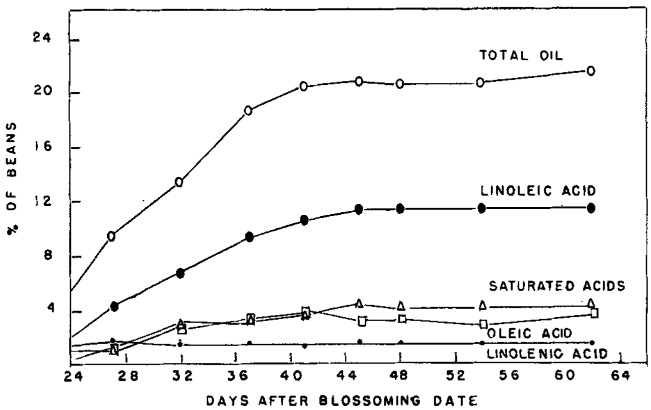

FIG. 1. Increases in oil and its constituent fatty acids with maturity of Lincoln soybeans (1948).

days after the blossoming date. Linoleic acid and total oil reached a constant level at approximately 45 days after the beans were tagged. The ratios of the different acids remained essentially constant after 50 days from the tagging date.

The results obtained from the dehydrogenation experiments were erratic. Some sample data are shown in Table V. In the cases in which added lipid substrate increased the rate of decolorization of methylene blue, the decolorized solution was acidified and extracted with ether and iodine adsorption values obtained on the extract. One of the potassium stearate substrates and one of the monostearin so treated showed increases in iodine values from 0.6 to 3.3 and 0.3 to 3.2, respectively. However, since no increase in iodine value was noted in 11 other trials with potassium stearate and three with monostearin, these small changes were considered insignificant. The possibility of dehydrogenation with subsequent cleavage was investigated by determining the change in neutralization equivalent of the substrate by the method of Marcali and Reiman (8). No change was noted in stearic or myristic acid neutralization equivalent.

Summary

Lincoln soybeans harvested at successive stages of maturity showed continuous increases in amounts present of each of the fatty acids: saturated, oleic, linoleic, and linolenic.

The iodine value and linolenic acid percentage of the oil decreased somewhat during the early stages of seed development. The linoleic acid and total oil percentage in the bean increased continuously until the 50th day then remained constant. Oleic and saturated acids fluctuated.

No evidence for dehydrogenation of saturated fatty acids was obtained either in the oil analyses or in tests of soybean tissues for dehydrogenase activity.

REFERENCES

1. Beadle, B. W., Oil and Soap, *23*, 140 (1946).
2. Brice, B. A., Swain, Margaret L., Schaffer, B. B., and Ault, W. C., Oil and Soap, *22*, 219 (1945).
3. Daubert, B. F., Longenecker, H. E., and Fricke, H. H., J. Am. Chem. Soc., *65*, 2142 (1942).
4. Grande, F., Shand. Arch. f. Physiol., *69*, 189 (1934).
5. Hilditch, T. P., "The Chemical Composition of Natural Fats," 2nd ed., pp. 340-346, John Wiley and Sons Inc., New York (1947).
6. Kleinzeller, A., "Advance in Enzymology," vol. B., Interscience Publishers Inc., New York (1947).
7. Official and Tentative Methods of the American Oil Chemists' Society, 39 (1941).
8. Marcali, K., and Reiman, W., Ind. Eng. Chem., Anal. Ed., *18*, 144 (1946).
9. Painter, Edgar Page, Arch. Biochem., *5*, 337 (1944).
10. Rewald, B., and Reide, W., Biochem. Z., 147 (1933).
11. Sosa-Bourdouil, C., Brunorel, A., and Sosa, A., Compt. Rend., *212*, 1049 (1941).
12. Wolfe, Alvin C., Park, J. B., and Burrell, R. C., Plant Physiol., *17*, 289 (1942).
13. Schoenheimer, R., and Rittenberg, D., J. Biol. Chem., *120*, 155 (1937).

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The Use of Thiobarbituric Acid as a Measure of Fat Oxidation[1,2]

C. G. SIDWELL, HAROLD SALWIN, MILADA BENCA,[3] and J. H. MITCHELL JR., Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

OBJECTIVE tests for food product deterioration are highly desirable to facilitate research investigations on stability and to aid in specifying product quality. Recently 2-thiobarbituric acid has been proposed as a reagent with potentialities of partially fulfilling these requirements (4, 6, 13). During oxidation of fats, compounds are formed which can be reacted with 2-thiobarbituric acid (TBA) to give red-colored products. Patton and Kurtz (13) investigated the reaction of TBA with milk fat, and Dunkley and Jennings (6) published a procedure for oxidized fluid milk. Biggs and Bryant (4) modified the method for milk and extended its application to milk powder, cheese, and butter. Inasmuch as these investigators confined the use of TBA to milk fat, it was thought that its application to other fats would be of interest and value. Modifications of the TBA tests were developed to meet the objectives of this investigation.

Fats stored at —20, 0, 72, and 100°F.(—29, —18, 22, and 38°C.) and fats oxidized by the active oxygen method (A.O.M.) (14) or by ultraviolet irradiation were examined. Higher TBA values were obtained for soybean oil than for cottonseed oil at comparable peroxide values. This is of interest because of the greater tendency of soybean oil to develop oxidized flavors. The volatile products of oxidation of lard, cottonseed, and soybean oil were examined also, and at comparable peroxide values soybean oil volatiles developed the greatest intensity of color.

Experimental

When oxidized fat samples were dissolved in an organic solvent such as carbon tetrachloride, chloroform, or benzene, reactive material could be extracted

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[2] This paper represents research undertaken by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 450 in the series of papers approved for application. The views and conclusions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[3] Present address: Custom Chemical Laboratories, Chicago, Ill.

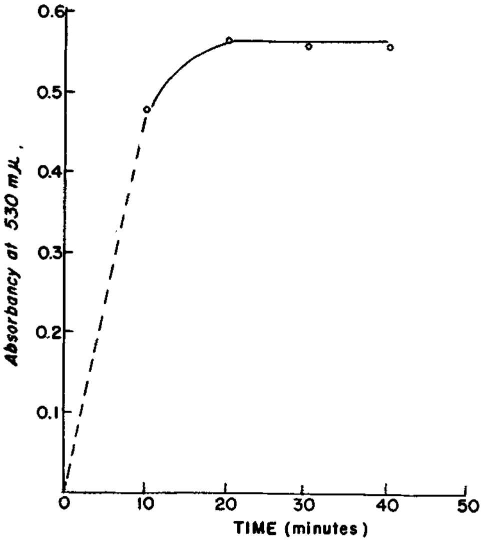

FIG. 1. Rate of color development at 100°C. of the TBA extract from oxidized soybean oil.

with an acid solution of TBA. An advantage of this procedure is that since the fat is not heated, it is presumably left in its original state of oxidation. Published procedures require heating the reagent in the presence of the fat. Two modifications of the extraction procedure were developed. The first effected nearly complete extraction of the reactive material by successive extractions with an hydrochloric acid solution of TBA and subsequent concentration of the developed color with isoamyl alcohol; the second consisted of a single extraction of the fat solution with an acetic acid solution of TBA.

The details of the single extraction procedure with acetic acid are given since it proved to be the more satisfactory of the two modifications.

Reagents

a) Glacial acetic acid, C.P.
b) 2-thiobarbituric acid (TBA), reagent grade. Material should be a white powder.
c) Benzene, thiophene-free, or carbon tetrachloride, C.P.
d) TBA solution. Dissolve 0.67 g. of thiobarbituric acid in distilled water with the aid of heat from a steam bath. Transfer solution to a 100-ml. volumetric flask, cool, and make to volume.
e) TBA reagent. Mix an equal volume of the TBA solution with an equal volume of glacial acetic acid.

Apparatus

a) Maisel-Gerson tubes or equivalent.
b) Suitable rack for holding Maisel-Gerson tubes.
c) Variable speed shaking machine, reciprocating type, approximately 1¾-in. oscillation stroke.
d) Spectrophotometer, Coleman Universal Model 14[4], or equivalent.
e) Boiling water bath.

Method

Pipette or weigh a 3.00-g. sample of the melted fat into a glass-stoppered Maisel-Gerson tube and dissolve the sample in 10 ml. of benzene or carbon tetrachloride. Pipette 10 ml. of the TBA reagent into the Maisel-Gerson tube and shake the tube in a horizontal position for a period of 4 minutes at approximately 125 oscillations per minute. Transfer the contents to a separatory funnel and withdraw the aqueous layer into a 25- x 200-mm. test tube. Immerse the tube in a boiling water bath for 30 minutes, cool and transfer a portion of the contents to a cuvette. Read the absorbancy at 530 mμ against distilled water.

Standardization of Procedure. All TBA procedures (4, 6, 13) are empirical. The procedure, developed as described above, was adopted because it gave consistent and reproducible results as well as clear test solutions with the fats under study. No organic solvents such as petroleum ether (13) or isoamyl alcohol-pyridine solution (4, 6) were necessary to obtain turbid-free test solutions.

A gradual increase in TBA values of an oxidized soybean oil sample (A.O.M.) was obtained for extraction periods of 2½, 5, and 10 min.; and the oxidized material was not completely removed from fats by extraction for time intervals up to one hour. The extraction time of 4 min. was selected because it gave consistent and reproducible results.

Figure 1 shows the rate at which the color developed when an extract from oxidized soybean oil was heated with the TBA reagent in a boiling water bath. This behavior was nearly analogous to that shown for butterfat by Biggs and Bryant (4). Two investigators (6, 13) indicated a continuous increase in density values with increase in heating time. Satisfactory results were obtained with a 30-min. heating period, and this was adopted.

An absorption maximum was obtained at 530 mμ when either the acetic or hydrochloric acid extraction was used. The position of this maximum agrees closely with wave lengths of 532 mμ (13) and 535 mμ (6), reported previously.

Examination of Oxidized Fats

The TBA, peroxide (14), and total carbonyl values (8) for lard, soybean, and cottonseed oils oxidized in the A.O.M. apparatus are shown in Figure 2. The total carbonyl values were all determined by measurement at 440 mμ and so do not differentiate between the saturated and unsaturated compounds. Similar iodometric peroxide value curves for this range of peroxide values of cottonseed oil were also shown by Moore and Bickford (11) and by King, Roschen, and Irwin (9). The peroxide values for cottonsed oil did not reflect the superior resistance toward oxidation that

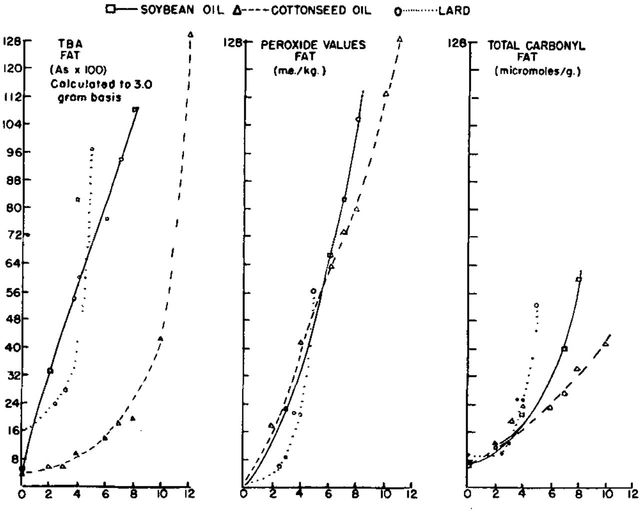

FIG. 2. Comparative TBA, peroxide, and total carbonyl values from the A.O.M. oxidation of lard, soybean oil, and cottonseed oil.

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[4] The mention of this and other commercial products does not imply that they are endorsed or recommended by the Department of Defense over other similar products not mentioned.

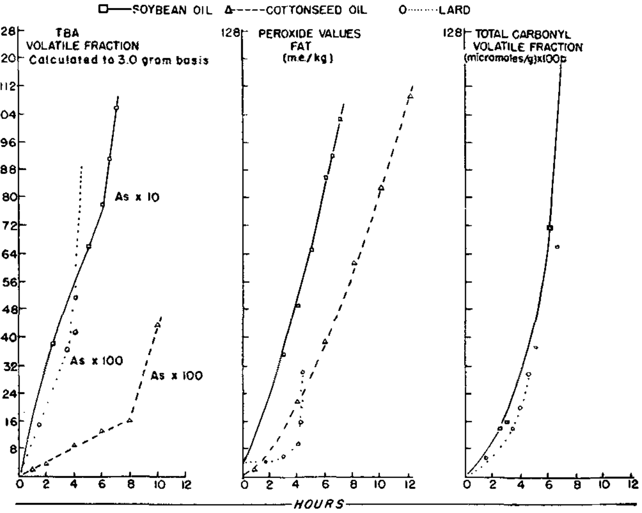

FIG. 3. Peroxide values of the fat, and TBA and total carbonyl values of the volatile products during A.O.M. oxidation of lard, soybean oil, and cottonseed oil.

this oil usually has when compared to soybean oil. The flavor changes which develop during the A.O.M. oxidation of these two oils are quite different despite the similarity of their peroxide curves. Soybean oil has been reported to develop unacceptable flavors at a peroxide value of 2.5 whereas cottonseed oil, an oil not subject to flavor reversion (7), does not usually become rancid until a peroxide value approximately 50 times that value is reached (2). The TBA test differentiated these two oils on the basis of stability more effectively than did either the peroxide or carbonyl tests. For instance, at peroxide values of 80 and 84 m.e./kg. for cottonseed and soybean oil and with total carbonyl values at this point of 34 and 41 micromoles per gram, the corresponding TBA values were 0.19 and 0.95 absorbancy units, respectively. At peroxide values of 60 and 66 and with carbonyl values of 23 and 33, the TBA values of these two oils were 0.14 and 0.78, respectively.

The behavior of the TBA test appeared to be associated with the linolenic acid content of the fat. The linolenic acid contents of lard, cottonseed oil, and soybean oil have been reported as 0.8, 0.0, and 8.0% (3) respectively. Figure 2 shows that the fats of low linolenic acid content had induction periods when the course of oxidation was followed with the TBA test.

Examination of Volatile Products from Oxidizing Fats. Preliminary investigation disclosed that a large fraction of the TBA reactive material formed during fat oxidation was volatile. The oxidized products in the effluent air from a 20-g. sample in the A.O.M. apparatus were collected in 10 ml. of carbonyl-free toluene, and subsequently the TBA extraction procedure was applied to this solvent as described in detail in a previous section of this paper. The toluene was purified as described by Lappin and Clark (10) except that trichloroacetic acid was used in place of hydrochloric acid. The test tubes containing the toluene were immersed in a Dewar flask containing a mixture of dry ice and ethanol. The air used for oxidation was pre-dried by passing it through a liquid air trap in a dry ice ethanol bath. Within the periods of oxidation used a single receiving tube was sufficient to collect all of the volatile products evolved from each sample. At predetermined periods of oxidation the collecting tubes were removed and the contents transferred to 25-ml. volumetric flasks. After warming to room temperature, the contents were diluted to the mark with thiophene-free benzene. The TBA-acetic acid procedure was applied to aliquots of this solution.

The aliquots were so chosen that the range of absorbancy values was from approximately 0.1 to 1.0 for each series. TBA values equivalent to 3 g. of fat were calculated.

The extraction of TBA reactive material from the fat-free toluene-benzene solution was nearly quantitative. The incomplete recovery in the extraction procedure described previously for the examination of oxidized fats is probably not due to physical interference by the fat. The addition of non-oxidized butter oil to a toluene-benzene solution of the volatiles from oxidized butter oil did not interfere with the efficiency of the extraction.

Data obtained from the A.O.M. oxidation of lard, soybean, and cottonseed oil are shown in Figure 3. Again the differences in TBA values of cottonseed oil and soybean oil were greater than the differences in peroxide values. Soybean oil always produced the highest concentration of TBA volatile material. The TBA curves for the volatile oxidation products (Figure 3) have the same characteristics as those for the fats (Figure 2). Figure 3 represents a different series of samples from those used in Figure 2.

Butter oil exhibited such a different behavior from other fats that peroxide and total carbonyl values for the fat and TBA and total carbonyl values for the volatile products were plotted in a separate figure (Figure 4). The slopes of all of the curves are not as great as those of the other fats and oils. The TBA values of the volatiles increased progressively with time of oxidation whereas the corresponding carbonyl values reached a constant level early in the oxidation period, possibly suggesting that TBA reacts with both carbonyl and non-carbonyl compounds. The peroxide values also showed a continuous increase with time of oxidation, but were quite low. No TBA values for the fat were shown since only the 23½-hr. sample gave a red color.

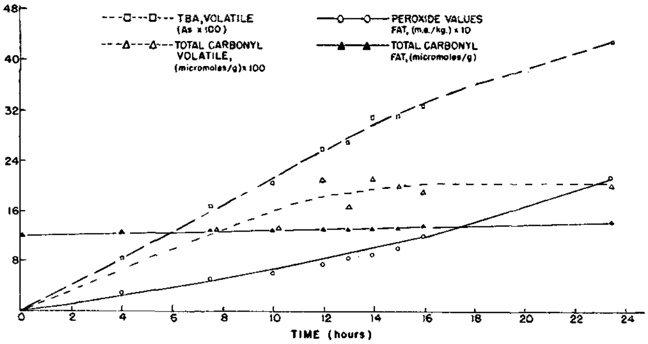

FIG. 4. Peroxide and total carbonyl values of the fat, and TBA and total carbonyl values of the volatile products during A.O.M. oxidation of butter oil.

Butterfat Oxidized by Prolonged Storage

The TBA acetic acid procedure was applied to a series of butterfat samples, which had been stored approximately 2 years at —20, 0, 72, and 100°F.

(—29, —18, 22, and 38°C.). A modification of the procedure was necessary for butterfat. In order to obtain a red color it was necessary to heat the samples before applying the TBA extraction procedure. The results shown in Table I were obtained from samples preheated in a boiling water bath for 60 minutes. The 530 mμ absorbancy value of the 100°F.(38°C.) sample increased 560% after such treatment whereas the corresponding carbonyl value increased 11%. The high free fatty acid content of this sample is apparently not responsible for its high TBA value since tests on a sample of butyric acid (Technical) were negative. Iodometric peroxide and free fatty acid determinations (1) were also made on these samples.

TABLE I

TBA Values, Peroxide Values, and Free Fatty Acid Values of Butterfat, Stored Approximately 2 Years at —20, 0, 72, and 100°F. (—29, —18, 22, and 38°C.)

Storage temperature of sample (°F.)	TBA values[a]	Peroxide values	Free fatty acid values as oleic
		m.e./kg.	%
—20	0.29	2.00	0.67
0	0.30	1.33	0.60
72	0.51	4.32	0.69
100	0.87	3.33	1.42

[a] Samples were preheated in a boiling water bath for 60 min. before applying the TBA test.

It may be seen that the TBA values were more directly related to storage temperatures than peroxide values. Sensory evaluations made by an expert panel of laboratory personnel showed a direct relationship between TBA values and off-tastes and odors.

Butterfat Oxidized by Ultraviolet Irradiation

Butterfat was irradiated by exposure to the ultraviolet rays of a Mineralight lamp. In contrast to the stored butterfat samples no preheating of the fat was necessary to obtain a red color upon subsequent application of the TBA extraction procedures. Although the test solutions were turbid, they were easily clarified by centrifugation. Values thus obtained were directly related to iodometric peroxide values.

When the butyric acid sample, which had given a negative TBA test, was exposed to ultra-violet irradiation for a 19-hr. period, it also reacted positively to TBA. The absorption spectrum was similar to that obtained from oxidized fat samples. The oxidation of butyric acid by photochemical means has been reported (5, 12).

Summary

An evaluation of thiobarbituric acid (TBA) as an agent for the measurement of fat oxidation was made by the application of several empirical procedures to animal and vegetable fats. An extraction procedure was used for removing the products of oxidation. The reaction with TBA was conducted in a boiling water bath to produce a red color, which was then estimated spectrophotometrically.

Fats stored at —20, 0, 72, and 100°F.(—29, —18, 22, and 38°C.) and fats oxidized by the active oxygen method (A.O.M.) and by ultraviolet irradiation were examined. It was found that the TBA test might be of value in following the course of oxidation of cottonseed oil and soybean oil in the A.O.M. apparatus. Higher TBA values were obtained for soybean oil than cottonseed oil at comparable peroxide values. This is of interest because of the greater tendency of soybean oil to develop oxidized flavors. The volatile reaction products of oxidation were collected in toluene, and a comparison of the TBA values at comparable peroxide values of lard, cottonseed and soybean oils showed that the soybean oil volatiles developed the greatest intensity of color.

REFERENCES

1. A.O.C.S. Official Method, Ca 5a-40, Official and Tentative Methods of the American Oil Chemists' Society, 2nd ed, 1946.
2. Bailey, A. E., "Cottonseed and Cottonseed Products," p. 389, New York, Interscience Publishers Inc., 1948.
3. Bailey, A. E., "Industrial Oil and Fat Products," pp. 144, 152, 172, New York, Interscience Publishers Inc., 1951.
4. Biggs, D. A., and Bryant, L. R., Can. J. Technol., *31*, 138-145 (1953).
5. Canteni, R., Z. Wiss. Phot., *36*, 90-95 (1937); C. A. *31*, 8381.
6. Dunkley, W. L., and Jennings, W. G., J. Dairy Sci., *34*, 1064-1069 (1951).
7. Dutton, H. J., Lancaster, Catherine R., Evans, C. D., and Cowan, J. C., J. Am. Oil Chemists' Soc., *28*, 115-118 (1951).
8. Henick, A. S., Benca, M. F., and Mitchell, J. H. Jr., J. Am. Oil Chemists' Soc., *31*, 88-91 (1954).
9. King, A. E., Roschen, H. L., and Irwin, W. H., Oil & Soap, *10*, 105-109 (1933).
10. Lappin, G. R., and Clark, L. C., Anal. Chem., *23*, 541-542 (1951).
11. Moore, R. N., and Bickford, W. G., J. Am. Oil Chemists' Soc., *29*, 1-4 (1952).
12. Mukherjee, S., Indian Chem. Soc., *27* (11), 589-598 (1950). From Dairy Sci. Abst., *14*, 379 (May 1952).
13. Patton, S., and Kurtz, G. W., J. Dairy Sci., *34*, 669-674 (1951).
14. Report of the Committee on Analysis of Commercial Fats and Oils, Oil and Soap, *22*, 101-107 (1945).

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Pilot-Plant Application of Filtration-Extraction to Soybeans[1]

E. L. D'AQUIN, J. J. SPADARO, A. V. GRACI JR., P. H. EAVES, L. J. MOLAISON, N. B. KNOEPFLER, A. J. CROVETTO, H. K. GARDNER, and H. L. E. VIX, Southern Regional Research Laboratory,[2] New Orleans, Louisiana

PILOT-PLANT development of a new solvent process called filtration-extraction for extracting cottonseed and rice bran has been reported in previous papers (1, 5) by this laboratory. Extension of the process on a pilot-plant scale to the processing of soybeans is warranted by the fact that during the 1951-52 crushing season there were 58 mills outside the North Central States that processed soybeans and other oilseeds (9) and most of these were cottonseed oil mills that crushed soybeans after their supply of cottonseed had been exhausted. Since practically all of these were small to medium-sized mills, application of the process to soybeans is further warranted because the new process holds definite promise of cost feasibility (6) for low capacity installations, together with versatility, and may for the first time bring direct solvent extraction within the economic reach of the small processor who can predicate his installation on a longer crushing season by processing rice bran,

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[2] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture.