

TABLE 7
 2-Stearoyl Oleoyl Palmitin (OSP)

Pretreatment	Melting and transition points	Polymorphic form	Long spacing	Principal short spacings
a) Melted, chilled at -3°C . for 10 min.....	25.5 T 25.5-28.3 30.6 T 30.6-32.3 37.2	sub α -3 ?	79	4.18 (VS), 3.81 (M+)
b) Melted at 60°C . and allowed to cool slowly to R.T. during 16 hrs.....	30.9 T 30.9-33.0 37.1	β' -2 ?	44.0	4.20 (VS), 3.85 (M), 4.33 (M)
c) Melted, crystallized at 28°C . for 16 hrs.....	37.2	β' -2 β' -2	44.0	4.20 (VS), 3.85 (M), 4.33 (M)
d) Melted, crystallized at 28°C . for 45 min.....	30.7 T 30.7-32.3 37.9	? β' -2	-
e) Crystallized from acetone	36.1	β' -3 (trace β' -2)	68.9	4.23 (S), 4.03 (MS), 3.82 (S) 3.56 (W), 5.48 (MW)

the present apparatus any change in the specific heat of the sample during a measurement will influence the shape of the heating curve.

In our investigation all the α forms were unstable and changed into a higher polymorphic form. This new form, except for POP and OSP, did not transform readily into a third form unless held for some considerable time just below the melting point of this third form. With POP a third form was just indicated at a heating rate of $1.5^{\circ}\text{C}/\text{min}$. while at $0.5^{\circ}\text{C}/\text{min}$. it was very obvious.

Although OSP showed three melting zones, it was not comparable with POP. In these three zones only two polymorphic forms could be detected; whereas POP and the other glycerides existed in an α form, OSP had a sub- α form and there was no evidence of an α form. To determine the pattern of the change on heating the sub- α form, a sample was prepared and its X-ray pattern determined at 15-min. intervals while it slowly (in six hrs.) warmed up to 32°C . Only the sub- α and β' -2 polymorphic forms were detected. Since, with the instrument used, only strong lines were detected in this way, it seemed possible that some transient intermediate form might have been missed.

It may well be that a second-order transition takes place in OSP although a transition of this kind has never been recorded in the literature for any triglyceride.

On chilling the OSP melt, the sub- α -3 form was always obtained whereas Lutton (2) claims the most likely form to be sub- α -2.

In general, the melting points of the various forms agreed reasonably well with those published by other workers, notably Lutton. It must be remembered however that in this apparatus melting points can-

not be determined with high precision, with the result that in a few cases there was a variation of one or two degrees in our melting points and the literature values. But it seemed significant that the melting point of the same polymorphic form, obtained in two different ways, differed considerably in at least two cases.

For example, Table IV shows that the β' -3 form of OPP had melting points of 31.2°C . and 34°C . Table III shows the β' -3 form of OSS with melting points of 40.1 and 42.2°C .

The great diversity of results, obtained by different workers in the field of polymorphism of glycerides, may result either from variations in the treatment of the glycerides or from the presence of impurity. Of these two, the presence of impurity presents the greater problem for it is not easy to determine which of the samples described in the literature is the most nearly pure.

Acknowledgment

The author wishes to express his thanks to E. T. Sanders, who constructed the apparatus, and to T. Shuttleworth who carried out the X-ray analyses. He would also like to thank the directors of Unilever Ltd. for permission to publish this paper.

REFERENCES

- Lutton, E. S., *J. Am. Chem. Soc.*, **68**, 676 (1946).
- Lutton, E. S., and Jackson, F. L., *ibid.*, **72**, 3254 (1950).
- Lutton, E. S., *ibid.*, **73**, 5595 (1951).
- Malkin, T., and Wilson, B. R., *J.C.S.*, 369 (1949).
- Daubert, B. F., and Clarke, T. H., *J. Am. Chem. Soc.*, **66**, 690 (1944).
- Daubert, B. F., and Clarke, T. H., *Oil & Soap*, **22**, 113 (1945).
- Chapman, D., Crossley, A., and Davies, A. C., *J.C.S.*, 1502 (1957).
- Chapman, D., private communication, to be published.
- Ubbelohde, A. R., *Trans. Farad. Soc.*, **34**, 282 (1938).

[Received August 12, 1957]

Detection and Measurement of Hydroperoxides by Near Infrared Spectrophotometry¹

RALPH T. HOLMAN, CHRISTENSE NICKELL, and ORVILLE S. PRIVETT, Hormel Institute, University of Minnesota, Austin, Minnesota, and PAGE R. EDMONDSON, Department of Medicine, University of Minnesota, Minneapolis

IN A STUDY of the near infrared spectra of fatty acids and related substances (2), unusual absorption maxima were noticed in the spectrum of methyl linoleate hydroperoxide. In order to test

¹This work was aided by grants-in-aid from the Hormel Foundation, the Atomic Energy Commission (Contract AT-11-1-108), the Office of Naval Research (Contract N8 onr66218), the National Live Stock and Meat Board, and the National Dairy Council. Hormel Institute Publication No. 170.

whether these maxima are specific for hydroperoxides, spectra of a series of hydroperoxides and related substances have been measured. All the evidence gathered agrees with the assignment of the maxima at 2.07 and 1.46μ to hydroperoxidic groups. These maxima, although weak, have proven to be useful in studies of the oxidation of unsaturated

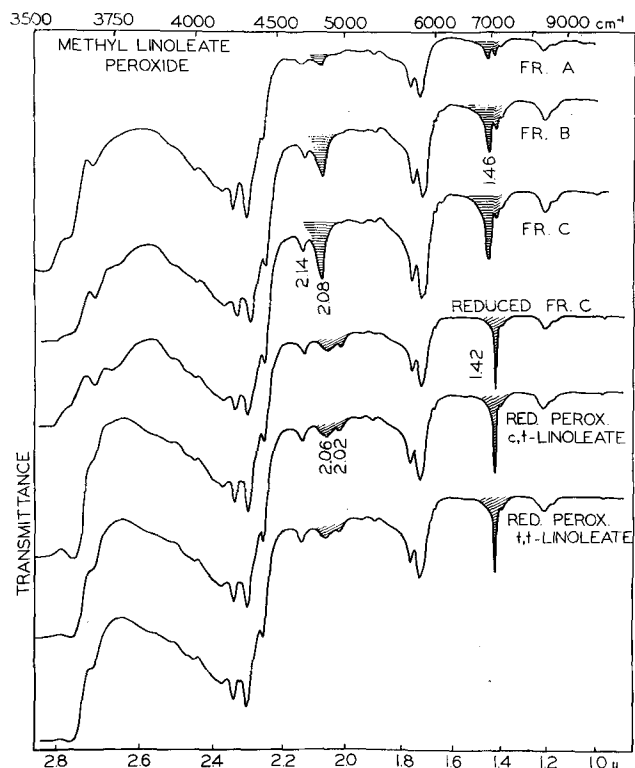


FIG. 3. Methyl linoleate peroxide A—50 g./L.; B—34 g./L.; C—41 g./L.; reduced fraction C—41 g./L.; reduced peroxide from *cis*, *trans* linoleate 53 g./L.; reduced peroxide from *trans*, *trans* linoleate 49 g./L.

89% of the theoretical value. The near-infrared spectrum of this peroxide and the parent methyl oleate are shown in Figure 2. The conversion of methyl oleate to its hydroperoxide caused the disappearance of the absorption maxima at 2.15 and 2.19 μ due to *cis* unsaturation, and new maxima appeared at 1.42, 1.46, 2.08, 2.77, and 2.82 μ . The hydroxyl maxima at 1.42 and 2.77 μ are relatively weak, indicating that this preparation has only a small amount of hydroxy substances which appear with hydroperoxides and are difficult to separate from them.

Octadecadiene was autoxidized at 0–2°C. to a peroxide value of 1,510 meq./kg., and its hydroperoxide fraction was concentrated by countercurrent distribution (6). The preparation had a peroxide value of 6,950 (theo. 7,080) and its k_{234} was 77.0. The spectra of octadecadiene and its hydroperoxide shown in Figure 2 are very similar to those of methyl oleate and its hydroperoxide. Octadecadiene offers the advantage over methyl linoleate that it contains no oxygen in the molecule, therefore the appearance of hydrogen-bonded oxygen-containing groups as a consequence of its oxidation is not obscured. The conversion of the hydrocarbon to its peroxide removed the *cis* absorption at 2.15 and 2.18 μ , and new maxima appeared at 1.42, 1.45, 2.07, 2.77, and 2.82 μ . The hydroxyl absorption at 1.42 and 2.77 μ was strong in this preparation, indicating low purity despite the high apparent peroxide value.

Methyl linoleate was allowed to oxidize under air at 0–2°C. to a peroxide value of 935 meq./kg., and the peroxides were concentrated by countercurrent distribution (6). The preparation was divided into three fractions according to polarity. Fraction A, the most polar, had a peroxide value of 3,730 and $k_{234} =$

31.6. Fraction B had a peroxide value of 6,040 and $k_{234} = 55.0$. Fraction C, the least polar, had a peroxide value of 6,150 and $k_{234} = 73.3$. The highly polar substance present in greatest proportion in Fraction A and least in C represents secondary products of oxidation. The near infrared spectra of these preparations, shown in Figure 3, indicate that the absorption maxima at 1.46 and 2.08 μ are in proportion to the peroxide values and that no *cis* unsaturation remained. The maximum at 2.14 μ is caused by the methyl ester.

Methyl linoleate hydroperoxide and hydroperoxides prepared similarly from methyl-*cis*-9, *trans*-12 linoleate and methyl *trans*-9, *trans*-12 linoleate were converted to the corresponding hydroxy compounds by reduction with stannous chloride. In all cases the peroxide values were reduced to less than 200, and the conjugated diene system remained intact as judged by ultraviolet absorption spectra. The near-infrared spectra of these reduced hydroperoxides, shown in Figure 3, show intense hydroxyl absorption at 1.42 and 2.77 μ and two accompanying weak broad hydroxyl absorptions at 2.02 and 2.06 μ . The maxima at 1.46 and 2.08 μ present in the peroxides disappeared after reduction.

In all samples tested thus far in which hydroperoxides are known to occur, maxima appear near 1.46, 2.07, and 2.82 μ . Conversely, related substances in which hydroperoxide groups are not present fail to have these absorption maxima. These evidences allow the assignment of these maxima to the O-H bonding of a hydroperoxide group.

Because of the difficulty of obtaining pure hydroperoxides, and of the measurement of their purity, molecular extinction coefficients cannot now be calculated with certainty. However, from the preparations described here, the following approximate values are estimated:

	ϵ 2.07 μ	ϵ 1.46 μ
Cyclohexene hydroperoxide.....	0.45	1.46
Methyl oleate hydroperoxide.....	0.64	0.73
Octadecadiene hydroperoxide.....	0.40	0.48

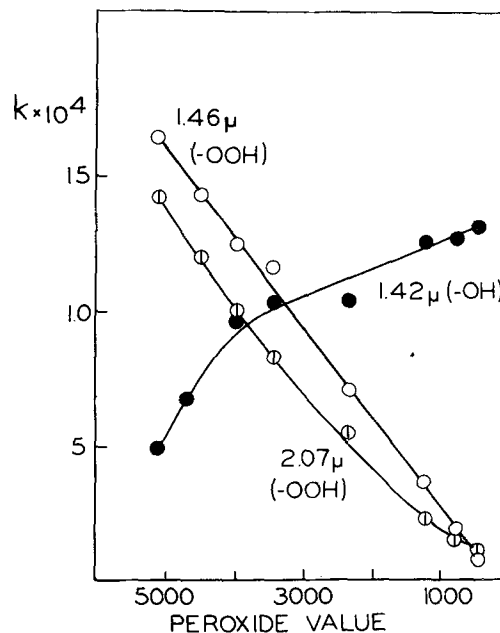


FIG. 4. Thermal decomposition of methyl oleate hydroperoxide at 100°.

In an effort to verify that absorption at 1.46 and 2.07 μ is proportional to peroxide content, a study of the thermal decomposition of methyl oleate hydroperoxide was made. A sample of methyl oleate hydroperoxide, P. V. = 5,110, was divided in eight portions and sealed in vacuum ampoules. The ampoules were placed in a flask of water, which was allowed to boil under reflux. At appropriate intervals ampoules were removed, the peroxide values were determined, and the near-infrared spectra were measured. The effect of thermal decomposition upon the spectral absorption is shown in Figure 4. The extinction at 2.07 and 1.46 μ diminished linearly with the decrease in iodometric peroxide value. However hydroxy substances (1.42 μ) increased as the peroxides were decomposed.

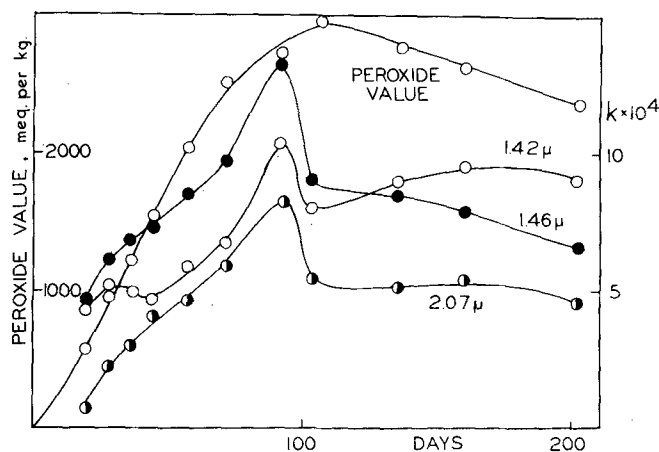


Fig. 5. Autoxidation of methyl linoleate at room temperature.

When a sample of methyl linoleate was allowed to oxidize in air, the extinction at 1.46 and 2.07 μ increased in parallel fashion (Figure 5). Hydroxyl absorption (1.42 μ) likewise increased as the peroxide value increased. When maximum peroxide value was attained, discontinuities in the curves for absorption at 1.42, 1.46, and 2.07 μ were observed. Thereafter, as iodometric peroxide values decreased slowly, hydroperoxide absorption at 1.46 and 2.07 μ decreased slowly whereas hydroxyl absorption at 1.42 μ increased, indicating peroxide decomposition.

The detection and measurement of hydroperoxides by their absorption at 2.82 μ is possible by use of conventional infrared spectrophotometers, but resolution by rock salt prisms in that region is poor, making it difficult to differentiate between the hydroperoxide maximum at 2.82 μ and the hydroxyl maxi-

mum at 2.77 μ . Some advantage may be gained by the use of a quartz prism, but the region of 2.7–2.9 μ is the location of absorption maxima of aldehydes, ketones, acids, and esters as well as hydroxyls and hydroperoxides. For this reason the present study centered on the maxima at 1.46 and 2.07 μ , which are sufficiently removed from other absorption maxima to make interference least likely. In these two selected regions the resolution of the quartz prism is sufficient to distinguish the neighboring O-H and OO-H absorptions. A disadvantage is that these maxima are not expressions of the fundamental modes of vibration. The maximum at 2.07 μ is probably a combination absorption and that at 1.46 μ is a harmonic of the fundamental mode at 2.82 μ . Consequently these absorptions are of low intensity, and they must therefore be measured in high concentrations or in long cells. The solutions used in measurements reported here varied between 3 and 10% in carbon tetrachloride. The measurement of most of the spectra required 100 to 200 mg. of sample. This can be a limiting factor in chemical studies. In the present investigation hydroperoxides could not be detected by spectra until the peroxide value was about 500 meq./kg. Detection of lower concentrations is possible with cells of longer light path.

Summary

Near-infrared spectra have been measured on a group of hydroperoxides of fatty acid esters and related substances. Only those substances having an -OOH group were found to absorb at 1.46 and 2.07 μ . Dialkyl peroxides and ozonized unsaturated substances had no such maxima in their near infrared spectra although they had high iodometric peroxide values. In a study of the thermal decomposition of methyl oleate hydroperoxide and a study of the autoxidation of methyl linoleate, the intensity of absorption at 1.46 and 2.07 μ paralleled the iodometric peroxide value.

Acknowledgment

The authors are indebted to F. J. Pusch for the preparation of 9-octadecene and 9,12-octadecadiene used in this work.

REFERENCES

1. Deatherage, F. E., *J. Am. Chem. Soc.*, **61**, 630 (1939).
2. Holman, R. T., and Edmondson, P. R., *Anal. Chem.*, **28**, 1533 (1956).
3. Jackson, J. E., Paschke, R. F., Tolberg, W. E., Boyd, H. M., and Wheeler, D. H., *J. Am. Oil Chemists' Soc.*, **29**, 229 (1952).
4. Kass, J. P., and Burr, G. O., *J. Am. Chem. Soc.*, **61**, 1062 (1939).
5. Lightelm, S. P., von Rudloff, E., and Sutton, D. A., *J. Chem. Soc.*, **1950**, 3187.
6. Privett, O. S., Lundberg, W. O., Nickell, Christense, *J. Am. Oil Chemists' Soc.*, **30**, 17 (1953).

[Received March 3, 1958]

The Bleaching of Soybean Oil. A Spectrophotometric Evaluation

M. R. ARMSTRONG, James Millikin University,¹ and C. E. IRELAND, A. E. Staley Manufacturing Company, Decatur, Illinois

THE QUANTITATIVE STUDY of bleaching agents and/or methods requires information on the amount of coloring matter in the initial and partially decolorized oil. Since the eye is an integrating organ, similar color responses can be caused by different

compositions of pigmentation. Lovibond glasses numerate what the eye sees and hence are not suited for determining the amounts of the different pigments. Spectrophotometric evaluation was chosen because of the intrinsic suitability of this method and the availability of good instruments.

¹ Now with E. I. du Pont de Nemours Company Inc., Kinston, N. C.