THE 2-THIOBARBITURIC ACID (TBA) METHODOLOGY FOR THE EVALUATION OF WARMED-OVER FLAVOUR AND OXIDATIVE RANCIDITY IN MEAT PRODUCTS

FEREIDOON SHAHIDI

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9, CANADA

SUMMARY

Although warmed-over flavour and rancidity are primarily organoleptic characteristics of foods, chemical methods for their quantitation have been developed. Malonaldehyde (MA) is one of the several decomposition products generally estimated as a marker of lipid oxidation. An extraction/filtration or a distillation procedure for the isolation of malonaldehyde followed by colour development with the 2-thiobarbituric acid reagent is often used. Each evaluation method has its own advantage(s)/disadvantage(s). The filtration procedure often affords more realistic results and prevents overestimation of the TBA reactive substances (TBARS). However, presence of coloured additives such as the cooked cured-meat pigment, as well as turbidity of the extracts, may interfere with accurate determination of the coloured chromogen of TBARS-MA. On the other hand, the distillation method generally affords higher values of TBARS due to further breakdown of labile hydroperoxides. Addition of antioxidants to the mixtures prior to distillation proved beneficial in some cases. Fur cured meats, the residual nitrite may react with malonaldehyde at elevated temperatures and this would result in underestimation of the TBARS. Addition of sulfanilamide to the distillation mixture prevented the nitrosation of malonaldehyde. However, sulfanilamide itself gave rise to the formation of condensation products with malonaldehyde. Thus, the TBA determination of oxidative rancidity in meat products may give rise to different results and interpretations.

INTRODUCTION

The 2-thiobarbituric acid (TBA) test was first used by Kohn and Liversedge (1944). They observed that animal tissues, upon aerobic incubation, gave a pink-coloured compound with the 2-thiobarbituric acid (TBA reagent). During autoxidation of polyunsaturated fatty acid (PUFA) components of lipids, malonaldehyde (MA) is produced. This secondary oxidation product is highly reactive and remains bound to other food ingredients. An acid/heat treatment of food would presumably release the bound malonaldehyde (Tarladgis *et al*. 1960).

Malonaldehyde is the major substance reacting with the TBA reagent as formulated in Figure 1 (Nair and Turner, 1984). The pink-coloured chromogen so produced has an absorption maximum at 532 nm. Spectrophotometric determination of this complex is the method usually employed for quantification of malonaldehyde and other TBA reactive substances. In addition to MA, 2,4-alkadienals, and to a lesser extent, 2-alkenals also produce a pink-coloured pigment which absorbs at 532 nm (Marcuse and Johansson, 1973).

The TBA test may be performed directly on a food product (Wills, 1965), and this may be followed by the extraction of the coloured pigment into butanol or butanol-pyridine mixture (Placer *et al*., 1966; Uchiyama and Mihara, 1978). The

test may also be carried out on a aliquot of an acid extract of food (usually 7.5 to 28% trichloroacetic acid, TCA, solution) (Siu and Draper, 1978) or on a portion of a steam distillate of the sample under investigation (Tarladgis et al., 1960, 1964). The latter, the distillation procedure developed by Tarladgis et al. (1960) is the method frequently used. The extraction in TCA is also another procedure used by many researches.

The TBA number which expresses warmed over flavour or lipid oxidation, defined as mg of malonaldehyde per kg of sample (Sinnhuber and Yu, 1958), is calculated by multiplying the absorbance of the TBA-MA complex at 532 nm by a constant. This constant is in turn obtained by the use of standard precursors of malonaldehyde such as 1,1,3,3-tetramethoxypropane (TMA) or 1,1,3,3-tetraethoxypropane (TEP).

Several modified procedures have been reported to improve the standard method of Tarladgis et al. (1960). These include the coupling of the procedure with an HPLC method in place of colourimetry or extraction of the TBA-MA into an organic solvent to avoid possible interference from other coloured impurities (Kakuda et al., 1981).

The present paper reports on the advantages and disadvantages of each of the two major methods employed in the determination of the TBA numbers of meat products, mainly the distillation method and the extraction/filtration procedure. Effect of minor components present in the meat or added to the samples prior/during quantification will also be presented.

MATERIALS AND METHODS

Materials

All chemicals used in these studies were reagent-grade, and were used without any further purification. The cooked cured-meat pigment was prepared as described previously (Shahidi and Pegg, 1988).

The meat, loin pork, was deboned and trimmed of most of its surface fat. It was then ground twice using an Oster meat grinder. Additives, along with 20% of distilled water were added to meat samples prior to cooking. It all cases the mixtures were thoroughly mixed to obtain homogeneous samples. The addition level of different additives are given in corresponding tables where they appear.

Homogenized meat samples were cooked in a thermostated water bath for 40 minutes to reach an internal temperature of 75°C. After cooling to room temperature, they were homogenized and stored in plastic bags at 4°C until use.

The TBA number of meat samples were determined by a distillation and/or an extraction procedure. The distillation procedure was essentially that of Tarladgis et al. (1960) with minor modifications as described elsewhere (Shahidi et al. 1986). A 10 g meat sample was placed into a 500 ml round-bottom flask containing 97.5 ml distilled water and 2.5 ml 4N HCl, along with few drops of Dow Antifoam A and several glass beads. For cured meats sulfanilamide was added to the mixture, in some cases, as described elsewhere (Shahidi, 1989). The mixture was then heated for approximately 20 min to collect 50 ml of distillate. A 5 ml aliquot of the distillate was pipetted into a 50 ml vial containing 5 ml 0.02 M aqueous solution of 2-thiobarbituric acid reagent. The vial was then capped and

heated in a boiling water bath for about 35 min to obtain the TBA-MA chromogen. After cooling the vial to room temperature, the absorbance of the complex was read at 532 nm on a DU-8 spectrophotometer. Using 1,1,3,3-tetramethoxypropane standard, a conversion factor of 8.1 was obtained for converting the TBA-MA absorbance readings to TBA numbers.

For the extraction procedure, a 2 g meat sample was placed in a vial along with 5 ml 10% (W/V) TCA solution. The mixture was then vortexed for 2 min and then 5 ml 0.02 M aqueous solution of 2-thiobarbituric acid was added to it and vortexed again for 2 min. The mixture was then centrifuged and the resultant supernatant was pipetted off for colour development and measurement of the TBA-MA absorbance reading. A factor of 3.6 was employed for converting the absorbance units to TBA numbers.

In some experiments an antioxidant or a chelator was added to meat samples prior to distillation/extraction. The level of addition of these additives are given in Tables where they appear.

RESULTS AND DISCUSSION

The absorption spectra of the TBA-malonaldehyde is depicted in Figure 2. The absorption maxima of the TBA-MA from a sample of meat distillate and from malonaldehyde prepared directly from its precursor 1,1,3,3-tetramethoxypropane was 532 nm. Both distillation and extraction methods showed similar absorption patterns.

Table 1 summarizes the results for the TBA numbers of meat systems prepared by addition of certain additives such as butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), sodium tripolyphosphate (STPP), disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), sodium nitrite ($NaNO_2$) and the performed cooked cured-meat pigment (CCMP), with or without the addition of TBHQ. A close scrutiny of the results indicated the following trends: a) the TBA numbers of meat systems determined by the distillation method generally gave results which were numerically higher than those obtained by the extraction procedure; b) storage of the meat samples at 4°C for nearly 3 weeks resulted in a substantial increase in the TBA numbers; c) presence of sodium nitrite in the systems gave results which varied in different direction when sulfanilamide was added to the mixture prior to distillation; d) addition of CCMP to meats gave substantially higher TBA values when extraction procedure was employed; however, this trend was reversed when meats where were stored for nearly 3 weeks; and e) addition of TBHQ to the meat system containing CCMP gave results which were always higher when the extraction procedure was employed.

The above results could be interpreted in the following manner. Breakdown of labile hydroperoxides during the distillation process is responsible for larger TBA values when this method was employed. As expected, storage of meat for an extended period of time results in the production of a larger amount of malonaldehyde in most of the systems. For nitrite-treated samples nitrosation of malonaldehyde by the residual nitrite present in the systems was responsible for their underestimation. However, addition of sulfanilamide (Zipser and Watts, 1962) resulted in a significant increase in the TBA values when meats were cured with 150 ppm of sodium nitrite. Sulfanilamide intercepted nitrite molecules, resulting in better quantification of the malonaldehyde in the meat sample. For

meat systems devoide of nitrite or treated with 25 ppm nitrite, addition of sulfanilamide resulted in a decrease in the TBA numbers. Formation of adducts between sulfanilamide and the malonaldehyde were found to be responsible as it has already been documented elsewhere (Shahidi et al. 1987).

Presence of CCMP in the meat systems in freshly cooked meat systems, gave rise to larger TBA values by the extraction procedure. This was due to the dissolution of this pigment in the TCA extracts and thus, their interference with the determinations. However, as the samples were stored and rancidity developed, the effect of the interference from CCMP was overwhelmed by the production of large amounts of malonaldehyde in the systems and also due to further breakdown of labile hydroperoxides during the distillation process. Presence of an antioxidant such as TBHQ in the system prevented the development of rancidity and thus the trend remained unchanged.

A further scrutiny of the results show that for samples treated with strong antioxidants such as BHA and TBHQ, or powerful chelators such as EDTA, somewhat larger TBA numbers were obtained when extraction procedure was employed. This is due to the dispersion of some lipid particles in the aqueous extracts, thus causing turbidity. Turbidity of the extraction solutions result in artificially high TBA values.

In another set of experiments the effect of addition of antioxidants/ chelators during the distillation or extraction process on the TBA values of the samples was monitored. Results summarized in Table 2 indicate that the addition of antioxidants had a slight effect in lowering the TBA values by the distillation procedure. However, little effect was observed when the extraction process was employed. Presence of Na_2EDTA, alone or in combination with an antioxidant, resulted in a slight increase in the TBA numbers. This may be due to the fact that iron in the systems prior to its release would be kept by EDTA in its more powerful pro-oxidant state of Fe(II) rather than being converted to its less potent pro-oxidant state of Fe(III). (The pro-oxidant activity of ferrous and ferric ions, as quantified by their effect on the TBA number of meats, have been given in Table 2). Furthermore, EDTA-Fe(II) may cause the decomposition of hydroperoxides, thus giving rise to artifically high TBA values.

ACKNOWLEDGEMENTS

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Kakuda, Y., Stanley, D.W. and van de Voort, F. 1981. Determination of TBA number by high performance liquid chromatography. J. Am. Oil Chem. Soc. 7:773.

Kohn, H.I. and Liversedge, M. 1944. On a new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine, epenephrine and menadione. J. Pharmacol. 82, 292.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50:387.

Nair, V. and Turner, G.A. 1984. The 2-thiobarbituric acid test for lipid peroxidation: Structure of the adduct with malonaldehyde. Lipids 19:804.

Placer, Z., Cushman, L.L. and Johnson, B.C. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16:359.

Shahidi, F. 1989. Validity of the 2-thiobarbituric acid (TBA) test for the evaluation of oxidative rancidity in cured meat products. In proceedings, 35th International Congress of Meat Science and Technology. Copenhagan, Denmark. August 20-25. Vol. II. p. 563.

Shahidi, F. and Pegg, R.B. 1988. Synthesis of cooked cured-meat pigment, dinitrosyl ferrohemochrome, and its colour characteristics. In proceedings, 34th International Congress of Meat Science and Technology. Brisbane, Australia. August 29 - September 2, p. 357.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987. Control of lipid oxidation in cooked meats with antioxidants and dinitrosyl ferrohemochrome. J. Food Sci. 52:564.

Sin, G.M. and Draper, H.H. 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43:1147.

Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol. 12:9.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R., Jr. 1964. Chemistry of the 2-thiobarbituric acid for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid heat treatment. J. Sci. Food Agric. 15:602.

Tarladgis, B.G., Watts, B.M., Younathan, H.T. and Dugan, L.R., Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44.

Uchiyama, M. and Mihara, M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86:271.

Wills, E.D. 1965. Mechanisms of lipid peroxide formation in tissues. Role of metals and haematein proteins in the catalysis of the oxidation of unsaturated fatty acids. Biochem. Biophys. Acta 98:238.

Zipser, M.W. and Watts, B.M. 1962. A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. Food Technol. 16:102.

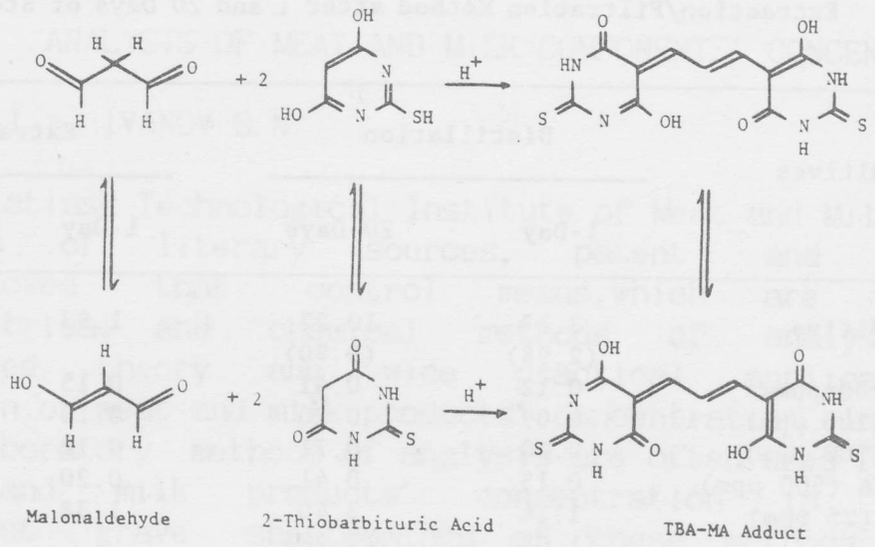

Figure 1. Adduct formation between malonaldehyde and the 2-thiobarbituric acid reagent.

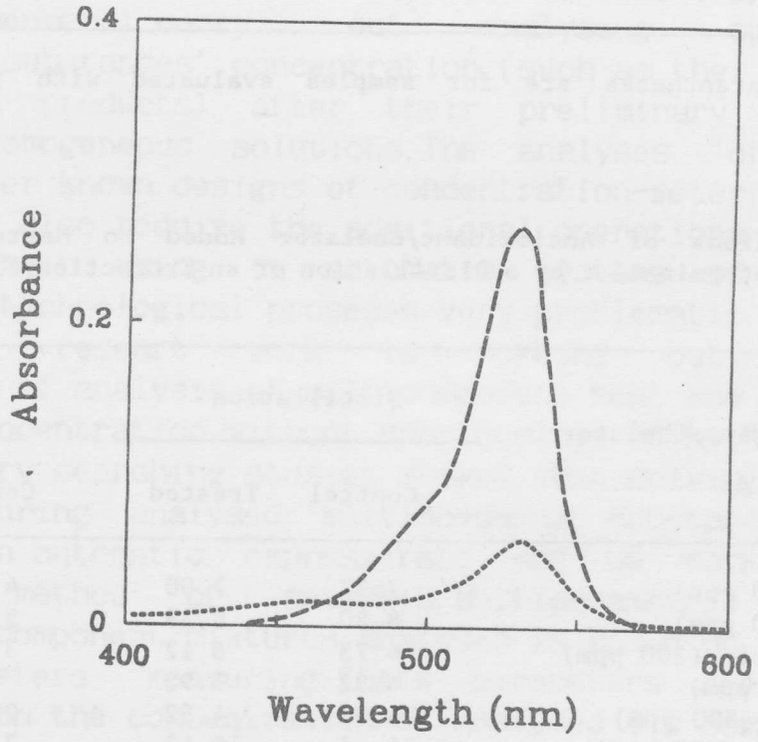

Figure 2. Spectra of TBA-malonaldehyde complex for: ------, meat distillate; and — — —, standard TMP solution.

Table 1. TBA Numbers of Meat Systems Determined by a Distillation or Extraction/Filtration Method after 1 and 20 Days of Storage at 4°C.[a]

Meat Additives	Distillation		Extraction	
	1-Day	20-Days	1-Day	20-Days
No Additive	3.17 (2.98)	10.22 (9.80)	1.83	5.33
BHA (200 ppm)	0.12	0.41	0.15	0.37
TBHQ (200 ppm)	0.09	0.20	0.18	0.25
STPP (500 ppm)	0.10	0.71	0.12	0.48
Na_2EDTA (500 ppm)	0.15	0.41	0.20	0.41
$NaNO_2$ (25 ppm)	1.14 (1.08)	3.40 (3.28)	1.38	2.93
$NaNO_2$ (150 ppm)	0.01 (0.29)	0.10 (0.36)	0.28	0.26
CCMP (12 ppm)	0.36	8.32	1.09	4.09
CCMP (12 ppm) + TBHQ (200 ppm)	0.16	0.17	0.33	0.34

[a]Values in parantheses are for samples evaluated with the addition of sulfanilanide.

Table 2. Effect of Antioxidant/Chelator Added to Meats Prior to TBA Determination by a Distillation or an Extraction/Filtration Method.

No.	Antioxidant/Chelator	Distillation		Extraction	
		Control	Treated	Control	Treated
1	BHA (500 ppm)	7.37	7.00	4.92	4.96
2	BHT (500 ppm)	8.80	8.39	5.48	5.42
3	Ethoxyquin (200 ppm)	6.73	6.12	3.28	3.34
4	PG (500 ppm)	4.32	3.92	-	-
5	Na_2EDTA (500 ppm)	4.32	4.52	2.92	3.33
6	Tenox II (200 ppm)	11.46	10.43	7.52	7.43
7	Tenox A (200 ppm)	8.80	7.71	-	-
8	1 + 5	7.37	7.40	-	-
9	Fe(II) (10 ppm)	3.20	4.93	-	-
10	Fe(III) (10 ppm)	3.20	4.70	-	-