Fat Oxidation at Low Oxygen Pressure: III. Kinetic Studies on Linoleic Acid Oxidation in Emulsions in the Presence of Added Metal Salts[1]

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ABSTRACT

Earlier studies on lipid oxidation at low oxygen pressure were continued to cover the effect of low oxygen pressure in the presence of added metal salts. Addition of Cu^{2+} and Fe^{3+} salts was shown to exert a catalytic effect, which, on increasing metal concentration, may reach a maximum and then decrease. This so called conversion is favored at low oxygen pressure (e.g., corresponding to 1% O_2 at atmospheric pressure). Under certain conditions it may result in an antioxidative effect. The phenomenon is more pronounced at pH 5 than at pH 7, and it is dependent on the anions present and the ratio between the concentration of the catalyst and that of the substrate. A tentative explanation of these effects of Cu^{2+} and Fe^{3+} salts at low oxygen pressure, which are not given by Fe^{2+} and Co^{2+} salts, is offered.

INTRODUCTION

This investigation is a further link in a series of studies on lipid oxidation at low oxygen pressure (1,2). The dependence of fat oxidation on oxygen pressure is of interest with respect to the increasing importance of packaging at reduced pressure and the use of inert gas for protection of food liable to deterioration by fat oxidation. Development of rancidity is considerably influenced by metal catalysis. Our earlier studies were therefore extended to cover the presence of metal salts. The metals responsible for a catalytic effect may occur in various physico-chemical states, e.g., ions, undissociated salts and chelates. An easily handled, well defined state is the one achieved by the addition of salts at stable pH.

Heavy metals, especially those with two or more valences, are known to increase rancidification by either reducing the length of the induction period or increasing the rate of oxidation. They may, however, under certain conditions, act as stabilizers or inhibitors (3-5). Their overall effect can be retraced to individual effects upon initiation, propagation and termination, as shown and reviewed by Ingold (6,7).

The effect of metals upon fat oxidation may be particularly critical in systems consisting of a water and a lipid phase with metallic salts or complexes in the water phase and metal soaps in the lipid phase. Such systems exist in most foods. Typical of these conditions are emulsions. In spite of the great importance of emulsions for biological systems and food, few investigations have been published upon the effect of metal catalysts on lipid oxidation in such circumstances and practically nothing is known on the significance of oxygen pressure in this case.

EXPERIMENTAL PROCEDURES

Emulsions of linoleic acid, generally obtained from Fluka, Switzerland (puriss., peroxide value 2-10) were used as substrate. The results were checked by experiments with linoleic acid from Mann (peroxide value near 0). No significant difference was observed.

The metals studied were Cu^{2+}, Fe^{3+}, Fe^{2+} and Co^{2+}. Their effect was examined in a broad range of concentration, generally 10^{-4} to 10^{-2}M, except Co^{2+}, which was examined in the range 10^{-6} to 10^{-2}M. The salts were dissolved in water and added to emulsions of linoleic acid at the beginning of the experiment. The molarity was calculated for the total emulsions.

The emulsions were prepared as described previously (1) and buffered at pH 5 to pH 7, generally with phosphate, or, in some cases, with phthalate. The buffer molarity in the emulsions was 0.1 M. At high metal salt concentrations pH adjustment sometimes required addition of NaOH.

Oxidation was followed by determination of oxygen consumption in a modified type of Warburg apparatus permitting automatically recorded studies at constant pressure of oxygen (8).

The reaction vessels were flushed with mixtures of oxygen and nitrogen prepared in a mixing equipment (1). The experiments were carried out at atmospheric pressure and the partial pressure of oxygen was expressed as percentage of oxygen (v/v). The results are given in terms of oxygen uptake as a function of time.

RESULTS

Addition of Cu^{2+} ($CuSO_4$)

Addition of Cu^{2+} generally accelerates the rate of lipid oxidation (Fig. 1a). With increasing concentration this accelerating effect may, however, become converted into a less accelerating one. Such conversion happened under the given experimental conditions for 10^{-2}M Cu^{2+} and at pH $<$ 7 (Fig. 1b). Addition of 10^{-3}M Cu^{2+} under the same experimental conditions (experiments in air) did not show any conversion. When, however, 10^{-3}M Cu^{2+} was added at low O_2 pressure (e.g. corresponding to 1% O_2), its effect could, under certain conditions, become rate decreasing (Fig. 2). Also this inhibitory effect of 10^{-3}M Cu^{2+} was dependent on pH; it became increasingly pronounced with pH decreasing from 7 to 5 (Fig. 3).

The effect and its conversion were also dependent on the ratio between the concentrations of catalyst and substrate. This was shown by experiments comparing the effect of Cu^{2+} addition in different concentrations of the substrate and giving rise to different rates of oxidation. It was established that the threshold concentration of O_2, at which conversion from a prooxidative to an antioxidative effect occurred, varied with the concentration of the substrate. At a higher concentration (0.107 M) the threshold was between 1% and 5% O_2 (Fig. 4a), at a lower concentration (0.036 M) between 5% and 10% O_2 (Fig. 4b).

Thus, under certain conditions, Cu^{2+} was shown to have an antioxidative tendency, which is enhanced at low O_2 pressure and dependent on pH and the ratio of the concentrations of the catalyst and the substrate.

Addition of Fe^{3+} [$Fe_2(SO_4)_3$]

As for addition of Fe^{3+}, the catalytic effect was less and

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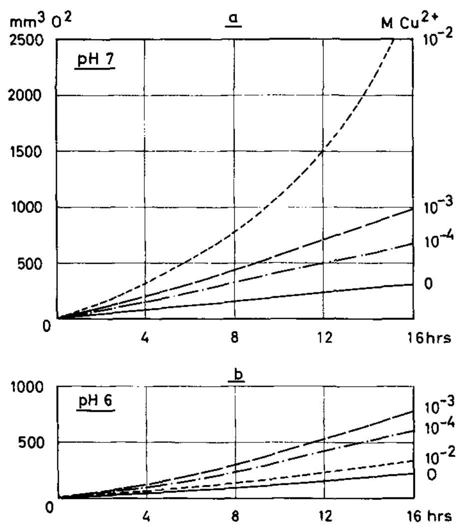

FIG. 1. Effect of Cu^{2+} at pH 7 and 6 in air.

the conversion was more pronounced than on addition of Cu^{2+}. In general, on increasing the concentration, a maximum rate was reached at about 10^{-3}M addition. Further increase of the concentration did not promote, and sometimes depressed, the rate as compared to the maximum rate. At low O_2 pressure, e.g., an O_2 pressure corresponding to 1% O_2, the conversion of the effect with increasing Fe^{3+} concentration was relatively more pronounced than in air (Fig. 5).

Also, in the case of addition of Fe^{3+}, the formation of a conversion was more expressed at pH 5 (Fig. 6) than at pH 7 (Fig. 5), at least at low O_2 pressure. While at pH 7 the effect of 10^{-2}M Fe^{3+} addition at low O_2 pressure was less accelerating than that of 10^{-3}M, although not inhibiting, it decreased the rate at pH 5 as compared to the reference without Fe^{3+} addition.

At still lower O_2 pressure, corresponding to 0.1% or 0.2%, the tendency of the rate to decrease with increasing concentration of Fe^{3+} was still more evident. The rates of oxidation were, however, very low and the experimental error was relatively large.

The effect of Fe^{3+} upon the rate of lipid oxidation was found to be dependent upon the anions present. When experiments were carried out without addition of phosphate, the effect of Fe^{3+} was exclusively more prooxidative with increasing concentration. There was no indication of conversion in air or at 1% O_2. However, due to the absence of a buffer, the reproducibility of the results was unsatisfactory. When using phthalate as a buffer, increasing Fe^{3+} concentration in experiments in air again showed only an increasing prooxidative effect. At low oxygen pressure, however, the effect could be converted at increasing Fe^{3+} concentration (Fig. 7).

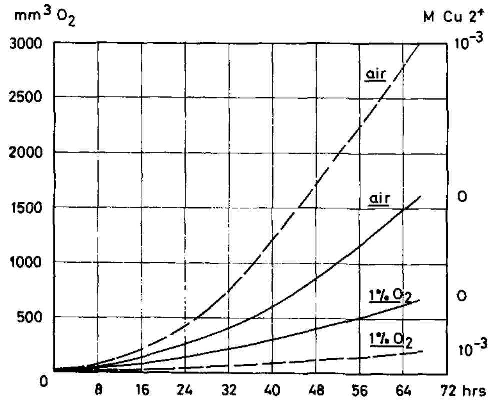

FIG. 2. Effect of Cu^{2+} at pH 5 in air and in 1% O_2.

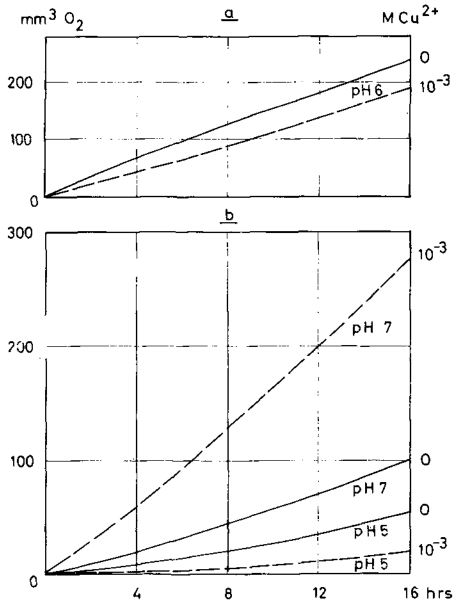

FIG. 3. Effect of Cu^{2+} in 1% O_2 at pH 7, 6 and 5.

Thus, also in the case of Fe^{3+} addition, an antioxidative tendency was established which was shown to be dependent on O_2 pressure, pH and the anions present.

Addition of Fe^{2+} [$Fe(SO_4)$]

In contrast to the effects observed for Fe^{3+}, addition of Fe^{2+} did not lead to any conversion. The prooxidative effect increased with increasing concentration of Fe^{2+} and there was no tendency for the influence of Fe^{2+} to decrease at higher concentration (e.g., 10^{-2}M) in air or at low oxygen pressure (Fig. 8).

Addition of Co^{2+} [$Co(NO_3)_2$]

Similar results, as in the case of Fe^{2+}, were obtained after addition of Co^{2+}. The prooxidative effect increased with Co^{2+}

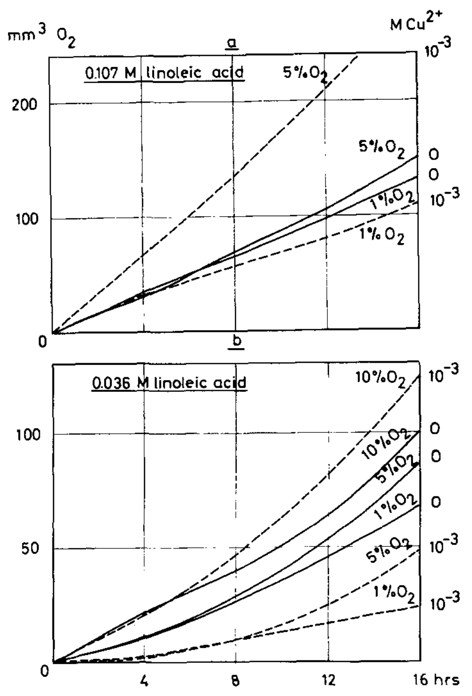

FIG. 4. Effect of Cu^{2+} for different concentrations of linoleic acid in 1, 5 and 10% O_2.

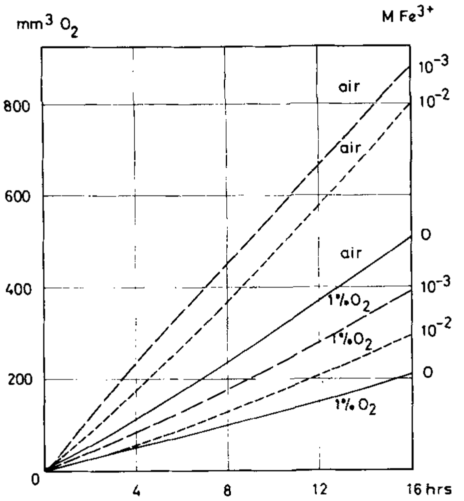

FIG. 5. Effect of Fe^{3+} at pH 7 in air and in 1% O_2.

concentration without any formation of a conversion at higher Co^2 concentration in air or at low oxygen pressure (Fig. 9).

DISCUSSION

The prooxidative effect of added metal salts (Cu^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}) on the oxidation of linoleic acid in emulsions was relatively low at low oxygen pressure when compared with the effect in air. Under extreme conditions, especially in the case of Cu^{2+}, the effect could be antioxidative. In the case of Fe^{3+}, a similar but less pronounced dependency on oxygen pressure was observed. Both metals demonstrated the phenomenon of converted effects with increasing concentration (i.e., the prooxidative effect increased until a certain maximum and thereafter decreased). This conversion was favored by low oxygen pressure; more in the case of Cu^{2+}, less with Fe^{3+}, while Fe^{2+} and Co^{2+} did not show the phenomenon.

Inhibitory effects of metal catalysts have been described in the literature; Cu^{2+} in particular has been reported as having an inhibiting effect on the oxidation of both hydrocarbons (9,10) and lipids (11). As a rule, Cu^{2+} at lower concentration reacts as a prooxidant, whereas at higher concentrations it may behave like an inhibitor (12). Such an antioxidant effect has generally been ascribed to a catalytic effect upon chain termination. However, a complex formation of free radicals with Cu^{2+} during propagation may also, according to Ingold (6), result in reduced rate.

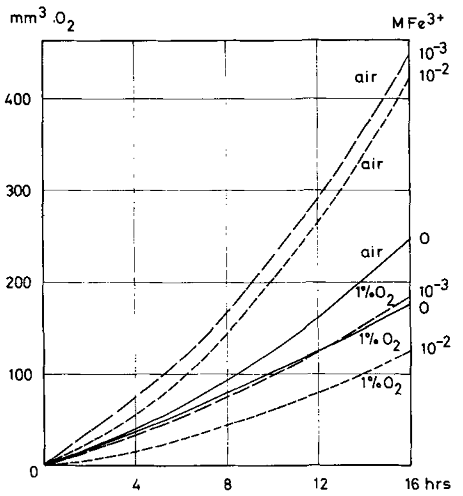

FIG. 6. Effect of Fe^{3+} at pH 5 (phosphate buffer) in air and in 1% O_2.

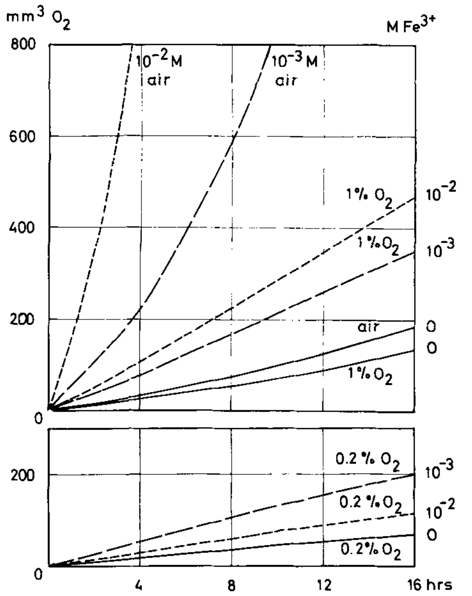

FIG. 7. Effect of Fe^{3+} at pH 5 (phthalate buffer) in air and in 1% and 0,2% O_2.

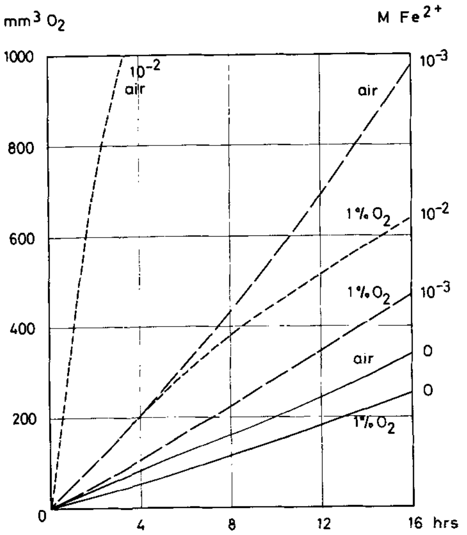

FIG. 8. Effect of Fe^{2+} at pH 5 in air and in 1% O_2.

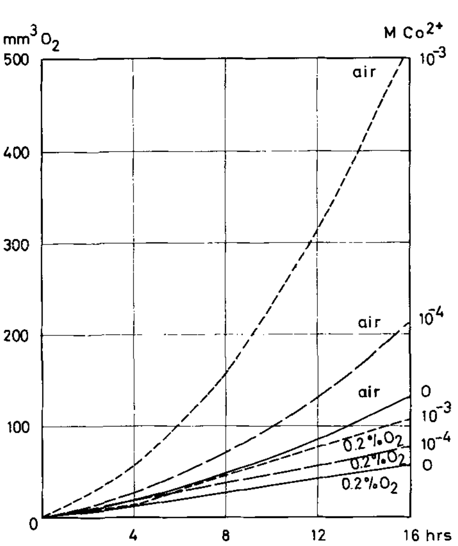

FIG. 9. Effect of Co^{2+} at pH 5 in air and in 0,2% O_2.

The phenomenon of the conversion of a metal catalyst into an inhibitor by an increase in concentration may, according to Betts and Uri (3-5), be explained by the assumption that a relatively inefficient initiation reaction (first order with regard to the metal) competes with a termination reaction (second order with regard to the metal). Therefore, above a certain metal concentration, inhibition is bound to exceed initiation.

The experiments reported in this paper have shown that this conversion of the catalytic effect is dependent on oxygen pressure and, further, that this dependence varies with the metals tested. In some cases low oxygen pressure only decreased the strength of the prooxidative effect, in other cases it caused an antioxidative conversion.

It is logical that a prooxidative effect is dependent on oxygen pressure, at least in a certain range of this pressure, and that such dependence at low oxygen pressure will be further enhanced by slow diffusion of oxygen into the oxidizing system, as shown by Pokorný (13). The effect of a metal catalyst usually accelerates the rate of oxidation when studied in air, while at low oxygen pressure a constant rate is reached which cannot be surpassed due to limitation by slow diffusion of oxygen. This means that at low oxygen pressure the prooxidative effect of metal salt addition can be expected to be relatively low as compared to the reference oxidation in air.

Yet, the fact that the catalytic effect of different metals at low oxygen pressure differs, and especially the converted effects obtained by addition of Cu^{2+} and Fe^{3+}, show that the above mentioned relationships alone cannot be responsible for the low rate of the process at low oxygen pressure. While certain metals, such as Co^{2+} and Fe^{2+}, had a relatively strong prooxidative effect also at low oxygen pressure, the catalytic effect of Fe^{3+} and Cu^{2+} at low oxygen pressure was only weakly prooxidative or even antioxidative. Thus, besides being prooxidants these metals also react as antioxidants. Under suitable conditions, e.g., low oxygen pressure, the inhibiting effect, usually hidden due to dominance of the prooxidative influence, can become more effective or even dominant.

The dependence of the catalytic effect of metals on oxygen pressure may be traced back to the chain reaction mechanism with both oxidative reactions, leading to the formation of, e.g., peroxides and radicals, and terminating reactions independent of oxygen. At low oxygen pressure, the oxidizing reactions are relatively slow and the terminating ones may consequently become more dominant.

A greater influence of terminating effects at low oxygen pressure was confirmed for Cu^{2+} and Fe^{3+}. Such antioxidative tendencies were, however, not observed after the addition of Fe^{2+} or Co^{2+}, transition metals in their lower valency. There should, consequently, be a special mechanism involved in the effect of Cu^{2+} and Fe^{3+} addition, both representing transition metals in their higher valency.

The special mechanism for Cu^{2+} and Fe^{3+} might be the rapid reaction of Cu^{2+} with alkyl radicals, described by Kochi (14) and a similar less pronounced effect of Fe^{3+}. According to Ingold (7), Cu^{2+} ions at concentrations of $10^{-3}M$ and higher can be supposed to compete with the oxygen present for the $R\cdot$ radicals and to interfere with the chain reaction. The lower the oxygen pressure, the more the reaction with the metal is favored and the greater is the influence of the metal-catalyzed terminating reaction.

There was, however, also a considerable difference between the effect of Cu^{2+} and that of Fe^{3+} This difference may be due to the different catalytic power of these metals since a powerful catalyst like Cu^{2+} strongly enhances both promoting and terminating reactions and thereby can give rise to the formation of either a strongly prooxidative or a strongly antioxidative effect; in the case of a weaker catalyst, the promoting and terminating reactions are more balanced. In the latter case, both prooxidative effects and antioxidative tendencies are, therefore, less pronounced. The antioxidative tendency of Fe^{3+} addition was demonstrated mainly by the converted effects obtained with increasing metal concentration. This tendency was, like the effect of Cu^{2+}, enhanced at low oxygen pressure.

Very little is known about the dependence of the catalytic effect on the physico-chemical state of the catalyst, a question of great practical importance. In the experiments with the addition of Fe^{3+} the tendency for conversion was influenced by the anions present, phosphate and phthalate. In experiments in air with phthalate as a buffer, no conversion occurred with increasing metal concentration, in contrast with the effect in the presence of phosphate. At low oxygen pressure, however, such antioxidative tendencies occurred also with phthalate. These observations indicate that the anions present may more or less favor or suppress the conversion.

Finally, as shown by Ingold (7), the ratio between metal catalyst and substrate concentration may be decisive for the results. This was also found in our experiments at low oxygen pressure reported above. The antioxidative tendency at low oxygen pressure was shown to be drastically influenced by the ratio between the concentrations of metal and substrate, an observation which may become of practical interest in the storage of food liable to fat oxidation.

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REFERENCES

1. Marcuse, R., and P. -O. Fredriksson, JAOCS 45:400-407 (1968).
2. Marcuse, R., and P.-O Fredriksson, Ibid. 46:262-268 (1969).
3. Betts, A.T., and N. Uri, Makromol. Chem. 95:22-39 (1966).
4. Betts, A.T., and N. Uri, Advan. Chem. Ser. 76; Amer. Chem. Soc. 1968: 160-181.
5. Uri, N., Is. J. Chem. 8:125-139 (1970).
6. Ingold, K.U., "Metal Catalysis," AVI Publishing Co., Westport, Conn., 1962, p. 93-121.
7. Ingold, K.U., SIK-Rapport 240:11-42 (1968).
8. Marcuse, R., K. Remi and P. -O. Göthe, Fette Seifen Anstrichm. 66:992-997 (1964).
9. Ingold, K.U., Chem. Rev. 61:563-589 (1961).
10. Denisov. E.T., and M. Emanuel, Usp. Khim. 29:1409-1438 (1960).
11. Kirjakka, P., and M. Nieminen, Suom. Kemistilehti 27A:207-215 (1954).
12. Kharasch, M.S., and A. Fono, J. Org. Chem. 24:72-78, 606-614 (1959).
13. Pokorný, J., SIK-Rapport 240:89-96 (1968).
14. Kochi, J.K., and F.F. Rust, J. Amer. Chem. Soc. 83:2017-2018 (1961).

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