Peroxide Value Determination—Comparison of Some Methods

G. BARTHEL and W. GROSCH, Deutsche Forschungsanstalt für Lebensmittelchemie, 8 München 40, Germany

ABSTRACT

A comparison between the determination of the peroxide value by the methods of Wheeler and Sully (iodometric titration) and that of Stine, et al. (ferric thiocyanate method) was made. Some oxidized vegetable oils, H_2O_2, t-butyl hydroperoxide, cumene hydroperoxide, methyl oleate hydroperoxides, and methyl linoleate hydroperoxides were used as substrates. One-hundred percent of the methyl linoleate hydroperoxides were recovered by the Wheeler reduction, 85% by the Sully method. The Wheeler method was used to reduce the methyl linoleate hydroperoxides to the corresponding hydroxy acids. In the Sully procedure, the hydroxy acids are only intermediates which are dehydrated to octadecatrienoic acids. One equivalent methyl linoleate hydroperoxide oxidized two equivalents of I^- (Wheeler) and four equivalents of Fe^{2+} (Stine, et al.). By way of contrast, H_2O_2 needs only two equivalents I^- or Fe^{2+} for reduction. The excess consumption of reduction equivalents in the ferric thiocyanate method probably is caused by secondary reactions of the methyl linoleate hydroperoxide acyl residue.

INTRODUCTION

Numerous analytical methods under the heading, "procedures for peroxide value determination," for the quantitative determination of the primary products of oxidative deterioration have been developed. Of these, iodometric titration plays an important role. Comparative experiments, according to Lea (1), Franzke (2), Hadorn, et al., (3), Thaler and Kleinau (4), among others, show that the results of analysis depend upon the experimental conditions and upon the reducing agent used. It is not yet clear how differences in experimental procedure affect the complete evaluation, the course of reaction, and the stoichiometry of the reduction. An answer to this question is possible if hydroperoxides or peroxides, normally found in the oxidative deterioration of fats and whose structures are known, are analyzed by the recommended methods.

A mixture of methyl-13-hydroperoxy-9,11-octadecadienoate and methyl-9-hydroperoxy-10,12-octadecadienoate (LMPO) which arise from the autoxidation of methyl linoleate was used as substrate in the following experiments. Comparisons were made between iodometric titration according to Wheeler, modified by Hadorn, et al. (3), iodometric titration of Sully according to the method of the Dectsche Gesellschaft für Fettwissenschaft (5), as well as the reduction with Fe^{2+} measured photometrically according to Stine, et al. (6). The preliminary data from these experiments were published earlier as a short communication (7).

MATERIALS

The following materials were obtained from the source given in parentheses: linoleic acid (Nu Chek Prep, Elysian, Minn., 99%); Tween 20 (Schuchardt, Darmstadt, Germany); Lipoxygenase (Sigma St. Louis, Mo.; Type I, 50,000 E/mg); Silica Gel HF_{254} (Merck, Darmstadt, Germany) palladium/charcoal (Merck); N-methyl-N-nitroso-p-toluolsulphonamide (Schuchardt); carbitol (Schuchardt); and t-butylhydroperoxide and cumene hydroperoxide (Elektrochemische Werke, München, Germany). All other reagents were of reagent grade.

Corn, sunflower, and olive oils were obtained commercially; hazelnut and walnut oils were extracted from the kernel with n-heptane, and the solvent was removed under vacuum.

Autoxidation of the oils: A thin layer of each sample of oil was exposed to UV light (λ = 254 nm) at room temperature until a certain peroxide value was reached. For walnut oil, this was 90 min and for hazelnut oil 180 min.

LMPO: Linoleic acid hydroperoxides were formed using soybean lipoxygenase after Grosch, et al. (8). The hydroperoxy acid was purified using thin layer chromatography (TLC) (Silica Gel HF_{254}; developing solvent: heptane/ether/glacial acetic acid 50 + 50 + 1 v/v/v). The hydroperoxy acids were extracted from the TLC scrapings with absolute methanol and then methylated with diazomethane as per the method of Schlenk and Gellerman (9). Purification of the LMPO was carried out in the same way using TLC (Silica Gel HF_{254}, developing solvent: isooctane/ether 2 + 3 v/v). The LMPO viewed under UV light were extracted with absolute methanol.

Autoxidized methyl oleate (OMPO): A thin layer of methyl oleate was exposed to UV light for 180 min at room temperature.

Mixture of methyl-13-hydroxy-9,11- and methyl-9-hydroxy-10,12-octadecadienoate (LOH): Reduction of LMPO with $NaBH_4$ was carried out according to Zimmerman and Vick (10).

METHODS

Determination of LMPO Concentration

The LMPO concentration was determined from the diene absorption of methanolic LMPO solution with A_{234} = 28,000 $l \cdot mol^{-1} \cdot cm^{-1}$ (11).

For the determination by the iodometric method of Wheeler, modified by Hadorn, et al. (3), 8 μmole LMPO was dissolved in 10 ml glacial acetic acid/chloroform 3+2 (v/v). Ca. 1.2 mmole potassium iodide (KI) dissolved in 0.2 ml H_2O was used as reducing agent, and this was titrated against a 0.01 N solution of thiosulphate.

For the determination by the iodometric method of Sully (5), 8 μmole LMPO was added to 20 ml boiling glacial acetic acid/$CHCl_3$ (1:1 v/v) and was reduced with 6.1 mmole KI, dissolved in 1.3 ml H_2O. This was titrated against a 0.01 N solution of thiosulphate.

The reduction of the LMPO with $FeSO_4$ was carried out according to Stine, et al. (6); $1.6 \cdot 10^{-2}$ μmole LMPO dissolved in 2 ml methanol and 0.1 ml water were diluted with benzol/methanol (7 + 3 v/v) to 10 ml. The reduction took place using 3.6 μmole $FeSO_4$ dissolved in 0.02 ml 3.6% HCl. Thirty sec after the addition of the $FeSO_4$ solution, 0.02 ml 30% potassium thiocyanate (KSCN) solution was added. The mixture was warmed for 2 min at 50 C, and, in the following 2 min, cooled under running water to room temperature. The color intensity was measured at 505 nm against benzene/methanol (7 + 3 v/v) and the values calculated from a calibration curve of $NH_4Fe(SO_4)_2 \cdot 12\ H_2O$.

Gas Chromatographic Analysis of Oils

The oils were purified chromatographically over neutral Al_2O_3 (activity grade 1) with petroleum ether/ether 7:3 v/v

TABLE I

Fatty Acid Composition of Oils Expressed in Percent Wt.

Fatty acid	Sunflower oil	Corn oil	Walnut oil	Hazelnut oil	Olive oil
16:0	6.18	10.4	6.6	5.0	12.2
18:0	4.02	1.8	1.7	2.13	2.0
20:0	—	0.22	—	—	0.27
16:1	—	—	—	—	1.36
18:1	22.1	25.3	19.7	82.04	75.1
18:2	67.7	61.4	61.0	10.8	8.3
18:3	—	0.94	11.0	0.2	0.85

and transesterified with sodium methoxide after Shehata, et al. (12). The resulting methyl esters were analyzed with a Hewlett-Packard gas chromatograph (conditions: 15% diethyleneglycol succinate [DEGS] Varaport 30 (70-80 mesh); 10 ft; 1/8 in. ϕ; detector temperature, 235 C; injector temperature, 240 C; and column temperature, 185 C).

Peroxide Value of Autoxidized Oils

Each 1 g oil was analyzed as per Wheeler and Sully. For the Fe^{2+} test, 4 mg each oil was dissolved in 9.9 ml benzol/methanol (7 + 3 v/v). The determination followed as per the method of Stine, et al., after the addition of 0.1 ml H_2O.

Iodometric LMPO Determination: Isolation of Reaction Products

At the end of the reduction, the organic phase was separated and washed with H_2O until it gave a neutral reaction. The solution was dried over $CaCl_2$, concentrated, and separated by TLC (Silica Gel HF_{254}, developing solvent: isooctane/ether 2 + 3 v/v). The bands visible under UV light were extracted with ether.

Structural Determination

Hydrogenation of double bonds: The solvent containing the substances isolated by TLC was evaporated and the residue taken up in 5 ml absolute ethanol and hydrogenated after Zimmerman and Vick (10) with palladium/activated charcoal in absolute ethanol.

Ozonolysis of double bonds: Ca. 20 mg substance was dissolved in 5 ml $CHCl_3$ and treated with ozone at -20 C as per Hamberg (13) until the blue color of the indicator solution (KI, H_2SO_4, starch) showed that the reaction was complete. The solution was left to stand for 10 min at room temperature and the solvent then evaporated. The residue was dissolved in 7.5 ml glacial acetic acid, mixed with 2 ml 30% H_2O_2, and, after 18 hr at 50 C, the solvent was evaporated at 30 C under vacuum. The residue was dissolved in ether/methanol (9 + 1 v/v) and methylated after Schlenk and Gellerman (9). The methyl esters were analyzed by gas liquid chromatography (GLC) using 15% DEGS (Varaport 30, 70-80 mesh) as stationary phase. Working conditions for monomethyl ester were: column temperature, 80 C; injector temperature, 140 C; and detector temperature, 140 C. Working conditions for dimethyl ester were: column temperature, 185 C; injector temperature, 235 C; and detector temperature, 235 C.

IR spectroscopy: The substances were dissolved in carbon tetrachloride to take the spectra.

Mass spectrometry: The spectra were made with an AEI mass spectrometer 30 at a temperature of 250 C (ion source) and an energy of 70 ev.

Course of Dehydration Reactions—Sully Method

The hydroxy acids resulting from the reduction with $NaBH_4$ of LMPO (after reference 10) were methylated after Schlenk and Gellerman (9).

Ca. 8 μmole methyl hydroxyoctadecadienoate mixture was dissolved, as described in the method of Sully, in $CHCl_3$/glacial acetic acid and heated with added KI. A control without KI also was included. The reaction products from both samples were isolated and separated by TLC, as described above.

TABLE II

Peroxide Values of Autoxidized Oils Determined by Methods of Wheeler, Sully, and Stine, et al.

Oil	Method		
	Wheeler	Sully	Stine, et al.
Sunflower	18.2	16.0	31.4
	57.0	51.6	108.3
Corn	6.6	6.5	11.5
	19.3	19.2	35.8
Walnut	27.7	24.5	58.6
Hazelnut	27.7	27.1	57.5
Olive	24.0	24.1	45.5

Stability of LMPO in Solvent Systems and Temperatures According to Sully and Wheeler

The experiments were carried out as described above, only without the addition of KI. The organic phase was separated, the methyl ester of palmitic acid was added as internal standard, the solution washed with water until it gave a neutral reaction, and dried over $CaCl_2$. After evaporation of the solvent, the residue was dissolved in absolute ethanol and reduced with $NaBH_4$ according to Zimmerman and Vick (10). After removal of the unused $NaBH_4$ and of the solvent, the reaction products were dissolved in 1 ml pyridine, silylated by the method of Graveland (14) and analyzed using gas chromatography. In a control experiment, LMPO was reduced, and the hydroxy groups were silylated as described above and then determined by GLC with the aid of the methyl ester of palmitic acid as internal standard.

RESULTS AND DISCUSSION

Peroxide Values of Some Autoxidized Oils

Five vegetable oils, whose fatty acid compositions are given in Table I, were autoxidized rapidly by exposure to UV light. The peroxide values of these oils were at the same time determined by the three methods previously introduced. Two different oxidation states were tested for corn oil and sunflower oil (Table II).

To show the differences in consumption of reduction equivalents in the three methods, the results given in Table II were worked out in the following way. For Wheeler's method, the following stoichiometry is postulated: LMPO + $2e^- + 2H^+ \rightarrow LOH + H_2O$. The results of the Sully and the Stine, et al., methods were related to the two electron transfer assumed for Wheeler's method. This relationship is shown in Table III.

As Tables II and III for iodometric procedures show, the values after Wheeler and Sully agree for hazelnut, olive, and corn oils; however, different results were found for sunflower and walnut oils. Here the iodide consumption for the "hot" iodometry was smaller than that of Wheeler's

TABLE III

Relationship between Peroxide Values Determined by Three Methods[a]

Oil	Method		
	Wheeler	Sully	Stine, et al.
Sunflower	2	1.77	3.46
		1.81	3.80
Corn	2	2.0	3.45
		2.0	3.71
Walnut	2	1.76	4.20
Hazelnut	2	2.0	4.24
Olive	2	2.0	3.78

[a]The results of Sully's and Stine's methods were related to the two electron transfer assumed for Wheeler's method.

TABLE IV

Quantitative Determination of Mixture of Methyl-13-Hydroperoxy-9,11-Octadecadienoate and Methyl-9-Hydroperoxy-10,12-Octadecadienoate

Method	Percent
Diene absorption	100
Wheeler[a]	100
Sully[a]	88.5
Stine, et al.	181

[a]The basis for the conversion was taken as the diene absorption.

method. The measured peroxide values for the two different oxidation states of corn and sunflower oils show that the difference between the results obtained by Wheeler's method and those of Sully was affected little by the peroxide concentration. If the Fe^{2+} test after Stine, et al., is included in the comparison, then it can be seen that the reduction with Fe^{2+} with a transfer of ca. four electrons/peroxide group shows a different stoichiometry from that shown by iodometric procedures, as observed by Lea (1). In comparison to the Wheeler method, the Fe^{2+} consumption varied from oil to oil (Table II).

Analysis of Particular Hydroperoxides

The three peroxide procedures were checked with peroxidized methyl linoleate (LMPO). The LMPO was not obtained through autoxidation but through lipoxygenase catalysis. This enzymatic oxidation, kept at 1-3 C, offered the advantage that only small amounts of secondary oxidation products were formed besides the hydroperoxides. By the use of this soy preparation, as opposed to the autoxidation, the relationship between the 13- and the 9-hydroperoxides was displaced from 1:1 to 4:1.

The evaluation of the peroxide value determination was carried out on the basis, previously mentioned, that one LMPO needs two electrons for reduction. The basis for the conversion was taken as the diene absorption at 234 nm.

Table IV shows that, with the Wheeler method, the expected LMPO concentrations were found. However, "hot" iodometry yields values which are too low, as already observed for autoxidized oils with a high linoleic acid content. After titration, no more LMPO was found through TLC (Silica Gel HF_{254}—isooctane:ether 2 + 2 v/v). Under the severe conditions of Sully, ca. 10% of the hydroperoxides were decomposed before being able to react with the reducing agent.

The conversion that is assumed to occur in the iodometric method in the most part depends upon the medium in which the reduction takes place. The reduction of the LMPO took place in a mixed solvent of glacial acetic acid/$CHCl_3$, after Sully and Wheeler as previously stated, together with added methanol. The experiment shows a 76% conversion in the "cold" iodometric procedure and a

TABLE V

Influence of Methanol on Iodometric Determination of Mixture of Methyl-13-Hydroperoxy-9,11-Octadecadienoate and Methyl-9-Hydroperoxy-10,12-Octadecadienoate

Method	Percent
Diene absorption	100
Wheeler[a]	76
Sully[a]	96

[a]The basis for the conversion was taken as the diene absorption. The determinations were carried out as described in "Methods"; however, each sample contained an additional 2 ml methanol.

TABLE VI

Reduction of Various Hydroperoxides (Electrons/Molecule ROOH)

Hydroperoxide	Wheeler	Stine, et al.
H_2O_2	2	2.0
t-Butyl-OOH	2	2.52
Cumene-OOH	2	3.18
OMPO	2	3.98
LMPO	2	3.66

96% conversion by the "hot" iodometry (Table V). A TLC separation of the samples by Wheeler's method showed after titration that, in spite of the excess KI (1:75) at room temperature in the presence of methanol, ca. 25% of the LMPO still remained unreduced. Compared with iodometric procedures, the consumption of reduction equivalents for the LMPO determination with Fe^{2+} was raised to nearly double the value, as with autoxidized oils (Table IV). This higher consumption came from the further reactions of the acyl chain. An analysis of different hydroperoxides gave higher consumption of Fe^{2+} ions as compared with "cold" iodometry (Table VI). H_2O_2 showed, both for the iodometric and for the Fe^{2+} methods, a two electron transfer, while the Fe^{2+} consumption arises from the reduction of t-butylhydroperoxide; the I^- consumption was unchanged. The occurrence of aromatic systems or double bonds in the molecule makes a further relative increase in the Fe^{2+} conversion.

Reaction Products of LMPO

After iodometric titration, the resulting products from the LMPO were isolated through TLC. The "cold" iodometry by Wheeler's method gave a substance having an R_f = 0.41, and its structure determinations showed, by IR spectrum absorption, bands for a hydroxy- (3600 cm^{-1}), a methyl ester group (1730 and 1175 cm^{-1}), and for *cis,trans* conjugated double bonds (3040, 980, and 950 cm^{-1}). The UV spectrum (λm; 234 nm; methanol) showed that conjugated diene systems are in the proximity of the hydroxy groups. The substance was hydrogenated, using a palladium/charcoal catalyst until the diene absorption disappeared. The mass spectrum of the hydrogenated compound was identical with that published by Zimmerman and Vick (10) for a mixture of methyl-13-hydroxystearate and methyl-9-hydroxystearate. On the basis of these spectroscopic data, the isolated substance was found to be a mixture of methyl-13-hydroxy-9,11-octadecadienoate and LOH.

The "hot" iodometry by Sully's method gave, through TLC, a substance of R_f = 0.67 whose structure determined by UV spectrum (λm = 268 nm; methanol) indicates a triene system. The substance was hydrogenated using a palladium/charcoal catalyst until the disappearance of the triene absorption and then identified as methyl stearate. The oxidative ozonolysis of the triene system using O_3/peracetic acid and GLC analysis of the methyl and dimethyl esters of the mono- and dicarboxylic acids gave

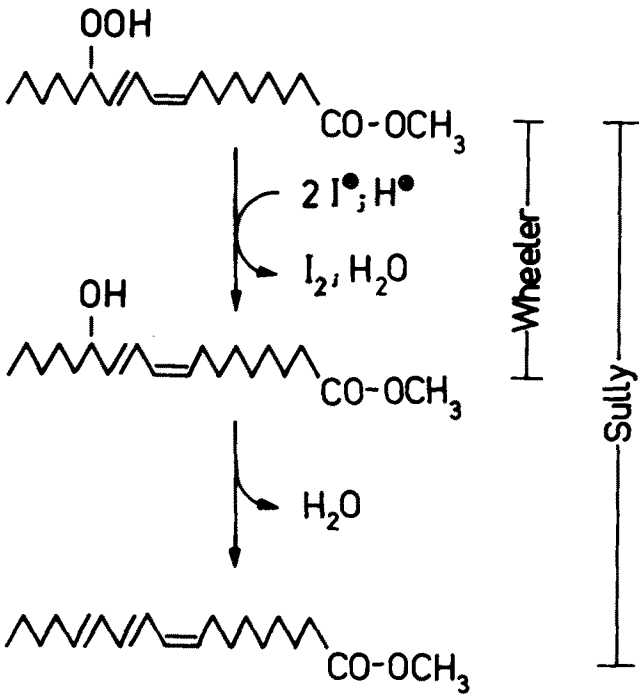

FIG. 1. Course of the reduction by the methods of Wheeler and Sully.

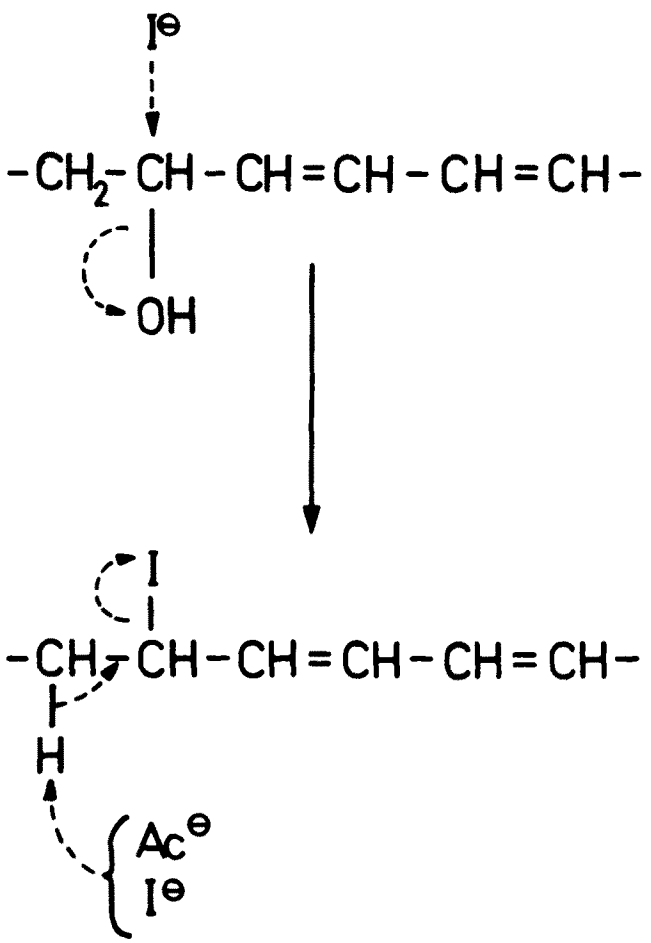

FIG. 2. Mechanism of the dehydration of Sully's method.

valeric acid, caproic acid, azelaic acid, and suberin acid. The isolated substance was, therefore, found to be a mixture of methyl 9,11,13-octadecatrienoate and methyl 8,10,12-octadecatrienoate.

The iodometric methods showed, from the isolated

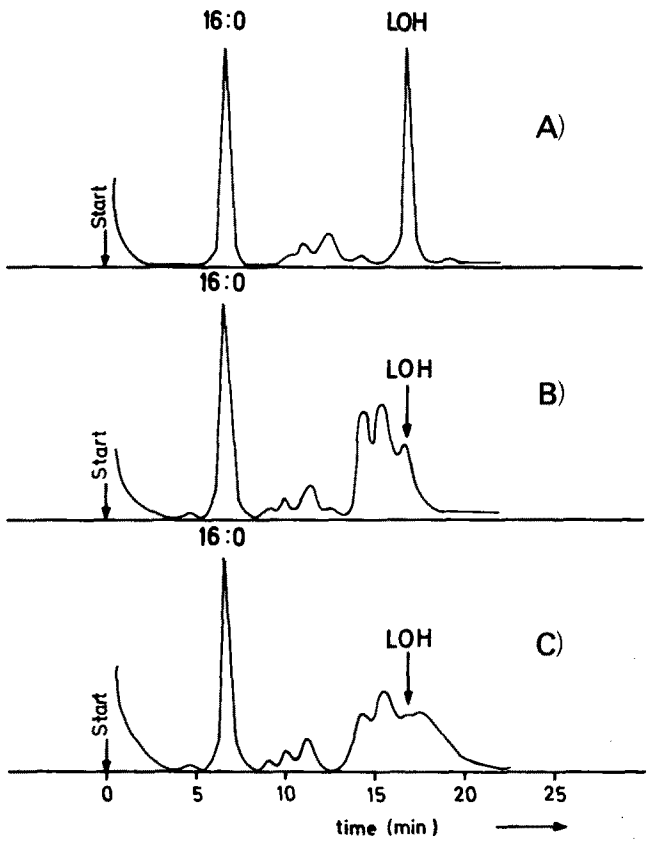

FIG. 3. Stability of peroxidized methyl linoleate (LMPO) under the conditions of Wheeler's and Sully's methods. Gas liquid chromatography of the reduced and silylated products (as per text). A. Control experiment, B. conditions of Wheeler, and C. conditions of Sully. LOH = mixture of methyl-13-hydroxy-9,11-octadecadienoate and methyl-9-hydroxy-10,12-octadecadienoate.

reaction products (Fig. 1), that the course of the reduction under Wheeler conditions was as expected; only the hydroperoxy group was reduced to the hydroxy group. With the severe experimental conditions of Sully, however, a dehydration of hydroxy diene to a triene system took place. The course of the H_2O removal was studied. Just heating the methyl hydroxy octadecadienoate dissolved in $CHCl_3$/glacial acetic acid (1:1 v/v) (produced by reduction of LMPO with $NaBH_4$) did not remove water from the reaction. What is important is the presence of a quantity of KI, as described by Sully. As shown in Figure 2, we assume that the dehydration was initiated by a nucleophilic substitution of the hydroxy group brought about by the presence of iodide, and the hydrogen iodide group then leaves the molecule.

Stability of Peroxidized Methyl Linoleate in Solvent Systems and Temperatures According to Sully and Wheeler

The solvent mixture $CHCl_3$/glacial acetic acid (3:2 v/v Wheeler; 1:1 v/v Sully) is too severe for the already described reduction of the hydroperoxides, as it promotes the breakdown of the peroxides. To show the possible differences between "cold" and "hot" iodometry, the LMPO was dissolved in $CHCl_3$/glacial acetic acid 3:2 v/v at room temperature (Wheeler) and in boiling $CHCl_3$/glacial acetic acid 1:1 v/v (Sully). The reducing agent KI was left out. After 1 min (Wheeler) and 4 min (Sully), the acetic acid was removed and the remaining hydroperoxides reduced with $NaBH_4$. The product was silylated and analyzed by GLC. When untreated LMPO was reduced with $NaBH_4$, then the methyl esters of the hydroxy fatty acids resulting from the LMPO predominated (Fig. 3A). Most of

the LMPO decomposed when treated with the medium indicated by both Wheeler and Sully before reduction with $NaBH_4$. The peaks for the methyl esters of the hydroxy fatty acids were small in comparison with that for the internal standard (methyl ester of palmitic acid) (Figs. 3B and 3C). A difference between "cold" and "hot" iodometry is indicated. The peak for the methyl ester of the hydroxy acid is clearly larger by Wheeler's than by Sully's method. The heating in Sully's method brings about an almost complete disruption of the LMPO. In the iodometric determination of the hydroperoxides, two processes compete with one another: the reduction of the hydroperoxy group and the breakdown of the hydroperoxides. During the "cold" iodometry of Wheeler, the reduction is much faster than the breakdown, so that all of the hydroperoxides can be determined by this analysis. The higher reaction temperature of Sully's method accelerates not only the reduction but also the hydroperoxide destruction. This is possibly the reason why, in this test, some part of the hydroperoxides break down and cannot, therefore, be reduced with KI. The peroxide values determined by Sully are, thus, somewhat lower in comparison with those found by the Wheeler method. Nothing final can be said about the very sensitive Fe^{2+} test, since the structure determinations of the products from the LMPO are still being worked on.

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