E. F. Herb from the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, has resigned as his work is not now in the field of Spectroscopy. He has been replaced by Paul Magidman from the same organization. Donald Wheeler, General Mills, Inc., has also resigned as his work is now no longer closely related to spectroscopy. He has been replaced by Seymore Goldwasser, Lever Brothers Company. In addition, we have added one new member, David Firestone from the Food and Drug Administration of the Department of Health, Education, and Welfare. Dr. Firestone is a referee for official methods of the Association of Official Agricultural Chemists. His appointment to the Spectroscopy Committee of the A.O.C.S. will be another step in standardizing official methods of the two societies. We welcome Dr. Firestone, Dr. Magidman, and Dr. Goldwasser to the Committee. We regret the necessity for Drs. Herb and Wheeler to leave us, but we look forward to the opportunity to consult with them as they join the illustrious members of our alumni.

Acknowledgments

The Spectroscopy Committee is ever aware that, particularly in the collaborative testing program, it is indebted to several individuals for assistance in making spectral measurements, compiling data, and offering suggestions.

The chairman, in particular, wishes to acknowledge the considerable assistance of Elizabeth R. McCall in compiling results of polls of the members and in distributing the secondary standards for the A.O.C.S. tentative method "Isolated *Trans* Isomers—Infrared Spectrophotometric Method."

Robert R. Allen — Ralph E. Kelly
J. R. Chipault — William E. Link
Robert O. Crisler — Paul Magidman
David Firestone — B. N. Rockwood
Seymore Goldwasser — Hans Wolff

Robert T. O'Connor, chairman

REFERENCES

1. A.O.C.S. Spectroscopy Committee 1958–59, JAOCS *36*, 627–631 (1959).
2. A.O.C.S. Spectroscopy Committee 1959–60, *Ibid.*, *38*, 180–184 (1961).
3. Schlenk, Hermann and Gellerman, Joanne L., Anal. Chem., *32*, 1412–1414 (1960).

The Chemistry of the 2-Thiobarbituric Acid Test for the Determination of Oxidative Rancidity in Foods. I. Some Important Side Reactions[1,2]

BASIL G. TARLADGIS,[3] A. M. PEARSON, and L. R. DUGAN JR., Department of Food Science, Michigan State University, East Lansing, Michigan

Evidence is presented by UV, visible, and IR spectra as well as by paper and column chromatography of variously treated TBA-acid reagents that the structure of TBA is altered upon acid-heat treatment. A more pronounced but similar effect results from the treatment of the TBA with hydrogen peroxide. Some of the degradation products of TBA absorb at the same wavelength as the TBA-malonaldehyde complex, as do many compounds which are reported in the literature to react with TBA. The significance of these findings in respect to the quantitative aspects of the test for the determination of rancidity in food products is discussed.

THE 2-thiobarbituric acid (TBA) test is widely used for measuring oxidative changes in foods containing unsaturated fatty acids. The red pigment produced after reacting TBA directly with oxidized foods or their distillates has been identified to be a condensation product of one molecule of malonaldehyde with two molecules of TBA (22). As malonaldehyde is believed to be derived from some decomposition product of the oxidized unsaturated fatty acids, spectrophotometric measurement of the TBA-malonaldehyde complex gives what is thought to be a quantitative measure of fat oxidation (18, 19,21–23,30).

In recent years various methods have been developed for performing the TBA test on food products. These methods can be classified under two categories. a) A solution of TBA in a strong acid is added to the food product, and the whole mixture is heated for periods of 10–35 min. in a water bath to obtain maximum color development. The red pigment is then extracted with a suitable solvent and measured in a spectrophotometer. b) The food product is first steam-distilled with acid, and the TBA-acid solution is added to a portion of the distillate, which is then heated for 35 min. for maximum color development. The red pigment is measured directly in a spectrophotometer.

The two methods are similar in that they both employ heating of the food at a low pH (0.9–1.5). This step is claimed to be essential for the liberation of malonaldehyde from some precursor as well as for the condensation of malonaldehyde with TBA (1–3,10–12, 18–23,26,29,30).

Their differences however are many. The distillation method appears to have advantages in that prolonged heating of the food product is avoided, thus keeping to a minimum any further oxidative or decomposition changes during the test. Furthermore only the volatile constituents of the food are distilled over, thus avoiding any reaction of the TBA with nonvolatiles of the food which may react with it. Finally the acid of the TBA reagent is diluted as the reagent is added to the distillate in equal amounts before the heating begins.

All methods for performing the TBA test invariably employ the addition of TBA dissolved in acid. TBA is an amide, thus acid-heat treatment can be expected to hydrolyze TBA to thiourea and malonic acid. The same treatment may also hydrate the $-C-SH$ group of the TBA molecule, thus yielding

[1] Journal Article No. 2818, Michigan Agricultural Experiment Station, East Lansing, Mich.
[2] Presented at the 52nd annual meeting, American Oil Chemists' Society, St. Louis, Mo., May 3, 1961.
[3] Present address: Department of Food Technology, University of New South Wales, Kensington, N.S.W., Australia.

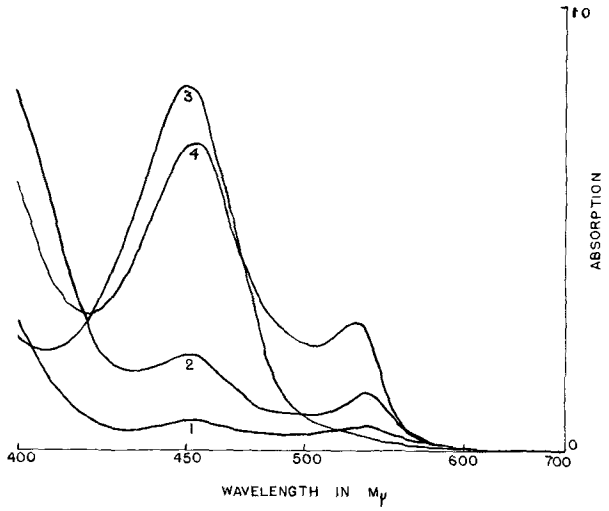

FIG. 1. Effect of different acids upon the absorption spectra of TBA:

1. TBA-TCA.
2. TBA-HCl.
3. TBA-acetic acid.
4. TBA-acetic acid, heated for 35 min. (20:1 dilution).

barbituric acid, which also may undergo subsequent hydrolysis to urea and malonic acid.

Acid-heat treatment may also accelerate the oxidation of the mercapto group as well as the $-CH_2$ group of TBA. The latter is located in between two carboxyl groups and, as such, is very susceptible to oxidation, yielding alloxan. These possible changes of TBA upon acid-heat treatment and their significance for the quantitative aspects of the TBA test have never been reported.

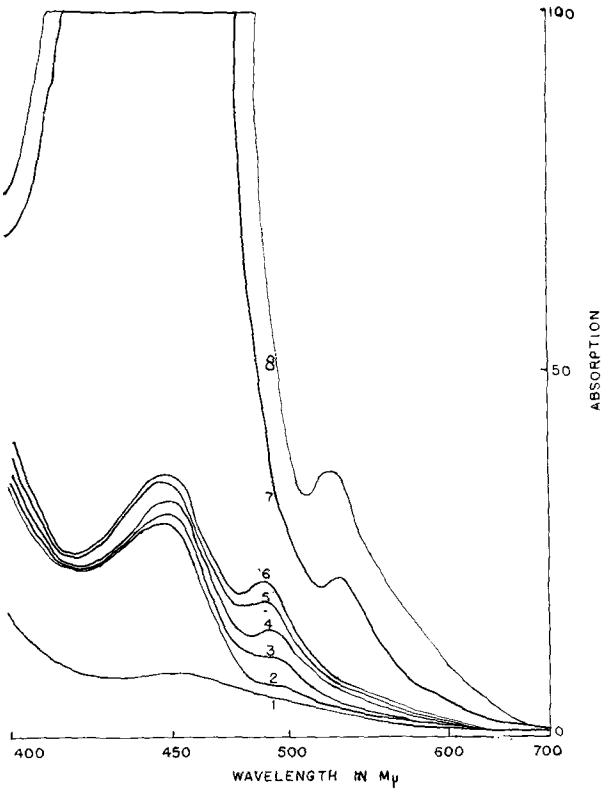

FIG. 2. The effect of time of heating upon the absorption spectra of TBA in acetic acid:

1. TBA-acetic acid, freshly prepared.
2. TBA-acetic acid, heated for 5 min.
3. TBA-acetic acid, heated for 10 min.
4. TBA-acetic acid, heated for 15 min.
5. TBA-acetic acid, heated for 20 min.
6. TBA-acetic acid, heated for 25 min.
7. TBA-acetic acid, heated for 30 min.
8. TBA-acetic acid, heated for 35 min.

The optical density of the red TBA-malonaldehyde complex is measured in a spectrophotometer against a blank containing TBA-acid reagent and treated the same as was the sample, or against unheated TBA-acid reagent (18,19), or against unheated TBA-water reagent (4–9,16,17,27,28). If some or all of the above-mentioned side-reactions occur, then the preparation of a blank in such a way as to ensure cancelling out of these side-effects becomes imperative in order to obtain the best quantitative results.

The purpose of this study is to investigate some of the conditions under which the structure of the TBA may be altered because of hydrolytic or oxidative changes, as well as to ascertain the significance of these changes in the quantitative aspects of the test for the determination of malonaldehyde in rancid foods.

Methods

The experiments reported below were repeated, using TBA (Eastman Organic Chemicals) from at least three different lots.

TBA Reagents. 0.02 M TBA solutions in water, 90% glacial acetic acid, 12% hydrochloric acid (HCl), and 20% trichloroacetic acid (TCA).

The TBA reagents were freshly prepared in all experiments unless otherwise stated. The heating tests were performed in such a way as to simulate the actual treatment of the blanks for the various TBA tests reported in the literature (20,21,23,24,29,30). The TBA reagents were therefore appropriately diluted with distilled water. A boiling water bath was used for heating purposes.

In one series of experiments the TBA reagents were heated in the water bath for periods of time varying from 5–35 min. In another series, hydrogen peroxide was added to the TBA reagent before heating. The concentrations of H_2O_2 varied from 1×10^{-3} to 5×10^{-5} M. Thiourea and malonic acid at 0.02 M concentrations were also heated alone or together with the TBA reagents.

The absorption spectra of all pigments produced by the above treatments were either recorded or measured in a Beckman DK-2 or DU spectrophotometer, respectively. Distilled water or the various acids used for the preparation of the different TBA reagents were used as blanks for the recording of all visible spectra. The blanks were not heated unless otherwise stated. The TBA-malonaldehyde complex was prepared as described by Sinnhuber *et al.* (22).

Column and Paper Chromatography. The column was prepared with cellulose powder and filled with isoamyl alcohol-acetic acid (98:2 v/v). The solvents, from the different treatments of the TBA previously described, were removed in a vacuum oven over sulfuric acid. The solids left behind were redissolved in 2 ml. of 10% acetic acid and were added to the column. A solution of 10% acetic acid was used as an eluting solvent.

TBA crystals were twice recrystallized from water and 95% ethyl alcohol. In a few hours after they were placed in the oven for drying at 100°C., it was noticed that the crystals had turned yellow-pink in color. These crystals were repeatedly run through the column after being dissolved in 10% acetic acid.

The same method and solvents were used for paper chromatography, as reported by Sinnhuber *et al.* (22), with the difference that an ascending rather than a

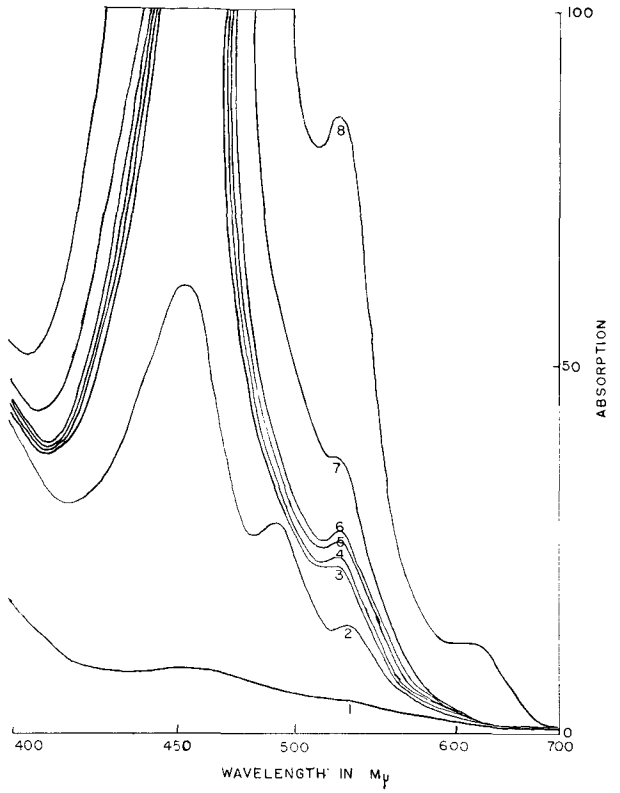

FIG. 3. The effect of time of heating upon the absorption spectra of TBA in acetic acid, in the presence of 5×10^{-5} M H_2O_2:

1. TBA-acetic acid, freshly prepared.
2. TBA-acetic acid + H_2O_2, heated for 5 min.
3. TBA-acetic acid + H_2O_2, heated for 10 min.
4. TBA-acetic acid + H_2O_2, heated for 15 min.
5. TBA-acetic acid + H_2O_2, heated for 20 min.
6. TBA-acetic acid + H_2O_2, heated for 25 min.
7. TBA-acetic acid + H_2O_2, heated for 30 min.
8. TBA-acetic acid + H_2O_2, heated for 35 min.

descending technique was employed. The solvent front traveled the distance to the top of the paper in 10 hrs.

The various colored solutions obtained after heating the different TBA reagents were spotted on the filter paper (Whatman No. 1) alone or side-by-side with the various eluents obtained from the column chromatography or with purified TBA-malonaldehyde complex.

UV Spectra. The UV spectra of TBA and the various TBA reagents treated as above were recorded in a Beckman DK-2 spectrophotometer. The spectra of TBA-malonaldehyde complex, barbituric acid, and thiourea were also recorded. Distilled water was used as a blank for the recording of all UV spectra.

Infrared Spectra. Since the various TBA derivatives prepared above are soluble only in acids and bases, KBr pellets were prepared from the crystals that were isolated by the various procedures described above, and their infrared spectra were taken with a Perkin-Elmer recording infrared spectrophotometer. In some cases spectra of impure compounds were taken for the purpose of comparison.

Results and Discussion

Effects of Heat and Hydrogen Peroxide on the TBA Reagents. Fig. 1 shows the absorption spectra of freshly prepared TBA reagents and that of TBA in acetic acid after heating for 35 min. The spectrum of the latter was recorded after a 20:1 dilution with distilled water. When TBA in TCA and HCl were heated for 5 and 30 min., respectively, their spectra had a diffused line. The spectra of TBA in acetic acid, heated from 5–35 min., are shown in Fig. 2. The absorbance at 450 mμ increased with the heating time while the peak at 490 mμ was obscured because of the increased intensity of the 450 mμ peak. Also a peak at 530 mμ was visible when the mixture was heated for 30 and 35 min.

Fig. 3 shows the absorption spectra of TBA in acetic acid heated for 5–35 min. after the addition of 5×10^{-5} M of H_2O_2. The intensity of the 450 mμ peak was magnified many-fold as was that of the 530 mμ peak. TBA-TCA and TBA-HCl reagents, treated the same way, gave discolored, cloudy solutions. When higher concentrations of hydrogen peroxide were used, the TBA-acetic acid reagent also gave discolored, cloudy solutions. Fig. 4 shows the absorption spectra of the TBA-malonaldehyde complexes prepared by heating 2×10^{-8} moles of 1,1,3,3-tetraethoxypropane (TEP) and 0.02 M TBA in the following solvents (1,14,18,20,21,23,28,29): water, freshly prepared 90% glacial acetic acid, 90% glacial acetic acid prepared two weeks in advance, 12% HCl, and 20% TCA.

The molecular extinction coefficients of the above TBA-malonaldehyde complexes at 530 mμ are compared in Table I. It should be noted that they were

TABLE I

Molecular Extinction Coefficients of the Various TBA-Reagents-Malonaldehyde Complexes

Colored complexes	Absorption	Molecular extinction coefficients	%
Malonaldehyde-TBA in			
Water	.308	$1,54 \times 10^7$	100
90% Acetic acid	.279	$1,40 \times 10^7$	90
90% Acetic acid[a]	.271	$1,36 \times 10^7$	88
12% Hydrochloric acid	.252	$1,26 \times 10^7$	81
20% Trichloroacetic acid	.229	$1,15 \times 10^7$	75

[a] Prepared two weeks in advance.

measured against blanks containing the respective solvents which were treated the same way as the samples. When the absorbance of the same samples was measured against a distilled water blank, they were found to be approximately the same in respect to each other. This clearly indicates the degradation of the TBA upon acid-heat treatment.

Column Chromatography. Using a 10% solution of

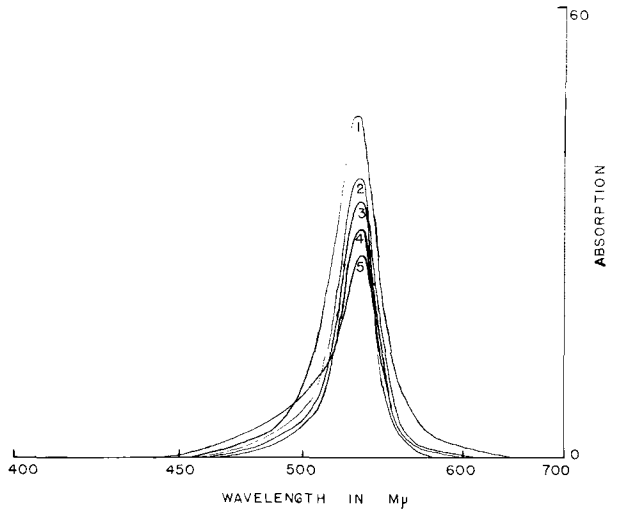

FIG. 4. Absorption spectra of malonaldehyde-TBA complexes, prepared by heating 2×10^{-8} moles of TEP with:

1. TBA in water.
2. TBA in 90% glacial acetic acid.
3. TBA in 90% acetic acid prepared 2 weeks in advance.
4. TBA in 12% HCl.
5. TBA in 20% TCA.

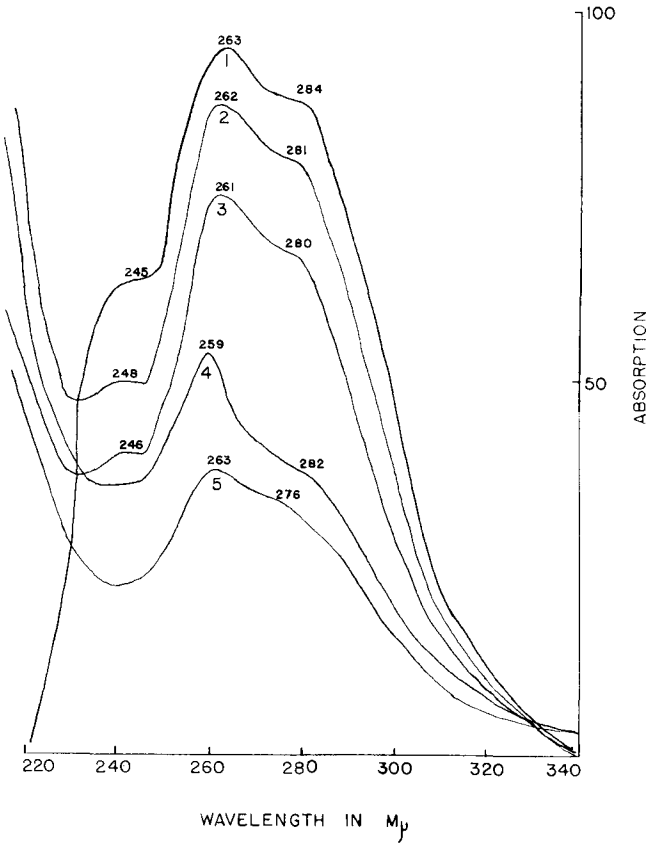

FIG. 5. UV spectra of:
1. TBA in water.
2. TBA in 90% glacial acetic acid.
3. TBA in 12% HCl.
4. Barbituric acid in water.
5. Barbituric acid in 90% glacial acetic acid

acetic acid as an eluting solvent, two fractions were obtained from all TBA reagents, one yellow in color and one orange. The absorption spectra of these fractions showed peaks at 452 and 490 mμ, respectively. A pink-colored band stayed at the top of the column after the elution of these bands. This was eluted with a solution of 10% ammonium hydroxide, and its absorption spectrum showed a peak at 530 mμ. Recrystallized TBA eluted from the column gave the same colored components with absorption maxima at 452, 490, and 530 mμ. Elution from the column of the reaction products of TBA with hydrogen peroxide gave the same three colored components, each absorbing at the same wavelength, respectively.

The colored components, isolated through column chromatography, change color rapidly when left at room temperature. Their color is preserved a little longer under nitrogen atmosphere or at refrigerated temperature. The yellow pigment changes progressively to orange-pink while the orange changes to pink. The pink fades. Their absorption maxima gradually shift from 452 mμ to 530 mμ, respectively. Repeated elutions of these pigments through the column yielded decreased amounts of yellow pigment and more pink pigment. Evaporation of the solvents in a vacuum oven over sulfuric acid yielded yellow and orange crystals and only traces of pink crystals although the color of the latter solution was very deep. These pink crystals were soluble in water.

Paper Chromatography. In all cases in which the yellow component was present, one yellow spot was visible with an Rf value of 0.67. No other visible spot was observed. When the pink component was present, a spot with an Rf value of 0.23 was observed under UV radiation. No visible spot or fluorescence was obtained for the orange component under the conditions of the chromatography.

One spot was visible under UV radiation with an Rf value of 0.23 when recrystallized TBA, TBA-malonaldehyde complex, or the pink component of TBA obtained from the column chromatographic elution of the various TBA-reagents heated alone or with H_2O_2, were spotted side-by-side. When the two pigments were mixed, only one spot resulted.

UV Spectra. Fig. 5 shows the UV spectra of TBA in water, acetic acid, and HCl, together with the UV spectra of barbituric acid in water and in acetic acid. Fig. 6 shows the spectra of the pink, orange, and yellow pigments isolated by column chromatography of TBA heated in acetic acid and that of the same pigment after treatment with hydrogen peroxide. Comparison of Figs. 5 and 6 shows that most spectra exhibit three peaks at about 280, 260, and 240 mμ. The peak at 240 mμ appeared to be the same as that of the orange pigment while the pink material gave a peak at about 280 mμ. Thiourea gave only one peak located at 240 mμ. The spectrum of the pink pigment, isolated after heating TBA and H_2O_2, was different from that of the heated TBA-acetic acid reagent while those of their yellow components were very similar. Barbituric acid in water gave a sharp peak at about 260 mμ, but when it was heated in acetic acid, a spectrum was obtained similar to that of TBA-acetic acid but without the peak at 240 mμ.

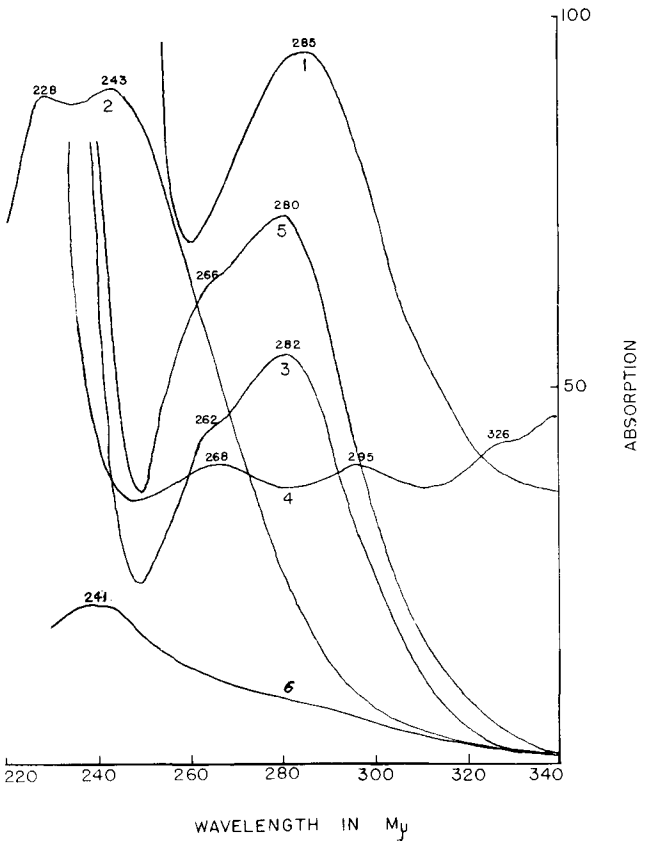

FIG. 6. UV spectra of the TBA degradation components isolated by column chromatography:

TBA in 90% glacial acetic acid, heated 35 min.:
1. Pink
2. Orange
3. Yellow

TBA in 90% glacial acetic acid, heated 35 min., after addition of 5×10^{-5} M H_2O_2:
4. Pink
5. Yellow

Thiourea in distilled water.

In general, the UV spectra of the various TBA reagents, treated in different ways, were similar to the structure of untreated TBA in water, but the peaks were shifted and new ones appeared, indicating new structures or structural differences. In the case of the various TBA-malonaldehyde complexes, the general structure of the TBA spectrum was retained, probably because it was present in such a great excess as compared to malonaldehyde.

Infrared Spectra. Table II compares the IR spectra of the various TBA derivatives. The alteration of the structure of TBA after the different treatments is quite obvious, as indicated by the appearance of new bands.

The presence of bands at 3.1 and 5.9 microns in the spectrum of TBA corresponding to −C−OH and −C=O groups, respectively, indicates that TBA exists in both the keto and enol form.

The IR spectrum of the TBA-malonaldehyde complex generally agrees with the chemical formula proposed by Sinnhuber *et al.* (22), as confirmed by Schmidt (18). In view of the bands at 2.9, 3.2, 3.45, 6.1, 6.7, 8.4, and 8.9 microns, corresponding to −NH, =CH, $-CH_2$, −C=O, and −C−OH groups, the actual chemical formula would be a resonance hybrid of many forms. There seems to be no water associated with the molecule as postulated by Sinnhuber (22) and measured by Schmidt (18,19). Water gives sharp bands at about 2.6 and 5.8 microns. These bands are not observed in the IR spectrum of the TBA-malonaldehyde complex.

The results of the experiments performed show that the structure of TBA is altered by acid-heat treatment. Evidence is presented by the UV, visible, and IR spectra as well as by the paper and column chromatography of the various TBA reagents.

It cannot be said with certainty whether these changes in the structure of TBA are caused by hydrolysis alone or by some other concurrent reactions. No evidence of hydrolysis of the TBA to malonic acid and thiourea was shown by the various spectra. This was not surprising since both thiourea and malonic acid may decompose upon acid-heat treatment to yield hydrogen sulfide, carbon dioxide, and ammonia; and acetic acid and carbon dioxide, respectively.

It has also been shown that the structure of TBA is altered when heated with an oxidizing agent. As noted before, TBA can be oxidized in two sites, the −C−SH and $-CH_2$ groups. When mercaptans are oxidized, disulfides, sulfoxides, and sulfones may be formed, depending on the degree of oxidation. The mercapto group may also be hydrated and lose H_2S, and the TBA may thus be converted to barbituric acid. On the other hand, if the $-CH_2$ group is oxidized, alloxan should be the end-product, which possibly could condense with an unoxidized TBA molecule.

Presence of such compounds in the TBA-reagents treated in various ways has not been detected, perhaps because of the incomplete purification of the samples. Continued attempts are being made to identify the various side-products.

In general, the results of this work indicate that TBA should not be heated with acids or in the presence of oxidizing agents. It has been taken for granted that, when the optical density of a colored complex is read against a blank treated the same way as the sample, all side-effects are cancelled out. It should be remembered however that when a TBA reagent is added directly to the oxidized food product and the whole mixture is heated for long periods of time, the blank is not necessarily treated the same way. The oxidized food product contains hydroperoxides which are expected to affect TBA the same way as hydrogen peroxide while the blank does not contain hydroperoxides.

The visible spectra of the TBA-reagents treated in various ways show peaks at 450, 490, and 530 mμ. The latter is located at the same wavelength as the

TABLE II

Infrared Spectra of TBA Derivatives

Compounds	Wavelength in microns											
	2	3	4	5	6	7	8	9	10	11	12	13
TBA		3.10 3.25 3.45	4.00 4.35	5.90 5.95	6.00 6.55 6.95	7.00 7.70 7.30 7.85 7.50	8.10 8.75	9.80	10.20 10.70	11.25 11.45 11.70	12.50	13.00 13.10 13.80
TBA-acetic acid		3.20 3.95 3.30 3.45	4.10 4.65 4.30 4.45	5.50 5.90	6.10 6.45 6.15 6.55 6.35	7.00 7.80 7.25 7.50	8.05 8.70 8.45 8.80 8.60 8.90	9.45 9.60 9.80	10.00 10.20 10.75	11.15 11.30 11.80	12.25	13.00 13.70 13.35 13.80 13.50 13.95
TBA-HCL		3.00 3.50 3.15 3.80 3.25	4.00 4.95 4.35 4.75	5.50 5.75 5.90	6.05 6.55 6.20 6.70 6.30	7.05 7.70 7.35 7.90 7.50	8.20 8.55 8.80	9.00 9.85 9.20 9.50	10.10 10.80 10.40 10.55	11.25	12.45	13.90
TBA-malonaldehyde complex	2.80 2.90	3.05 3.20 3.45	4.00 4.30		6.10 6.70 6.20 6.75 6.45	7.25 7.70 7.40 7.85 7.65	8.40 8.90	9.20 9.90 9.30 9.50	10.05 10.85 10.40 10.60	11.10 11.75 11.95	12.75	13.15 13.80
Yellow pigment		3.30 3.40 3.55	4,35	5.40	6.10 6.95 6.30 6.65	7.25 7.70	8.10 8.25 8.45	9.75 9.95	10.20 10.75	11.25 11.70 11.45 11.60	12.00 12.20 12.70	13.20 13.95
Orange pigment		3.30 3.50		5.00	6.35 6.70 6.95	7.25 7.75	8.25 8.45 8.70	9.95	10.75 10.50 10.75	11.30 11.95	12.25 12.75	13.80
Pink pigment	2.95	3.05 3.45 3.90	4.10 4.40	5.50 5.75	6.30 6.45 6.95	7.20 7.55 7.25 7.75 7.50 7.85	8.10 8.45 8.80	9.55 9.90	10.75	11.50	12.05 12.45 12.70	13.40 13.85
Barbituric acid	2.95	3.20 3.40 3.30 3.45 3.35 3.55	4.15 4.35	5.75 5.95	6.10 6.55 6.25 6.65 6.30 6.90	7.10 7.50 7.30 7.75 7.40 7.85	8.05 8.15 8.60	9.40 9.75	10.80		12.50	
		3.15 3.75 3.25 3.85 3.45	4.15 4.70 4.30 4.80 4.50	5.35 5.75 5.85	6.95	7.10 7.60 7.25 7.80 7.40 7.95	8.20 8.50	9.80	10.75 10.90	11.15	12.70	13.00 13.70
Malonic acid Thiourea	2.95	3.05 3.55 3.15 3.75 3.30 3.90	4.30 4.95 4.60 4.75	5.50 5.80	6.15 6.25 6.80	7.10	8.30 8.95 8.45 8.70	9.25 9.75	10.05 10.95 10.55 10.75			13.70

absorption maximum of the TBA-malonaldehyde complex. Paper chromatography has shown that both compounds give identical spots. Until this compound derived from degradation of TBA is identified, it cannot be said with certainty whether it is malonaldehyde or other 3-carbon fragment or decomposition products of either thiourea or malonic acid interacting with TBA to give a compound similar to the TBA-malonaldehyde complex and absorbing at the same wavelength. It should be remembered that even traces of a compound with a highly chromophoric group can give a color with a distinct visible spectrum, which may not be detected by infrared if it is present in concentration less than 5% of the mixture.

It has been reported in the literature that many compounds react with TBA to give complexes absorbing at 450 and 490 mμ (2,3,4–13,16–19,23–25,28,29). In view of the fact that pure TBA heated with acids and even twice recrystallized TBA absorbs at the same wavelength, it has to be determined whether the reported compounds really react with TBA or whether changes in the structure of TBA have been assumed to be other compounds, especially when the blanks were treated differently from the samples.

In view of the fact that highly significant correlations have been obtained between TBA numbers and taste-panel results on various oxidized foods, the results of this work should not be construed as a suggestion to discard the TBA test for determination of rancidity in oxidized foods. They merely point out that more care should be taken in the conditions of the test and in the treatment of the blank so that the results obtained are really quantitative.

Work is under way in this laboratory for the development of a TBA test without the need of acid-heat treatment for the determination of malonaldehyde in rancid foods.

Acknowledgments

The authors wish to express their gratitude to J. R. Brunner and P. A. Markakis of the Food Science Department for the use of various instruments. The help of G. J. Karabatsos of the Chemistry Department for the interpretation of the IR spectra is gratefully acknowledged.

REFERENCES

1. Bernheim, F., Bernheim, M. I. G., and Wilbur, K. M., J. Biol. Chem., *174*, 257 (1948).
2. Dunkley, W. L. and Jennings, W. G., J. Dairy Sci., *34*, 1064 (1951).
3. Kohn, H. I., and Liversedge, M., J. Pharmac., *82*, 292 (1942).
4. Landucci, J. M., Pouradier, J., and Pimont, M., Bull. Soc. Chem. France, *20*, 1072 (1953).
5. Landucci, J. M., Bull. Soc. Chem. France, *21*. 120 (1954).
6. Landucci, J. M., Bull. Soc. Chem. France, *21*. 124 (1954).
7. Landucci, J. M., Ph.D. Thesis, University of Paris, France, 1954.
8. Landucci, J. M., Pouradier, J., and Durante, M., Bull. Soc. Chem. France, *22*, 857 (1955).
9. Landucci, J. M., Pouradier, J., and Durante, M., Compt. Rendue Seance Soc. Biolog. France, 919 (1955).
10. Patton, S., Keeney, M., and Kurtz, G. W., J. Am. Oil Chemists' Soc., *28*, 391 (1951).
11. Patton, S., and Kurtz, G. W., J. Dairy Sci., *34*, 669 (1951).
12. Patton, S., and Kurtz, G. W., J. Dairy Sci., *38*, 901 (1955).
13. Patton, S., Food Research, *25*, 554 (1960).
14. Plaisance, G. P., J. Biol. Chem., *29*, 207 (1917).
15. Powick, W. C., J. Agr. Res., *26*, 323 (1923).
16. Saslaw, L. D., and Waravdekar, V. S., Arch. Biochem. Biophys., *90*, 239 (1960).
17. Saslaw, L. D., and Waravdekar, V. S., Arch. Biochem. Biophys., *90*, 245 (1960).
18. Schmidt, H., Fette und Seifen Anstrichm., *61*, 127 (1959).
19. Schmidt, H., Fette und Seifen Anstrichm., *61*, 881 (1959).
20. Sidwell, C. G., Salwin, H., and Mitchell, H. J., J. Am. Oil Chemists' Soc., *32*, 13 (1955).
21. Sinnhuber, R. O., and Yu, T. C., Food Technol., *12*. 9 (1958).
22. Sinnhuber, R. O., and Yu, T. C., Food Research, *23*. 626 (1958).
23. Tarladgis, B. G., Watts, Betty M., Younathan, M. T., and Dugan, L. R. Jr., J. Am. Oil Chemists' Soc., *37*, 44 (1960).
24. Tarladgis, B. G., and Watts, Betty M., J. Am. Oil Chemists' Soc., *37*, 403 (1960).
25. Taufel, K., and Zimmermann, R., Fette und Seifen Anstrichm., *61*, 836 (1959).
26. Turner, E. W., Paynter, W. D., Montie, E. J., Bessert, M. W., Struck, G. M., and Olson, F. C., Food Technol., *8*, 326 (1954).
27. Waravdekar, V. S., and Saslaw, L. D., Biochem. Biophys. Acta, *24*, 439 (1957).
28. Waravdekar, V. S., and Saslaw, L. D., J. Biol. Chem., *243*, 1945 (1959).
29. Wilbur, K. M., Bernheim, F., and Shapiro, O. W., Arch. Biochem. Biophys., *24*, 305 (1949).
30. Yu, T. C., and Sinnhuber, R. O., Food Technol., *11*, 104 (1957).

[Received June 13, 1961]

Application of Infrared Spectroscopy to the Analysis of Primary Fatty Amide Mixtures

W. E. LINK and K. M. BUSWELL, Archer-Daniels-Midland Company, Minneapolis, Minnesota

The composition of fatty amide mixtures, containing mostly simple primary amides, has been studied by means of an infrared method utilizing the Amide I carbonyl absorption band. In dilute chloroform solution the amides, $CH_3(CH_2)nCONH_2$, absorb consistently at about 5.95 μ and do not display apparent association or enolization. The concentration of unsubstituted amides has been quantitatively related to the intensity of the Amide I band throughout the range from 1 to 100%. With the scale expansion the sensitivity of the method may be extended to 0.03% without difficulty.

The influence of certain impurities on the intensity of the Amide I band is discussed. Carbonyl absorption from fatty acids, ketones, and esters constitutes an interference. *N*-monosubstituted amides also contribute to the absorption observed at 5.95 μ, but their presence is indicated by additional absorption at about 6.60 μ.

The determination of small amounts of amides in amines may be accomplished by measuring the total Amide I absorption of a sample dissolved in tetrachloroethylene and relating this absorption to that of known mixtures run under identical conditions.

CHEMICAL METHODS, which provide dependable and precise analyses with fatty amines, are less successful with fatty amides, for the usual basicity of the nitrogen, upon which many functional analyses are based, is neutralized by the presence of the acyl group. A variety of methods are available, nevertheless, for the study of primary amide mixtures.

Vigorous treatment with acid (8) or alkali (17) will hydrolyze amides so that the resulting amine or ammonia may be liberated, distilled, and finally titrated as in a Kjeldahl determination. Olsen (13) has presented a saponification value procedure which eliminates the interference of esters. The reduction of amides to amines with lithium aluminum hydride is feasible. In this procedure, by Siggia (18), the amine is steam distilled and titrated with standard acid. Nitriles are a serious interference. A number of investigators (2,14,19,21) have studied the reaction of hydroxylamine with amides to form hydroxamic acids which can be estimated by color reaction