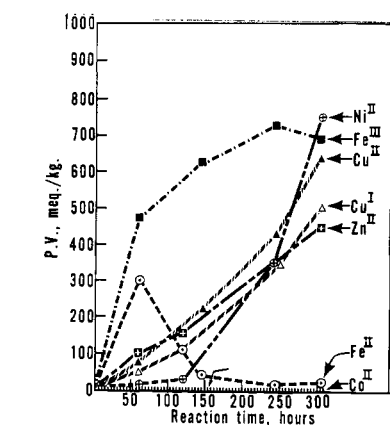


Figure 2. Autoxidation of methyl oleate catalyzed by SaEn chelates

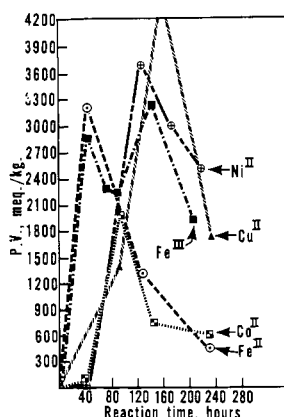


Figure 3. Autoxidation of methyl linoleate-linolenate mixture catalyzed by SaEn chelates

TLC, it was crystallized from ethyl acetate until analytically pure before use. When a column fraction was not found pure by TLC, the most abundant fraction was purified by TLC.

Soluble chelates of low stability were prepared directly on a silicic acid column by reacting the corresponding Schiff's base with metal salt that had been adsorbed previously on the column.

Like the Calvin chelates, the soluble chelates exhibit differentiated catalytic activity that varies widely with metal, as

shown in Figures 4 and 5. Activity is influenced remarkably by chain length and degree of saturation of *R* radical, as shown in Figures 6 and 7. Figures 6 and 7 show also that the soluble chelates are more active than the SaEn chelates (Figure 2).

A study of oxidation kinetics was made with a Warburg respirometer. In general, results on both SaEn (Figure 8) and

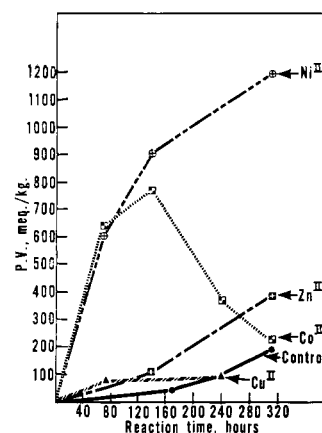


Figure 4. Autoxidation of methyl oleate catalyzed by soluble chelates with lauryl side chains

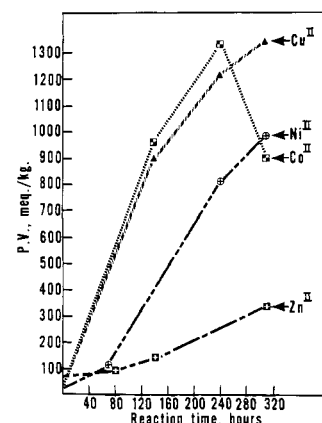


Figure 5. Autoxidation of methyl oleate catalyzed by soluble chelates with oleyl side chains

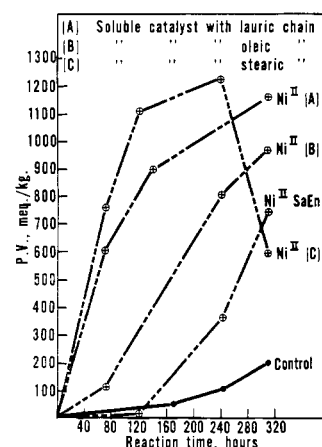


Figure 6. Influence of organic moiety on catalytic behavior of Ni^{II} in autoxidation of methyl oleate

soluble (Figure 9) chelates confirm data obtained earlier with air—i.e., the data show that widely varying activities can be obtained and that soluble chelates are more active than SaEn chelates.

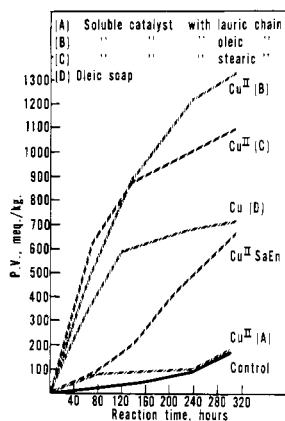


Figure 7. Influence of organic moiety on catalytic behavior of Cu^{II} in autoxidation of methyl oleate

In Table I, P.V. data on methyl oleate oxidized in the Warburg respirometer are compared with theoretical values calculated from oxygen absorbed. Soluble Cu^{I} and Cu^{II} chelates with lauryl side chains apparently have similar autoxidation mechanisms, as shown by their identical ratios of calculated-to-found P.V. values. Soluble Fe^{II} and Fe^{III} chelates also give nearly equal ratios, an indication of similar autoxidation mechanisms. In general, with soluble chelates, only a fraction of the absorbed oxygen appears as peroxide.

With SaEn catalysts, absorbed oxygen appears to be peroxidic only in oxidations of low velocity such as autoxidations without catalyst or with Cu^{II} , where the ratio between calculated and found P.V. is about 1. Although Fe^{II} and Fe^{III} yield distinctly different curves, their oxidation mechanisms appear to be similar, based on the similarity of their P.V. ratios. The lack of similarity between activated and nonactivated Co^{II} chelates (7, 4) probably is due to different chelate structures. The difference in activity becomes smaller after the induction period (Figure 8). The ratios of about

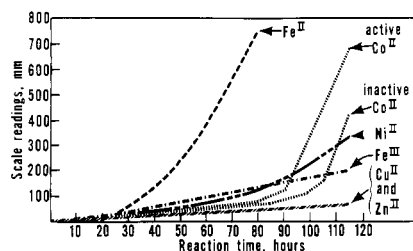


Figure 8. Oxidation of methyl oleate in Warburg respirometer catalyzed by SaEn chelates

2 for Fe^{II} , Fe^{III} , and Ni^{II} indicate that for each peroxide formed there is formation of a nonperoxidic product.

Comparison of Calvin and Soluble Chelates

A given chelated metal differs in catalytic action according to its presence as a Calvin or a soluble chelate. Among Calvin chelates, the sequence of decreasing activity appears to be as follows: Fe^{II} , activated Co^{II} , nonactivated Co^{II} , Ni^{II} , Fe^{III} , Cu^{II} , Zn^{II} , blank. In the case of soluble chelates, the sequence with saturated side chains with $R = \text{C}_{12-14,16,18}$ is Cu^{II} , Cu^{I} , Ni^{II} , Fe^{II} , Fe^{III} , Co^{II} , Zn^{II} , blank; and with $R = \text{oleyl}$ is Fe^{II} , Fe^{III} , Cu^{II} , Ni^{II} , Co^{II} , Zn^{II} , blank.

Stability Constants

A separate study is underway on the stability constants of SaEn and soluble chelates to establish a relationship between structure and activity of catalysts. Two approaches have been adopted: spectrophotometric determination of relative concentrations of metal, chelating agents, and chelates through measurement of their specific absorption in dioxane; and determination of electrical conductance.

Both methods are being used concurrently so that each can be used to

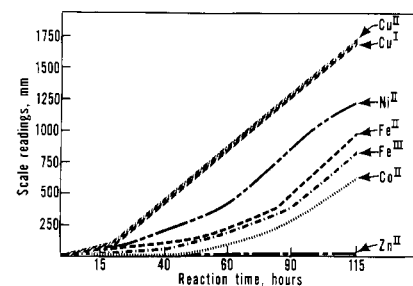


Figure 9. Oxidation of methyl oleate in Warburg respirometer catalyzed by soluble chelates with lauryl side chains

confirm the results of the other. For studies on the soluble chelates, conductance measurements were not feasible because of the low conductances encountered. Therefore, a third method has been evolved in which radioactive salts and chelates of a given labeled metal are used to determine the distribution between the two phases of the species (metal, chelating agent, and chelate) present at equilibrium. Of course, highly purified compounds are required for the determination of stability constants.

Preliminary results show a decreasing stability of chelates in the order Cu^{II} , Ni^{II} , Co^{II} , and Fe^{II} . IR analysis, which supplies information about ionic structure, gave evidence of close analogies in the structures of Cu^{II} , Ni^{II} , Fe^{II} , and Co^{II} chelates. Detailed results will be published later.

Oxidation Products

Studies on products formed during autoxidation were complicated by the low stability and concentration of the different oxidation products and their similar polarities. A first attempt to study fractions from air-oxidized methyl oleate was abandoned in favor of oxidations with the Warburg respirometer.

Because of the instability of the autoxidation products and the possibility

Table I. Oxidation of Methyl Oleate in Warburg Respirometer

Metal	Type of Chelate ^a	Reaction Time, Hours	Reacted Oxygen, Ml.	P.V. Calculated from Reacted Oxygen, Meq./Kg.	P.V. Found, Meq./Kg.	Ratio, Calcd./Found P.V.
Fe^{II}	SaEn	82	27.1	1610	750	2.15
	Soluble	125	34.3	2050	109	18.75
Fe^{III}	SaEn	116	8.15	485	225	2.16
	Soluble	125	30	1800	87.9	20.3
Cu^{II}	SaEn	116	2.26	135	108	1.25
	Soluble	125	61	3640	139.2	26.1
Cu^{I}	Soluble	125	70	4150	159	26.1
Co^{II}	SaEn active	116	30.7	1825	448	4.08
	SaEn inactive	116	15.5	922	294	3.14
	Soluble	125	17.8	1065	105	10.12
Ni^{II}	SaEn	116	14.1	842	379	2.22
	Soluble	125	50.5	3000	97.6	30.8
Zn	SaEn	116	0.67	40	78	0.51
	Soluble	125	1.14	68	16.4	4.15

^a R radical of soluble chelates is C_{14} .

of further autoxidation during fractionation, the oxidized substrates were stabilized by reduction prior to analysis. Each substrate from a Warburg autoxidation was divided into two parts. One part was reduced with Pt/C catalyst to give methyl stearate, oxygenated methyl stearate, and other oxidation products. The second part was reduced with lithium aluminum hydride to give oleyl, oxygenated oleyl, and stearyl alcohols, along with other products. Both reduced fractions were concentrated by TLC to remove unoxidized products that migrated faster, such as methyl stearate in one case and oleyl alcohol in

the other case. Further fractionation can be achieved by TLC with less polar solvents. This research is being continued.

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Received for review February 23, 1965. Re-submitted May 17, 1966. Accepted August 22, 1966. Division of Agricultural and Food Chemistry, Symposium on World-Wide Research Programs in Agricultural Chemistry, 148th Meeting, ACS, Chicago, Ill., September 1964. Supported by a Public Law 480 grant from the U. S. Department of Agriculture, sponsored by the Northern Regional Research Laboratory (NRRL), Peoria, Ill.

CUSTARDS

Viscous Behavior of Custard Systems

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For rapid cooling of menu items in quantity food service, agitation should be employed unless undesirable changes occur. Fluid properties of custards as affected by ingredients and heating were studied. Systems containing milk, egg, and starch were non-Newtonian and time-dependent. During shearing, viscosity losses increased as rate of shear increased, and were not recovered upon resting. Omitting egg or starch caused marked decreases in viscosity. When sugar was omitted, or when the system was overheated, protein-starch aggregates resulted which produced crossed hysteresis loops. For a custard of typical consistency, milk, egg, starch, and sugar have to be present; a cooking temperature of 185° F. cannot be exceeded. The role of sugar is to suppress protein flocculation during heating. The role of starch is to supply surfaces for protein-starch aggregate formation. Slow speed, short-time agitation of these systems is recommended.

BECAUSE of possible rapid multiplication of bacteria in large batches of warm food, the food should be cooled quickly to shorten the time in the hazardous zone of 140° to 45° F. (7). Previous work (6) has shown that agitating soft custards and puddings during cooling shortened cooling times considerably. However, the rate at which foods were agitated affected their apparent viscosity and, hence, their culinary acceptability.

Information on viscous behavior of food systems should be useful in suggesting speed of agitation during cooling and predicting cooling times. The present study was undertaken to define the viscous behavior of soft custards representing milk-egg-starch-sugar systems as affected by ingredients and heating.

Experimental Procedure

The formulas used, except where designated otherwise, are given in Table I.

Throughout the cooking period the heating temperatures were controlled by

the use of a rheostat and recorded by a potentiometer.

Procedure. Portion I (Table I), heat in top of double boiler. Portion II (Table I), sift dry ingredients together, add reconstituted egg and milk (when used), and mix 1 minute with electric beater. Add portion II to portion I

at 195° F. within 45 seconds, using 20 strokes with a small wire whip. At 5-minute intervals, agitate for 20 seconds during cooking, using a rubber spatula; keep the mixture covered except when stirred. Total cooking time 30 minutes; average cooking temperature 175° to 185° F. Remove a 150-ml. sample from the cooked mixture, cover, and cool at

Table I. Formulas for Systems and pH of Cooked Systems

System	Abbreviation	Portion I		Portion II				pH of Cooked Systems (Range)
		Milk, ^a g.	Sugar ^b , g.	Milk, ^a g.	Sugar ^b , g.	Starch ^c , g.	Egg ^d , g.	
Milk-egg-starch-sugar	MESSt ^e	2041	113		113	42	184	6.4-6.6
Milk-egg-sugar	MES	2004	113	37	113		184	6.5-6.6
Milk-starch-sugar	MStS	1880	113	161	113	42		6.3-6.4
Milk-sugar	MS	1843	113	198	113			6.4-6.5
Milk-egg-starch	MESSt	1956		85		42	184	6.4-6.6
Milk-egg	ME	1919		122			184	6.5-6.6
Milk-starch	MSt	1797		244		42		6.4-6.6
Milk	M	1760		281				6.4-6.5

^a Spray-dried, low-heat, nonfat milk solids, reconstituted in the proportion of 227 grams of solids to 1814 ml. of distilled water.

^b Commercial granulated sucrose.

^c Commercial cornstarch.

^d Stabilized dry egg solids, reconstituted 1:3, egg to distilled water, by weight.

^e System MESSt was a complete soft custard.