a good deal of the experimental analytical work connected with this research.

Grass Meal

Total quantity of fatty matters, extracted with solvents from 500 grams dried meal:

Acetone extract	= 23.0 gr.	= 4.6%
Petrolether extract	= 2.05 gr.	= 0.41%
Alcohol and Benzol extract	= 11.7 gr.	= 2.34%
	36.75 gr.	= 7.35%

Acetone Extract

Total ash	1.5%
Silica	nil
Iron	nil
Copper	nil
Ca.	0.105%
Mg.	0.180%
P.	0.50%

Petrolether Extract

a.) Acetone insoluble part (Phosphatide) contains 3.00% P. (80% Phosphatide).

b.) Acetone soluble part (fat) contains 1.11% P. (28% Phosphatide).

Alcohol and Benzol Extract

Petrolether Extract:

P.	2.80%
N.	1.72%
SiO_2	nil
Ca.	0.25%
Mg.	0.18%
Total ash	6.88%

REFERENCES

(1) Chibnall and Channon, Bioch. Jour., *21*, 233 (1927).
Smith and Chibnall, Bioch. Jour., *26*, 218 (1932).
Chibnall and Sakai, Annals of Botany, *45*, 499 (1931).

(2) Hillstätter and Stoll, Investigations on Chlorophyll. The Science Press Printing Co., Lancaster, Pa., 1927, page 131.

Formation and Decomposition of Peroxides of Unsaturated Fat Esters

R. F. PASCHKE and D. H. WHEELER[1]
U. S. Regional Soybean Industrial Products Laboratory[2]
Urbana, Illinois

Introduction

The reaction of molecular oxygen with unsaturated fats, fat acids, or esters to form peroxides has in recent years become the most generally accepted mechanism for the primary reaction of oxygen upon the unsaturation present in these compounds. This autoxidation is a fundamental factor in reactions such as rancidification, film formation in paints, and the formation of blown oils for technical uses. The present study was undertaken as a preliminary investigation to determine the effect of temperature on the formation and decomposition of these peroxides. It was felt that a knowledge of optimum conditions for formation and of the rate of decomposition at various temperatures was necessary before detailed studies of their decomposition products, or their possible isolation and characterization, could be undertaken. Distilled methyl esters of soybean fat acids were used because they were easy to prepare and could be readily purified by distillation. A similar study using the esters of pure individual unsaturated fat acids would obviously be highly desirable. However, the difficulty of preparing the pure esters in sufficient quantities for the exploratory work led us to use the mixed methyl esters prepared from soybean oil, with the expectation that data on such a mixture would be of considerable value in planning future work on pure compounds. Since the study on pure compounds has been postponed, due to the urgency of other work, the data on the mixed esters are presented at this time.

Experiments and Discussion

Hydroxyl numbers were determined by the acetic anhydride-pyridine method of West, Hoagland, and Curtis (1), with 24-hour reaction time at room temperature. Butanol was not added and aqueous alkali was used, with vigorous shaking of the resulting emulsion at the end-point. The phenolphthalein end-point was easier to observe in the emulsion than in the red-brown colored homogeneous solution ordinarily obtained. Highly peroxidized esters always developed this color with the pyridine reagent.

Acid numbers were calculated from the blank titration (pyridine with no anhydride) of the hydroxyl number determination.

Iodine number. Four different methods for determination of iodine number were tested on a sample of fresh esters and on one which had been blown with oxygen for 24 hours at 55° C. The results are shown in Table 1.

The Kaufmann method (200 percent excess and 4-hour reaction) agreed well with the Wijs method and was subsequently used because of convenience in preparation of the reagent and in handling a large number of samples at one time. It may be of interest to note that with oxidation there was a two- or three-fold increase in the difference between the Hanus and Kaufmann iodine numbers.

Peroxidized esters showed a "repeating" end-point to a degree proportional to the peroxide content. Titrations were made immediately after the addition of the potassium iodide solution and water to the reagent to minimize the liberation of iodine by the peroxides before completion of the titration. On an oxidized sample, titration of the excess bromine by arsenious acid solution (5) gave a value for iodine number (93.7) very close to that obtained by the usual iodide-thiosulfate method (93.9). This would indicate that the liberation of iodine from iodide in the latter method was negligible, or that the peroxides oxidized

---

[1] Present address: General Mills, Inc., Minneapolis, Minnesota.

[2] The Chemical and Engineering Sections of the Soybean Industrial Products Laboratory, Urbana, Illinois, were merged with the Northern Regional Research Laboratory, Peoria, Illinois, July 1, 1942.

the arsenious acid to exactly the same extent to which it oxidized iodide in the former method.

Peroxide determinations. Morrell (6) has shown that the presence of mineral acids (H_2SO_4 or HI) in iodometric peroxide determination results in the liberation of iodine by oxido and hydroxy-keto groups, whereas these groups liberate no iodine in the acetic acid-potassium iodide methods. He concluded that the latter methods "give only unpolymerized peroxides." Since the interest in this study was in the primary peroxide formed by autoxidation of double bonds, the latter method was studied.

When the method of Wheeler (7) was used with 30 ml. of solvent mixture, a pronounced increase in apparent peroxide value was observed as the sample size was decreased below 0.5 gm. In the determinations a 1 gm. sample was used unless otherwise stated. The effect of sample size was much less pronounced when oxygen-free conditions prevailed. Oxygen was excluded by weighing the sample in a 2-ml. beaker, placing the beaker upright in a CO_2-flushed 250-ml. glass-stoppered flask, and adding 30 ml. of the solvent (40 percent chloroform, 60 percent acetic acid, by volume) and 1 ml. of freshly prepared saturated potassium iodide solution carefully so as not to mix it with the sample. The solvent was flushed with CO_2 for 10-15 minutes before use. The flask was then carefully flushed again for 3 to 5 seconds with a vigorous stream of CO_2 and stoppered. Swirling of the liquid in the flask upset the sample beaker and mixed the sample and reagents. The solution was then stored in the dark for the given reaction time, after which 75 ml. of distilled water was added and the iodine titrated with 0.03 to 0.1 N thiosulfate, using a starch indicator.

For a more detailed study of the effect of reaction time on peroxide determination, mixed methyl esters of soybean fat acids were blown with oxygen at 35° for various periods, as follows: Sample A, 96 hours; B, 120; C, 173; D, 309; and E, 479. The effect upon apparent peroxide values of the oxidized esters caused by varying periods of reaction with the peroxide reagent in the absence of oxygen is shown in Figure 1. Samples A and B, which were relatively less oxidized and contained smaller amounts of peroxide decomposition products, reacted rapidly, while Samples C, D, and E, which had increasing quantities of peroxide decomposition products, reacted progressively slower. Thus, compared to the one-hour values, B was 84 percent complete in 1 minute, and 91 percent complete in 5 minutes, while E was only 50 percent complete in 1 minute, and 65 percent complete in 5 minutes. These phenomena would indicate the presence of two or more substances of different reactivity. They might be peroxides of different activity, decomposition products which react slowly, or both. In all subsequent data, peroxide values were determined in the absence of oxygen with a one-hour reaction time in the dark, using the technique described above.

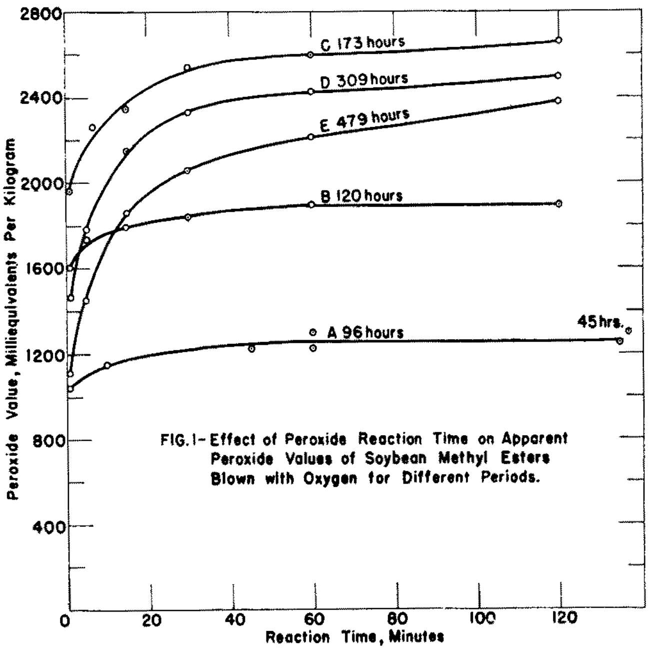


FIG. 1—Effect of Peroxide Reaction Time on Apparent Peroxide Values of Soybean Methyl Esters Blown with Oxygen for Different Periods.

Peroxide accumulation and decrease in unsaturation in soybean methyl esters at various temperatures. Methyl esters were prepared from alkali-refined soybean oil by alcoholysis, using sodium methylate catalyst. The esters were distilled twice at a pressure below 1 mm., and a small first fraction of 1 to 2 percent, which was usually somewhat colored, was discarded. The main fraction used had an iodine number of 130 to 132, and a negligible peroxide value. The esters were blown with oxygen at a rate of 10 ml. per second in all-glass vessels 40 mm. by 170 mm. with a 2-mm. capillary blowing tube which extended to within one-fourth inch of the bottom. A thermostatically controlled oil or water bath was used to obtain the various temperatures ± 0.5°. The blowing was done in the absence of light.

The results of oxidation at 100°, 75°, 55°, 35°, and 15° C. are shown in Figures 2, 3, 4, 5, and 6.

Samples were removed at intervals for peroxide and iodine number determinations. The samples were quickly cooled and immediately analyzed, or in some

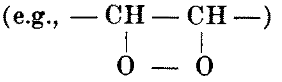
TABLE 1

Iodine Number. Comparison of Methods

	Wijs (2)		Hanus (3)		Rosenmund Kuhnhenn (4)		Kaufmann (5)	
	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized
Iodine number	132.1	117.6	129.8	111.8	....	106.4	129.9	116.7
Reaction time	30 min.	30 min.	30 min.	30 min.	....	5 min.	2 hrs.	2 hrs.
Excess reagent (percent)	200	200	200	200	....	150	200	200
Iodine number	130.3	116.8	128.4	109.1	121.9	104.2	130.4	116.4
Reaction time	30 min.	30 min.	30 min.	30 min.	5 min.	5 min.	2 hrs.	2 hrs.
Excess reagent (percent)	100	100	100	100	33	33	100	100
Iodine number	130.6	117.2	131.3	113.9	122.0	108.6	130.1	117.8
Reaction time	60 min.	60 min.	60 min.	60 min.	10 min.	10 min.	4 hrs.	4 hrs.
Excess reagent (percent)	200	200	200	200	150	150	200	200
Iodine number	130.2	116.7	130.0	111.5	122.3	105.5	130.5	117.3
Reaction time	60 min.	60 min.	60 min.	60 min.	10 min.	10 min.	4 hrs.	4 hrs.
Excess reagent (percent)	100	100	100	100	33	33	100	100

cases stored in the refrigerator in stoppered tubes overnight.

On the generally-accepted assumption that one mole of oxygen saturates one double bond to form one mole of peroxide (e.g.,



$$-\underset{\displaystyle O}{\underset{|}{CH}}-\underset{\displaystyle O}{\underset{|}{CH}}-$$

) capable of liberating two equivalents of iodine in the peroxide determination, it is a simple matter to calculate a theoretical peroxide value from the decrease in iodine number during peroxidation if this is considered to be the only reaction. The formula which relates the decrease in iodine number, ΔI, to the "theoretical peroxide," T.P. (milliequivalents per kilogram), for an olefinic unsaturated compound is as follows: $T.P. = \dfrac{62{,}500\,\Delta I,}{793 + I_B}$ where I_B is the original iodine number of the material before peroxidation.

The ordinates for decrease in iodine number in Figures 2 to 6 have been made such that the ΔI number

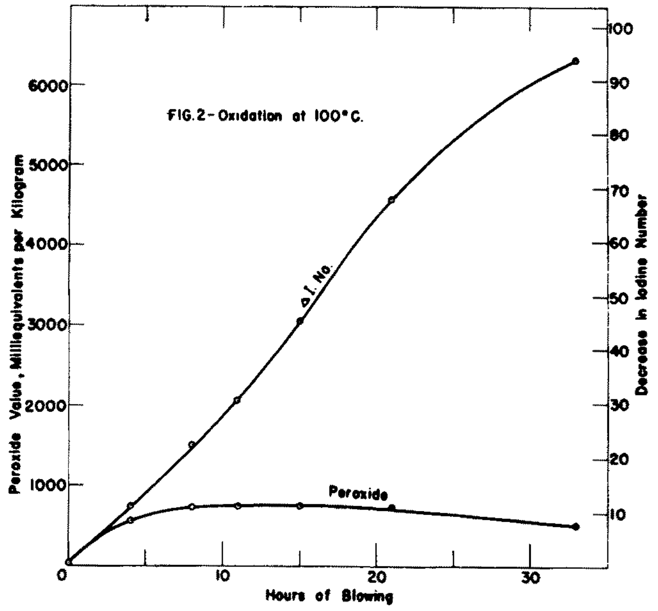


FIG. 2-Oxidation at 100°C.

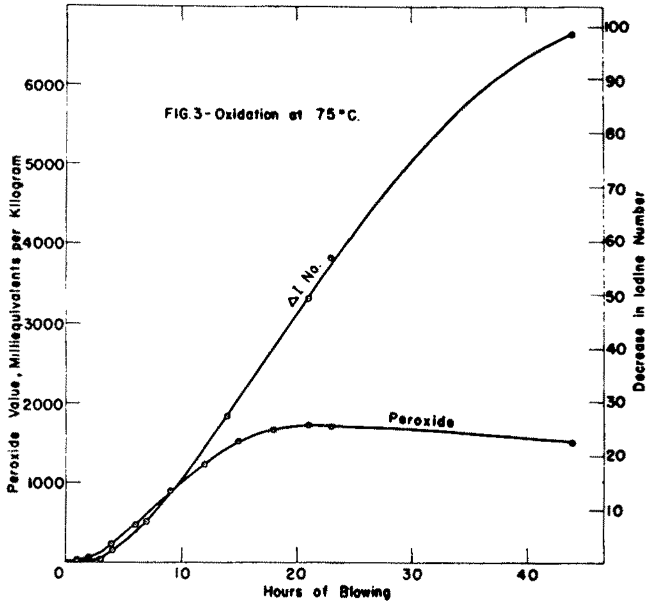


FIG. 3-Oxidation at 75°C.

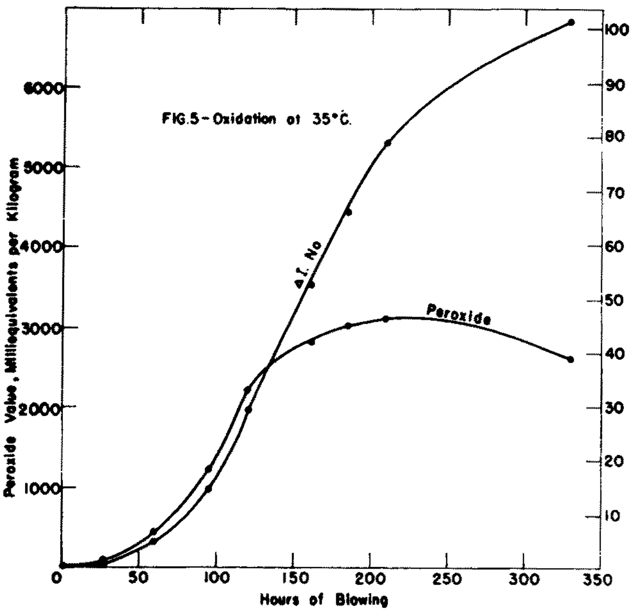


FIG. 5-Oxidation at 35°C.

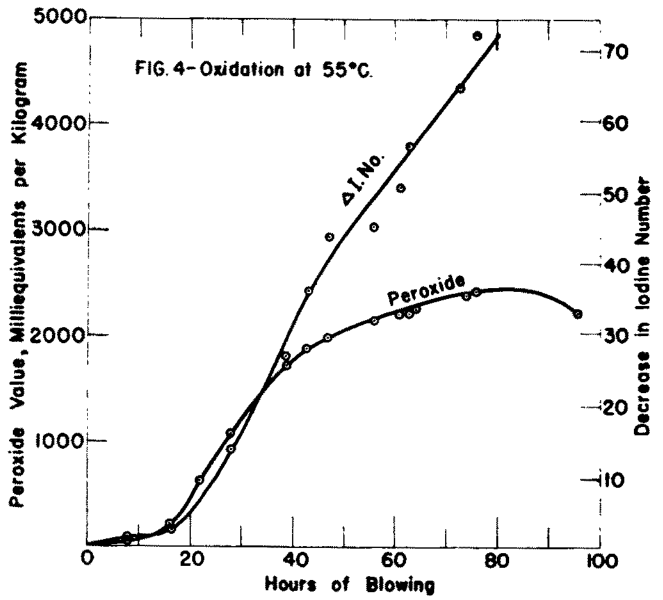


FIG. 4-Oxidation at 55°C.

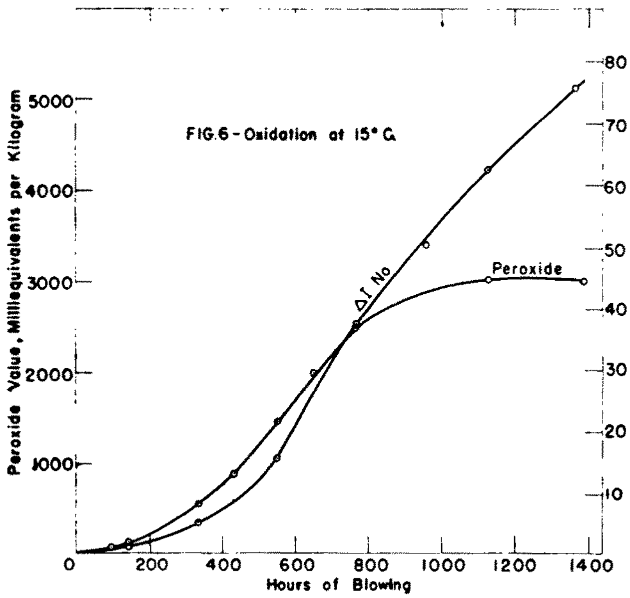


FIG. 6-Oxidation at 15°C.

curves are also "theoretical peroxide" curves when the peroxide ordinates are used. Considering all decrease in iodine number to be due to peroxide formation, one would expect the "theoretical" and actual peroxide curves to coincide (1) if the methods of analysis were valid, (2) if no volatile material were lost, and (3) if no decomposition of peroxides occurred. As regards the first condition, it is believed that the peroxide and iodine numbers are reasonably correct. As for the second, it was found that only a fraction of a percent of volatile material was evolved up to the maximum peroxide value at 55°. The difference between the actual and the ΔI number, or "theoretical peroxide," curves can therefore be considered a measure of peroxide which has decomposed. The "theoretical peroxide" would be about 9,000 if all the double bonds were converted to peroxides.

Examination of the curves shows that at 100° the ΔI number and peroxide curve deviate at relatively low values. The peroxide curve reaches a maximum of 750 and then slowly decreases, while the ΔI number curve continues to increase. At 75° the ΔI number curve and the peroxide curve are close, up to a peroxide value of 1,000, and the maximum peroxide value is 1,700. The 55° curves show this agreement to a greater degree, deviations beginning at about 1,700, with a maximum of 2,400 in the peroxide curve. At 35° the two curves are quite close, up to a peroxide value of 2,600, and then diverge rapidly. The maximum peroxide value is about 3,100, which is over 30 percent of the maximum possible theoretical value. The fact that in the early stages the actual peroxide curve is slightly above the ΔI number curve in this case (to a much less degree at 75° and 55°, and to a greater degree at 15°) is possibly explained by the observations of Farmer and Sutton (8) who found that in the early stages of the autoxidation of methyl oleate the double bond was unchanged but that a hydroperoxide was formed on either of the adjacent active methylene groups when the autoxidation was catalyzed by ultraviolet light at 35° C. They propose that such a substitutive peroxidation is general for all of the non-conjugated unsaturated fat acid esters, and that decrease in unsaturation is caused by a secondary reaction between the hydroperoxide and a double bond. However, the fact (see below) that unsaturation, OH number, and acid number change very little when peroxides decompose at various temperatures is not readily explained on the latter assumption. The 15° curves are very similar to those at 35°, except that the time required to reach a maximum peroxide value is about six times greater at 15°. The maxima and the points of deviation of the two curves are very nearly identical for these two temperatures. The actual peroxide curve in the early stages at 15° is relatively higher than the ΔI curve to an extent greater than was observed at 35°.

Figure 7 shows the peroxide curves at the various temperatures on the same abscissa of time, and demonstrates more clearly the differences due to temperature. The maximum peroxide value progressively increases as the temperature is lowered to 35°, but at 15° the maximum is the same as at 35°, and these two maxima represent over 30 percent of the maximum possible "theoretical peroxide."

Peroxide decomposition. A preparation of mixed methyl esters was blown with oxygen at 100° for 4 hours to eliminate the induction period, and then at 20° for four weeks. Samples were taken to represent three stages of oxidation. Sample A (after 4 hours at 100°) had an actual peroxide value of about 400, and a theoretical value of about 500 (ΔI = 7.6). Sample B (after 1 week at 20°) had an actual peroxide value of about 2,400 (ΔI = 37.2) and a theoretical peroxide value of 2,500. These two samples represent a low and a high value, respectively, for peroxides in which relatively little decomposition has occurred, compared to Sample C (4 weeks at 20°) which had an actual peroxide value of about 2,600, and a theoretical peroxide value of about 4,700 (ΔI = 69.6). Samples were placed in glass tubes, evacuated, sealed, and stored in the dark for various periods at 100°, 75°, 55°, and 35°. The results of the analyses of these samples are shown in Figures 8 to 12. In all cases the low peroxide sample, A, decomposed most slowly, while B and C decomposed rapidly, and C more rapidly than B in the earlier stages. This difference in stability is shown in Table 2, where the time required for 25 percent decomposition of the original peroxide is given. This table also illustrates the pronounced effect of temperature upon the decomposition of the peroxides at different degrees of oxidation.

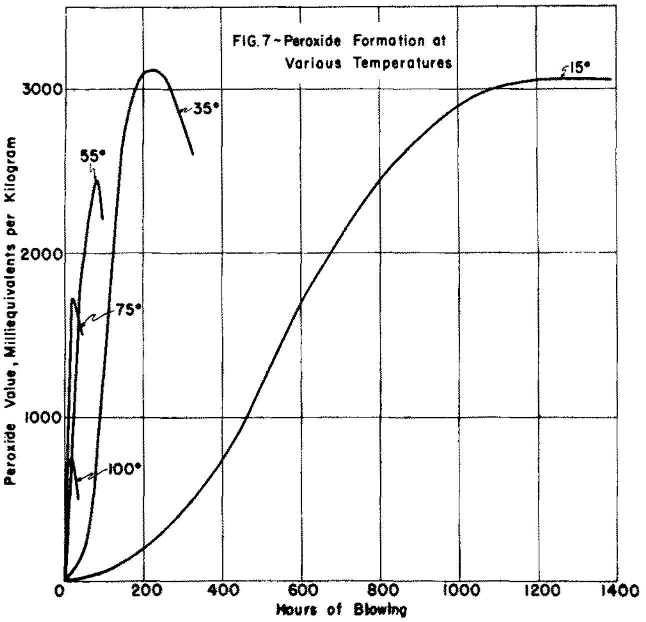


FIG. 7—Peroxide Formation at Various Temperatures

TABLE 2

Hours Required for 25 Percent Decomposition of Peroxide at Various Temperatures

Sample	100°	75°	55°	35°
	Hours	*Hours*	*Hours*	*Hours*
A	3.4	4.6	290*	1,325*
B	.8	4.5	27	230
C	.4	1.5	9	176

* By extrapolation.

Attempts were made to determine the order of the peroxide decomposition reaction by graphical methods (9). The data show best agreement with the second order rate equation, indicating that the decomposition may be a bimolecular reaction between two molecules of peroxide. However, at 100°, Sample C became slower; at 75°, B and C became faster; and at 55°, C became faster in decomposition as the reaction pro-

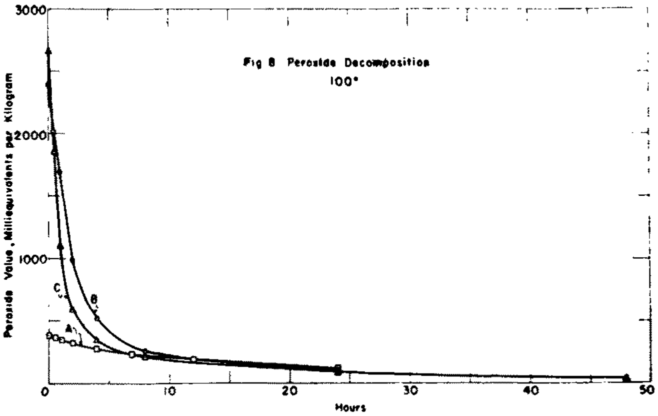


Fig 8 Peroxide Decomposition 100°

gressed than should a second order reaction. In view of these exceptions, and in view of the probable complexity of the decomposition reaction, the agreement of the data with a bimolecular reaction should be interpreted with caution. However, the fact that unsaturation, OH number, and acid number changed very little (see below) during the decomposition would be in accord with a bimolecular reaction in which the principal reaction was between two molecules of peroxide. The polymerization of the peroxide groups in autoxidized fat products has been previously proposed by Morrell and Davis (10), who concluded that the reaction was of a dimeric character. Morrell and Phillips (loc. cit.) consider that the "remote" double bond of β-eleostearin forms a peroxide which polymerizes rapidly, while the "near" double bond forms a more stable peroxide which is partly transformed to a ketol group.

The results of the analysis of the decomposed esters for unsaturation, OH number, and acid number are shown in Figure 12. Similar data were obtained for the other temperatures, but they were qualitatively so similar to those shown that they are not presented. It will be seen that the changes in these values are all quite small compared to the magnitude of change in peroxide values. The possible significance of this fact has been previously mentioned. More complete analysis of the decomposed samples for carbonyl and oxido groups might have shown results which would have thrown more light on the decomposition mechanism.

The data presented are not inconsistent with the theory that the disappearance of peroxide in the samples studied is due principally to a polymerization reaction between two peroxide groups. However, other reactions are also undoubtedly involved since there are minor changes in unsaturation, OH number, and acid number. Furthermore, the highly oxidized oils after long periods of decomposition were observed to have evolved volatile products of sufficient quantity to show considerable positive pressure when the sample tubes were opened. In some cases, the gas was qualitatively observed to be inflammable. Andrews (11) showed that when cottonseed oil peroxides decomposed the gas evolved had a high content of hydrogen. More work would be necessary to permit a complete

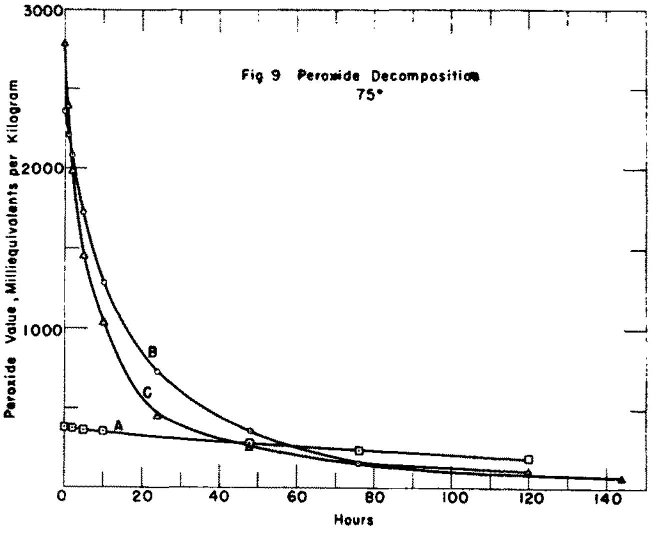


Fig 9 Peroxide Decomposition 75°

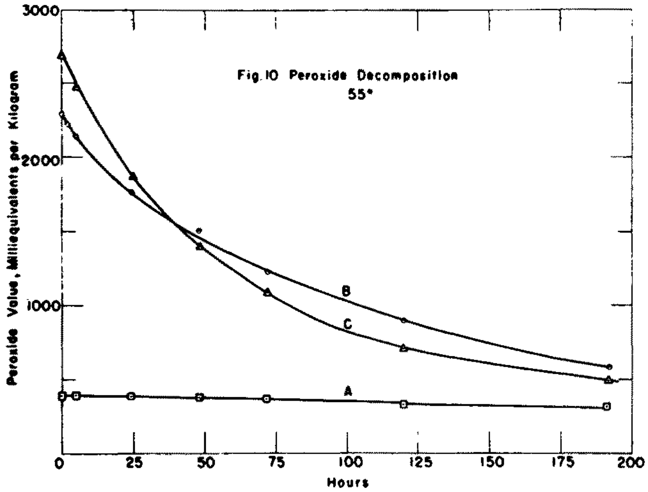


Fig. 10 Peroxide Decomposition 55°

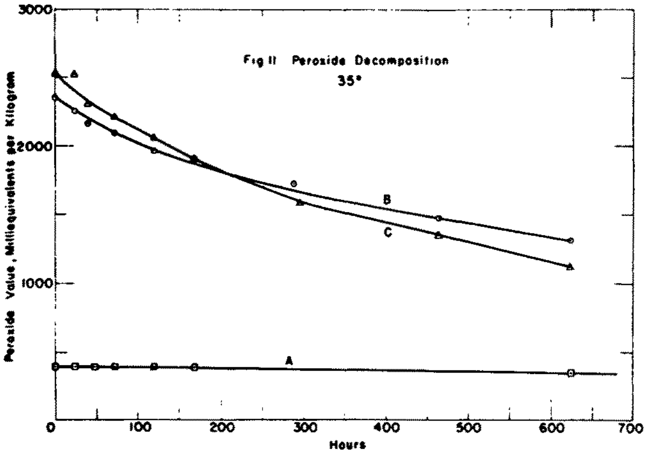


Fig 11 Peroxide Decomposition 35°

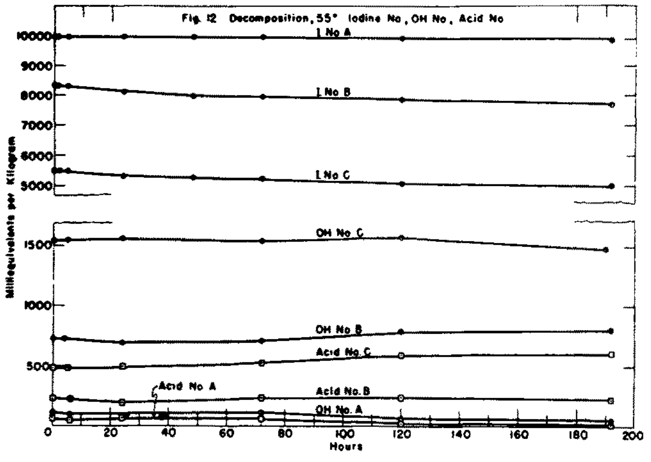


Fig. 12 Decomposition, 55° Iodine No, OH No, Acid No

explanation of all of the reactions occurring as peroxides form and decompose.

Summary

1. A study of reaction time and the effect of oxygen on determination of peroxide by the acetic acid-potassium iodide method shows that a one-hour reaction time in the absence of oxygen is necessary, especially on samples of high peroxide.

2. The peroxide-formation curves at 100°, 75°, 55°, 35°, and 15° show a progressive increase in maximum peroxide value reached as the temperature is lowered to 35°. At 15°, the maximum is the same as at 35°; this value is over 30 percent of what it would be if all the double bonds were converted to peroxide.

3. The agreement between actual peroxide and "theoretical peroxide" calculated from decrease in iodine number extends to higher peroxide values as the temperature is decreased.

4. The rate of decomposition or disappearance of peroxide at 100°, 75°, 55°, and 35° agrees best with that of a bimolecular reaction, but definite exceptions exist.

5. The speed of decomposition of peroxide becomes progressively greater as the degree of oxidation is increased.

6. The changes in unsaturation, OH number, and acid number are small compared to the decrease in peroxide value as the peroxide decomposes. The formation of volatile and inflammable products was qualitatively observed in the decomposition of highly oxidized esters.

REFERENCES

(1) West, E. S., Hoagland, C. I., and Curtis, G. H., J. Biol. Chem. *104*, 627-634 (1934).

(2) Methods of Analysis A. O. A. C., 4th Ed., 1935, p. 412.

(3) *Ibid.*, p. 410.

(4) Rosenmund, K. W., and Kuhnhenn, Z. F., Unter. Nahr. u. genussm. *46*, 154-8 (1923).

(5) Kaufmann, H. P., "Studien auf Dem Fettgebiet," Verlag chemie. g.m.b.H., Berlin, 1935, p. 23.

(6) Morrell, R. S., and Phillips, F. O., J. Soc. Chem. Ind. *58*, 159-63 (1939).

(7) Wheeler, D. H., Oil and Soap *9*, 89-97 (1932).

(8) Farmer, H. E., and Sutton, D. A., J. Chem. Soc. *1943*, 119-122; 122-125.

(9) Getman, F. H., and Daniels, F., Outlines of Theoretical Chemistry, 5th Ed., 1931, p. 331, Wiley and Sons, Inc., New York, New York.

(10) Morrell, R. S., and Davis, W. R., Analyst *54*, 503 (1929).

(11) Andrews, J. T. R., Oil and Soap, *12*, 105 (1935).

Determining Recoverable Fat Losses In Plant Waste Effluents

E. N. MORTENSON

Swift & Company, Chemical Engineering Division, Research Laboratories

Introduction

This problem has always been a serious interest to all primary producers of fats and oils and to those engaged in processing such material into products for commerce. The required operations are of such nature that losses are bound to occur in many ways if due care is not taken. It isn't the purpose of this discussion to elaborate on or even attempt to list all the places or manners in which fat and oil can be lost, or vice versa, salvaged in industrial operation. Many of them are better recognized by the readers of this journal than by any other single technical group.

From a number of years active experience in trying to reduce losses of fats down the waste lines in meat packing plants, and to recover what does tend to get away, sufficient problems have presented themselves to give us a fair idea what constitutes adequate recovery and how to recognize it.

Before proceeding, I wish to acknowledge and give credit to the man who outlined, directed and enthused our interest in this work, namely Mr. M. D. Sanders, now serving as a captain in the United States Army.

Losses of fat and oil that are usually considered irretrievable are those that occur when material is hauled to the dump, carried away in water that goes to the sewer, or becomes incorporated in product whose value is not determined by the fat content. Of these, the one about which something can be done is the loss to the sewer beyond the plant area.

Regardless of ceaseless repetition, of the maxim "Save at the source," it still deserves everyone's constant concern. That means to the fullest possible extent, keep the fat from getting started off on water carry. By taking adequate care at this point, recovery facilities can be adequate without being too numerous or too large. If fat or oil gets into water which is flowing to waste, hundred per cent recovery is not possible thereafter, and any recovered fat will be of poorer quality than the original.

Considering that, in spite of all precautions, some fat does get into the waste water flow, some equipment should be provided for its salvage or interception. When we speak of waste water flow, we mean all clean up water, process water, condenser water, etc., which leaves a plant. This means the installation of intermediate catch basins, traps and sumps followed by, or rather, discharging into one or two final settling basins for one last attempt to insure minimum carry away to outgoing sewer lines.

At this point, it may be well to interject the thought that fat and oil salvage from plant effluents is of interest not only directly from a dollar return on product recovery. The indirect advantages also are large. Excessive fat losses may result in blocked sewers, severe interference with sanitary waste treatment facilities and the pollution of streams into which the sewers discharge.

There is only one practical way in which to recover fats and oils from waste flows. That is by adequate detention, or more correctly, flow velocity reduction in some form of tank, basin or flume so that fat can rise to the surface and be skimmed off.