Fat Oxidation at Low Oxygen Pressure: II. Kinetic Studies on Linoleic Acid Oxidation in Emulsions in the Presence of Antioxidants

R. MARCUSE and P.-O. FREDRIKSSON, Swedish Institutet for Food Preservation Research, Göteborg, Sweden

Abstract

Earlier reported kinetic studies on the dependence of lipid oxidation on oxygen pressure in emulsions were continued by studying this relationship in the presence of antioxidants. The substances tested represented two types of antioxidants, phenolic inhibitors (α-tocopherol, BHA, PG) and amino acid-retarders (glycine, alanine, histidine, tryptophane). The inhibiting effect of the first mentioned group, i.e., the formation of an induction period was, in general, not dependent on oxygen pressure, while the retardation caused by amino acids was stronger at low oxygen pressure than in air. The effect of lowering oxygen pressure was practically the same, when phenolic inhibitors were added as without such addition. It was, however, enhanced by the addition of amino acid-retarders. When representatives of these two types of antioxidants were added in combination, their synergistic effect was considerably enhanced at low oxygen pressure.

Introduction

The role played by certain parameters in the dependence of fat oxidation on oxygen pressure has been discussed in a previous paper (Marcuse and Fredriksson, (1)). Kinetic measurements of lipid oxidation in emulsions indicated that, under the conditions prevailing in oil-in-water emulsions, the dependence of the rate of oxidation on oxygen pressure is influenced by the rate of oxygen diffusion as well as by certain principles inherent in the chain mechanism of lipid oxidation.

In general, however, also other mechanisms are involved in lipid oxidation, e.g., the action of antioxidants. Fundamental differences in dependence on oxygen pressure may exist between autoxidation and antioxidation.

Lundberg et al. (2) reported the antioxidative effect of α-tocopherol to be limited by the oxidative breakdown of the antioxidant. This oxidative breakdown can be supposed to be influenced by oxygen pressure which, consequently, might affect the duration of the induction period. This has received attention by Bolland and ten Have (3,4) who studied the thermal oxidation of ethyl linoleate. However, it was found that the oxidative breakdown and, consequently, the antioxidative effect in certain cases, e.g., with hydroquinone, was independent of oxygen pressure, while in other cases, e.g., with some hydroquinone derivatives, a relationship could be demonstrated to exist between the oxygen pressure and the antioxidative effect. This difference in behavior was ascribed to difference in redox-potential (3–5), low redox-potential meaning relatively greater liability for dependence on oxygen pressure. Further, Shelton (6), in experiments on the oxidation of rubber materials, found the rate of oxidation in the presence of certain amines to be approximately proportional to the square root of the oxygen pressure. He states that retarded oxidation in the presence of an antioxidant differs from non-retarded autoxidation in that the rate of the former is dependent on oxygen concentration, while that of the latter is generally not. The different results obtained by these authors seem to depend upon the different types of antioxidants studied.

This paper reports research in this regard, i.e.,

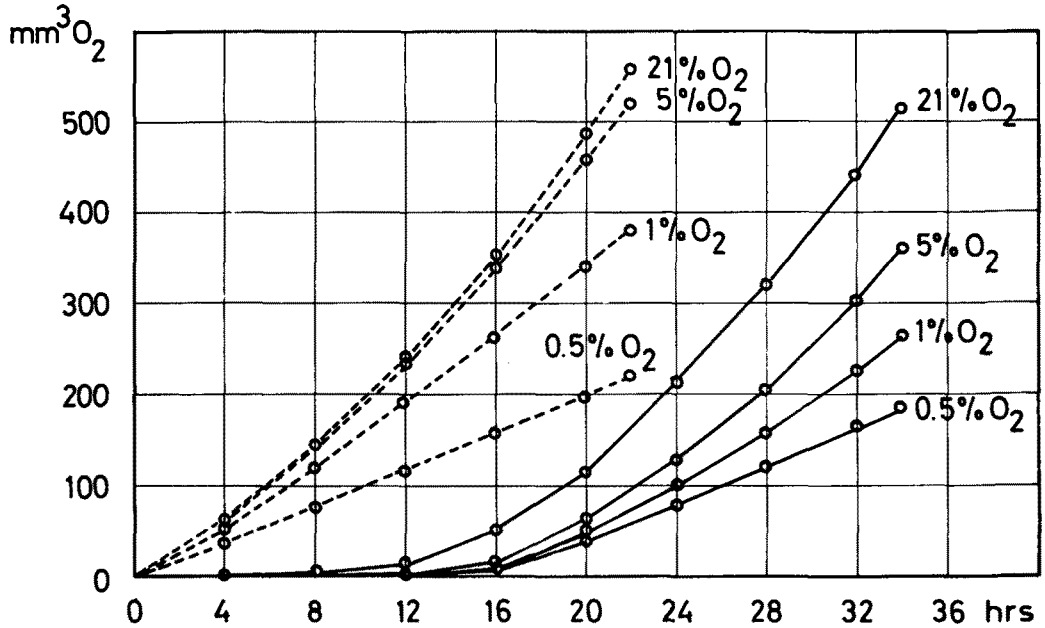

FIG. 1. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.07 M linoleic acid, pH 6, --- reference (no addition of antioxidant), —— 0.005% α-tocopherol added.

studies of the dependence on oxygen pressure of the rate of linoleic oxidation in emulsions in the presence of various antioxidants.

With respect to the type of their effect antioxidants may be grouped into two main classes: inhibitors and retarders.

Inhibitors give rise to an induction period (or a prolongation of such period) with little or no consumption of oxygen. The rate of oxidation after the end of the induction is in general the same as in the absence of an inhibitor. Retarders, on the other hand, decrease the rate of oxidation without causing any substantial induction period.

Inhibitors operate by a radical chain-breaking mechanism, while retarders prevent the introduction of chain-initiating radicals (7). Examples of inhibitors are compounds of phenolic type, e.g., tocopherol.

The induction period caused by an inhibitor may, as mentioned above, last until the antioxidant has been more or less oxidized, and may in this case be dependent upon oxygen pressure. This, however, is not generally representative. In other circumstances a large amount of antioxidant may still be present at the end of the induction period.

Retarders lowering the rate of oxidation are, e.g., amines as studied by Shelton (6) and amino acids, especially histidine and tryptophane, earlier studied by Marcuse (8–10).

The mechanism of the antioxidative action of amino acids has recently been studied by Mitsuda et al. (11), who found it probably to depend on electron donation. Further, chelating with metal traces is involved, as shown by Marcuse (12,13).

Experimental Procedures

The antioxidants of inhibiting type studied were α-tocopherol, BHA, BHT and PG (propyl gallate), those of retarding type were some amino acids: glycine, alanine, histidine and tryptophane. The experiments were carried out either with addition of one of these substances or with the combined addition of one representative of each group, such a combination being regarded as synergistic. The kinetics of the dependence of the rate of oxidation on oxygen pressure were studied in linoleic acid emulsions.

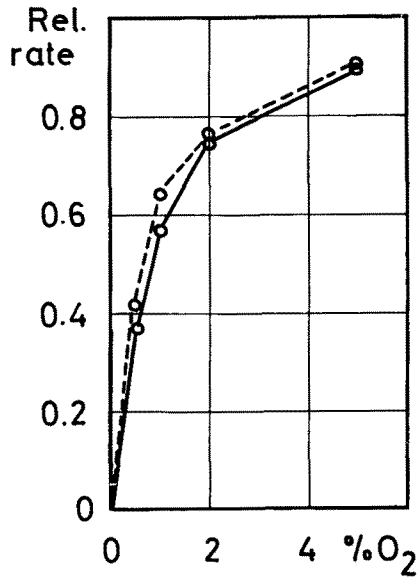

FIG. 2. Relative rate (to air) of linoleic acid oxidation as a function of O_2 concentration, 0.07 M linoleic acid, pH 6, - - - reference, —— 0.005% α-tocoprerol added.

The course of oxidation was determined by measurement of oxygen uptake in a previously described modified Warburg apparatus (14) with automatic electrolytic substitution of oxygen consumed. All experiments were carried out at atmospheric pressure, the range of oxygen pressure studied generally cor-

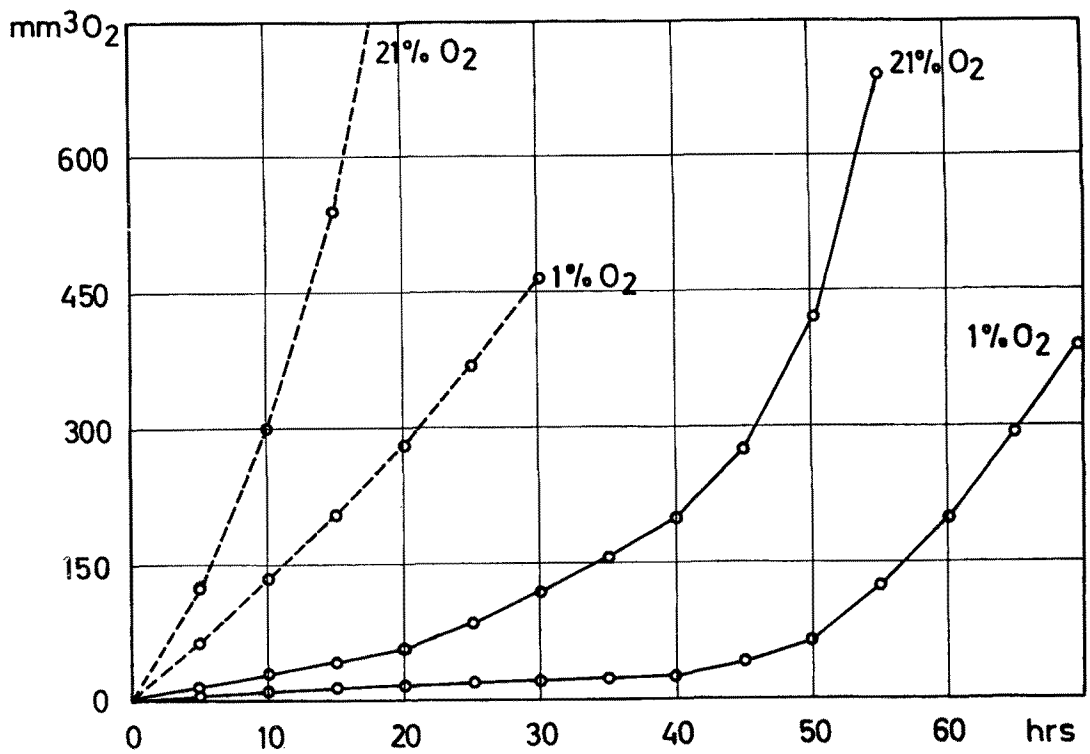

FIG. 3. O_2 consumption of linoleic acid oxidation in air and in 1% O_2 as a function of time, 0.28 M linoleic acid, pH 6, - - - reference, —— 0.01% α-tocopherol added.

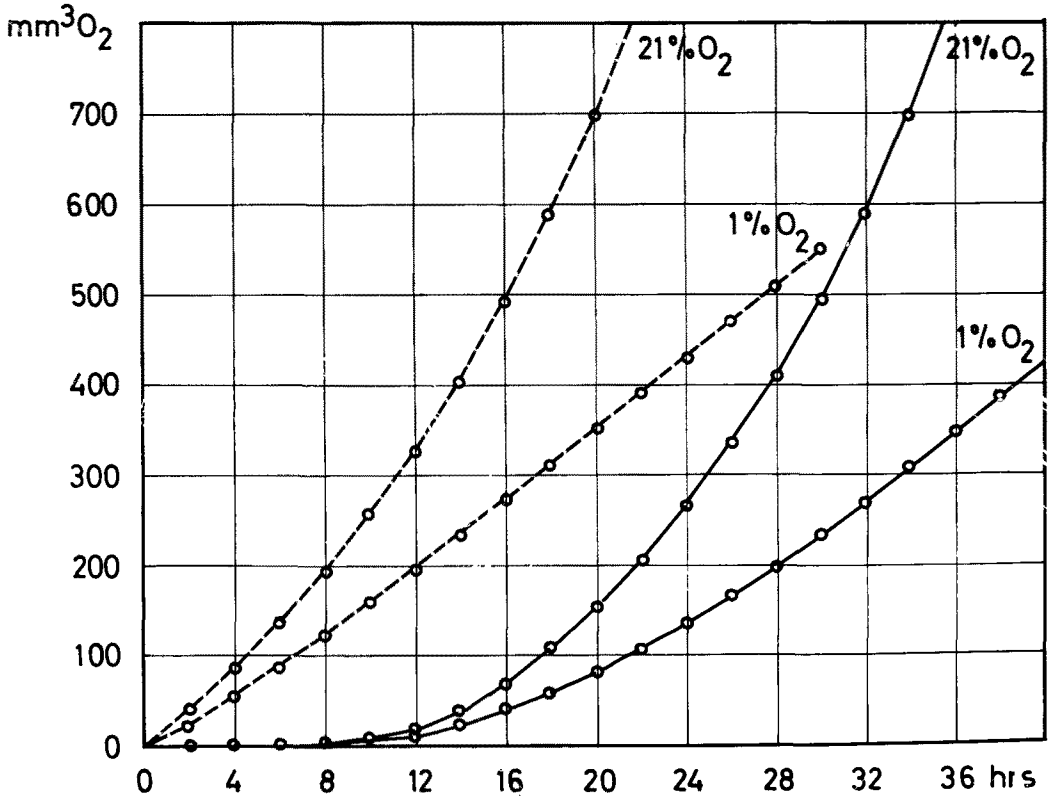

FIG. 4. O_2 consumption of linoleic acid oxidation in air and in 1% O_2 as a function of time, 0.214 M linoleic acid, pH 6, - - - reference, —— 0.0002% BHA added.

responding to 0.5–5%. The results were recorded as oxygen uptake as a function of time.

As discussed earlier (1) the rates at low oxygen pressure were further calculated relative to that in air and then recorded as a function of the oxygen pressure. As a rule, the pH in the experiments reported was 6. Exceptions will be mentioned. The duration of the induction period is expressed as the time for a certain consumption of oxygen, to be reached, usually 100 mm^3.

Results

Experiments With Antioxidants of Inhibiting Type

Figures 1 to 3 give the results of experiments with the addition of α-tocopherol, bringing about the formation (or prolongation) of an induction period. The effect of low oxygen pressure, corresponding to 1% oxygen at atmospheric pressure, was studied for its influence upon the length of the induction period and the post-induction rate of oxidation. The former was generally not prolonged—provided the antioxidative effect was strong enough to inhibit oxygen con-

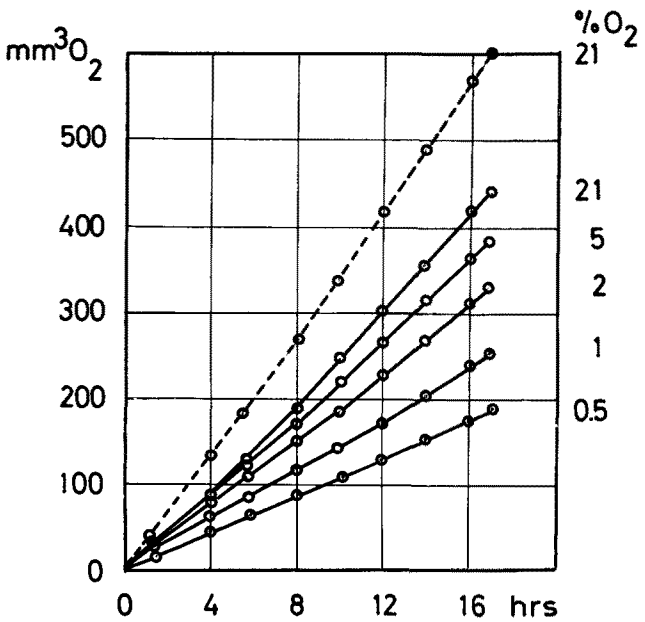

FIG. 5. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.11 M linoleic acid, pH 7, - - - reference, —— 0.05 M glycine added.

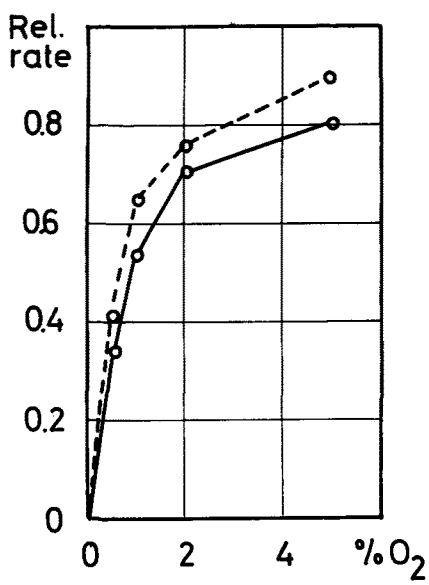

FIG. 6. Relative rate (to air) of linoleic acid oxidation as a function of O_2 concentration, 0.11 M linoleic acid, pH 7, - - - reference, —— 0.05 M glycine added.

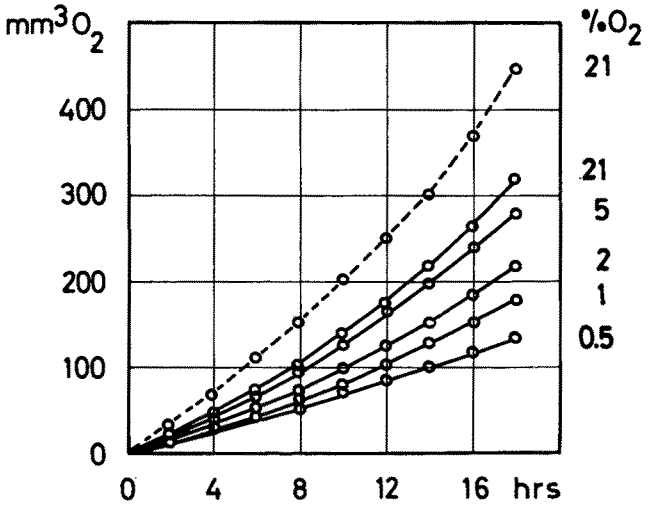

FIG. 7. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.07 M linoleic acid, pH 6, - - - reference, —— 0.01 M histidine added.

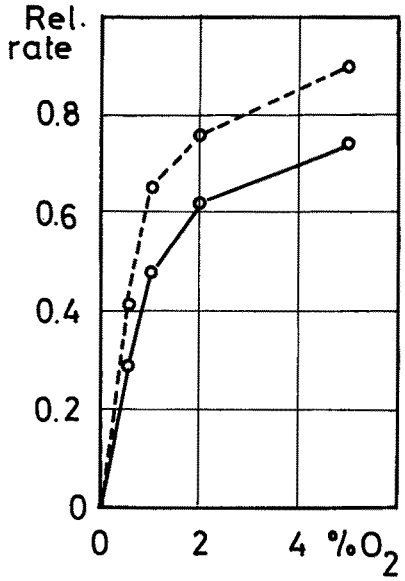

FIG. 8. Relative rate (to air) of linoleic acid oxidation as a function of O_2 concentration, 0.07 M linoleic acid, pH 6, - - - reference, —— 0.01 M histidine added.

sumption during the induction period more or less completely; the latter, i.e., the post-induction rate, was decreased to practically the same extent as in the absence of an antioxidant (Fig. 1 and 2).

At a ratio of antioxidant to substrate concentration not sufficient for complete cessation of oxygen uptake during the induction in experiments in air, the residual inductional oxygen consumption was more or less suppressed at low oxygen pressure and the induction period somewhat prolonged thereby (Fig. 3).

Similar results were obtained after addition of PG or BHA (Fig. 4). In other experiments the effect of low oxygen pressure corresponding to only a few tenths of 1% of oxygen was studied. Similar results were obtained to those reported above.

Experiments With Antioxidants of Retarding Type

The experiments with the addition of amino acids were carried out in emulsions added with glycine or alanine, known to have a weak retarding effect, and histidine and tryptophane which retard oxidation more effectively (8–10).

Addition of glycine (Fig. 5 and 6) or alanine lowered the relative rate of oxidation at low oxygen pressure. The influence of glycine and alanine was studied at pH 7, as it had been shown earlier that the antioxidative effect of these amino acids is rather small at pH < 7. Addition of histidine (Fig. 7 and 8) or tryptophane (Fig. 9 and 10) lowered the relative

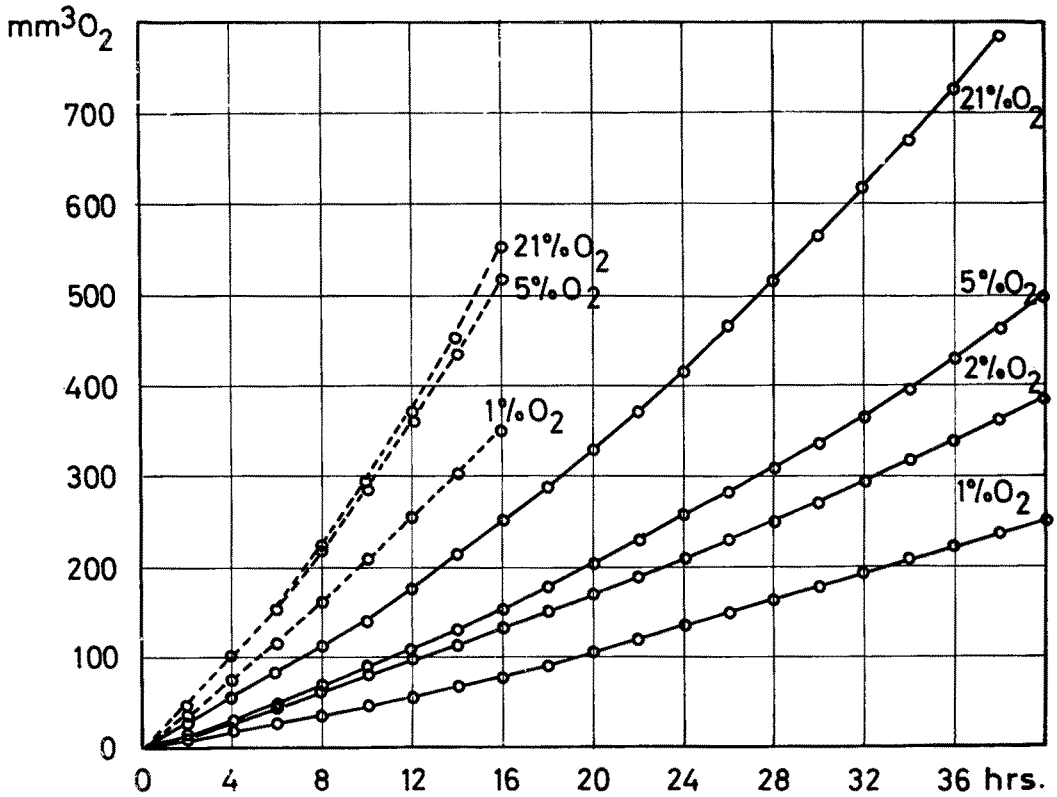

FIG. 9. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.07 M linoleic acid, pH 6, - - - reference, —— 0.01 M tryptophane added.

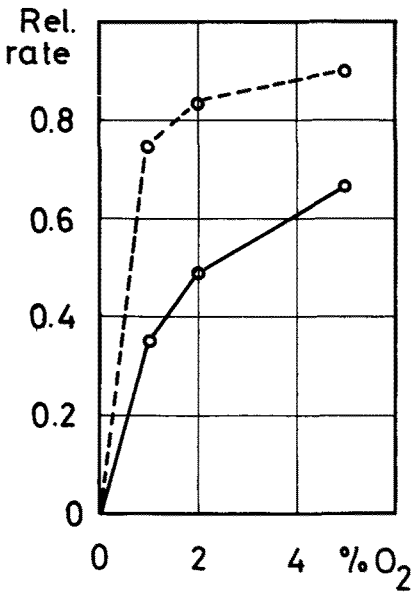

FIG. 10. Relative rate (to air) of linoleic acid oxidation as a function of O_2 concentration, 0.07 M linoleic acid, pH 6, --- reference, —— 0.01 M tryptophane added.

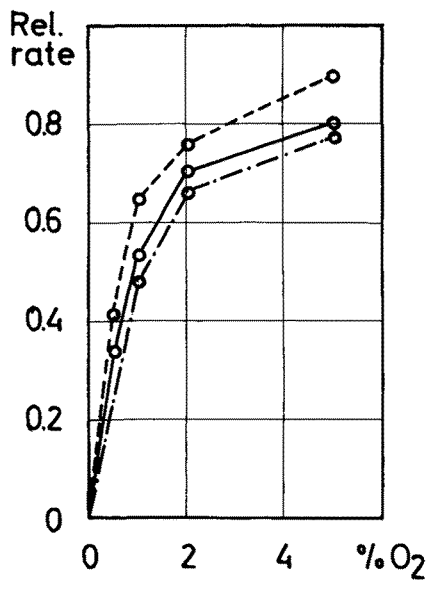

FIG. 12. Relative rate of linoleic acid oxidation as a function of O_2 concentration 0.11 M linoleic acid, pH 7, --- reference, —— 0.05 M glycine added, -.-.- 0.005% α-tocopherol + 0.05 M glycine added.

rate of oxidation at low oxygen pressure considerably more than did glycine or alanine.

Combined Addition of Antioxidants of Inhibiting and Retarding Type

Since amino acids are known as syngerists enhancing the antioxidative effect of antioxidants of phenolic type, further experiments were carried out to study the dependence on oxygen pressure in the presence of both phenolic antioxidants and amino acids. Low oxygen pressure caused a certain prolongation of the induction as well as a larger decrease of the relative rate of oxidation than in reference with the addition of amino acids alone. This is shown in Figures 11

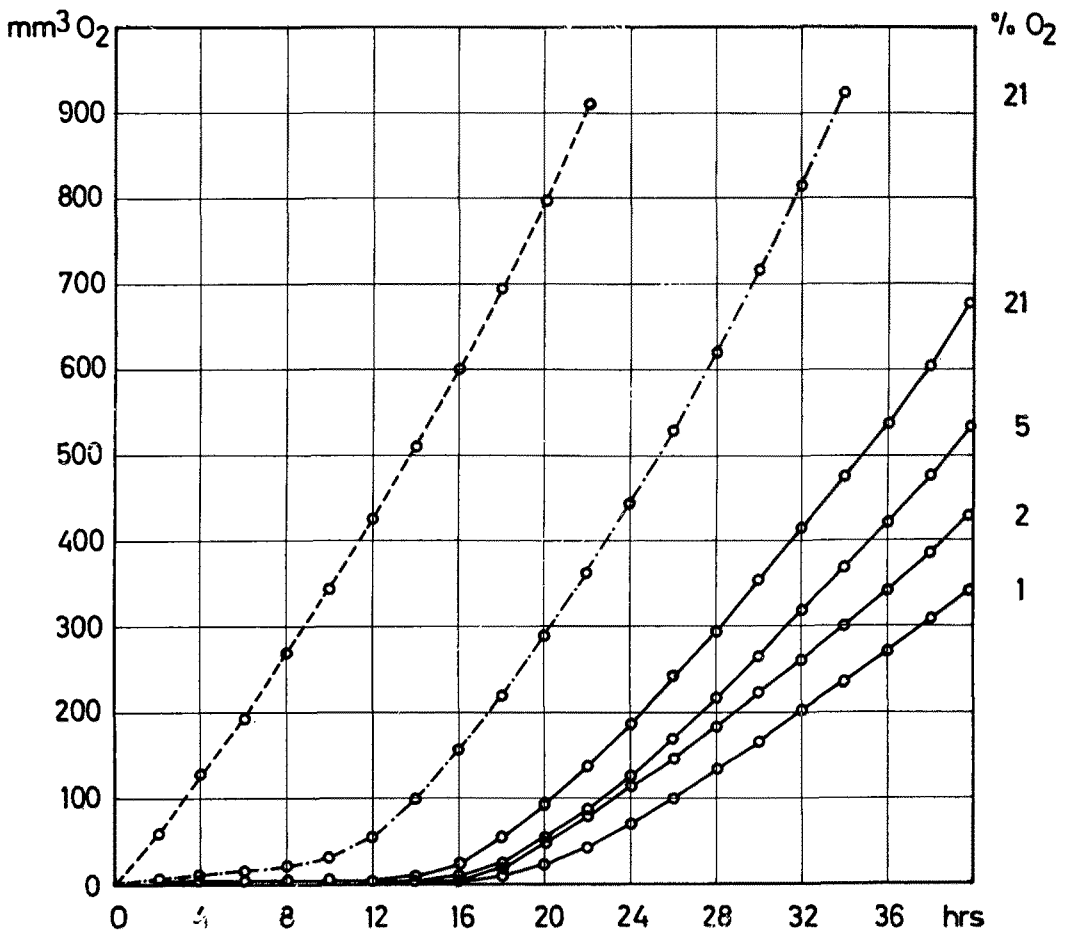

FIG. 11. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.11 M linoleic acid, pH 7, --- reference, -.-.- 0.005% α-tocopherol added, —— 0.005% α-tocopherol + 0.05 M glycine added.

TABLE I

Linoleic Acid Oxidation With Addition of α-Tocopherol and Histidine (0.07 M Linoleic Acid Emulsion, 0.005% Tocopherol, 0.01 M Histidine). Hours for 100 mm³ O_2-Consumption

% O_2	Reference	With addition of		
		Histidine	Tocopherol	Histidine & tocopherol
21	5.5	6.5	18.0	29.0
0.5	8.5	11.0	23.0	43.0
Retardation at 0.5% O_2	3.0	4.5	5.0	14.0
relative (to air)	1.0	1.5	1.7	4.7

TABLE II

Linoleic Acid Oxidation With Addition of BHA and Histidine (0.07 M Linoleic Acid Emulsion, 0.0002% BHA, 0.001 M Histidine). Hours for 100 mm³ O_2-Consumption

% O_2	Reference	With addition of		
		Histidine	BHA	Histidine & BHA
21	4.5	6.0	17.5	22.0
1	6.5	9.5	22.0	33.0
Retardation at 0.5% O_2	2.0	3.5	4.5	11.0
relative (to air)	1.0	1.8	2.3	5.5

and 12 for α-tocopherol and glycine (pH 7) and in Figures 13 and 14 for α-tocopherol and histidine (pH 6). The prolongation of the induction period at low oxygen pressure after combined addition of tocopherol and histidine or of BHA and histidine is demonstrated in Tables I and II.

Discussion

The studies reported above showed that the two types of antioxidants studied, inhibitors and retarders, differ in their effect upon the dependence on oxygen pressure of linoleic acid oxidation in emulsions. Inhibitors give rise to the formation (or prolongation) of an induction period, but do not affect the rate (post-induction rate) of oxidation. Retarders, on the other hand, decrease the rate of oxidation but do not produce an induction period. This difference is sometimes pointed out in the literature. Often, however, the terms antioxidant, inhibitor and retarder are used without differentiation.

As to antioxidants of the inhibiting type, the induction period brought about by the addition of the substances studied, mainly tocopherol, was on the whole not prolonged by lowering of the oxygen pressure; neither was the post-induction rate of the oxygen uptake decreased to a higher degree than the rate of uninhibited autoxidation. This shows the antioxidative effect of these substances not to be dependent on oxygen pressure within the range of oxygen pressure studied. This conclusion is in good agreement with the results of other research, e.g., work by Bolland and ten Have (3,4) who found substances such as hydroquinone to react only with radicals in the termination process of the chain mechanism and not to be directly attacked by molecular oxygen, provided the normal oxidation reduction potential is >0.6 V. At a normal redox potential of <0.6 V the antioxidant may be directly attacked by molecular oxygen. Substances of this type, besides being less effective as antioxidants, will be more or less dependent on oxygen pressure.

The normal oxidation reduction potential for α-tocopherol was given by Golumbic (15) as 0.77 V.

The radicals formed during propagation may be of alkylperoxy and alkyl-type. According to Bateman and Morris (16), the type formed is dependent on oxygen pressure: in air alkyl-peroxy radicals are preponderant; at low oxygen pressure, (a few mm Hg) this preponderance is changed in favor of alkyl-radicals. Since the length of the induction period in the experiments reported above is practically the same in air and at low oxygen pressure, the efficiency of the inhibitors tested appears to be the same for both types of radicals. However, oxygen pressures still lower than those studied in these experiments are required for a real preponderance of alkyl radicals and definite conclusions.

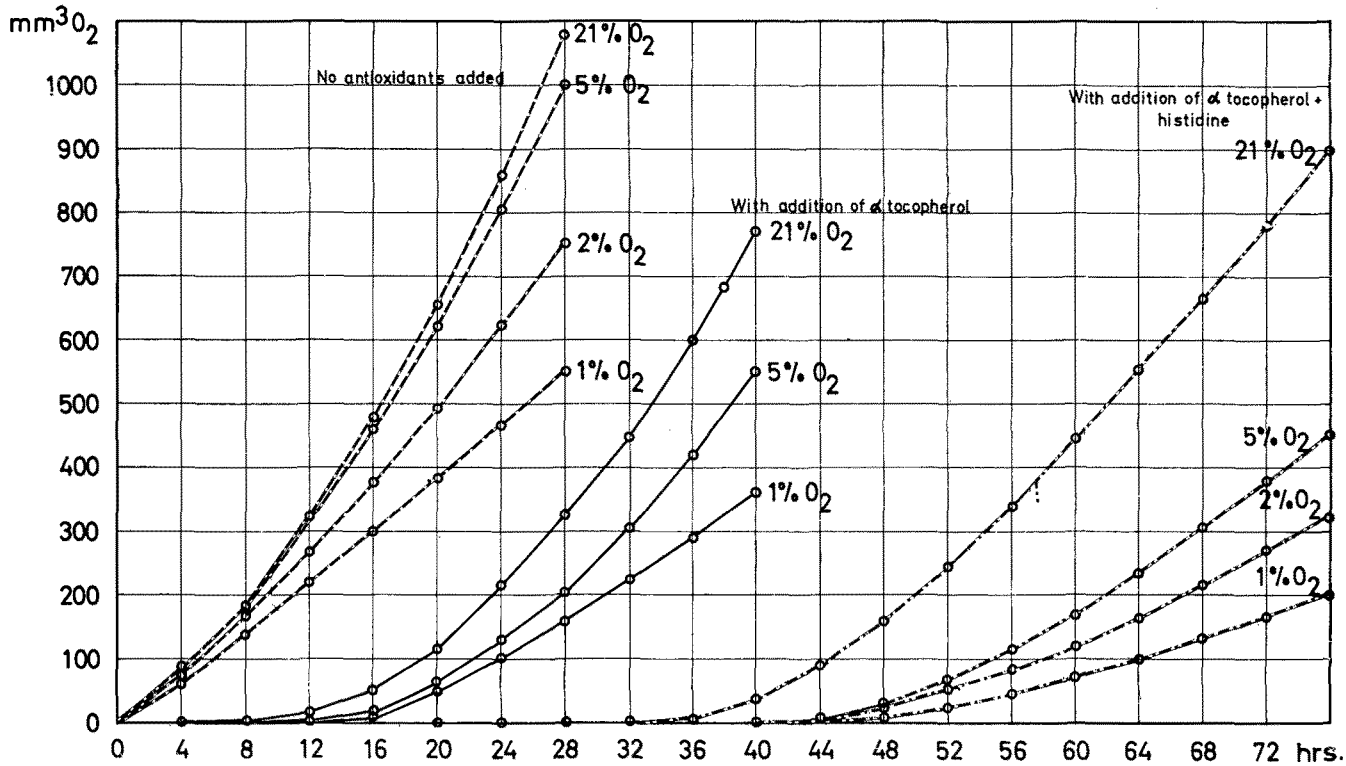

FIG. 13. O_2 consumption of linoleic acid oxidation at various O_2 concentrations as a function of time, 0.07 M linoleic acid, pH 6, --- reference, —— 0.005% α-tocopherol added, -.-.- 0.005% α-tocopherol + 0.01 M histidine added.

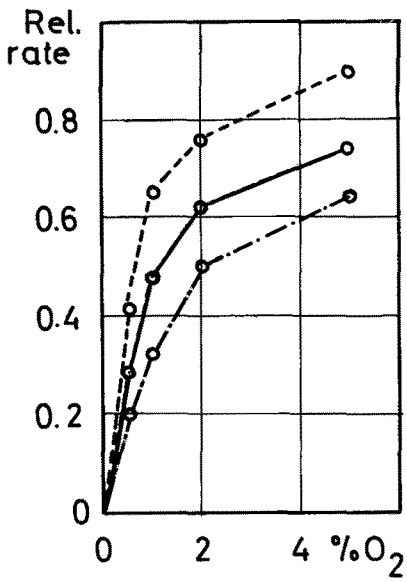

FIG. 14. Relative rate of linoleic acid oxidation as a function of O_2 concentration, 0.07 M linoleic acid, pH 6, --- reference, —— 0.01 M histidine added, -.-.- 0.005% α-tocopherol + 0.01 M histidine added.

As for amino acid-retarders the rate lowering effect of low oxygen pressure was enhanced or, in other words, the antioxidative effect of addition of amino acid was stronger at low oxygen pressure than in air.

These results are similar to those reported by Shelton (6), mentioned above. He found the retarding effect of certain amines on the rate of oxidation of rubber to be dependent on oxygen pressure. A direct attack of oxygen upon the substrate and upon the retarder was shown to play a dominant role in chain initiation in retarded oxidation, while the role played by peroxide decomposition is more or less limited, this implying dependence upon oxygen pressure.

The mechanism of the enhanced antioxidative effect of amino acid addition at low oxygen pressure is, however, not yet fully understood. It might be tentatively explained by oxidative destruction of the retarder during its antioxidant action.

ACKNOWLEDGMENTS

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