Fat Oxidation at Low Oxygen Pressure. I. Kinetic Studies on the Rate of Fat Oxidation in Emulsions[1]

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Abstract

Oxidation was measured by oxygen consumption in a Warburg apparatus, modified to maintain constant partial oxygen pressure by automatic electrolytic generation of oxygen with automatic recording of the oxygen consumed.

The decrease in rate of oxygen consumption on the lowering of partial oxygen pressure at atmospheric pressure was found to depend on a) the varying influence of the nonoxygen-dependent and the oxygen-dependent reactions of the propagation which may vary with the conditions such as the reactivity of the substrate, the temperature, and the pH value but which is not affected by light irradiation; b) the varying rate-limiting effect of slow oxygen diffusion, depending on the ratio between the rate of oxidation and the rate of oxygen diffusion.

Introduction

One way to protect fat and fatty food against oxidative rancidity is to prevent its exposure to oxygen. Exclusion of oxygen means mainly storage (e.g., packaging) in "vacuum" or in a protecting gas (generally nitrogen or carbon dioxide), i.e., in reality at low oxygen pressure. However exact threshold values of oxygen pressure which are tolerable under certain conditions are not known.

Oxygen pressures normally present in vacuum or gas packages are those which can be obtained under normal technical conditions with regard to the pumping system, the procedure of exhausting or gassing, and the packaging material, for instance. They are generally said to correspond to a few per cent of oxygen. Lower oxygen concentrations require special technical arrangements, which means an extra economic burden.

In order to elucidate autoxidation at low oxygen pressure, investigations of the rate of oxidation at low oxygen pressure were carried out under various conditions. Qualitative examinations of the composition of the products of oxidation at low oxygen pressure will be the subject of a future paper.

Earlier Research

Earlier research on such kinetic aspects has been published by Henderson and Young (2), especially by Bolland et al. (3–10), and recently by Karel (11,12). The significance of low oxygen pressure was studied at low total pressure. Little has been done on the significance of low partial pressure of oxygen at atmospheric total pressure because of the lack of suitable apparatus for such examinations at constant partial pressure of oxygen.

In the work published by Bolland et al., esters of unsaturated fatty acids, generally linoleic acid, were oxidized during effective shaking, i.e., under conditions said to eliminate the limiting influence of diffusion of oxygen on the rate of reaction. Under such conditions the oxidation, expressed as oxygen consumption, was accelerated without any preceding induction period; the rate of oxygen uptake varied linearly with the total amount of oxygen uptake. Bolland et al. were able to elucidate the part played by oxygen in the mechanism of autoxidation as well as to express the dependence of the rate of oxidation on oxygen pressure by a rate equation. The effect of oxygen pressure was calculated in terms of the relative values of oxygen uptake at such pressure compared with the oxygen uptake under comparable standardized conditions, e.g., at an oxygen pressure of 17 mm Hg.

Karel studied the dependence on oxygen pressure in two systems: linoleic acid in bulk and dispersed on a supporting substance with a large surface (representing conditions prevailing in dried food). For an in-bulk system, the course of oxidation expressed as oxygen consumption consisted of an induction period, followed by a period of constant rate of oxygen uptake. The nonaccelerated post-induction course of oxidation results from the influence of the diffusion of oxygen into the sample which limits the rate of oxidation. In these experiments by Karel the rates of oxygen uptake at different oxygen pressures could be compared directly, and expressions were derived for the dependence of the rate of oxidation on the pressure of oxygen under certain conditions, especially with regard to the size of the surface of the samples exposed to oxygen. In the other system studied by Karel, freeze-dried emulsions, oxygen has practically unlimited access to the oxidizable material. Consequently the course of oxidation is accelerated as in the system studied by Bolland. But the dry system shows certain complicating features, such as dependence on humidity and on the nature of the supporting material. Such aspects will be discussed in a later paper in connection with research under similar conditions.

Plan of Study

To begin with, the dependence of oxidation on the pressure of oxygen was investigated in emulsions of fatty substances. Such studies in emulsions are of interest as emulsions play an important part in food. Further, such a system allows investigation of the significance of certain conditions, e.g., the effect of water-soluble additives, such as antioxidants and pro-oxidants, on the dependence on oxygen pressure. It is also of interest with respect to studies on the effect of diffusion. Investigations in other systems, such as fatty material in bulk and especially fatty material dispersed on solid supporting substances, will be the subject of later papers. These studies were carried out at atmospheric total pressure. Comparisons at low total pressure are planned.

The following factors were investigated for their influence on oxidation at low pressure of oxygen: 1) the absolute rate of oxidation, influencing the significance of diffusion of oxygen, 2) the reactivity of the substrate, 3) temperature, 4) light, 5) pH, 6) antioxidants, 7) metal salts known as pro-oxidants and 8) conditions inherent in dry systems. In this paper the results of investigations of Items 1 to 5 are presented. Items 6 to 8 will be treated in following papers.

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[1] A preliminary report was read at the 4th Scandinavian Fat Symposium at Åbo, Finland, September 1965. (See reference 1.)

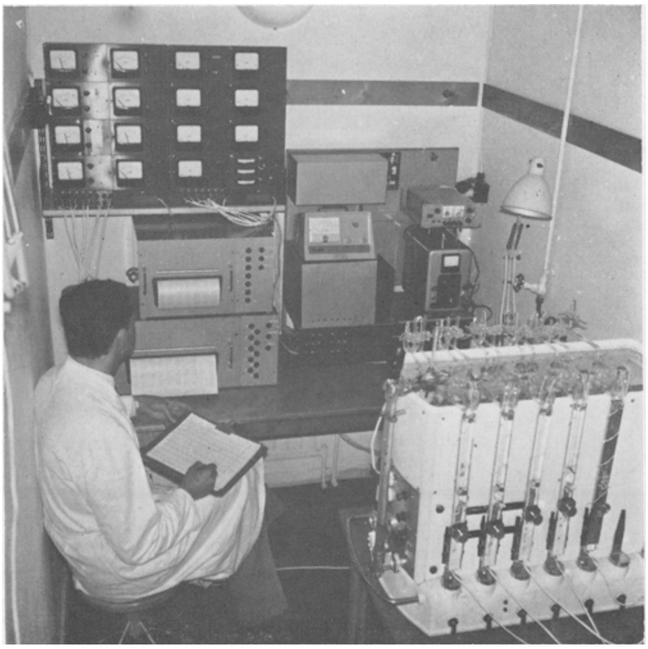

FIG. 1. Modified Warburg apparatus for automatic recording of O_2-consumption at constant pressure of oxygen.

Experimental Procedures

Apparatus

The course of oxidation at constant low pressure of oxygen was determined by measurement of the oxygen uptake. For this purpose it was necessary to develop a suitable apparatus. In the conventional manometric Warburg apparatus the oxygen concentration in the reaction vessel decreases during the experiment. At low oxygen pressure such a decrease of oxygen concentration excludes the use of this apparatus. A new type of Warburg apparatus with automatic electrolytic substitution of oxygen consumed was developed (Fig. 1). The electrolytic current is recorded automatically as a measure of oxidation at certain time-intervals. The amount of oxygen is calculated in accordance with the law of Faraday. Results can be recorded directly as mm^3 oxygen. The apparatus represents a completely closed system independent of atmospheric pressure. A description has been published earlier (13), and letters of patent have been granted. A paper with further details on the apparatus is being prepared.[2]

The reaction vessels were shaken at a speed of 80 strokes/min and an amplitude of 3 cm. Shaking of the reaction vessel can generally eliminate the limiting effect on the rate of oxidation because of the slow diffusion of oxygen. Under the experimental conditions of these studies this was however not always the case, as will be shown below.

Gas-Mixing Equipment

The oxygen-nitrogen mixtures were prepared in a gas-mixing equipment consisting of two 5-liter bottles vertically adjustable and connected to each other as shown in Fig. 2, one serving as the reservoir of the gas mixture, the other as pressure-balancing vessel. The gas reservoir is calibrated and connected at its top to a calibrated cylinder of some hundred ml volume for smaller amounts of gas to be mixed. A U-shaped manometer at the top of the cylinder checks the constancy of the pressure in the gas reservoir.

The effect of low oxygen pressure was studied at levels ranging from some tenths of 1% to 10% oxygen. Usually, for comparing the effect of certain conditions, a standard concentration of 1% oxygen was chosen.

Oxygen Analysis

The oxygen content of the mixture was controlled by conducting the gas through a Beckman paramagnetic oxygen analyzer. The efficiency of gassing the Warburg vessels with the gas mixture was checked by a Beckman polarographic oxygen electrode, mounted in a Warburg vessel and connected to the last one of a series of manometers to be flushed with the gas mixture.

In order to control the constancy of oxygen pressure in the reaction vessel during the experiments, samples were taken by a syringe and injected into a gas chromatograph (2S 0.91 molecular sieve column with 5Å pores; He, 200 ml/min; room temp.). Satisfactory results were obtained; the deviation was not more than 5–10 rel. % of the original concentration of oxygen (approx. 10% at low concentrations, approx. 5% in air).

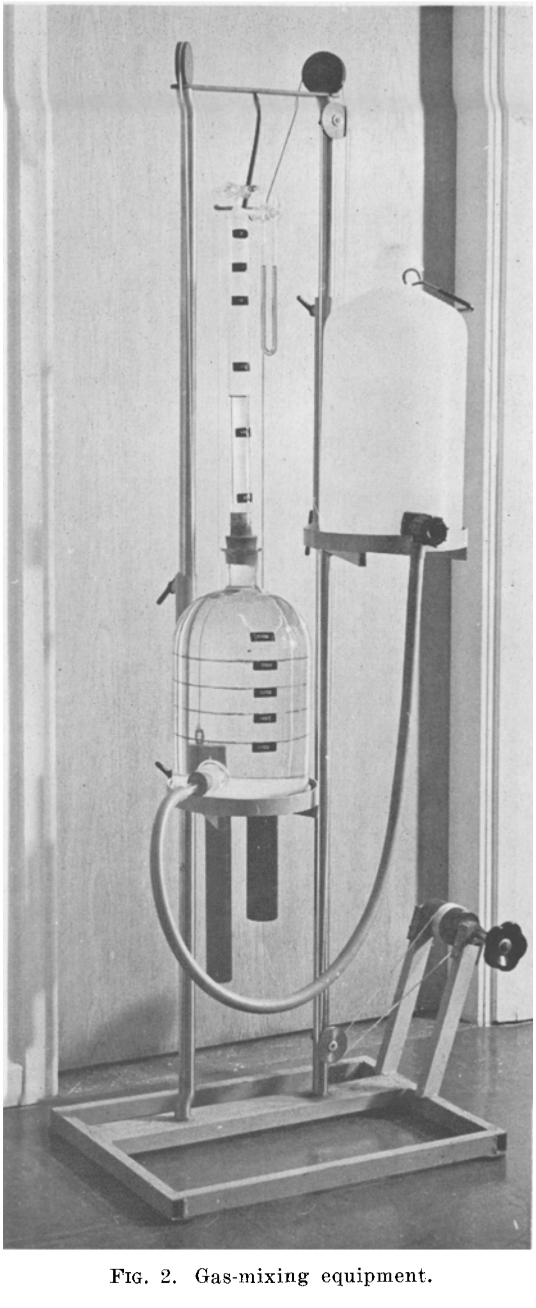

FIG. 2. Gas-mixing equipment.

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[2] The apparatus will be manufactured by Shandon Ltd., England.

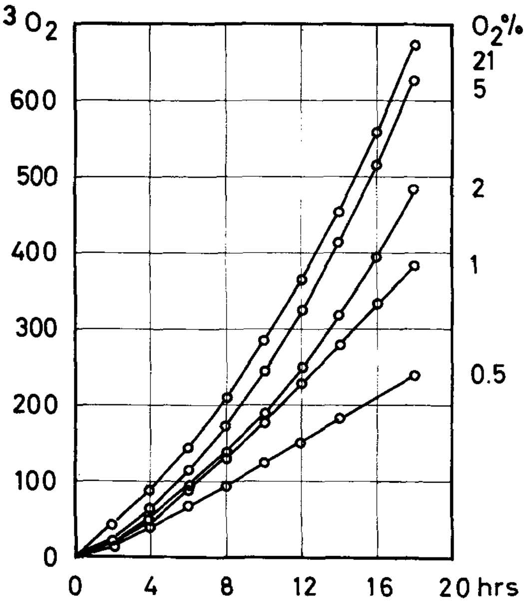

FIG. 3. The O_2-consumption of linoleic acid oxidation as a function of time at various O_2-concentration, 0.214 M linoleic acid, 25C, pH 6.

Substrates

The substrates used in these experiments were emulsions of fatty acids and esters, emulsified by ultrasonic treatment after addition of 0.17% of Tween 20 and 0.1 M phosphate buffer (pH 5–pH 7).

The fatty acids and esters were obtained from Fluka, Buchs, Switzerland, and from Mann, New York. For preliminary experiments, linoleic acid, "Fluka, natürlich" was used. This product was obtained in 100-ml bottles, filled in 1–10 ml ampoules under nitrogen, and stored at refrigerator temperature in the dark. Before the experiments the peroxide value was checked by iodometric titration. No higher peroxide value than 10 was tolerated. In such a higher quality substrate as "Fluka, puriss" the peroxide value was at the most only a few units, and the substances obtained from Mann showed at the most only a slight positive reaction.

Evaluation of the Results

The course of oxidation in a system as described, shaken in the Warburg apparatus, is, as a rule, more or less accelerated and without any appreciable induction period (Fig. 3). In can be influenced by a limiting effect on account of the rate of diffusion of oxygen into the sample, especially at low oxygen pressure (Figs. 3 and 4).

A simple way of expressing the effect of lowering of the oxygen pressure upon the rate of fat oxidation is to calculate the relative value of oxygen consumption at low oxygen pressure during a certain period of time relative to the oxygen consumption in air. The duration of the experiments referred to was generally 18 hours.

Owing to the accelerated course of the oxidation, at least in air, and the increasing influence of diffusion of oxygen with time in the case of low oxygen pressure, the relative values thus obtained are dependent upon the time reference chosen.

Comparing different systems on the basis of the same oxygen consumption in air (during a certain period of time), it was found that, in spite of the identical amount of oxygen consumed, the course of the oxidation during this period, especially the rate of oxygen consumption at the end of the period, might be different.

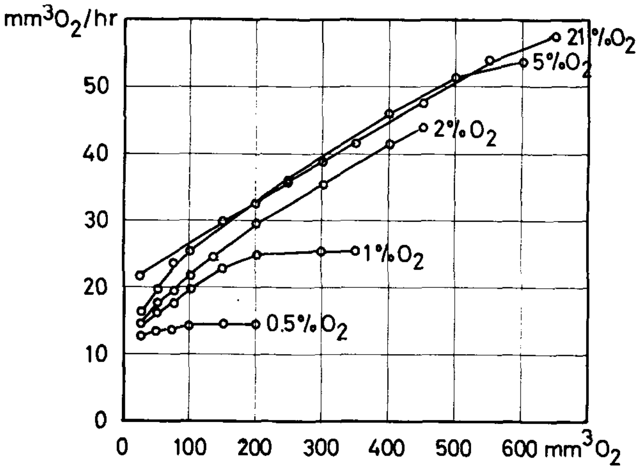

FIG. 4. Rate of oxidation of linoleic acid as a function of total O_2-consumption. 0.214 M linoleic acid, 25C, pH 6.

Therefore, with respect to the significance of the diffusion of oxygen shown below, it appeared preferable to base the evaluation upon the rates attained after a certain period of time and to calculate relative rates of oxygen consumption at low oxygen pressure, again relative to the rate in air. For comparisons of the dependence of oxygen pressure in different systems, these relative rate-values were calculated for the same rate in air. When evaluated on this basis, the results were found to be independent of the overall speed of the reaction (see below).

Rate values were obtained by approximating to a straight line the oxygen uptake for a short period (e.g., 2 hours) after a certain duration of the experiment.

In order to be able to base comparisons upon the whole course of the oxidation process, an attempt was made to apply the rate equation recently used by Quencer (14), for calculation of rate-constants. The rate of an autocatalyzed reaction, being a function of the degree of oxidation, i.e., oxygen consumption per unit of time (y) being a function of total oxygen consumption (x), it can be expressed by $y = k \cdot x^a$ (k and a = constants). On log-log-paper the rate constant k is given by the intercept on the y-axis. The value of *a*, represented by the slope of the curve, expresses the acceleration of the reaction. The values for *a* and k can be found either by drawing a best-fit straight line on log-log-paper or by calculation by means of the least-squares method. For this purpose a SAAB-D 21-computer was used.

Results

Expressing the experimental data by means of the rate equation $y = k \cdot x^a$, satisfactory results were obtained for oxidation in air, i.e., the experimental and the calculated values were in good agreement. However, in the case of low oxygen pressure, the equation is less suitable (Fig. 18).[3] It consequently appeared more appropriate to base evaluations and comparisons upon the calculation of relative rates discussed above.

The Influence of the Absolute Rate of Oxidation upon the Relative Rate at Low Oxygen Pressure. The relative rate of oxidation at low oxygen pressure in the emulsions is dependent upon the diffusion of oxygen. The connection with the phenomenon of diffusion could easily be demonstrated. Although the speed and amplitude of shaking did not considerably affect the oxygen uptake in air, it had a distinct effect

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[3] Discussed on page 407.

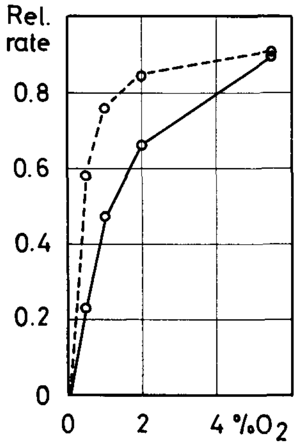

FIG. 5. Relative rate of oxidation of linoleic acid as a function of O_2-concentration for ——— 0.214 M linoleic acid, - - - - 0.035 M linoleic acid (25C, pH 6), calculated for the rate in air during the period 16–18 hr: 0.035 M: 15 mm³ O_2/hr, 0.214 M: 55 mm³ O_2/hr.

upon the rate of oxidation at low oxygen pressure.

The limiting effect of the diffusion of oxygen upon the oxidative process depends upon the ratio between the rate of diffusion and the rate of reaction. The rate of diffusion can be taken as constant for a certain pressure of oxygen and standardized conditions of shaking. The influence of the absolute rate of reaction upon the relative rate at low oxygen pressure was studied by variation of the fatty acid content of the emulsions.

The relative rate of oxygen consumption at low oxygen pressure was found to decrease with increasing concentration of the substrate, i.e., increasing rate of reaction, as shown in Fig. 5 for a slower and a more rapid reaction. The relative rates shown in Fig. 5 were calculated for the period 16–18 hours when the rates in air were 15 and 55 mm³/hr respectively.

When these two reactions were compared for equal rates in air (30 mm³/hr), attained after different time-periods (36–38 and 7–9 hours respectively), the values for the relative rate at low oxygen pressure were the same, as demonstrated in Fig. 6. The relative rate value, thus, is independent on the over-all speed of the reaction. Practically the same relative rates were obtained for identical rates in air of the emulsions of different fatty acid content.

Fig. 7 shows the dependence of the relative rate on the rate in air at an oxygen level of 1% for emulsions of varying fatty acid content.

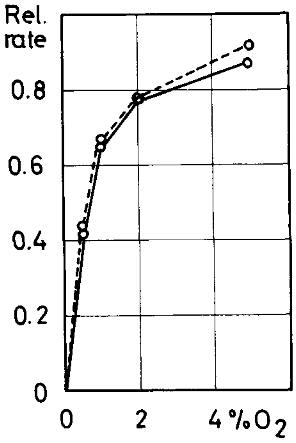

FIG. 6. Relative rate of oxidation of linoleic acid as a function of O_2-concentration for ——— 0.214 M linoleic acid, - - - - 0.035 M linoleic acid (25C, pH 6), calculated for the same rate in air (30 mm³ O_2/hr), attained for 0.214 M after 7 hr, for 0.035 M after 36 hr.

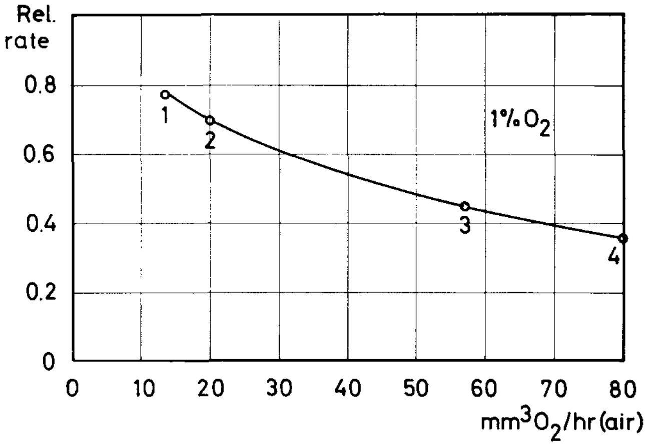

FIG. 7. Relative rate of oxidation of linoleic acid in 1% O_2 as a function of the rate in air (16–18 hr). Concentration of linoleic acid: 1) 0.035 M, 2) 0.053 M, 3) 0.214 M, D) 0.280 M.

The Influence of the Reactivity of the Substrate upon the Rate at Low Oxygen Pressure. Consequently, in a study of the effect of low oxygen pressure on the oxidation of substrates of various reactivity such as oleic, linoleic, and linolenic acid as well as their esters, the varying influence of diffusion at different rates of reaction had to be taken into account. The various substrates were investigated as far as possible at the same rate of oxidation in air by adjusting the concentration of the fatty acids and esters in the emulsion. Linoleic acid and linolenic acid were compared at the concentration of 0.214 M (Fig. 3) and 0.036 M (Fig. 8) respectively. These emulsions had practically the same consumption of oxygen in air during 18 hours, but the shape of the curves was not the same, as can be seen by comparison of Figs. 3 and 4, on one hand, and Figs. 8 and 9, on the other. As compared with linoleic acid, the oxidation of linolenic acid is relatively slower in the beginning, but more accelerated later on.

The relative rates of oxidation at low oxygen pressure were found to be different for linoleic and linolenic acid. This is demonstrated in Fig. 10, which shows relative values for the oxidation of linoleic and linolenic acid at low oxygen pressure, calculated on

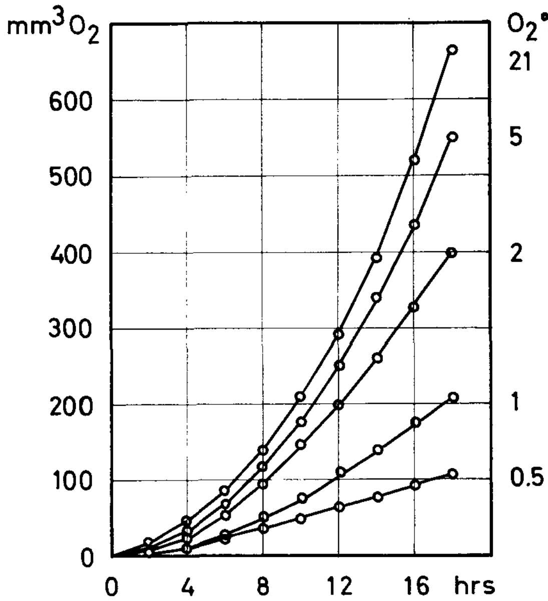

FIG. 8. The O_2-consumption of linolenic acid oxidation as a function of time at various O_2-concentration, 0.033 M linolenic acid, 25C, pH 6.

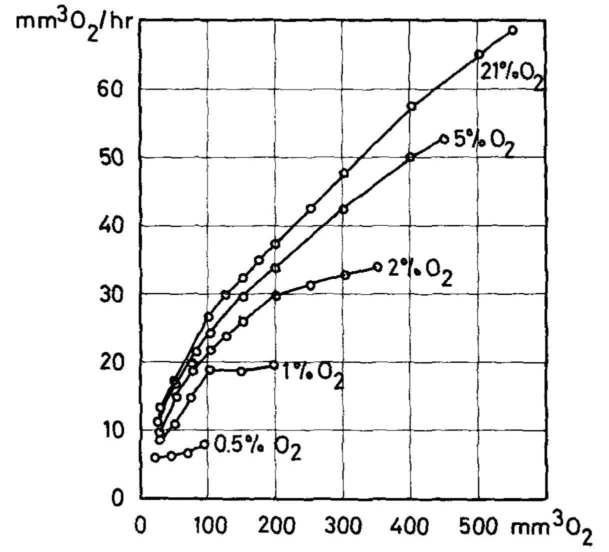

FIG. 9. Rate of oxidation of linolenic acid as a function of total O_2-consumption. 0.033 M linolenic acid, 25C, pH 6.

the basis of the same rate in air. The dependence on oxygen pressure is greater for linolenic acid.

In experiments with oleic acid emulsions it was found to be smaller. Relative values for substrates, such as oleic acid, naturally had to be compared at a lower rate level. In Fig. 11 the relative rates at low oxygen pressure of 2 substrates with different reactivity—linoleic and linolenic acid—are compared on different rate levels, due to different concentration of the substrate.

Similar observations were made regarding the dependence of the rate of oxidation of fatty acid esters on oxygen pressure. The reactivity of the ethyl or methyl esters of linolenic acid is less than that of the acid and their oxidation, consequently less dependent on oxygen pressure (Fig. 10).

The Influence of Temperature upon the Rate at Low Oxygen Pressure. The experiments were usually carried out at 25C. In order to study the significance of temperature for the relative rate of oxidation at low oxygen pressure, investigations were made at temperatures between 25C and 40C. The difference in the influence of the pressure of oxygen at different rates of reaction had to be considered, and the reaction rates of oxidation at low oxygen pressure had to be compared at the same rate in air. Again the shape of the curves of oxidation in air was somewhat different in spite of comparable substrate concentration (Fig. 12): at higher temperatures relatively slower in the beginning but more accelerated later on. The dependence on oxygen pressure increased with increasing temperature (Figs. 12 and 13).

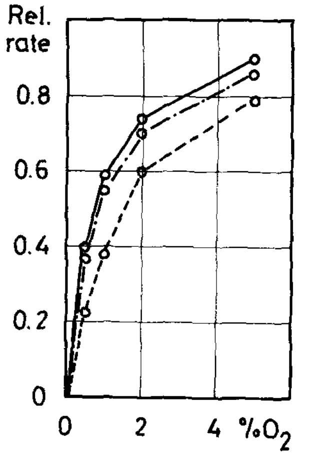

FIG. 10. Relative rate of oxidation as a function of O_2-concentration for ——— linoleic acid, –.–.–. linolenic acid ethyl ester, –––– linolenic acid, calculated for the same rate in air (40 mm³ O_2/hr), 25C, pH 6.

The Influence of Light upon the Rate at Low Oxygen Pressure. Further, the effect of lowering oxygen pressure was studied in the case of light irradiated oxidation. In general, the experiments were carried out with the samples protected as far as practically possible against the influence of light. No considerable difference in oxygen consumption was observed when the samples were shielded more effectively against light.

The reaction vessels were irradiated by light from incandescent lamps (Lumalampa SW 53 40W 3000 lx). Such irradiation doubled the rate of oxidation in air. When the relative rate of oxidation at low oxygen pressure was compared in pairs of non-irradiated and irradiated samples with the same rate of oxidation in air, these pairs showed practically the same dependence on oxygen pressure (Fig. 14). The difference between the results obtained for thermally accelerated oxidation on one hand and photo-oxidation on the other is discussed below.

The Influence of pH upon the Rate at Low Oxygen Pressure. The significance of the pH value for the effect of low oxygen pressure was studied in the range of pH 5 to 7. The standard value in these experiments was pH 6. Oxygen consumption as a function of pH shows a maximum at about pH 6 (Fig. 15a). The oxygen consumption at the different pH-values is however not exactly comparable as the course of the oxidation is somewhat different (Fig. 15b). At pH 5, oxidation is at first slower than at pH 6; later on, it is more accelerated. This phenomenon is reminiscent of the different course of oxidation obtained for a relatively more reactive substrate, e.g., linolenic acid, and oxidation at a higher temperature. As in all such cases of a more pronounced induction, the effect of lowering the oxygen concentration on the rate of oxidation was stronger at pH 5 (Fig. 15b).

Also at pH 7, oxidation is slower that at pH 6, and the shape of the curve for oxygen consumption is different. After a relatively rapid initial period, the uptake of oxygen is somewhat retarded; afterwards it is accelerated again.

At pH 7, at at pH 5, the decreasing effect of lowering the oxygen pressure upon the rate of oxidation is more pronounced than at pH 6 (Fig. 15b).

The relative rate at different pH, obtained in the experiment shown in Fig. 15b, was at pH 5: 0.58,

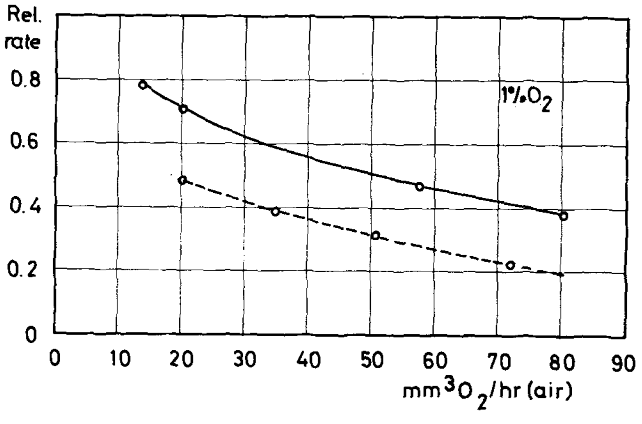

FIG. 11. Relative rate of oxidation in 1% O_2 as a function of the rate in air (16–18 hr) for ——— linoleic acid (same as Fig. 7), –––– linolenic acid (25C, pH 6).

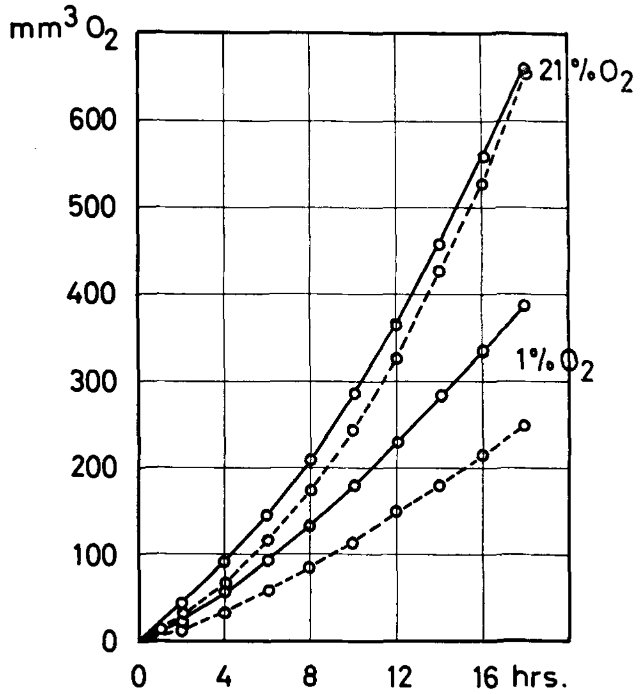

FIG. 12. The O_2-consumption of linoleic acid oxidation in air and in 1% O_2 as a function of time at 25C ——— 0.214 M linoleic acid, at 40C ---- 0.011 M linoleic acid, pH 6.

at pH 6: 0.69, and at pH 7: 0.56 (calculated on the same rate in air: 18 mm³/hr).

Discussion

These results show that the dependence of the rate of fat oxidation on oxygen pressure in emulsions is in part attributable to conditions inherent in the chain-reaction system of autoxidation and in part attributable to the limiting effect of diffusion of oxygen. The influence of oxygen diffusion in the system under study varied from insignificant to strong, depending upon the circumstances.

For systems not influenced by oxygen diffusion, the rate of oxidation can-according to Bolland-be expressed as a function of oxygen pressure (p_{O_2} = partial pressure of oxygen) by the equation:

$$\frac{d(O_2)}{dt} = k\,(RH)\,(ROOH)\,f(p_{O_2}),\ \text{where}$$

$$f(p_{O_2}) = \frac{(p_{O_2})}{n + (p_{O_2})}$$

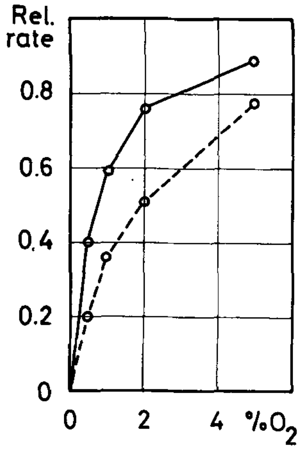

FIG. 13. Relative rate of oxidation of linoleic acid as a function of O_2-concentration at 25C ———, at 40C ----, calculated for the same rate in air (40 mm³/hr) pH 6.

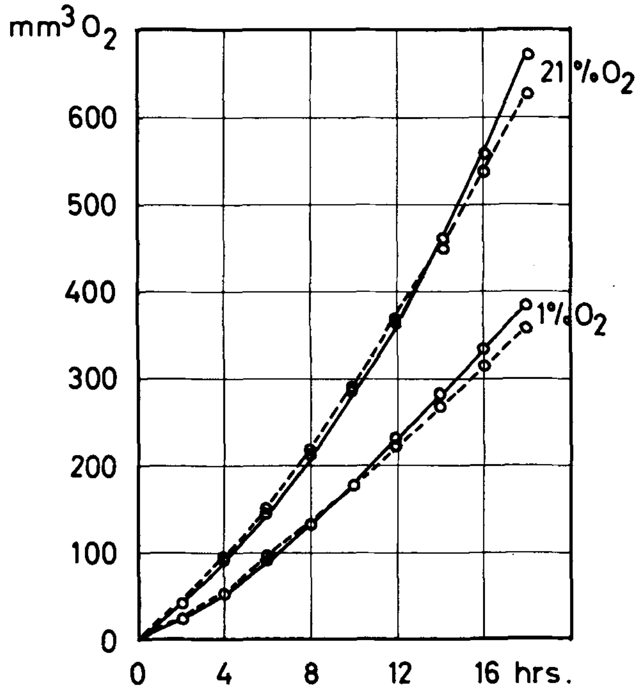

FIG. 14. The O_2-consumption of oxidation of linoleic acid in air and in 1% O_2 as a function of time without illumination ——— 0.214 M linoleic acid, with illumination ---- 0.035 M linoleic acid, pH 6.

k and n being experimental constants.

The relation between oxygen pressure and rate of oxidation is dependent especially upon the two main reactions of chain propagation and their activation energy:

			Activation energy
I .	R*	$+ O_2 \longrightarrow$ ROO*	4– 5 kcal/mole
II.	ROO*	$+$ RH $\longrightarrow$ ROOH + R*	8–14 kcal/mole

Under ordinary conditions, propagation is controlled by Reaction II, which is not dependent on oxygen pressure. Generally, Reaction I is much faster than Reaction II, and the reduction of the over-all rate of oxidation is relatively small when oxygen pressure is decreased. Under certain conditions however Reaction II is favored and Reaction I is getting a more rate-limiting character. In such cases the dependence on oxygen pressure is enhanced, and a reduction of the rate of oxidation may be obtained at relatively high values of oxygen pressure. In other words, the decrease of the relative rate of oxidation

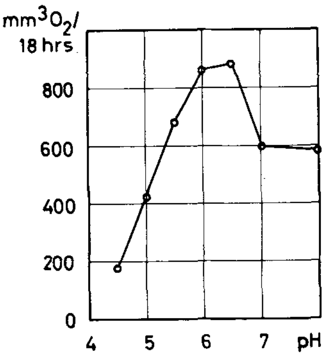

FIG. 15a. The O_2-consumption (18 hr) of oxidation of linoleic acid in air at various pH 0.214 M linoleic acid, 0.1 M phosphate buffer, 25C.

owing to the lowering of oxygen pressure is enhanced. Such an effect favoring Step II may be caused by the reactivity of the substrate (10). This is in conformity with the results reported above, showing a relatively greater rate decreasing effect of lowering oxygen pressure in the case of a more reactive substrate, e.g., linolenic acid, than a less reactive one, e.g., linoleic acid (Figs. 10 and 11).

Further, the dependence of the rate of oxidation on oxygen pressure was enhanced with increasing temperature, as shown in the experiments at 25C and 40C (Figs. 12 and 13). This phenomenon has also been reported by Bateman (10) for the system and conditions he studied. It can be explained by the relatively large temperature coefficient of Reaction II compared with the other steps of propagation and termination. It may also be a result more or less of oxygen solubility: the lower oxygen content of the emulsion at higher temperature reinforces the limiting influence of the diffusion of oxygen.

The greater rate-decreasing effect of lowering oxygen pressure in a more reactive substrate or at a higher temperature may be correlated to a certain change of the shape of comparable curves of oxygen uptake: at first slower, later on more accelerated. Similar changes were observed on variation of the pH, especially when the pH was lowered from 6 to 5. It may be supposed that the changed conditions at pH 5 have a similar influence upon the rate-decreasing effect of the lowering of the oxygen pressure as greater reactivity and higher temperature and thus explain the results reported above (Fig. 15b).

In the experiments on the influence of light, the relative values at low oxygen pressure were the same for irradiated as for nonirradiated samples (Fig. 14). According to Bateman and Gee (7), photo-oxidation and thermal oxidation differ in their mode of initiation. In photo-oxidation the chain mechanism proceeds with the photolytic generation of free radicals. However light irradiation has no effect upon the reactions of propagation and termination. Consequently acceleration of oxidation by illumination of the sample should not cause any change in the relative rate of oxidation at low oxygen pressure.

In contrast with Bolland, Karel worked under conditions of a well-defined limited surface area and consequently with diffusion of oxygen as a rate-limiting factor. Therefore constant rates of reaction were obtained. The relationship between these constant rates of oxidation (R_o) and the partial pressure of oxygen (pO_2) was expressed by the equation:

$$R_o = \frac{1}{k_1 + k_2/(p_{O_2})} = \frac{(p_{O_2})}{k_2 + k_1/(p_{O_2})}$$

The constants k_1 and k_2 vary with the surface area and with the temperature. A certain similarity exists between the expressions of Bolland and Karel for the dependence of the rate of oxidation on oxygen pressure.

Whereas, according to Bolland, the rate of oxidation in their experiments is not limited by the diffusion of oxygen and whereas the conditions studied by Karel are characterized by pronounced limitation because of more or less slow oxygen diffusion, the experiments on fat oxidation in emulsions herein reported are complicated by the varying limiting effect of diffusion of oxygen upon the rate of reaction.

Oxidation in air was not limited by diffusion of oxygen, but oxidation at a lower concentration of oxygen could be. The degree of limitation because of slow diffusion of oxygen increased with decreasing oxygen concentration. As a consequence, the course of oxygen uptake became increasingly less accelerated with decreasing oxygen pressure. This phenomenon is enhanced at higher concentration of the substrate and longer duration of the experiment, i.e., at higher

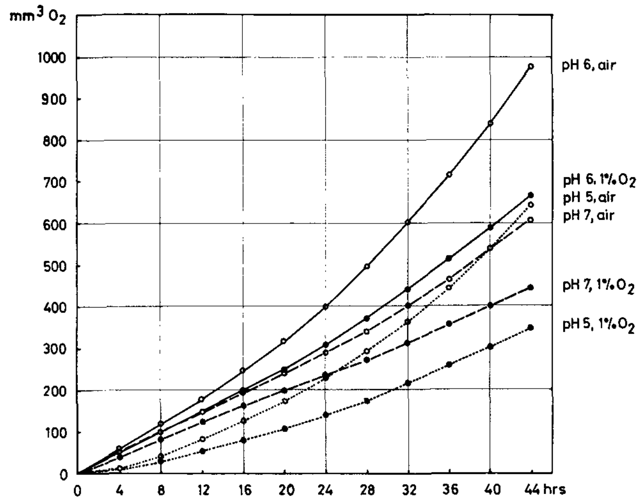

FIG. 15b. The O_2-consumption of oxidation of linoleic acid in air and in 1% O_2 as a function of time at pH 5 ----, pH 6 ———, pH 7 — — —, 0.035 M linoleic acid, 0.1 M phosphate buffer, 25C.

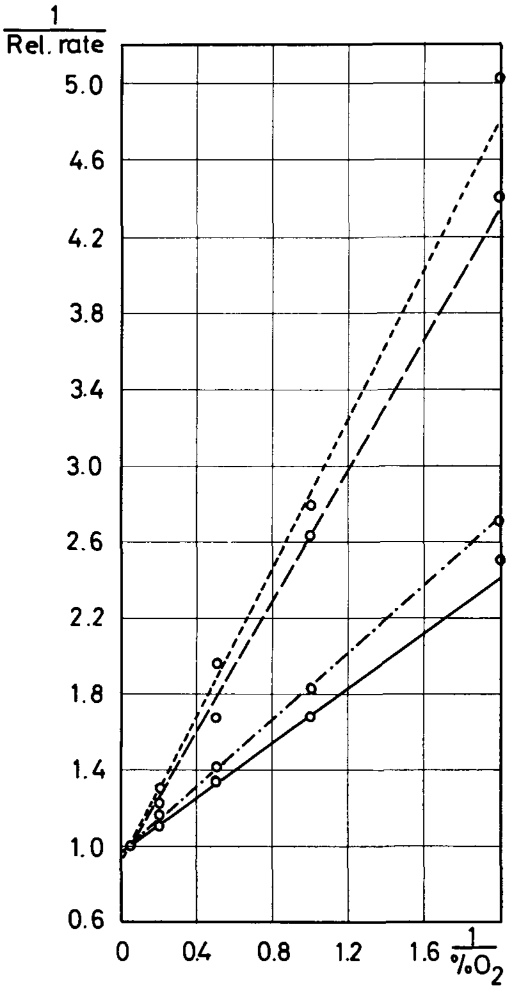

FIG. 16. Inverted values of relative rate of oxidation as a function of inverted values of O_2-concentration: ——— linoleic acid, 25C, –.–.– linolenic acid ethyl ester, 25C, — — — linolenic acid, 25C, ---- linoleic acid, 40C, calculated for the same rate in air (40 mm³/hr), pH 6.

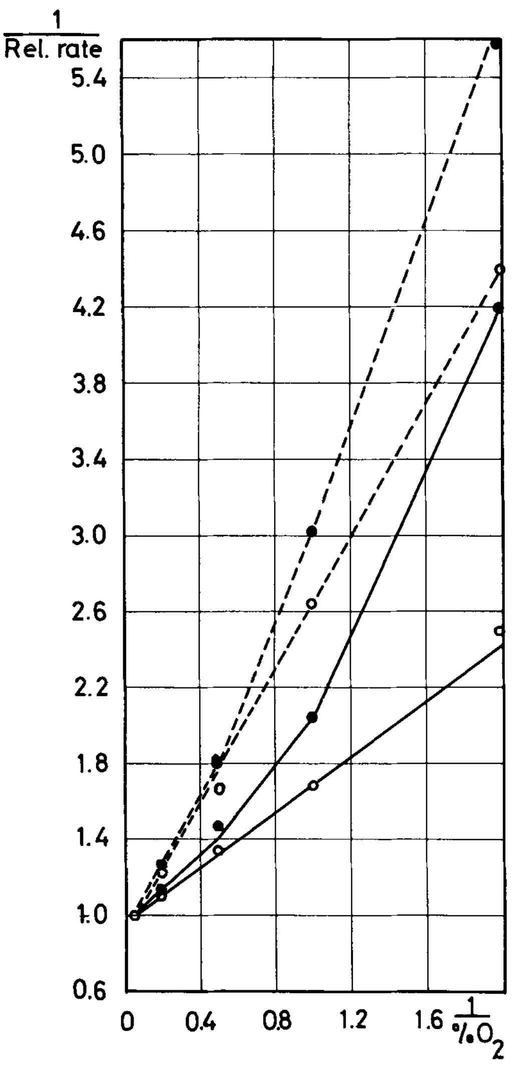

FIG. 17. Inverted values of relative rate of oxidation as a function of inverted values of O_2-concentration: ——— linoleic acid, – – – – linolenic acid, calculated for the same rate in air: 40 (○–○) and 55 (●–●) mm^3/hr respectively, 25C, pH 6.

rates of reaction when the ratio between the rate of reaction and the rate of diffusion is becoming more significant.

As the rate of diffusion is influenced by the efficiency of shaking, this factor was kept as constant as possible. A series of measurements of diffusion of oxygen into samples shaken in Warburg vessels was carried out by means of a Beckman oxygen electrode (results to be published elsewhere). The results showed that constancy of the shaking conditions is essential. Certain discrepancies of secondary importance may result from not completely comparable shaking.

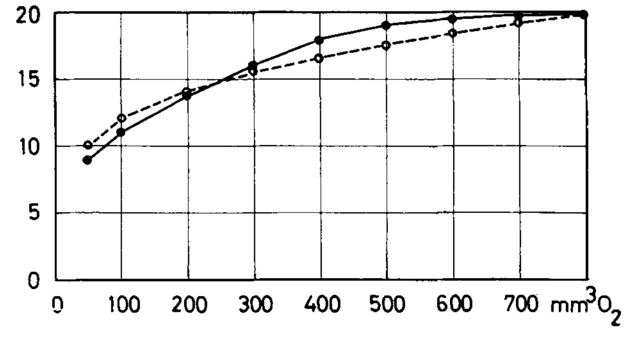

FIG. 18. Rate of oxidation of linoleic acid as a function of total O_2-consumption, in 1% O_2: ——— experimental data, – – – – calculated values ($y = k.x^a$), 0.035 M linoleic acid, 25C, pH 6.

TABLE I

a-Values of the Rate Equation $y = k.x^a$ Applied to the Experimental Data of O_2-Consumption of Linoleic Acid at Various O_2-Concentration[a]

O_2-Concentration	21%	10%	5%	2%	1%	0.5%
a-Value	0.34	0.36	0.33	0.22	0.07	0.02
Mean square deviation	0.035	0.026	0.039	0.037	0.081	0.106

[a] At low O_2-concentration the applied rate equation is less suitable, as shown by the increasing mean square deviation.

The similar expressions of Bolland and Karel for the dependence of the rate of oxidation on oxygen pressure, both representing hyperbolic curves, were obtained by plotting inverted values of relative rates or rate constants against inverted values for the pressure of oxygen: approximately straight lines could be drawn.

Such a procedure does not yield straight lines for an oxidation which increasingly is limited due to the influence of diffusion of oxygen. Only during an early period (e.g. up to a rate of 40 mm^3/hr) can an approximately straight line be obtained (Fig. 16). When the reaction proceeds (e.g. to a rate of 55 mm^3/hr), the limitation because of slow diffusion of oxygen causes a deviation from the straight line (Fig. 17).

As mentioned above, the expressing of the experimental data by means of the rate equation $y = k \cdot x^a$, which gives satisfactory results for oxidation *in air*, is less suitable for *low oxygen pressure.* This is caused by the rate-limiting influence of the slow diffusion of oxygen. The values calculated for k showed an apparent increase with decreasing oxygen pressure, which does not correspond to any such real tendency. In Fig. 18 experimental data are compared with values calculated by means of the equation: at low oxygen pressure the curve of the experimental values, at first accelerated, slowly passes into a course of constant rate on account of the rate-limiting influence of oxygen diffusion. The calculated values however show a certain deviation from the experimental ones; the complicating influence of oxygen diffusion is ignored with somewhat incorrect values for k consequently. As a positive result of these attempts, the values calculated for a, indicating the degree of acceleration, can serve as an expression for the influence of diffusion of oxygen: these values considerably decrease with decreasing oxygen pressure (Table I).

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