cated percentages are real or apparent only, and due to the limitations of the thiocyanogen method, as applied to highly hydrogenated fats. If the Bertram method is assumed to represent the true saturated acid contents of the samples, calculation upon this basis shows no linoleic acid. Common experience with the hydrogenation process would indicate the presence of an appreciable linoleic acid content in cottonseed oil hydrogenated to an iodine value of 25 to be improbable.

Summary

1. Selective hydrogenation, followed by fractional crystallization from a solvent, has been used to prepare from cottonseed oil a hard butter, very similar to cocoa butter.

2. The new product differs somewhat in composition from cocoa butter, due to an unavoidable content of iso-oleic acid.

3. Examination of the new product by a micro-penetration technique, by a standard solidification test, and by means of the dilatometer, reveals minor physical differences between it and cocoa butter.

4. The new fat has a slightly longer plastic range than cocoa butter. It supercools less strongly and contracts slightly less upon solidification. It exhibits the phenomenon of polymorphism to a less pronounced degree than cocoa butter.

5. A relatively low yield of the new fat is inherent in the process used for preparing it. In the case of the product most closely resembling cocoa butter, a yield of approximately 28 percent was obtained in the laboratory. The residual 72 percent is suitable for use as a hardening agent in shortening and similar products.

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The Application of the Ferric Thiocyanate Method to the Determination of Incipient Rancidity in Fats and Oils[1]

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A recent paper from this laboratory (1) describes a colorimetric method for determining fat-peroxides in whole milk powder. The method is based on the oxidation of ferrous to ferric iron by the peroxides present and the colorimetric determination of the ferric iron as ferric thiocyanate.

Subsequently the procedure was modified for use in the development of a practical antioxidant for lard and shortening (5). The details of the method, as applied to fats and oils, are given in this paper, and the results of a comparative study with other methods are also presented.

Description of the Method

1. *Preparation of the Reagent:* The reagent consists of a solution of 0.1% ferrous ammonium sulphate and 0.4% of ammonium thiocyanate in 96% acetone. The ammonium thiocyanate is weighed into a volumetric flask and an amount of distilled water equivalent to 4% of the final volume is added. The salt is dissolved, the solution allowed to stand for 5 to 10 minutes, and then brought almost to volume with anhydrous acetone and thoroughly mixed. (Neglect of this last operation may result in a cloudy solution when the final ingredient is added.) The ferrous ammonium sulphate is added, the solution diluted to the mark with acetone, and thoroughly shaken. The reagent is kept in the dark for two hours, shaking frequently during the interim. After filtering through an acetone washed filter paper, or decanting from the undissolved ferrous salt, the reagent is ready for use.

Acetone which has not already been used in the test must be carefully purified by distilling it from a small amount of ferric chloride, drying over calcium chloride, and redistilling before use. Spent solvent does not require any addition of ferric chloride since ferric-ions are already present, but it must be carefully dried and redistilled since the water content is critical.

2. *Preparation of the Calibration Curve:* A standard of reference curve (1) is prepared from readings obtained with standard solutions of ferric chloride (0.2 to 14.0 μg. per ml.) in purified anhydrous acetone. A 1 ml. aliquot of the solution is added to 9 ml. of reagent consisting of 0.4% ammonium thiocyanate in 96% acetone. The intensity of the color is measured with a Coleman spectrophotometer set at 485 mu. The color, which develops without heating, fades

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slowly so that the readings should be taken at once. The reagent, which must be colorless, is used as a blank in the initial setting of the instrument.

3. *Method:* A representative sample of about 0.10 g. of the fat is weighed into a small beaker and 25 ml. of anhydrous acetone (purified as in the preparation of the reagent) are added. To dissolve the fat completely it may be necessary to gently warm on a water bath. The remainder of the determination is carried out under red light since sunlight or strong electric light intensifies the color. If only comparative results are desired this precaution should not be necessary.

A 1 ml. aliquot of the acetone solution of the fat is transferred to a test-tube and 9 ml. of the reagent added. The tube is heated first at 70 to 80°C. until the first evolution of gas bubbles occurs and then for 10 minutes at 50°C. The intensity of the developed color is determined, as described above, in the Coleman spectrophotometer using pure acetone as a blank. A reading is also made with 9 mls. of the reagent and 1 ml. acetone treated in a manner identical with the test. The difference between the values for the determination and the reagent blank gives the ferrous iron oxidized to ferric iron by the peroxides in the fat. Total peroxides may be calculated as follows:

$$\frac{A \times B}{C \times 55.84} = \text{Milliequivalents of peroxide per kilogram of fat}$$

where

A = micrograms of Fe^{+++} in 10 ml. of test solution minus micrograms of Fe^{+++} in 10 ml. of reagent blank.
B = Volume of extract (25 ml.).
C = Weight of sample, in grams.
55.84 = Equivalent weight of iron.

Discussion of the Method

If prepared as outlined, the reagent has a faint pink tinge and will read 80 to 90 on the Coleman spectrophotometer with the instrument set at 485 mu. It must be kept in the dark in order to retard a gradual increase in the intensity of the color. Even when the reagent is protected from light, the color increases to some extent, but the same peroxide values are obtained when a fat is analyzed with samples of the reagent giving readings of from 50 to 90 on the galvanometer. The reagent may be used for three or four days although the range of the determination is considerably restricted if the color in the blank becomes too intense.

The optimum concentration of ammonium thiocyanate is found to be 0.4% although a range of 0.2% to 0.75% shows only a slight variation. A concentration of ferrous ammonium sulphate of 0.1% gives a maximum reading and the color is comparatively stable. However, the presence of water in the acetone has a marked effect on the reaction. Absolute acetone gives maximum color but rapid fading. The addition of 10% of water to the acetone improves the stability but reduces the color to only 50% of the former value; 96% acetone gives optimum sensitivity and stability.

If the red color developed in the test is too intense, it is recommended that the original acetone solution of the fat be diluted, rather than to dilute the final ferric thiocyanate solution. Traces of ferric iron might be present in the test fats but so far we have not encountered any such interference. The presence of ferric iron can of course be readily detected by dissolving a small amount of the fat or oil in 96% acetone, which contains only the ammonium thiocyanate, and heating in the usual manner. It is imperative that all glassware used in the test be scrupulously clean. Hot concentrated nitric acid is most satisfactory as a cleaning agent.

It is often difficult to obtain a representative sample for analyses since the surface fat may show a very much higher peroxide value than a sample taken from the interior. In studying the keeping quality of lard and shortening during storage we adopt the practice of sampling 1 lb. prints by cutting, from the corner, a block representing about 1/8 of the whole. This sample of about 50-60 g. is melted and from it is taken the sample for analysis.

When the stabilizing action of antioxidants on fats is compared by means of this peroxide test, the effect of each antioxidant on the reagent should be determined, and the amount of interference, if any, corrected for. Hydroquinone and α-tocopherol, although they are relatively strong reducing agents, do not interfere when added to the fat in amounts up to 0.1%. Citric acid, in common with other substances having a hydroxyl-group alpha to a carboxyl group, markedly reduces the color of ferric thiocyanate solutions. This interference by citric acid is of special interest since it has been recommended as an antioxidant (5), and may be present in fats or fatty foods.

Citric acid may be present in the fat in amounts up to 250 parts per million without affecting the test. While it is unlikely that citric acid will ever be present in amounts in excess of this we find that by adding acetic acid to the reagent the effect of much larger amounts of citric acid is eliminated. This is illustrated by the data in Table I which show that the results are not affected by the presence of a very large amount of citric acid (equivalent to 50 mg. per gm. of fat), when about 5 to 10% acetic acid has been added to the reagent. Furthermore, the use of acetone containing 10% acetic acid as the fat-solvent is advantageous in the case of oils, such as the cereal-germ oils, which are rich in phospholipids. However, acetic acid causes a gradual intensification of the red colour of ferric thiocyanate and its use is recommended only in these special cases.

TABLE I

Showing the Effect of Acetic Acid in the Reagent on the Interference Due to Citric Acid

Percentage of Acetic Acid	M. E. Peroxide per Kg.	
	No Citric Acid	Citric Acid*
0	3.54	0.82
1	3.46	1.61
3	3.28	2.27
6	3.46 } 3.66	3.17 } 3.47
10	3.86 }	3.77 }
20	4.41	4.90

* 20 micrograms of citric acid per ml. of reagent, i.e. equivalent to a concentration of 50 mg. of citric acid per gram of fat assuming 0.1 g. of fat used in the test.

Comparison with Iodimetric Methods

Peroxides in fats and oils are generally determined by methods based on iodimetry (4, 10). Stansby (8) has already drawn attention to some of the factors which seriously impair the accuracy and reproducibility of iodimetric procedures when applied to fish oils. Similar results are obtained in this laboratory

when the methods are applied to lard, shortening and butter. The moisture content of the reagent has a marked effect on the values obtained; the addition of 10% of water to the solvents reduces the peroxide value of a fat as determined by Lea's method (4), from 94.4 to 25.2. However, a certain minimum amount of water must be present to ensure sufficient ionization of the potassium iodide.

The results of an experiment designed to evaluate the changes in peroxide value under varying conditions are given in Table II. The lard was prepared

TABLE II

Peroxide Values Obtained Under Varying Conditions

Heating Conditions	Time in Contact with KI (minutes)	KI Reagent	Atmosphere	Peroxide Value***
1. Room temp.	1 minute	Sat.*	Air	73.8
2. Room temp.	1	Solid **	Air	89.0
3. Steam bath	1	Solid	Air	93.0
4. Steam bath	4	Solid	Air	123.4
5. Steam bath	4	Solid	Nitrogen	106.0
6. Steam bath	4	Sat.	Air	90.2
7. Room temp.	1	Solid	Air	64.8
8. Room temp.	1	Sat.	Air	76.8
9. Sand bath—300°C.	1	Solid	Nitrogen	97.6
10. Steam bath	1	Solid	Air	78.0
11. Steam bath	4	Solid	Air	71.6
12. Steam bath	4	Sat.	Air	95.4

* 1 ml. of a saturated solution of potassium iodide in water.
** Crystalline potassium iodide.
*** Milliequivalents per kilogram of fat, corrected for reagent blank. Reagent purity solvents used in samples 1-6; anhydrous chloroform and acetic acid in samples 7-12.

for analysis by dissolving 4 g. of the melted fat in 50 ml. of glacial acetic acid and diluting to 100 ml. with chloroform; the proportion of the reagents being the same as used by Smith (7). It is evident from these results, that varying the conditions of the test has a considerable effect on the peroxide values obtained. Comparison of the results with samples No. 4 and No. 5 shows that "the blank" in air does not adequately compensate for the error due to the reaction with atmospheric oxygen. The effect of heating and the length of time in contact with the reagents before adding water, is also evident.

Kokatnur and Jelling (3) have proposed a method employing 99% isopropanol as the solvent, to ensure a homogeneous medium during titration. No blank is required and there is no appreciable reabsorption of

TABLE III

Comparison of Peroxide Values on Lard and Shortening by Different Iodimetric Procedures

Material	Method	Peroxide Value (m.e. per kg.)
Shortening No. 1	Kokatnur and Jelling	108.6
	Wheeler	109.8
	Lea	112.3
Shortening No. 2	Kokatnur and Jelling	37.4
	Lea	40.6
Shortening No. 3	Kokatnur and Jelling	35.2
	Lea	40.0
Lard No. 1	Kokatnur and Jelling	75.2
	Lea	79.2
Lard No. 2	Kokatnur and Jelling	11.8
	Lea	15.8

the iodine. However, the end-point is difficult to detect with colored oils. A comparison of the results by this method with those obtained by Lea's (4) and Wheeler's methods (10) shows that the results tend to be considerably lower (Table III). The peroxide

TABLE IV

Effect of Sample Size on Peroxide Values

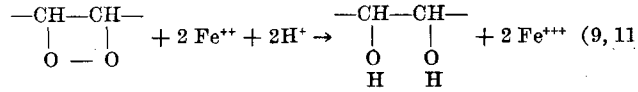
Method	Sample Taken	
	0.1 gram	1.0 gram
Wheeler (10)	62.0	36.6
Lea (4)	55.0	43.6
Stansby (8)*	32.0	27.4
Smith (7)	46.0	37.6

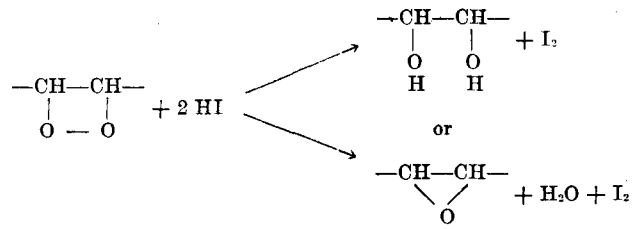
* Hydrochloric acid procedure.

value also increases as the sample size is decreased as shown in Table IV; an old sample of butter oil was used in this experiment and showed 93.5 m. e. of peroxide-oxygen per kg. by the ferric thiocyanate method.

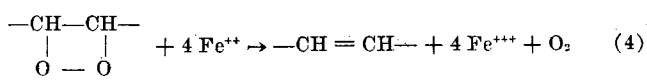
The colorimetric method indicates the presence of peroxides at a much earlier stage of rancidity than does the titration methods and the results are invariably higher by the ferric thiocyanate procedure (Table V). Furthermore, the colorimetric method is a truer index of the amount of oxygen absorbed by the fat, as shown in Table VI. The reason for this is somewhat obscure since the iodine-ion is more easily oxidized than the ferrous-ion under normal conditions. However, in acetone solution, ferrous-ion may react with oxidized products which will not act as oxidizing agents in glacial acetic acid and chloroform or in isopropanol. Sample size, instability of certain fat peroxides in the solvents employed for iodimetric titrations and iodine resorption may also be involved.

To calculate the oxygen absorption from the change in peroxide values during the manometric estimation, the ferric thiocyanate values in milliequivalents of peroxide-oxygen per kilogram were converted to grams of oxygen absorbed per kilogram of fat by using the factor 0.008, corresponding to an equivalent weight for peroxide-oxygen of 8. The equivalent weight of peroxide oxygen has, however, been regarded as 16, in accordance with the following reactions:





A reaction of the type:



would appear to be impossible since the peroxide of the organic compound, if acting as an oxidizing agent in a manner analogous to that of hydrogen peroxide, would gain only two electrons in a change to molecular oxygen.

The fact that the values for oxygen absorption according to the thiocyanate method agree closely with the weight of oxygen taken up, as determined manometrically, does not necessarily constitute a basis

TABLE V

Comparative Peroxide Values by Titration and Colorimetry

Material	Peroxide Value (m.e. per kg.)		Ratio Colorimetry ÷ Iodimetry
	Iodimetry	Colorimetry	
Hydrogenated* linseed oil	44.5 (Lea)	83.8	1.88
Hydrogenated* linseed oil	45.2 (Lea)	86.0	1.90
Hydrogenated* linseed oil	33.2 (Lea)	64.8	1.95
Hydrogenated* linseed oil	37.6 (Lea)	79.3	2.11
Hydrogenated* linseed oil	30.4 (Lea)	49.7	1.64
Hydrogenated* linseed oil	25.4 (Lea)	48.5	1.91
Hydrogenated* linseed oil	32.4 (Lea)	68.3	2.11
Hydrogenated* linseed oil	22.6 (Lea)	42.7	1.88
Hydrogenated* linseed oil	28.2 (Lea)	54.3	1.93
Hydrogenated* linseed oil	28.2 (Lea)	51.0	1.80
Hydrogenated* linseed oil	30.0 (Lea)	60.5	2.01
Triolein	220 (isopropyl alcohol)	498	2.26
Oleic Acid	30.0 (isopropyl alcohol)	53.2	1.77
Cod-liver oil	310 (isopropyl alcohol)	524	1.69
Linseed oil	30.0 (isopropyl alcohol)	66.5	2.22
Linseed oil	46.0 (isopropyl alcohol)	95.8	2.08
Linseed oil	21.0 (Lea)	36.0	1.71
Shortening	53.3 (Lea)	123	2.35
Lard	15.8 (Lea)	32.8	2.08

* This series of hydrogenated linseed oils at different stages of rancidity came from the same batch, which had an iodine number of 80.

for revision of the ideas concerning the equivalent weight of peroxide oxygen in these fats. Peroxide formation is complicated, particularly in the more unsaturated fats, by polymerization and by changes to hydroxy and oxy forms (6), which may react more completely in the colorimetric procedure than in the iodimetric methods. Nevertheless, such changes could not have been very marked with fats of low initial peroxide content at the relatively low temperatures of the experiments. The results emphasize the lack of knowledge concerning the actual mechanism of fat oxidation and of the reactivity of the compounds formed.

In a previous paper on the development of a practical antioxidant (5) we have presented the results of analysis of lard and shortening by a modification of the Swift Stability Test, and by the ferric thiocyanate method. The peroxide values of the fats after storage, as measured by the colorimetric method, are of the same order of magnitude as the difference in the stability time. A determination of the initial peroxide value of the sample by the colorimetric method may be a criterion of its keeping quality and the test might therefore be employed for grading samples.

Summary

The ferric thiocyanate method, previously described for the determination of fat-peroxides in milk powder, has been modified for use in the study of rancidity in fats and oils. The factors affecting the accuracy and reproducibility of the results obtained by various methods based on iodimetry, have been carefully investigated. Peroxide values determined by the ferric thiocyanate method are approximately twice those obtained by iodimetric procedures. The calculated oxygen absorption by various fats and oils, from the change in peroxide-values as determined by the colorimetric method, agrees closely with direct measurements in the Warburg-Barcroft apparatus, when the equivalent weight for peroxide-oxygen is assumed to be 8.

The proposed method is more sensitive and simpler than those previously described. It would appear to be specially applicable in the detection of incipient rancidity as, for example, in the study of "flavor reversion" in vegetable oil products.

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TABLE VI

A Comparison of Oxygen Absorption Measurements as Determined Manometrically and by Calculation from the Change in Peroxide-Value

Material	Temperature (°C.)	Time (hours)	Change in Peroxide Value (m.e. per kg.)		Oxygen Absorbed (gms. per kg.)	
			Iodimetric	Colorimetric	Determined Manometrically*	Calculated from Peroxide Values**
Linseed oil ***	50	8	15.4	29.3	0.241	0.234
Linseed oil ***	50	8	16.0	32.0	0.250	0.256
Linoleic acid ****	50	1½	13.1	27.0	0.252	0.216
Triolein ****	50	26	34.1	71.7	0.538	0.574
Linseed oil ***	60	6	20.7	41.2	0.353	0.330
Linseed oil ***	25	46	10.5	23.0	0.183	0.184
Linseed oil ***	25	47	12.9	26.0	0.197	0.208
Methyl oleate *****	55	22	22.2	39.1	0.302	0.313
Fatty acids of linseed oil	55	3½	15.3	37.1	0.407	0.297

* Employing a Barcroft-Warburg apparatus and a technique similar to that of Johnston and Frey (2) but using one gram of fat in a 50 ml. Erlenmeyer-type flask with ground glass joint and mercury seal. Reproducible results are obtained without shaking the flasks in the bath. Brodie's solution used as manometer fluid.

** Calculated from the change in peroxide values (ferric thiocyanate method), during the oxygen absorption determination.

*** Hot pressed linseed oil, refined by passing through a specially prepared silica gel-alumina adsorbent and deodorizing.

**** Refined by removing the material insoluble in petrol-ether and passing the petrol-ether solution through a silica-gel-alumina adsorbent.

***** Supplied by the Eastern Regional Research Laboratory, United States Department of Agriculture.