Pure & Appl. Chem., Vol. 61, No. 6, pp. 1165–1170, 1989.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION
COMMISSION ON OILS, FATS AND DERIVATIVES*

DETERMINATION OF 2-THIOBARBITURIC ACID VALUE: DIRECT METHOD

Results of a collaborative study and the standardised method

Prepared for publication by

J. POKORNY[1] and A. DIEFFENBACHER[2]

[1]Department of Food Chemistry, Institute of Chemical Technology, Prague 6, Czechoslovakia

[2]Quality Assurance Department, Avenue Nestlé 55, 1800 Vevey, Switzerland

*Membership of the Commission during the period of the collaborative study (1983–87) was as follows:

Chairman: 1983–85 M. Naudet (France); 1985–87 Ö. Levin (Sweden); *Vice-Chairman*: A. Hautfenne (Belgium); *Secretary*: W. D. Pocklington (UK); *Titular Members*: J. L. Beare-Rogers (Canada; 1985–87); É. Kurucz (Hungary; 1985–87); Ö. Levin (Sweden; 1983–85); J. Pokorný (Czechoslovakia; 1983–85); H. Wessels (FRG); *Associate Members*: J. L. Beare-Rogers (Canada; 1983–85); A. Dieffenbacher (Switzerland); E. Fedeli (Italy); J. Gracian-Tous (Spain; 1983–85); T. Hashimoto (Japan; 1985–87); G. Hølmer (Denmark; 1985–87); B. Jacobsberg (Belgium); A. Karleskind (France; 1985–87); F. H. Koelman (Netherlands); É. Kurucz (Hungary; 1983–85); R. G. Manning (USA); A. T. Møller (Denmark; 1983–85); R. Ohlson (Sweden; 1985–87); M. Teupel (FRG); A. Visapää (Finland); G. Zwerenz (Austria); *National Representatives*: A. M. Gad (Arab Republic of Egypt); N. G. Bazan (Argentina; 1983–85); R. R. Brenner (Argentina; 1985–87); A. R. Johnson (Australia); R. C. A. Lago (Brazil; 1983–85); D. G. Chobanov (Bulgaria); A. P. Tulloch† (Canada); J. Pokorný (Czechoslovakia; 1985–87); G. Hølmer (Denmark; 1983–85); J. V. Holm (Denmark; 1985–87); G. Ostermann (FRG); R. Linko (Finland); J. P. Wolff (France); V. M. Kapoulas (Greece; 1983–85); D. Boskou (Greece; 1985–87); M. Jeránek (Hungary; 1985–87); N. V. Bringi (India; 1983–85); A. G. Mathew (India; 1985–87); M. McCarthy (Ireland; 1983–85); A. M. Sharkey (Ireland; 1985–87); A. Dolev (Israel); E. Tiscornia (Italy); T. Hashimoto (Japan; 1983–85); S. Watanabe (Japan; 1985–87); A. S. H. Ong (Malaysia); P. W. Hendrikse (Netherlands); F. B. Shorland (New Zealand; 1983–85); L. Eyres (New Zealand; 1985–87); W. Zwierzykowski (Poland); G. D. Carr (Republic of South Africa); R. Stoica (Romania); M. Gassiot Matas (Spain); R. Ohlson (Sweden; 1983–85); H. Brüschweiler (Switzerland); R. Perin (Turkey; 1983–85); T. Tuğlular (Turkey; 1985–87); V. L. Roubaijlo (USSR); A. E. Waltking (USA).

†deceased 1987

Determination of 2-thiobarbituric acid value: direct method—results of a collaborative study and the standardised method

Abstract - The development, by collaborative study, of a standardised method for the direct determination of 2-thiobarbituric acid value in oils, fats and derivatives is described. Fats and oils are reacted, without previous isolation of volatiles, with 2-thiobarbituric acid, and the absorbancy of red-coloured condensation products is measured at 530 nm.

INTRODUCTION

The determination of the 2-thiobarbituric acid value is one of several analytical methods for the evaluation of the degree of oxidation of vegetable oils, fish oils and animal fats, particularly those containing linolenic acid and more unsaturated fatty acids. 2-thiobarbituric acid forms red-coloured products with malonaldehyde (1), some polyunsaturated aldehydes (2), dioxolanes and furan derivatives. The intensity of coloration is correlated with the rancidity degree of fats and oils.

In the modification described the direct determination of total reactive substances is used, without previous isolation of the volatile fraction. The 2-thiobarbituric acid (TBA)-value, measured at 530 nm, is particularly useful for measuring oxidative changes in fats and oils containing fatty acids of greater unsaturation than linoleic acid.

Secondary oxidation products react with TBA forming condensation products with absorption maxima at 450 nm and 530 nm. The latter maximum is measured in this direct procedure while the analysis of substances absorbing at 450 nm requires a different procedure (2).

The method was preliminarily tested in six laboratories, each laboratory using its own samples; on the basis of these experiments the final text was prepared, and the method submitted for interlaboratory testing.

1st COLLABORATIVE STUDY AND RESULTS

In the first interlaboratory study the following five samples of oils were analysed : sample A = crude zero-erucic rapeseed oil; sample B = refined high-linoleic sunflowerseed oil stored for 3 months at room temperature; sample C = refined zero-erucic rapeseed oil stored for 6 months at room temperature; sample D = identical with C (but not revealed as such to the collaborators); sample E = refined soybean oil stored 9 days at 60°C (peroxide value = 17.5).

A summary of the results submitted by 16 laboratories is given in Table 1 and the statistical evaluation (3) in Table 2.

The repeatability of the method was found to be satisfactory, and results for analyses for the two identical samples C and D were in good agreement. Reproducibility coefficients of variation were, however, rather high.

A supplementary set of experiments using several reagents of various origin showed the purity of reagents was not the main cause of the high interlaboratory variance.

2nd COLLABORATIVE STUDY AND RESULTS

For the second collaborative study the following five samples were distributed : sample A = refined zero-erucic rapeseed oil stored for 3 months at room temperature; sample B = partially hydrogenated zero-erucic rapeseed oil, 0.05% trienoic acids, 0.10% dienoic acids stored at room temperature for 1 month; sample C = identical with sample A, the fact not revealed to the collaborators; sample D = soybean oil oxidized at room temperature for 2 years; sample E = butter fat prepared from fresh winter butter, stored for 14 days at room temperature.

The results obtained from 20 laboratories are presented in Table 3. The statistical evaluation is given in Table 4. In agreement with the results of the first collaborative study, the repeatability was satisfactory while the reproducibility was substantially higher, and of the same order as in the first experiment.

TABLE 1. Results of the TBA value determination (1st interlaboratory study)

Lab code	Duplicate results of the TBA value determination									
	sample A		sample B		sample C		sample D		sample E	
01	0.180	0.164	0.022	0.026	0.150	0.155	0.163	0.135	0.974*	0.803*
02	0.149	0.153	0.013	0.013	0.065	0.063	0.076	0.074	0.182	0.193
03	0.196	0.198	0.025	0.027	0.094	0.106	0.089	0.104	0.448	0.471
04	0.199	0.194	0.030	0.028	0.108	0.107	0.102	0.095	0.264	0.254
05	0.164	0.158	0.028	0.026	0.113	0.118	0.103	0.095	0.228	0.225
06	0.187*	0.159*	0.030	0.027	0.110	0.114	0.098	0.099	0.240	0.223
07	0.136	0.143	0.030	0.034	0.105	0.112	0.103	0.103	0.176	0.171
08	0.206	0.213	0.033	0.031	0.090	0.102	0.101	0.093	0.163	0.173
09	0.101	0.095	0.011	0.010	0.047	0.048	0.052	0.054	0.062	0.059
10	0.090	0.091	0.009	0.008	0.047	0.048	0.054	0.057	0.107	0.099
11	0.130	0.130	0.008	0.008	0.043	0.044	0.042	0.041	0.111	0.110
12	0.068	0.072	0.006	0.006	0.038	0.036	0.030	0.030	0.056	0.057
13	0.073	0.072	0.004	0.003	0.050	0.049	0.045	0.050	0.166	0.206
14	0.167	0.174	0.043	0.046	0.111	0.110	0.099	0.113	0.154	0.144
15	0.058	0.053	0.005	0.005	0.021	0.022	0.013	0.012	0.053	0.053
16	0.151	0.158	0.017	0.015	0.050	0.048	0.053	0.055	0.082	0.082

Note : * results eliminated on basis of the Cochran or Dixon tests

TABLE 2. Statistical analysis of results (1st interlaboratory study)

Sample code	A	B	C	D	E
Mean value	0.138	0.020	0.079	0.076	0.167
Repeatability					
standard deviation	0.005	0.002	0.004	0.007	0.010
coefficient of variation	3.28 %	7.71 %	4.60 %	8.80 %	5.93 %
Reproducibility					
standard deviation	0.050	0.012	0.038	0.036	0.105
coefficient of variation	36.36 %	62.9 %	48.2 %	47.2 %	62.9 %
repeatability value r (95)	0.013	0.004	0.010	0.019	0.028
reproducibility value R (95)	0.142	0.035	0.108	0.102	0.297

CONCLUSIONS

1) The TBA value is a method for the analysis of certain carbonylic oxidation products present in stored fats and oils and causing their rancidity. The content of reactive substances is very small of the order of 10 - 2000 $\mu g\ kg^{-1}$. Therefore, the criteria of precision common in trace analysis should be applied. Moreover, oxidized rancid fats and oils are very complicated mixtures of many relatively unstable oxidation products which decompose in various ways on heating, or may interfere with the reaction.

2) The repeatability values determined from a statistical analysis of the results (see Tables 2 and 4) indicate that the determination of TBA value can be carried out at an acceptable degree of precision. The TBA value determination may be affected by hydroperoxides, oxygen, antioxidants, and trace metals. By observing the procedure carefully, and by using chemicals of high purity, most of these influences can be eliminated or remain constant, and fairly repeatable results are obtained.

TABLE 3. Results of the TBA value determination (2nd interlaboratory study)

Lab code	Duplicate results of the TBA value determination									
	sample A		sample B		sample C		sample D		sample E	
01	0.195	0.183	0.079	0.074	0.199	0.193	0.228	0.277	0.030	0.026
02	0.135	0.151	0.068	0.060	0.137	0.136	0.236	0.247	0.021	0.026
03	0.092	0.084	0.079	0.071	0.087	0.079	0.099	0.067	0.049	0.045
04	0.109	0.108	0.029	0.029	0.118	0.121	0.207	0.202	0.016	0.015
05	0.111	0.120	0.030	0.028	0.088	0.081	0.139	0.130	0.014	0.011
06	0.117	0.107	0.065	0.073	0.161	0.150	0.109	0.097	0.014	0.012
07	0.049	0.057	0.037	0.025	0.070	0.062	0.149	0.133	0.005	0.002
08	0.096	0.107	0.016	0.025	0.088	0.092	0.177	0.162	0.022	0.031
09	0.194	0.190	0.024	0.018	0.150	0.155	0.344	0.342	0.044	0.035
10	0.185	0.191	0.068	0.065	0.149	0.159	0.302	0.315	0.033	0.035
11	0.044	0.044	0.020	0.021	0.035	0.035	0.057	0.052	0.004	0.005
12	0.048	0.033	0.020	0.020	0.040	0.046	0.060	0.059	0.009	0.019
13	0.097	0.092	0.033	0.037	0.100	0.084	0.040	0.040	0.017	0.014
14	0.063	0.069	0.042	0.040	0.091	0.094	0.064	0.068	0.018	0.017
15	0.113	0.109	0.052	0.052	0.097	0.109	0.276	0.298	0.010	0.011
16	0.099	0.106	0.047	0.054	0.087	0.098	0.384	0.389	0.012	0.013
17	0.090	0.088	0.027	0.029	0.091	0.085	0.113	0.117	0.011	0.013
18	0.033	0.034	0.014	0.014	0.032	0.034	0.048	0.056	0.007	0.007
19	0.095	0.080	0.048	0.040	0.070	0.077	0.198	0.186	0.015	0.016
20	0.025	0.025	0.032	0.031	0.063	0.062	0.060	0.062	0.008	0.008

TABLE 4. Statistical analysis of results (2nd interlaboratory study)

Sample code	A	B	C	D	E
mean	0.099	0.041	0.098	0.165	0.018
Repeatability					
standard deviation	0.006	0.004	0.005	0.011	0.003
coefficient of variation	6.10 %	9.70 %	5.47 %	6.94 %	16.8 %
value r (95)	0.017	0.011	0.015	0.032	0.009
Reproducibility					
standard deviation	0.050	0.020	0.044	0.108	0.012
coefficient of variation	50.4 %	49.7 %	44.6 %	65.4 %	65.9 %
value R (95)	0.141	0.058	0.123	0.305	0.034

3) The results given in Tables 1 and 3 show that the ratios of the results for samples analyzed in the same laboratory are close to the ratios obtained in another laboratory even when the absolute values were different. This means that the reproducibility can be considerably improved by use of reference standards.

4) The reproducibility was substantially higher than the repeatability (see Tables 2 and 4). From the reasons given in 1), good reproducibility can be hardly expected; similarly this is one case with other methods for the estimation of oxidative rancidity; however, the determination of rancidity is mostly used for comparison of various samples within a laboratory. For this application the method is suitable.

Following the conclusions drawn from the results of the 2nd collaborative study the Commission decided to adopt the method for the direct determination of the TBA value. The text of the standardized method is given on the following pages.

2.531 DETERMINATION OF 2-THIOBARBITURIC ACID VALUE: DIRECT METHOD

1 SCOPE

This standard describes a method for the direct determination of the 2-thiobarbituric acid value (TBA value) in oils and fats without preliminary isolation of secondary oxidation products.

2 DEFINITION

The TBA value is defined as the increase of absorbance measured at 530 nm due to the reaction of the equivalent 1 mg of sample per 1 ml volume with 2-thiobarbituric acid determined by the present method.

3 FIELD OF APPLICATION

This standard is applicable to animal and vegetable fats and oils, fatty acids and their esters, partial glycol esters and similar materials (Note 1).

4 PRINCIPLE

Secondary oxidation products of oils and fats are reacted with 2-thiobarbituric acid forming condensation products the absorbance of which is measured at 530 nm, the wavelength of one of their absorbtion maxima.

5 MATERIAL

5.1. Volumetric flask 25 ml

5.2. Volumetric flask 100 ml

5.3. Pipette 5 ml

5.4. Ground test-tubes, I.D. 10-15 mm, with ground glass stoppers

5.5. Glass cells, 10 mm suitable for spectrometric measurements

5.6. Thermostated bath maintained at 95°C + 0.5°C

5.7. Spectrometer allowing the reading at 530 nm to the nearest 0.001

6 REAGENTS

6.1. Pure 1-butanol containing less than 0.5% water, (Note 3)

6.2. Chemically pure 2-thiobarbituric acid, (Note 3)

6.3. The TBA reagent is prepared by dissolving 200 mg 2-thiobarbituric in 100 ml 1-butanol. Leave the weighed amount with butanol overnight or use an ultrasonic apparatus, filter or centrifuge the suspension to remove the undissolved residue, make up the filtrate to 100 ml with 1-butanol; the reagent should not be stored for more than one week in the refrigerator.

7 PROCEDURE

7.1. Weigh accurately 50-200 mg of the sample (Note 4) into a volumetric flask (5.1.). Dissolve it in a small volume of 1-butanol and make up to volume with the same solvent.

7.2. Transfer, using a pipette (5.3.) 5.0 ml of the sample solution to a dry test tube (5.4); add by pipette (5.3) 5.0 ml of the reagent solution (6.3). Close the test tube with a ground stopper and mix thoroughly.

7.3. Place the prepared test tube into a thermostated bath (5.6.) at 95°C.

7.4. After 120 minutes, remove the test tube from the thermostated bath and cool it under running tapwater for about 10 minutes until it reaches room temperature.

7.5. Measure the absorbance of the reaction solution in a 10 mm cell (5.7.) at 530 nm (5.7.) using distilled water in the reference cell (Note 5).

7.6. Run at the same time a reagent blank. The reading of the blank determination should not exceed 0.1 in a 10 mm cell.

8 CALCULATION

8.1. Expression of results

$$\text{TBA-Value} = \frac{50 \times (\underline{A} - \underline{B})}{\underline{m}}$$

$\underline{A}$ is the absorbance of the test solution
$\underline{B}$ is the absorbance of the reagent blank
$\underline{m}$ is the mass in mg, of the test portion
50 is a factor valid if the volume of the volumetric flask is 25 ml (7.1.) and the cell width is 10 mm (7.4.) (Note 5)

8.2. Report of results

Report as the final results the mean of values obtained from two determinations, provided the requirements of repeatability (8.3) are met.

8.3. Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst using the same apparatus and the same reagents for the same test material, should not exceed 10% of the mean value of the two determinations in case of oils and 20% in case of milk fat.

9 NOTES

1. The method is not applicable to phospholipid concentrates, and to samples containing carbohydrates or proteins which could react either with the reagent or with TBA active substances. For the analysis of such samples the lipid fraction should be isolated by extraction before the analysis, or the volatile TBA active substances should be isolated by steam distillation.

Under the conditions of the method hydroperoxides and dioxolanes may be partially decomposed with formation of TBA active coumpounds, and the decomposition is catalyzed by a trace of heavy metals; on the other hand antioxidants may react with some TBA active substances thus decreasing the TBA value. Some substances present in the sample might react with TBA to form other colored complexes or reaction products of which the absorbance is not any more at 530 nm. This can be recognized by another colour tone.

2. The absorbance of the reagent blank measured according to procedure 7, but without test portion, should not exceed 0.1. If so, a new reagent solution should be prepared using TBA of better purity. In the case of reagent blanks higher than 0.1 the high blank value may be due to impurities in 1-butanol. They are removed by refluxing the solvent with 0.1% TBA for 2 h, and distilling. Water may be removed by distillation, and rejection of the first, opalescent fraction.

3. Solid samples are melted at not more than 10°C above the melting point and, if not entirely clear, filtered. Butter is melted at 40°C and the water removed on filtering with a hydrophilic filter.

4. If the absorbance falls outside the range of 0.8 - 0.2, the determination is repeated with more suitable cells or with more appropriate amount of sample.

5. In the case of low blank values (0.05) the absorbance of the test solution can be measured directly against distilled water

The value is then calculated as follows :

$$\text{TBA-Value} = \frac{50 \times \underline{A}}{\underline{m}}$$

Acknowledgements

The following collaborators participated in the interlaboratory studies :
H. Brüschweiler (Switzerland), H. Brüning-Pfaue (FRG), D. Chobanov (Bulgaria), A. Dieffenbacher (Switzerland), G. Hall (Sweden), T. Hashimoto (Japan), I. Hoffmann (Sweden), B. Jacobsberg (Belgium); M. Jeranek (Hungary), E. Kurucz (Hungary), C.A. Lago (Brazil), R. Marcuse (Sweden), M. McCarthy (Ireland), L.C.A. Parsons (United Kingdom), M. Pike (United Kingdom), M. Naudet (France), W.D. Pocklington (United Kingdom), M. Solère (France), R. Stoica (Romania), Z. Svoboda (Czechoslovakia), H. Valentova (Czechoslovakia), H. Wessels (FRG)

REFERENCES

1. R.O. Sinnhuber and T.C. Yu, Food Technol., 12, 9 (1958); 16, 115 (1962); Yukagaku, 26, 259 (1977).
2. R. Marcuse and L. Johansson, J. Amer. Oil Chem. Soc. 50, 387 (1973).
3. ISO 5725 : Precision of test methods - Determination of repeatability and reproducibility by interlaboratory tests (1986).