The Estimation of Peroxides in Fats and Oils by the Ferric Thiocyanate Method[1]

R. A. CHAPMAN [2] and K. MACKAY [3]

Introduction

A NUMBER of methods for the estimation of peroxides having been based on the oxidation of ferrous iron to the ferric form and the estimation of the latter by various procedures. Yule and Wilson (1) determined the peroxides in petroleum hydrocarbons by titrating the ferric iron with titanous chloride. Later Young, Vogt, and Nieuwland (2) modified the method by estimating the ferric iron as ferric thiocyanate. The method has also been used in the estimation of peroxides in rubber. Bolland et al (3) developed a colorimetric method using benzene-methanol as a solvent, and more recently Robey and Weise (4) have reported that a solvent composed of 20% ethanol in cholorform was more satisfactory for this purpose.

Chapman and McFarlane (5) adapted the method to the estimation of peroxides in whole-milk powders and later Lips, Chapman, and McFarlane (6) applied the procedure to fats and oils. Their reagent consisted of a 96% aqueous acetone solution containing ferrous ammonium sulphate and ammonium thiocyanate. An acetone solution of the fat was added directly to this reagent and the intensity of the colour determined in a photoelectric colorimeter. Hills and Thiel (7) have also used the method for the estimation of peroxides in oils, but these workers used benzene-methanol as a solvent. They also found that the stability of the reagent was improved by preparing separate solutions of ferrous chloride and ammonium thiocyanate.

However, when the ferric thiocyanate procedures are applied to fats and oils, peroxide values are obtained which are considerably higher than the results given by iodimetric methods although proportional to these values. Lea (8) has attributed this effect to the interference of atmospheric oxygen at the moment of contact between the reagent and the fat peroxide. He was able, by rigorously excluding all oxygen from the solvent and reaction chamber, to reduce the peroxide values obtained by the ferric thiocyanate method (6) to approximately one-quarter of the original value. The present investigation involves a study of the effect of atmospheric oxygen on the various modifications of the ferric thiocyanate procedure and an attempt to find some means of offsetting this interference.

Experimental

Bolland et al (3), when applying their method to rubber and rubber derivatives, have reported that peroxide values were obtained which corresponded to 80% of the oxygen uptake. Since the reagent employed by these workers contained an appreciable amount of sulphuric acid and there appeared to be no oxygen interference, it seemed advisable to determine the effect of increasing the acidity of the acetone reagent (6). The acidity of two reagents was therefore adjusted by the addition of sulphuric acid to pH's of 1.19 and 2.08. The original reagent without the addition of acid was found to have a pH of 5.48. Peroxide values were then determined, employing these reagents, and the results are shown in Table I. It is evident that the addition of acid did not eliminate the interference of the atmospheric oxygen; in fact the use of the acidified reagents resulted in higher values for linseed and soybean oil.

Hydroxyl derivatives of aromatic compounds have long been known as inhibitors of the oxidation of fats and oils. Since it has been assumed that oxygen interferes by oxidizing the unsaturated linkages in the fat molecule during the test, it appeared possible that a compound of this type might be effective in reducing or eliminating the interference of atmospheric oxygen. However, it was necessary to find a substance which reacted readily with dissolved oxygen but would not reduce the ferric iron. A number of substances were therefore tested by adding a small amount of each substance to a reagent containing an appreciable amount of ferric iron. If the colour of the undissociated ferric thiocyanate disappeared, it was assumed that the particular substance would not be satisfactory. This preliminary screening eliminated all substances tested with the exception of phloroglucinol, resorcinol, and α-naphthol.

TABLE I

The Effect of Increased Acidity on Peroxide Values

Oil Sample	Milliequivalents of peroxide per kg. of oil		
	pH 5.48	pH 2.08	pH 1.19
Linseed	149	160	173
Olive	1582	1582	1547
Soybean	72	97	85
Cottonseed	541	538	518

Preliminary tests were then conducted with reagents containing 0.1% of each of the foregoing substances on a sample of cottonseed oil. The results indicated that phloroglucinol and resorcinol had no effect since the values obtained were identical with those of the control. However the peroxide values obtained with the reagent containing α-naphthol were slightly reduced, and it was therefore decided to investigate the use of this substance more thoroughly.

In order to carry out the determination it was necessary to prepare a calibration curve with a reagent containing α-naphthol. It was found that when reagents were employed containing 0.1, 0.15, and 0.2% of α-naphthol, and ferrous chloride was also present, curves were obtained with all three concentrations which were almost identical with the curve given by an untreated reagent. However when the concentration was increased to 0.5%, there was appreciable reduction in the colour. The results obtained employing reagents containing α-naphthol at levels of 0.15, 0.20, and 0.25% are given in Table II.

---

[1] Contribution from the Faculty of Agriculture, McGill University, MacDonald College, Que., Canada. Journal Series No. 245. Presented before the Annual Conference of the Chemical Institute of Canada, Montreal, June, 1948.

[2] Assistant Professor of Chemistry. Present address, Food and Drug Divisions, Department of National Health and Welfare, Ottawa, Canada.

[3] Research Assistant in Chemistry.

TABLE II

The Effect of α-Naphthol on Peroxide Values

Oil Sample	Peroxide values[a]			
	Concentration of α-naphthol			
	Control	0.15%	0.20%	0.25%
Soybean	98 98	24 24	34 29	16 15
Linseed	212	135 135	209 —..	96 96
Olive	1692 1692	930 708	865 846	451 372
Cottonseed	583 572	362 343	345 321	161 161

[a] The peroxide values are reported as milliequivalents of peroxide per kilogram of oil.

It is evident from these values that there is an appreciable reduction when α-naphthol is present at the foregoing levels. The results however were erratic with poor agreement between duplicates. In addition, there was a possibility that the α-naphthol could interfere by reacting with the peroxides. In view of these facts, it was considered inadvisable to attempt to use α-naphthol or other compounds of this type to prevent the oxygen interference.

Lea (8) has suggested that further oxidation of the fat molecule was catalyzed by the ferrous ions present in the solution. This point was checked by preparing a solution of soybean oil in acetone to which had been added 0.1% ferrous ammonium sulphate. A control was prepared containing only soybean oil, and a reagent blank was also included in the experiment. Air was then bubbled through these solutions for periods up to 12 hours. There was only a very slight oxidation of the ferrous iron and the peroxide values obtained with the sample aerated in the presence of ferrous ammonium sulphate were identical with the control sample. It was therefore concluded that contact with the ferrous ion for long periods did not catalyze the oxidation of the triglyceride molecule. However, this experiment did not eliminate the possibility that a catalytic action was being exerted at the moment of the initial contact between the ferrous ions and the fat peroxides.

It was felt advisable at this point to investigate the extent of the oxygen interference in the Hills and Thiel procedure (7). In this modification benzene-methanol was employed as a fat solvent in place of acetone, which permitted the use of samples up to 1 gram and thus increased the sensitivity of the method. Aqueous solutions of ferrous chloride and ammonium thiocyanate were employed, and these solutions were kept in separate containers until the determination was made. These workers employed a visual colorimeter and compared the test solution with an artificial standard prepared from cresol red although a few measurements were also made with a Coleman spectrophotometer. It appeared that the method would be more valuable if a standard curve could be prepared and the peroxide values calculated from this curve. The calibration curve obtained by employing varying amounts of a ferric iron solution instead of a fat or oil is shown in Fig. 1. The curve obtained with the Lips, Chapman, and McFarlane method (6) is also given for comparative purposes. An Evelyn photoelectric colorimeter, equipped with a 520 mμ filter, was used for all readings. The details of the method are given in the following section.

The reagents employed were those recommended by Hills and Thiel (7) and the procedure differs only in minor details.

REAGENTS:

1. *Benzene-methanol.* The solvent is a mixture of 70 vol. of benzene and 30 vol. of absolute methanol. The benzene is redistilled prior to use, and the methanol is dried by refluxing during 4 hr. with bright magnesium ribbon and then distilled.

2. *Ferrous chloride solution.* Hydrated barium chloride (0.4 g.) dissolved in 50 ml. of water is added slowly with stirring to 0.5 g. of hydrated ferrous sulphate dissolved in 50 ml. of water and 2 ml. of 10N hydrochloric acid is then added. The precipitate of barium sulphate is allowed to settle, and the clear solution is then decanted into a bottle protected from light. This solution is stable for approximately a week.

3. *Ammonium thiocyanate solution.* The solution is made by dissolving 30 g. ammonium thiocyanate in water and diluting to a volume of 100 ml.

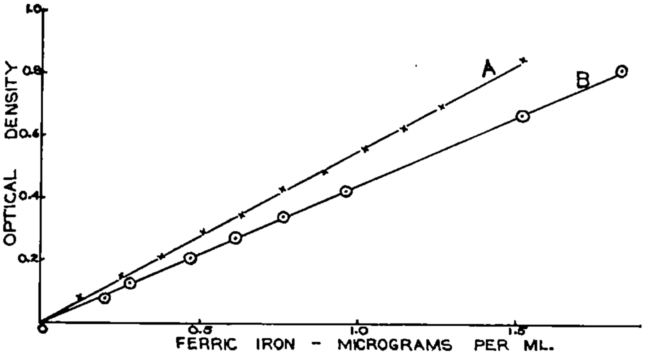

FIG. 1. The Calibration curves obtained with (A) Hills and Thiel Method (B) Lips, Chapman and McFarlane procedure.

4. *Standard ferric iron solution.* Hills and Thiel (7) prepared a solution containing 1 mg. of iron per ml. by dissolving 0.500 g. of bright iron wire in iron-free hydrochloric acid and then oxidizing with hydrogen peroxide. The excess of peroxide was then boiled off and the solution diluted to 500 ml. This solution was diluted and acidified to contain 10 mg. of iron and 1.0 ml. 10N hydrochloric acid per 100 ml. A further dilution was made from this aqueous solution by diluting with the benzene-methanol solvent. In the present investigation a solution of C.P. hydrated ferric chloride in the benzene-methanol solvent was found satisfactory as a standard if care were taken to prevent the absorption of moisture during the weighing of the ferric chloride. In the preparation of the standard curve 1 ml. of a solution containing the required amount of iron replaced the 1 ml. of the fat or oil solution, and all reagents were added including the ferrous chloride.

PROCEDURE:

A 0.1 to 1.0 g. sample of the fat or oil is weighed into a 50 ml. volumetric flask and diluted to volume with the benzene-methanol solvent. One ml. of this solution is then transferred to a 16 x 150 mm. test-tube. In the case of a fresh fat with a very low peroxide value no preliminary dilution may be necessary. Sufficient solvent is added to bring total volume to 9.9 ml. One drop of ammonium thiocyanate solution and one drop of ferrous chloride solution are then added, the tube is shaken and placed in a water bath at 50°C. for 2 minutes. It is then cooled to 25°C. and readings taken in an Evelyn colorimeter employing a 520 mμ filter. A blank containing all reagents except the fat or oil and subjected to the same treatment as the other tubes is included. The peroxide value is then calculated as milliequivalents per kg. of fat or oil as follows:

$$\text{P. V.} = \frac{A \times B}{C \times 55.84}$$

A = net micrograms of iron per 10 ml. i.e. micrograms of iron per 10 ml. of test solution less micrograms of iron in blank.

B = volume of the original dilution

C = weight of sample

It is evident that the intensity of the colour follows Beer's law in both methods, and the Hills and Thiel procedure (7) is slightly more sensitive. In view of these facts this curve and the Evelyn colorimeter were used in all further work.

In order to compare the values obtained by the Lips, Chapman, and McFarlane method (6) with those given by the Hills and Thiel procedure (7) a series of oils were examined. The results are given in Table III. It is evident from these data that the peroxide values were considerably lower when carried out in benzene-methanol but that atmospheric oxygen still interferes to a considerable extent. Lea (8) found that it was possible to decrease the ferric thiocyanate peroxide values to one-third or one-quarter of their original value by rigorously excluding all oxygen. A number of determinations were conducted, using an iodimetric method and the two ferric thiocyanate techniques, to confirm this statement. The results of the test are given in Table IV. These data indicate clearly that the exclusion of air markedly reduces the peroxide values by the ferric thiocyanate procedure. The anaerobic ferric thiocyanate method resulted in slightly lower values than the anaerobic iodimetric technique, possibly owing to the more complete exclusion of oxygen.

TABLE III

A Comparison of Peroxide Values by the Hills and Thiel and the Lips, Chapman and McFarlane Procedures

Oil Sample	Peroxide values in milliequivalents per kilogram		
	Lips, Chapman and McFarlane (6) Method	Hills and Thiel (7) Method	Ratio LCM/HT
Linseed	40	29	1.36
	40	29	1.36
Olive	649	506	1.28
	625	506	1.26
Soybean	13	8	1.65
	13	8	1.65
Cottonseed	591	418	1.41
	591	375	1.58
		Average	1.45

TABLE IV

Comparison of Peroxide Values by Iodimetric and Ferric Thiocyanate Procedures

Oil Samples	Peroxide Values in Milliequivalents per Kilogram			
	Lea "Cold" Iodimetric (9)	L.C.M. Anaerobic (6)	L.C.M. Aerobic (6)	Hills and Thiel (7)
Soybean	72	47	229	146
	72	47	229	146
Linseed	108	92	303	227
	108	92	303	227
Olive	804	810	1,922	1,382
	781	......	1,922	1,382
Cottonseed	203	......	570	473
	202	191	570	473

A further experiment was conducted to determine the effect of oxygen on the iodimetric method. Samples were analyzed employing the following procedures: 1. Lea's "cold" procedure (9), 2. Lea's "cold" procedure (9) without the use of nitrogen, 3. the foregoing iodimetric method but using carefully purified nitrogen in the apparatus designed by Lea (8) for the anaerobic ferric thiocyanate procedure. The data shown in Table V indicate that the exclusion of air in method 2 resulted in lower values by the iodimetric method and that the use of the special apparatus and carefully purified nitrogen resulted in a further slight reduction.

TABLE V

The Effect of Oxygen on the Iodimetric Procedure

Oil Sample	Peroxide Values in Milliequivalents per Kilogram		
	Method (i)	Method (ii)	Method (iii)
Soybean	75	83	69
	76	84	70
Linseed	117	135	109
	115	137	109
Cottonseed	197	229	194
	204	228	194

In a study of the oxidation products of methyl oleate, ethyl linoleate, ethyl linolenate, and squalene, Bolland and Gee (10) reported that they obtained peroxide values with their modification of the ferric thiocyanate procedure, which were in good agreement with the results obtained with the iodimetric method except in the case of ethyl linoleate. With this compound the results were absurdly high for no apparent reason. A number of workers have also reported that hydrogen peroxide solutions gave identical values by the ferric thiocyanate and iodimetric methods. An experiment was conducted to check these statements using hydrogen peroxide solutions, free fatty acids, esters, and two samples of oil. Bolland's method (3), which consisted of adding the fat or oil in benzene solution to the reagent containing ferrous ammonium sulphate and ammonium thiocyanate in acidified methanol, was included. Determinations were also carried out by the Lips, Chapman, and McFarlane (6), and Lea (9) "cold" iodimetric technique. The results are shown in Table VI. Since the values for the hydrogen peroxide solutions are in good agreement, it would appear that the high values are not the result of oxidation of the ferrous iron by atmospheric oxygen. However, considerable higher peroxide values were obtained by the ferric thiocyanate procedure on all the other compounds. This interference appears to have no connection with the structure of the fat or oil since fatty acids, esters, and triglycerides all gave the same results. These data are in contradiction of Bolland's (10) statement that peroxide values on methyl oleate were in good agreement with iodimetric values.

TABLE VI

A Comparison of Peroxide Values by Ferric Thiocyanate and Iodimetric Methods

Sample	Milliequivalents of Peroxide per Kg.		
	Bolland Method (3)	Lips, Chapman and McFarlane (6)	Iodimetric "cold" Method (9)
Hydrogen Peroxide	25	23	24
	25	23	24
Linoleic Acid	346	487	188
	357	487	187
Oleic Acid	454	688	256
	445	679	248
Erucic Acid	171	265	89
	177	265	....
Methyl Oleate	291	349	133
	291	344	....
Methyl Linoleate	4,190	5,150	1,880
	4,080	5,150	....
Cottonseed Oil	548	726	205
	548	717	206
Butter Oil	10	11	4
	....	11	4

A number of workers (6), (9), (11), (12) have reported that peroxide values by the iodimetric technique increased as the sample size was reduced. Lea (9) has suggested that this effect was caused by further oxidation of the fat molecule by dissolved oxygen. Paschke and Wheeler (12) were the first to point out that this effect could be largely eliminated when oxygen-free conditions prevailed, a fact which has been confirmed by Lea (9). In this investigation an experiment was conducted to determine the effect of reducing the sample size to the limit of the sensitivity of the iodimetric procedure. The method employed was the "cold" technique proposed by Lea (9) but without the use of an inert gas. Determinations weer conducted on a sample of cottonseed oil having a peroxide value of 548 m.e. per kilogram by the Bolland method (3) and 721 by the Lips, Chapman, and McFarlane (6) technique. The results are shown in Fig. 2.

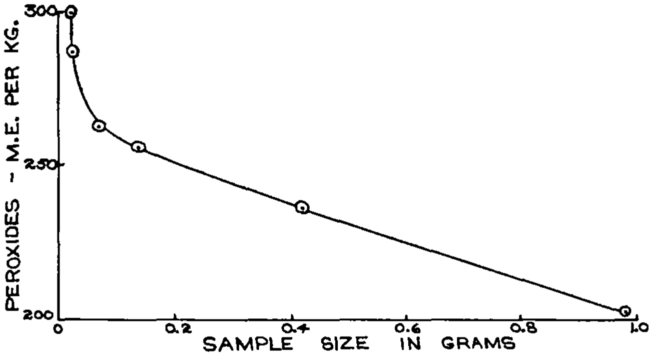

FIG. 2. The effect of sample size on peroxide values obtained with the "cold" iodimetric procedure without the use of an inert gas.

These data confirm the fact that in the presence of oxygen, peroxide values by the iodimetric technique increase rapidly as the sample size is reduced. The smallest amount of this particular oil which could be used with the iodimetric method was 25 mg. This amount is still much larger than the 4 mg. of oil or fat in solution, recommended as the optimum sample size for the ferric thiocyanate procedure (6). Paschke and Wheeler (12) observed that the increase in apparent peroxide value was most pronounced as the sample size was decreased below 0.5 g. However, when the ferric thiocyanate method was employed on most fats or oils, it was not possible to increase the sample size due to the intensity of the colour produced. Hills and Thiel (7), when working with fats with peroxide values of 0.5 to 5.0 m.e. per kilogram, used 0.1 ml. of the fat, and under these circumstances the ratio between the ferric thiocyanate values and the iodimetric figures was approximately 2.

Conclusions

All modifiactions of the ferric thiocyanate method investigated were affected by atmospheric oxygen. However, the interference in the Hills and Thiel (7) method and the Bolland (3) procedure was not as great as in the Lips, Chapman, and McFarlane (5) method. The results of Lea (8) were confirmed in respect to the lower values obtained when the atmosphere was rigorously excluded. Minimal results by the ferric thiocyanate procedure were possible only when carefully purified nitrogen was employed, and all possible precautions to prevent contact with air were observed. No other satisfactory method for offsetting this interference was found.

When the ferric thiocyanate procedures are conducted in the presence of air, the results, while high, are proportional to the iodimetric values. The former method, however, is much more sensitive than iodimetric procedures. Therefore under conditions where only comparative results are desired, such as in the assessment of the activity of antioxidants or in accelerated tests to predict the stability of a fat or oil, the ferric thiocyanate method should prove of considerable value. The Hills and Thiel (7) method was found to be the most satisfactory of those investigated since the use of benzene-methanol as a solvent permits the use of larger samples and the reagents are considerably more stable than in other methods.

REFERENCES

1. Yule, J. A. C., and Wilson, C. P. Jr., Ind. Eng. Chem., *23,* 1254 (1931).

2. Young, C. A., Vogt, R. R., and Nieuwland, J. A., Ind. Eng. Chem., Anal. Ed., *8,* 198 (1936).

3. Bolland, J. L., Sundralingham, A., Sutton, D. A., and Tristram, G. R., Trans. Inst. Rubber Ind., *17,* 29 (1941).

4. Robey, R. F., and Weise, H. K., Ind. Eng. Chem. Anal. Ed., *17,* 425 (1945).

5. McFarlane, W. D., and Chapman, R. A., Can. J. Research, B *21,* 133 (1943).

6. Lips, A., Chapman, R. A., and McFarlane, W. D., Oil and Soap, *20,* 240 (1943).

7. Hills, G. L., and Thiel, C. C., J. Dairy Research, *14,* 340 (1946).

8. Lea, C. H., J. Soc. Chem. Ind., *64,* 106 (1945).

9. Lea, C. H., J. Soc. Chem. Ind., *65,* 286 (1946).

10. Bolland, J. L., and Gee, G., Trans. Faraday Soc., *42,* 236 (1946).

11. Stansby, M. E., Ind. Eng. Chem., Anal. Ed. *13,* 627 (1941).

12. Paschke, R. F., and Wheeler, D. H., Oil and Soap, *21,* 52 (1944).