The Stability of Vegetable Oils
II. Apparatus for Determination of the Rate of Fading of Methylene Blue-Fat Systems

By W. G. BICKFORD and K. S. MARKLEY[1]
U. S. REGIONAL SOYBEAN INDUSTRIAL PRODUCTS LABORATORY, URBANA, ILLINOIS[2]

WHILE engaged in an investigation of the nature and cause of the flavor and odor deterioration of refined soybean oil, a number of methods, including the accelerated oxidation method of King, Roschen, and Irwin (1), were examined with respect to their applicability to the determination of the stability of this oil. None of these methods proved to be entirely satisfactory for determining the stability of soybean oil, but of the several methods which were examined, the so-called methylene blue test appeared to merit further consideration despite its empirical application by previous workers and the fact that the reactions involved in the reduction of the dye in the methylene blue-fat system were incompletely understood.

Whitehead (2) published an account of the phenomenon of the fading of methylene blue in milk which was exposed to sunlight, and about the same time Greenbank and Holm (3) reported the results of a comparative examination of a sample of milk fat at intervals of several weeks, by the direct oxygen absorption and by a photochemical method involving the reduction of methylene blue. These authors described an apparatus consisting of a source of radiation, reaction cell, photoelectric cell, and current amplifying unit. No effort was made to control the light intensity, infrared radiation, or temperature of the reaction cell. In operation the sample to be tested was mixed with 1 to 2 ml. of a 0.025 percent alcoholic methylene blue solution, after which it was placed in the reaction cell and the time recorded from the instant the light was applied until sufficient light passed the cell to actuate a relay and operate a buzzer signal. The length of this time interval was taken as a measure of the relative stability of the fat under examination. Although the authors determined *only the total time* required to effect a specified degree of dye reduction, they nevertheless concluded that "The *rate of reduction* of methylene blue in a fat or oil when catalyzed by light may serve as a measure of the rate of reaction of the initial oxidative processes and may, therefore, be utilized to determine the relative susceptibilities of fats and oils to oxidation."

Royce carried out a more extended investigation of the methylene blue-fat reaction and compared the results of the dye reduction method with several other accepted methods of determining the stability of fats and oils. In his original investigation of the dye reduction in the methylene blue-oil system, Royce (4) employed a photoelectric method but concluded in a later publication (5) that it was preferable to judge the end point of the dye reduction visually rather than by photoelectric means even though the accuracy in the former instance depends entirely upon the personal factor. This conclusion was based upon the observation that many natural oils and all hydrogenated oils gave secondary color changes which interfered with the precise determination of the point of total dye reduction, although various filters were tried in an attempt to obviate this difficulty.

Other workers, notably Davies (6) and Aikins and Fay (7), have investigated the reaction between methylene blue and various fat systems and have advanced several hypotheses concerning the probable nature and function of the several constituents of the fat-dye system. Since the work of these investigators is more pertinent to the reaction mechanism than it is to the method of measuring the rate of dye reduction, the discussion of this work has been omitted here and instead is included in another communication of this series which is concerned primarily with the various reactions involved in the dye reduction of the methylene blue-oil system.

Previous investigators have taken the entire time necessary to effect total visible dye reduction as a measure of the stability of a fat or oil, but it is obvious that it would be more advantageous to follow continuously the instantaneous changes occurring in the dye concentration and thereby obtain evidence regarding the rate, as well as the total time, of the dye reduction.

In order to measure the rates, as well as the relative total times of dye reductions of the various methylene blue-oil systems under investigation, it became necessary to refine and improve the methods and techniques previously described in the literature by controlling or eliminating certain variables, such as the fluctuation in line voltage across the light filament, the effect of temperature, infrared radiation, and the limitations imposed by visual observation.

Accordingly, an apparatus was designed and constructed which was equipped with a voltage regulator, a filter to remove all infrared radiation, a reaction chamber jacketed to control the temperature, a photovoltaic cell, and an indicating device for accurately determining the rate of fading of the dye.

The present communication is primarily concerned with the details of the construction of the apparatus and the technique of determining the time-light intensity curves of a number of methylene blue-fat systems. The effect of such factors as the substrate, temperature, dissolved gases, aldehydes, and peroxides on the time-light intensity curves of the methylene blue reduction are also reported. No attempt has been made here to establish the mechanisms of the various reactions involved, or to establish the relation between the time-light intensity curves and the organoleptic stability of fats and oils, but these subjects will be treated in

[1] Now associated with the Southern Regional Research Laboratory, U. S. Department of Agriculture, New Orleans, Louisiana.

[2] A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

detail in further communications of this series. However, it will become evident that the apparatus and method which are described in the present publication provide a means of evaluating the relative stability of fats and oils.

EXPERIMENTAL

Apparatus

The assembled apparatus is shown in figure 1, and the methylene blue photometer is shown diagrammatically in figure 2. It consists of three metal compartments or housings containing, respectively, the light source, the absorption cell, and the photoelectric cell. The three compartments are placed in a straight line and are connected with metal tubing forming a light-tight system.

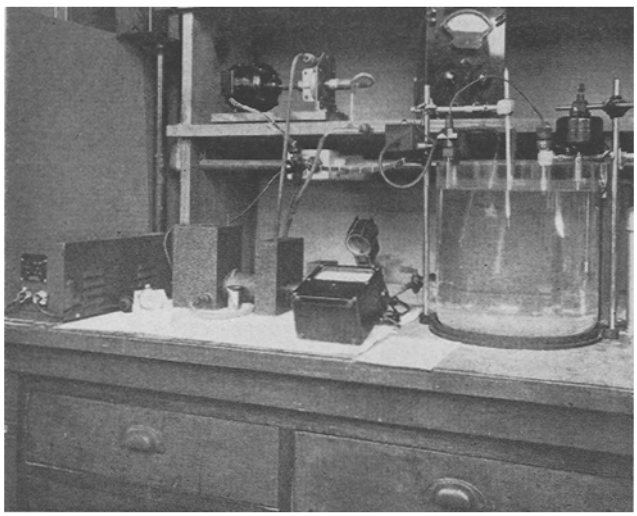

Figure 1.—Methylene blue photometer with auxiliary equipment.

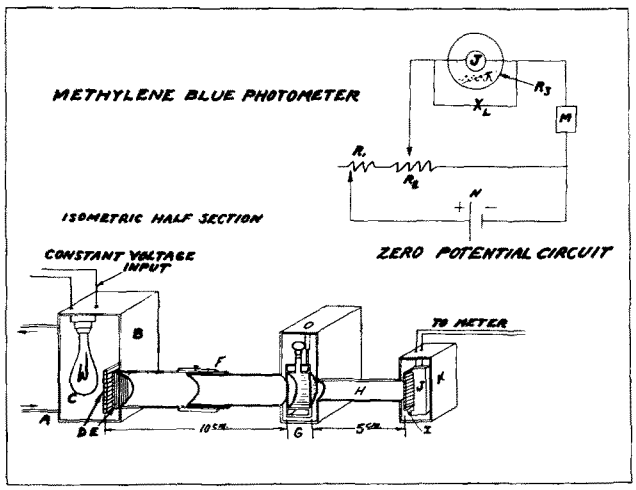

Figure 2.—Methylene blue photometer and zero potential circuit.

The ports of entry and exit for air used to cool the light source are located at A. B is the housing for the 100-watt frosted bulb C, the shutter D, and the infrared filter E (Corning No. 397). Tube F is divided into two sections and is provided with a sleeve so that its length may be varied, thus permitting adjustment of the light intensity at the absorption cell in compartment G. The diameter of this tube is such that it permits irradiation of the entire area of the methylene blue-oil solution in the absorption cell. The tube H communicates with the photocell compartment K, and is smaller than the area of the absorption cell facing the photocell J.

Filter I is a yellow shade glass (Corning No. 351) which absorbs very nearly the same band of frequencies as methylene blue transmits. This filter has a sharp cutoff at λ 5200 and absorbs the light below this wave length. The use of such a filter increases the percentage sensitivity of the system to changes in the red region in which methylene blue absorbs strongly. At the same time the photocell is protected from secondary color changes in the region below λ 5200 which may possibly occur in the oil during irradiation. This filter should not be placed before the absorption cell. In such a position it would exclude certain light which is essential for the proper progress of the fading reaction.

The line voltage supplying the 100-watt bulb was found to be subject to considerable variation and consequently affected the rate of dye reduction and the output of the photocell. In order to maintain constant intensity of the light emitted at C, the e.m.f. across the filament was maintained constant at 115 volts by means of a type VR3 Raytheon voltage regulator.

Instead of the potassium hydride type of photoemissive cell employed by Greenbank and Holm (3) and also by Royce (4) in their measurements of methylene blue reduction, a photovoltaic cell was used. The selection of the particular photovoltaic cell was predicated on the following considerations.

A spectrographic determination showed that maximum absorption of methylene blue is in the region λ 6100-6800, whereas the response of the potassium hydride cell is quite sharply peaked, with its major sensitivity in the spectral range λ 3500-4500. Furthermore, the sensitivity of the potassium hydride cell to white light, especially that from incandescent tungsten, is low (8). However, the metal oxide types of emissive cells are satisfactory for measurements in the red end of the spectrum.

The photovoltaic type of cell, with a threshold at λ 7400, has good response in the region of maximum absorption of methylene blue. Furthermore, it has the advantage of simplicity and compactness. Light intensity is not a limiting factor under the conditions prevailing in the apparatus since tungsten lamps have a high energy output in the red regions. For these reasons a type 1, Weston photronic cell, model 594, (9) was selected as being best suited for making the required measurements.

Although otherwise satisfactory for the measurement of the light transmitted by methylene blue or the methylene blue-oil system, the above-mentioned cell, in common with all barrier layer cells, suffers one disadvantage; namely, the internal resistance varies appreciably with changing light intensity. If a microammeter with an appreciable resistance serves as the load for such a cell, nonlinearity between light input and external current output results. With the cell which is employed in the present apparatus the effect is small at illumination intensities less than 50 footcandles at the photocell, with an external resistance of 500 ohms or less, but with increasing resistance or light intensity, departure from linearity becomes appreciable and seriously affects the measurement of the light falling on the photocell.

Microammeters of low internal resistance are expensive as well as delicate, and in order to overcome these handicaps it was necessary to select an instrument of relatively high resistance and compensate for the resulting nonlinearity. This was accomplished by selecting a model 430, d. c., Weston microammeter

having an internal resistance of 600 ohms. The non-linearity of current output as a function of light intensity at the photocell was compensated by means of a correction curve of the deviation which was established by measuring the current output as a linear function of light intensity with the secondary circuit described below.

This artifice, which was devised by Wood (10), consists essentially in reducing to zero the e.m.f. developed in the photocell by placing a counter e.m.f. across the cell terminals. Since the potential is zero, it follows that there can be no current leakage through the internal resistance of the photocell, and hence, there is no shunting effect on the resistance of the measuring device. The circuit is shown in figure 2. R_3 represents the internal resistance of the photocell. R_1 and R_2 are rheostats of 500 and 200 ohms, respectively. N is a 1.5-volt dry cell, and M is the microammeter. The circuit is balanced if closing and opening the shorting switch L causes no deflection in the meter reading.

This circuit, although not well adapted for following directly the fading of the methylene blue-oil system because of the necessity of balancing it each time a reading is taken, does make possible the construction of a correction curve for the meter readings taken with the primary circuit of the methylene blue photometer. The correction curve, whose values were found to remain accurate for long periods of time, is obtained by plotting the values of the current output of the photocell with the 600 ohm resistance microammeter in the circuit against the current output measured by the secondary circuit for a series of light intensities over the desired range of use. Reference to the curve thus established permits the correction of any observed set of readings made under ordinary conditions with the inexpensive but high resistance microammeter in the working apparatus.

It was found that the agreement between the corrected readings made with the high resistance microammeter and readings made directly with an expensive low resistance (6 ohms) microammeter in the circuit of the working apparatus was better than 99 percent. It should be noted that each photocell used must be individually calibrated and the correction curve used with the given photocell.

It was deemed essential for purposes of comparison that all samples receive an equivalent irradiation intensity. However, the tungsten lamps employed have "horseshoe" shaped filaments as a consequence of which the radiation is not uniform in all directions. Moreover, the efficiency of bulbs of the same rating is not constant. In order to ensure that the same irradiation intensity was always supplied to each sample, it was necessary to adopt a reproducible but arbitrary meter reading for purposes of standardizing the value of the incident light. A value of 180 microamperes (uncorrected) equivalent to 760 footcandles at the reaction cell was selected as the standardization factor.

The system was adjusted to the arbitrarily selected value by supplying current to the filament for a period of 20 minutes and allowing light to fall on the photocell after traversing the entire system, including both the infrared and yellow filters, but with the absorption cell omitted. At the end of 20 minutes the meter reading was adjusted to the selected value, namely, 180 microamperes, by adjusting the distance between the lamp and absorption cell housings (B and G, figure 2). Once this adjustment had been made, the light intensity supplied to the absorption cell was found to remain constant for very long periods of time and, in fact, required readjustment only when a light bulb or photocell was replaced.

The absorption cell is shown diagrammatically in figure 3. It was constructed entirely of pyrex glass. Optically flat disks were fused to cylinders of the indicated dimensions thus forming the reaction chamber and the water jacket. The distortion produced by the operation of sealing at the inner cylinder was small.

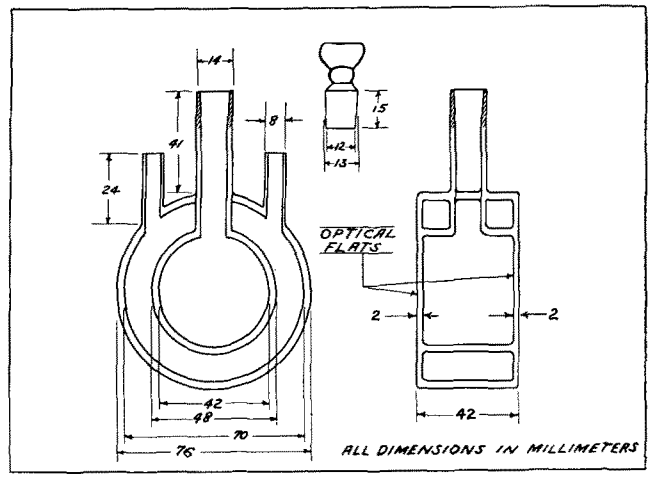

Figure 3.—Absorption cell.

Method and Technique

The apparatus described and illustrated above was applied in a study of the factors which affect the rate and capacity of methylene blue reduction in the presence of various lipoidal substances. Unless otherwise stated, the following procedure was used in determining the time-light intensity curves for the various dye systems described:

The photocell was given a preliminary irradiation for approximately 20 minutes in order to allow the system to approach equilibrium conditions and to reduce to a minimum the effects of photocell fatigue.

Two ml. of an alcoholic methylene blue chloride[3] solution (50 mg. of the dye in 100 ml. absolute ethyl alcohol) was added to 50 ml. of the substance under test which was contained in the absorption cell (figure 3). The cell was protected from light by a black felt cloth and was vigorously shaken for 2 minutes in order to ensure complete solution of the dye. The absorption cell was then placed in the apparatus, and water at 50° C. ± 0.5° C. was circulated through the jacket for a period of 20 minutes. Meanwhile the light was turned on, but the shutter between the lamp housing and absorption cell was kept closed to prevent exposure of the sample to radiation while it was being brought to proper temperature.

After the cell and its contents had been brought to the desired temperature, the shutter was opened and the sample exposed to the filtered radiation from the tungsten lamp. Microammeter readings corresponding to the light transmitted by the dye-oil system in the absorption cell were made at intervals and their corrected values plotted to give a time-light intensity curve. Under ordinary conditions with soybean oil in the cell, the methylene blue eventually was progressively reduced with the result that the percentage of

[3] The methylene blue employed was J. T. Baker Company's U.S.P. medicinal grade. It should be borne in mind that for comparative purposes, the use of methylene blue from one source is absolutely necessary, because dyes from different sources contain varying amounts of methylene blue chloride. The use of methylene blue chloride-zinc chloride double salt is not recommended, but methylene blue chloride (National Aniline and Chemical Company) for biological staining which contains 88 percent dye may be used.

the incident light transmitted by the system increased with increase in the time interval.[4]

For purposes of determining the approximate degree of dye reduction of a methylene blue-oil sample at any given instant, a curve (figure 4) of the corrected microampere readings as a function of dye concentration was constructed. The data for this curve were obtained by dissolving 2 ml. of a 0.05 percent alcoholic solution of methylene blue in 50 ml. of distilled water, and from this solution four additional solutions were prepared by dilution to yield dye solutions of 50, 25, 12.5, and 6.25 percent of the original concentration. Each of the five solutions was placed in the absorption cell in the manner described above, and the corresponding transmitted light intensities were measured in microamperes.

[4] Although the technique described here proved satisfactory for the present investigation, it was later found desirable to make certain changes when dealing with particularly unstable lipids. The improved technique differs from the one described here in the following details: The fatty material was placed in the reaction cell and maintained in the dark at 50° C. for 20 minutes. After the oil had come to temperature the dye solution was added, and mixing was accomplished in 2 minutes as before. The light was admitted to the system at the beginning of the third minute.

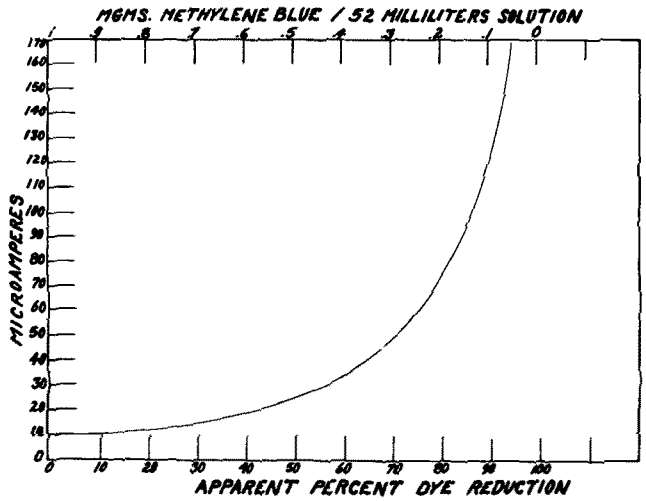

Figure 4.—Concentration-light intensity curve showing apparent percentage reduction of the standard methylene blue solution.

From the plot of the dye concentration vs. corrected microampere readings the approximate percentage of dye reduction of an unknown methylene blue-oil system could be determined from the observed microampere reading at any given moment.

It will be noted later that the first microampere reading obtained for a freshly prepared solution of 2 ml. of 0.05 percent methylene blue in soybean oil indicated a prereduction of the dye of nearly 50 percent at the time it was first exposed to radiation. When methylene blue solution was added to ethyl esters and alcohols under the same conditions, very little or no prereduction of the dye occurred. This prereduction, which is a dark reaction, is connected in some way with the susceptibility of the fat to deterioration but in what manner is not entirely clear at present.

Effect of Substrate

The behavior of methylene blue in the presence of ethyl alcohol, ethyl stearate, and ethyl oleate under the experimental conditions stated above are shown in curves 4, 5, 6, and 7 of figure 5. It is apparent from

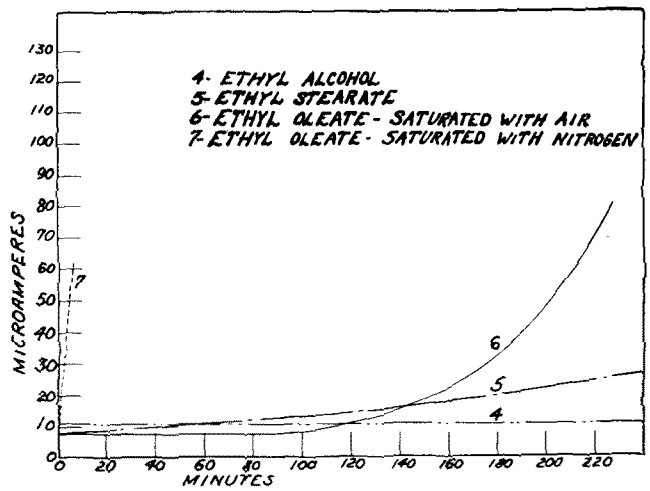

Figure 5.—Behavior of methylene blue in the presence of ethyl alcohol, ethyl stearate, and ethyl oleate.

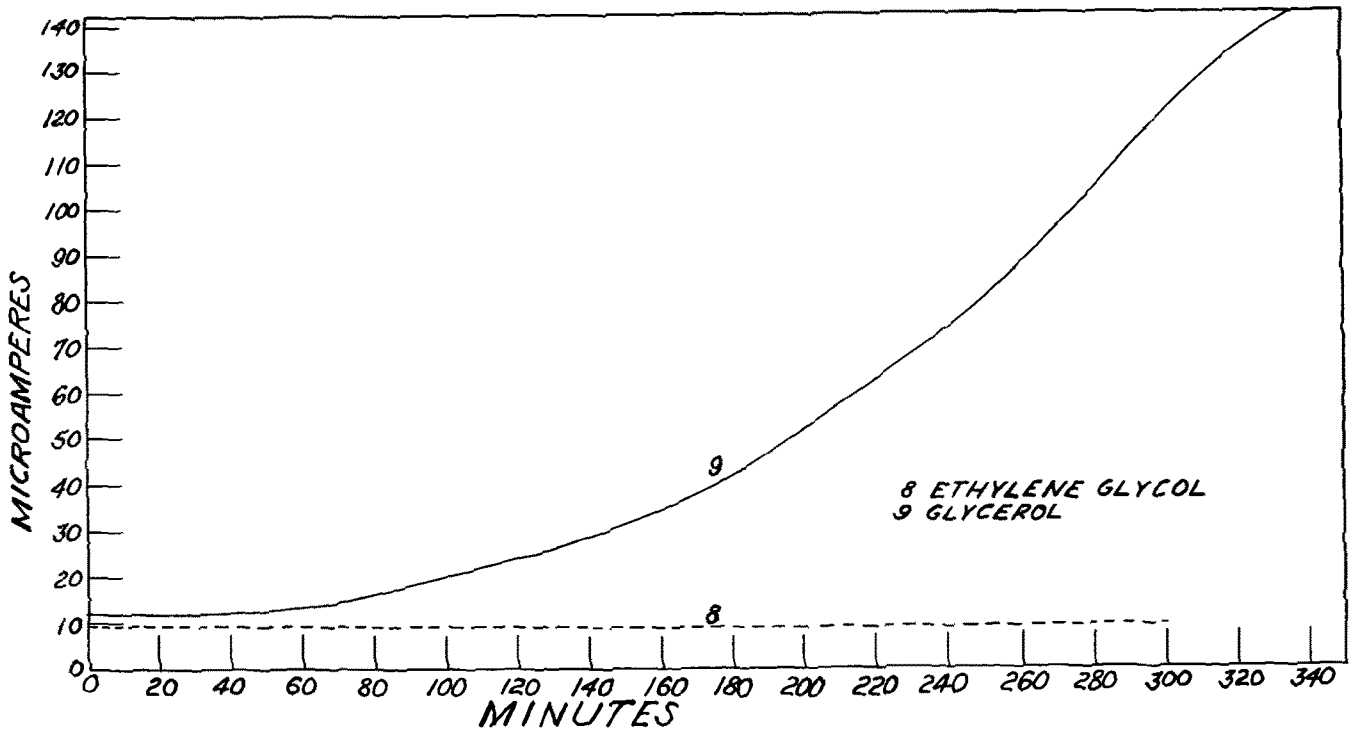

Figure 6.—Behavior of methylene blue in the presence of ethylene glycol and glycerol.

curve 4 that methylene blue in alcohol solution is quite stable in the presence of light.

In order to ensure the presence of oxygen, the samples of ethyl stearate and ethyl oleate were aerated prior to their examination in the apparatus. The slight but definite reduction of the methylene blue in the presence of ethyl stearate can be attributed to the lack of purity of the ester, since it represented a commercial product which was used without further purification.

The behavior of the methylene blue-ethyl oleate system [5] under the influence of the radiation is in sharp contrast to that of the methylene blue-alcohol and methylene blue-ethyl stearate systems. From the time-light intensity curve of the methylene blue-ethyl oleate it would appear that unsaturation is an important factor in determining the rate of dye reduction in a nonaqueous system as well as in aqueous systems as was first pointed out by Whitehead (2).

When the oxygen was removed from the ethyl oleate and replaced by nitrogen, reduction of the methylene blue took place with extreme rapidity as is evident from curve 7 of figure 5. Lasareff (11) and also Gebhard (12) have reported an analogous behavior for a gelatin-glycerol-methylene blue system in which they found that the methylene blue was reduced much more rapidly in a vacuum than in the presence of air during irradiation.

Methylene blue-ethylene glycol solutions behave quite similarly to methylene blue-ethyl alcohol solutions when irradiated under the same conditions, as is evident from a comparison of curve 8, figure 6, and curve 4, figure 5. Perrin and Choucroun (13) reported that an aqueous glycerol-methylene blue solution was reduced by light to the leuco form with the formation of an aldehyde. Reference to curve 9 of figure 6 indicates a similar reduction occurs in a nonaqueous methylene blue-glycerol system when irradiated under controlled conditions. The behavior of the three methylene blue-alcohol systems is in accord with the relative ease of oxidation of the corresponding alcohols.

[5] It was also noted that the increased light transmission was accompanied by the separation of a slight flocculent, colored precipitate in the absorption cell. Gebhard (12) has observed a similarly violet colored compound produced in a gelatin suspension of methylene blue and glycerol subsequent to *long* irradiation and reoxidation in the air. This compound was thought to be an oxidation product of methylene blue in which a $(CH_3)_2N-$ group had been replaced by a hydroxyl group.

Effect of Temperature

In order to evaluate the effect of temperature on the rate of dye reduction in the methylene blue-oil system, time-light intensity curves were made at four different temperatures. Four samples of the same refined and freshly deodorized soybean oil (50 ml.) and 2 ml. of alcoholic methylene blue chloride solution (25 mg. of dye per 100 ml. of absolute ethyl alcohol) were placed in the absorption cell as previously described, except that the jacket temperatures were maintained at 0°, 25°, 50°, and 70° C., respectively. The plots of the time-light intensity values for the four temperatures are shown in curves 10, 11, 12, and 13, respectively, of figure 7. It should be noted that the curves of the methylene blue reduction at 25° and 50° are quite close together and have nearly the same slope. This behavior was observed with all soybean oils of good quality which were examined at both temperatures. It should also be noted that the base level tends to be lower, the higher the temperature, i.e., in the first part of the reaction apparently less dye reduction occurs at higher than at lower temperatures. This is contrary to the expected behavior of such a system.

In order to determine the influence of dye concentration on the course of the methylene blue reduction, two samples of the same soybean oil (50 ml.) as employed in the previous experiments and 2 ml. of alcoholic methylene blue (50 mg. of dye in 100 ml. of absolute ethyl alcohol) were irradiated in the apparatus under standard conditions at 0° and 50° C.

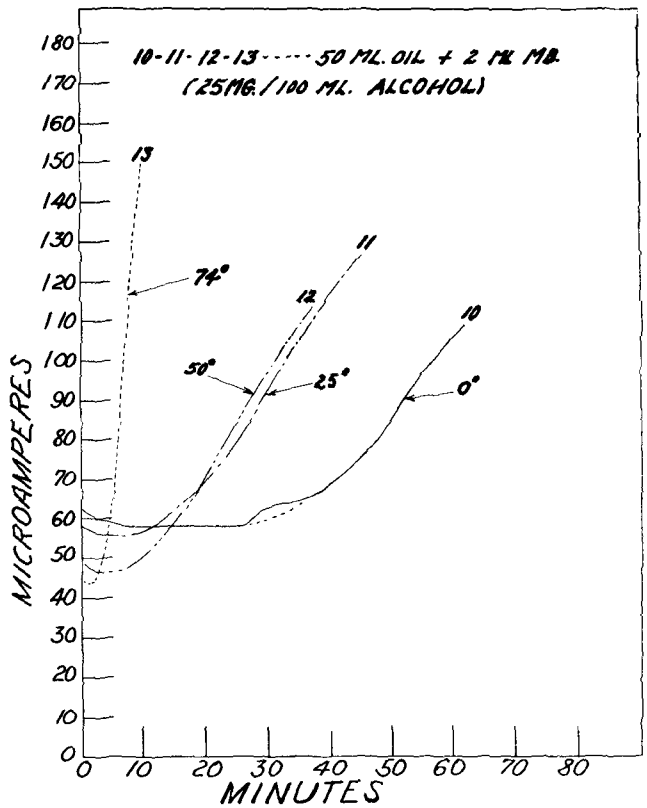

Figure 7.—Effect of temperature on the rate of dye reduction in the methylene blue-soybean oil system.

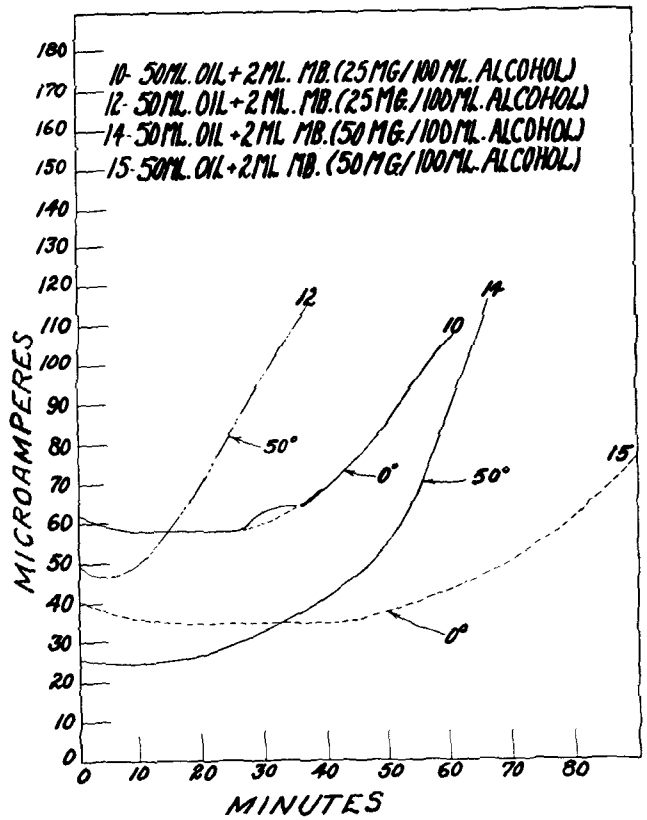

Figure 8.—Effect of dye concentration on the rate of dye reduction in the methylene blue-soybean oil system.

The time-light intensity curves (Nos. 10 and 12) for the two samples of soybean oil containing 2 ml. of alcoholic methylene blue (25 mg./100 ml.) and curves (Nos. 14 and 15) for the two samples of oil containing 2 ml. of alcoholic methylene blue (50 mg./100 ml.) are reproduced in figure 8. Reference to these curves indicates that doubling the dye concentration reduces the transmission threshold and increases the induction period, but it does not greatly affect the rate of the methylene blue reduction.

Examination of the curves in figure 7 indicates that a marked change in their slope (rate of dye reduction) occurs above 50° C. Between 0° and 50° the rate of reduction, judged by the slopes of the time-light intensity curves, are not greatly different after the end of the induction period. The slope of the time-light intensity curve for 74° C., however, indicates a marked acceleration of the rate of reduction.

Since the rates of reduction over the temperature range 0° to 50° were quite similar to each other but markedly different from that at 74°, it seemed evident that the rate shifted fairly sharply at some critical reaction temperature. Therefore, three additional 50-ml. samples of another completely refined, fresh soybean oil containing 2 ml. of alcoholic methylene blue solution (50 mg./100 ml. of alcohol) were irradiated in the apparatus at 50°, 55°, and 60° C., respectively, curves 16, 17, and 18 of figure 9. The slopes of the curves at 55° and 60° indicate a rate of reduction similar to that at 74° (curve 13, figure 7) and is greatly accelerated compared to the rate at 50° C.

Disregarding the induction period, it is evident that the rates of dye reduction of methylene blue-soybean oil system for all temperatures of 50° and below are of one order of magnitude and those at 55° and above are of a quite different order of magnitude. The exact temperature of the transition from one form of curve to the other was not located, but it is evident that greater precision of measurement can be obtained at temperatures below 55° C. On the other hand, since the rate does not vary appreciably at temperatures of 50° C. and below, this temperature was adopted as standard since most fats, as well as other lipids, are liquids at this temperature and can, therefore, be examined under comparable conditions.

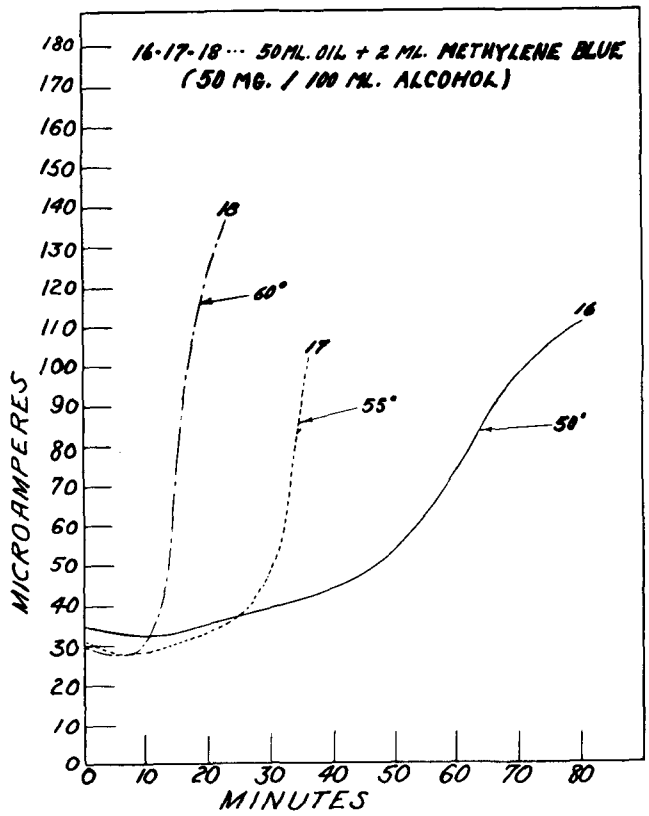

Figure 9.—Effect of temperature on the rate of dye reduction in the methylene blue-soybean oil system.

The results of the effect of temperature on the rate of dye reduction indicated that the reaction was partly thermal in character. In order to evaluate the relative importance of light and temperature, an attempt was made to separate the two effects. This was accomplished by comparing two portions of a methylene blue-edible soybean oil mixture, one of which was submitted to continuous irradiation (curve 19, figure 10) and the other to intermittent irradiation (curve 20, figure 10). Curve 19 was obtained in the usual manner and curve 20 by exposing the sample to irradiation for a period of 10 seconds every 20 minutes. Since curve 20 indicates very little dye reduction at the minimum radiation

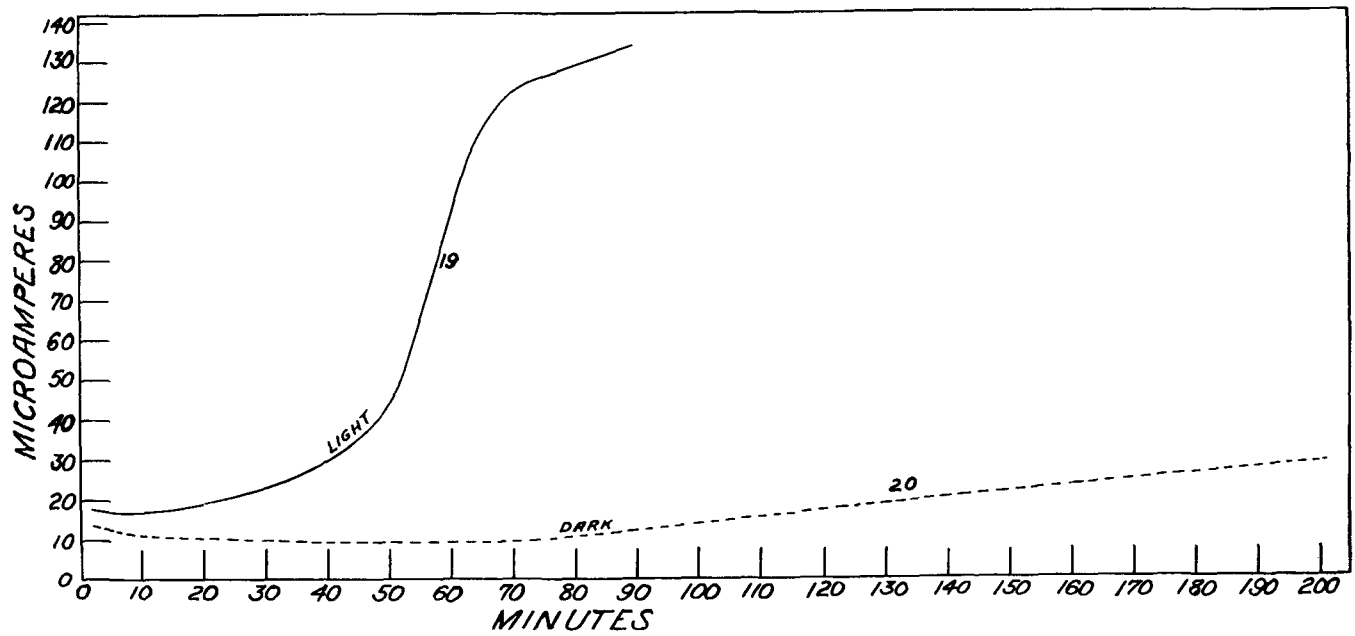

Figure 10.—Effect of light on the methylene blue-soybean oil system.

necessary for measurement, it would appear that the methylene blue-oil system would be stable at 50° in the complete absence of light. It should also be noted that the plot of the values in the near absence of light is very nearly a straight line function of time and, therefore, cannot represent an autocatalyzed reaction of the type observed in curve 19.

It is also quite apparent that this photochemical reaction is dependent on the presence of the soybean oil, or some constituent thereof, since it was previously shown that methylene blue in ethyl alcohol (curve 4, figure 5) and ethylene glycol (curve 8, figure 6) is perfectly stable at a temperature of 50° C. under prolonged irradiation at high light intensity.

Effect of Dissolved Gases

Examination of curves 4 to 20, inclusive, reveals the fact that wherever soybean oil is not a component of the reacting system the microampere readings never fall below the threshold (i.e., initial) value of light transmission. This condition obtains whether the system is examined in equilibrium with its dissolved oxygen, or after saturation with oxygen, or after evacuation of its dissolved air and saturation with nitrogen. On the other hand, when an edible quality soybean oil is a component of the reacting system the microampere readings always drop below the threshold value of light transmission. This drop may be very small or quite appreciable and may extend over a good portion of the total reaction period.

The distance along the Y-axis to the threshold reading indicates the relative extent of dye reduction (dark reaction) prior to irradiation, and the drop from the threshold reading to the lowest point of the curve following exposure of the system to irradiation measures the extent of the reoxidation of the dye prior to the onset of the final rapid reduction. This tendency toward reoxidation of the dye in the methylene blue-soybean oil system might be presumed to be associated with the presence of dissolved oxygen in the oil, consequently an effort was made to evaluate this factor.

Two samples of a high quality and quite stable edible soybean oil were examined in the apparatus. One sample (curve 21, figure 11) was examined under standard conditions, while the second sample (curve 22, figure 11) was first evacuated under an oil pump for 4 hours in the dark, and then saturated with and maintained under nitrogen freed from traces of oxygen by passage over hot reduced-copper oxide.

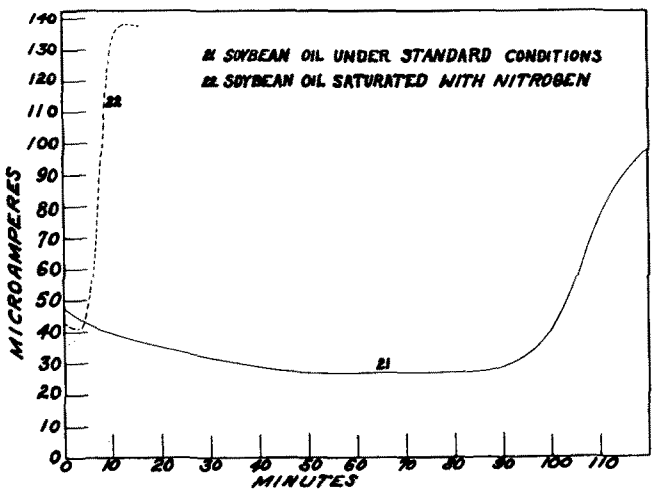

Figure 11.—Effect of dissolved oxygen on the rate of dye reduction in the methylene blue-soybean oil system.

Except for the extended induction period the curve of the untreated oil was quite similar to the other samples of soybean oil examined under the same conditions. The time-light intensity curve for the oxygen-free sample was quite similar to that observed with ethyl oleate (curve 7, figure 5) under the same conditions. Both oils exhibited very nearly the same pre-reduction of methylene blue prior to exposure to light (threshold value), and both of them showed definite evidence of reoxidation of methylene blue in the early stages of irradiation. On the basis of the behavior of these two oils it would seem necessary to conclude that the reoxidation of the dye in the methylene blue-soybean oil system could not be attributed to dissolved oxygen, *unless the evacuated sample was not entirely freed of its dissolved oxygen.*

In view of the behavior of the irradiated methylene blue-soybean oil system in the absence of oxygen, it was deemed advisable to determine the effect of aeration on the system. Examination of several fresh, commercially deodorized oils before and after aeration for various lengths of time showed no significant change in the time-light intensity curves. If, however, the soybean oil was allowed to age until it had reverted or until reversion was imminent, and it was then examined in the apparatus before and after aeration, the time-light intensity curves were found to be markedly affected by the aeration.

The effect of aeration on the behavior of an aged soybean oil is readily observed in curves 23 to 27 of figure 12. Curve 23 represents the control sample examined under standard conditions. Curves 24, 25, and 26 represent the behavior of the same oil after aeration in the dark with dry, carbon dioxide-free air for 20, 40, and 60 minutes, respectively. Curve 27 was obtained with the same oil after evacuation under the pump and resaturation with purified nitrogen.

Aeration of the aged oil sample resulted in a pro-

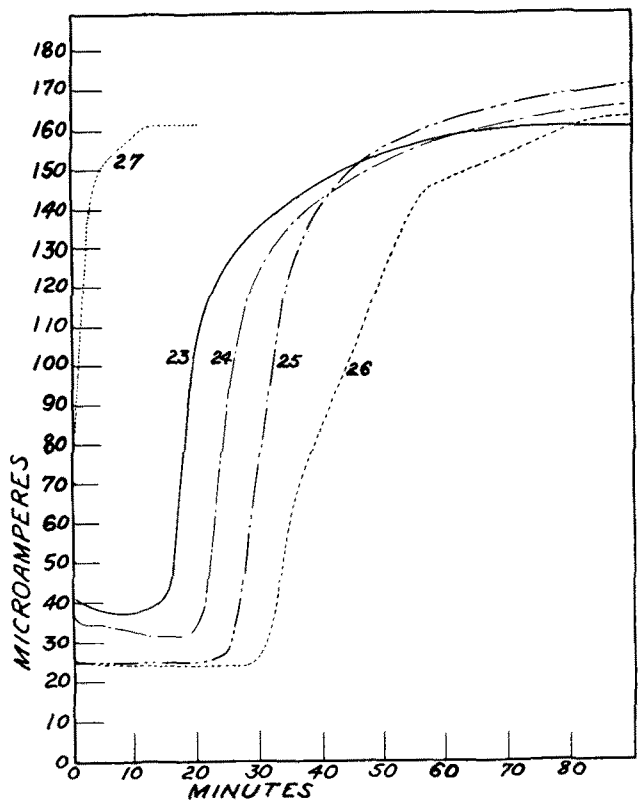

Figure 12.—Effect of aeration on the time-light intensity curves of a partially oxidized soybean oil system.

gressive lowering of the threshold value and in extending the induction period prior to reduction of the methylene blue. However, once dye reduction began, the rate, as well as the total reducing capacity, of the various aerated and the control samples were not markedly different. These results are in contrast to the behavior of the freshly deodorized oil which, as was stated above, was unaffected by aeration so far as dye reduction was concerned.

The behavior of the various fresh and aged oils before and after aeration and of the corresponding evacuated (oxygen-free) oils, curves 21 to 27, inclusive, may be explained by the assumption that the method employed is measuring the *rate at which an oil is consuming its dissolved oxygen* under the conditions obtaining in the methylene blue-oil system. This assumption would appear to be confirmed by the following experimental observations:

(1) The dye present in the methylene blue-soybean oil system entirely freed of oxygen undergoes extremely rapid reduction.

(2) Aeration of a freshly deodorized oil has little or no effect on the time-light intensity curve, probably because a freshly deodorized oil has not had time to consume any of its dissolved oxygen. Therefore, the oil is at its maximum oxygen tension and is unaffected by further aeration.

(3) An aged oil, i.e., one which has already consumed part or all of its originally dissolved oxygen, displays on aeration an increased induction period and a lower threshold value prior to the onset of rapid reduction.

As a corollary to the first assumption, it might further be assumed that the so-called induction period merely represents the interval during which reduction and reoxidation of the dye is occurring and which continues until the oxygen is entirely consumed or its tension becomes too low to affect the reverse reaction. This assumption would account for the lowering of the threshold of dye reduction below the initial value observed at the instant the system is first exposed to radiation. It will be recalled that part of the dye is always reduced in the dark prior to radiation, and in most cases it is partially reoxidized during the initial stages of the irradiation. During the interval that the time-light intensity curve is falling, the rate of reoxidation exceeds the rate of reduction, while toward the end of the reaction the reduction exceeds the reoxidation.

This interpretation is not all-exclusive and no doubt represents an over-simplification of the behavior of the methylene blue-oil system under the conditions to which it is subjected, since it does not provide a mechanism for the reaction or explain the function or effect, as the case may be, of:

(1) The components involved in the oxygen consumption,
(2) The natural antioxidants in the oil,
(3) The reducing of other substances present or formed in the reaction,
(4) The naturally occurring pigments of the oil,
(5) The added methylene blue in the reaction, and
(6) The radiation as the catalyst during dye reduction in the methylene blue-oil system.

Since the natural antioxidant of completely refined soybean oil has never been isolated in pure form and in sufficient quantity to permit its use experimentally, no direct evidence is available concerning the manner in which it functions to protect an oil, or the manner in which it would behave in the apparatus.

On the other hand it is possible to determine, either directly or indirectly, the effect of some of the reaction products, especially aldehydes and peroxides, which are presumed to form during the process of deterioration of an oil.

Effect of Aldehyde

Heptaldehyde is known to be one of the compounds which contributes to the off-flavor of rancid oils (12), and its effect on the rate of dye reduction in the methylene blue-soybean oil system was, therefore, determined experimentally. Two samples of otherwise identical edible soybean oil, except one sample contained one drop of heptaldehyde in 50 ml. of oil, were examined under identical conditions. The time-light intensity curves of the control oil and of the heptaldehyde-treated oil, curves 28 and 29, respectively, are reproduced in figure 13. Although the curves are slightly different in appearance, no marked effect on the rate of dye reduction is observable in the treated over the untreated oil.

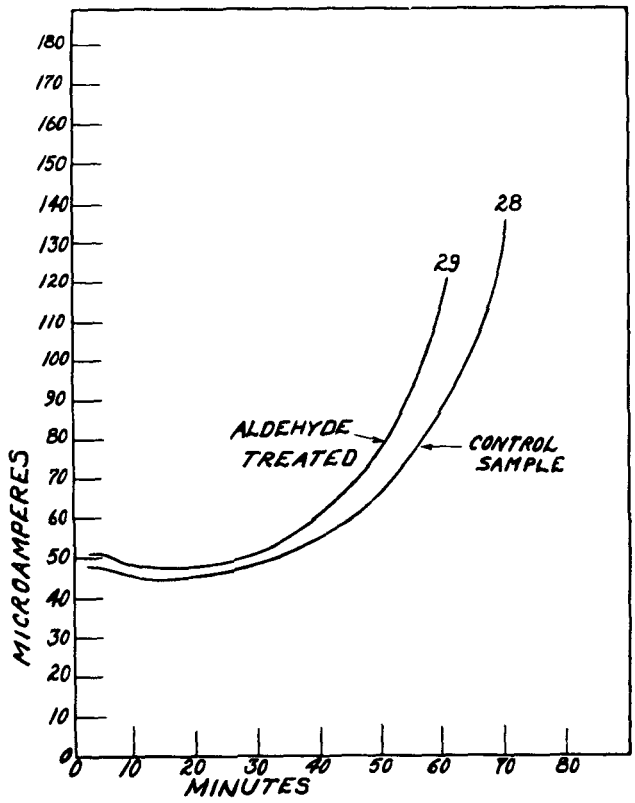

Figure 13.—The effect of heptaldehyde on the methylene blue-soybean oil system.

Greenbank (15) and Holm and Greenbank (16) reported that volatile oxidation products of fats have a positive accelerating effect on the induction of rancidity in fresh fats. However, Roschen and Newton (17) reported that heptaldehyde and other volatile oxidation products had no accelerating effect on the deterioration of a fat as judged by the peroxide test. The results of the single experiment mentioned above agree, so far as interpretation is possible, with the observations reported by Roschen and Newton.

Effect of Peroxides

In view of the demonstrated activity of dissolved oxygen in the role of dye reduction in the methylene blue-fat system, an effort was made to determine the

possible effect which preformed peroxides might have on the course of the dye reduction. Accordingly, a fat was assayed for its peroxide content in the presence of the same concentration of methylene blue as was employed in the reduction tests. The peroxide number determined in this way was found to be 9.9 millimols per kg. of fat.[6] A sample of the fat was then subjected to the methylene blue reduction test for a period of 90 minutes, at the end of which time the dye reduction was complete so far as visual observation of blue color was concerned. The sample, after reduction of the dye and removal from the apparatus, was protected from further oxidation by purified nitrogen while it was again assayed for peroxides. The peroxide number was found to be 11.2, which value under the test conditions is within the experimental limits of the previously determined value. A peroxide number of 11.2 is equivalent to combination of 12.5 ml. of oxygen with 50 ml. of fat. However, calculated from the data by Vibrans (18) 50 ml. of the fat saturated with air would contain only 1 ml. of oxygen.

The above-mentioned results are anomalous in several respects. Apparently the peroxide which is capable of oxidizing the iodide ion is incapable of oxidizing leuco methylene blue under the test conditions, despite the fact that methylene white is extremely sensitive to oxygen, while the peroxide reagent (potassium iodide in chloroform and glacial acetic acid) is only slowly oxidized by atmospheric oxygen. The coexistence of peroxides and leuco methylene blue thus appears to be inconsistent. That the methylene blue had not been destroyed under the test conditions was demonstrated by the fact that it could readily be regenerated by suitable means from the leuco form of the dye, in the presence of the fat.

It is possible that other reducible substances which are present in fats may be partly responsible for the apparent peroxide numbers of oils (19). So far as the authors are aware, no peroxide has ever been isolated from a fat or oil, and the existence of such substances in natural fats is merely postulated on their reactivity with the reagent commonly used for the determination of hydrogen peroxide and related compounds.

SUMMARY AND CONCLUSIONS

A stability apparatus which employs the principle of methylene blue reduction in a fat-dye system exposed to filtered radiation of a tungsten filament lamp is described. The apparatus is designed and constructed in such a manner that certain variables, such as the fluctuation in light intensity, temperature, infrared radiation, and the limitations of visual observation, are controlled or eliminated.

The effect of such factors as the nature of the substrate temperature, dissolved gases, aldehydes, and peroxides on the rate of dye reduction in the methylene blue-oil system have been determined.

On the basis of the shapes of the time-light intensity curves of a number of fresh and aged soybean oils before and after aeration, and of the correspondoing evacuated (oxygen-free) oils, it would appear that the apparatus and method is applicable to the determination of:

(1) The relative induction period of fats and oils,

(2) The relative rate at which a fat or oil is capable of consuming its dissolved oxygen under standardized conditions, and

(3) The extent of the prior consumption of dissolved oxygen at the time the fat or oil is subjected to examination, or stated in another way, the latent capacity of a fat or oil to consume its residual oxygen.

Since these factors are precisely those which it is desirable to know for the purpose of evaluating the stability of a fat or oil, the above-described apparatus and method appears to be well adapted for the determination of the relative stability of edible oils. Further evidence for this conclusion will form the subject of additional communications which will deal with the reaction mechanism of the methylene blue method and its correlation with organoleptic stability.

[6] This value was calculated by the usual formula for conversion of milliequivalents of peroxide into millimols. This equation assumes that 1 mol of peroxide yields 2 equivalents of iodine. Morrell and Phillips (19) state that a drying oil peroxide liberates 4 atoms of iodine according to the equation

$$\text{X-CH-CH-Y} + 4\text{HI} \longrightarrow \text{X-CH=CH-Y} + 2\text{H}_2\text{O} + 4\text{I}$$
$$\quad\;\; | \quad |$$
$$\quad\;\; \text{O} \quad \text{O}$$

In this event the millimols of peroxide and the computed value of oxygen involved would be only half of the amount stated.

BIBLIOGRAPHY

(1) King, A. E., Roschen, H. L., and Irwin, W. H., Oil and Soap, *10*, 105-109 (1933).
(2) Whitehead, H. R., Biochem. Jour., *24*, 579-584 (1930).
(3) Greenbank, G. R., and Holm, G. E., Ind. Eng. Chem., Anal. Ed., *2*, 9-10 (1930).
(4) Royce, H. D., Soap, *7*, (No. 9) 25-27, 38 (1931).
(5) Royce, H. D., Ind. Eng. Chem., Anal. Ed., *5*, 244-247 (1933).
(6) Davies, W. L., J. Soc. Chem. Ind., *47*, 185T-187T (1928); *53*, 148T-151T (1934).
(7) Aikins, G. A., and Fay, A. C., J. Agr. Res., *44*, 85-95 (1932).
(8) Strong, John. Procedures in Experimental Physics, p. 401. New York. Prentice-Hall, Inc., 1938.
(9) Bulletin 18-A, Technical Data on Weston Photoelectric Cells. Newark, N. J., Weston Electrical Instrument Corporation, 1938.
(10) Wood, L. A., Rev. Sci. Inst., *7*, 157 (1936).
(11) Lasareff, P., Z. physik. Chem., *78*, 661-666 (1911-1912).
(12) Gebhard, K., Z. physik. Chem., *79*, 639-640 (1912).
(13) Perrin, J., and Mlle. Choucroun, Compt. rend., *178*, 1401-1406 (1924); Perrin, J., Compt. rend., *177*, 612-618 (1923).
(14) Lea. C. H. Rancidity in Edible Fats. Food Investigation Board, Special Report No. 46, p. 112. London, His Majesty's Stationery Office, 1938.
(15) Greenbank, G. R., Oil and Soap, *13*, 140-141 (1936).
(16) Holm, G. E., and Greenbank, G. R., Ind. Eng. Chem., *15*, 1051-1053 (1923).
(17) Roschen, H. L., and Newton, R. C., Oil and Soap, *11*, 226-228, 236-238 (1934).
(18) Vibrans, F. C., Oil and Soap, *12*, 14-15 (1935).
(19) Morrell, R. S., and Phillips, E. O., Fette u. Seifen, *46*, 544 (1939).