Food Chemistry **28** (1988) 187–196

Some Further Observations on the TBA Test as an Index of Lipid Oxidation in Meats*

Rhonda L. Crackel, J. I. Gray,‡ A. M. Pearson, A. M. Booren & D. J. Buckley§

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA

(Received 20 May 1987; accepted 18 August 1987)

ABSTRACT

A modified TBA procedure utilizing an aqueous TBA solution to replace the acetic acid TBA reagent was tested with several meat samples. The modification prevented the formation of an interfering absorption peak at approximately 450 nm. The conversion factor for this method was determined to be 6·2. The method was further modified to allow the addition of TBHQ to raw and cooked samples prior to blending and heat distillation. The addition of 0·01% TBHQ (fat basis) significantly ($p < 0·05$) reduced TBA numbers of fish and chicken breast and raw chicken thigh meat, but did not significantly influence the TBA numbers for beef or cooked chicken thigh meat.

INTRODUCTION

One of the most commonly used methods for monitoring lipid oxidation in meat products is the 2-thiobarbituric acid (TBA) test (Gray, 1978; Melton, 1983). The steam distillation procedure of Tarladgis *et al.* (1960) in which a portion of the distillate is directly reacted with an acidic TBA reagent is

* Michigan Agricultural Experiment Station Journal Article No. 12314.

‡ To whom all correspondence should be addressed.

§ Present address: Department of Dairy and Food Science, University College, Cork, Ireland.

frequently used to follow lipid oxidation and development of warmed-over flavor (WOF) in poultry, beef and pork products (Melton, 1983). The applicability of the method has been questioned, however, because of the possibility of interfering side reactions and absorption peaks (Tarladgis *et al.*, 1964; Marcuse & Johansson, 1973) and the artifactual formation of TBA reactive substances (TBARS) during distillation (Rhee, 1978; Siu & Draper, 1978).

Tarladgis *et al.* (1964) demonstrated that use of acidic TBA reagent contributed to the appearance of absorption peaks at 390 and 452 nm. These researchers also found that the acid/heat treatment was not necessary for the condensation reaction of TBA with malonaldehyde, nor for maximum color development and proposed that acid may actually be responsible for the degradation of the TBA reagent. To minimize the interfering reactions, a modified distillation method was recommended in which distillates of samples were reacted with TBA reagent without the use of acid.

The possibility of further lipid oxidation and formation of TBARS during sample distillation has been investigated by several researchers. Rhee (1978) demonstrated that the addition of propyl gallate (PG) and ethylenediamine tetraacetic acid (EDTA) to distillation mixtures substantially reduced TBA numbers of catfish samples but had no significant effect on beef, pork and chicken samples. Pikul *et al.* (1983) evaluated the influence of butylated hydroxytoluene (BHT) on the TBA assay of fat from chicken breast and leg meat. Samples analyzed without added BHT yielded six-fold higher malonaldehyde concentrations than those treated with BHT before extraction and 75 μg BHT/mg of fat during the TBA assay.

The present study was designed to (1) evaluate the applicability of the modified aqueous TBA assay for various muscle samples and (2) to determine the effect of tertiarybutylhydroquinone (TBHQ) on the formation of TBARS during the TBA assay.

MATERIALS AND METHODS

Muscle samples

Chicken breasts and thighs and whitefish fillets were obtained from a local supermarket. Skin and visible external fat were removed and samples were ground twice through a 0·48 cm (3/16-in) plate. Experimental restructured beef steaks formulated to 15% fat were used as the source of beef (Crackel *et al.*, 1988). Samples were cooked in open-ended retort pouches in a boiling water bath to a final temperature of 70°C. Cook-out fat and juices were remixed with samples prior to TBA analysis.

TBA assay

Malonaldehyde calibration curves were established using aqueous solutions of pure tetramethoxypropane (TMP) (Sigma Chemical Co., St. Louis, MO) containing 0 to 2×10^{-8} moles/5 ml. Aliquots of the TMP solutions were reacted with both acidic and aqueous 0·02M TBA reagents, and absorbance was measured at 532 nm. The recovery of malonaldehyde during the TBA assay was determined by distilling aliquots of TMP using the traditional TBA method (Tarladgis *et al.*, 1960), developing the pink pigment and reading absorbance values from the appropriate calibration curve.

To investigate the appearance of any interfering pigments absorbing near 450 nm, distillates from muscle samples were divided into two lots, and identical samples were reacted with both acidic and aqueous TBA reagents. Absorbance was measured at both 532 and 450 nm. Spectral scans from 650 to 350 nm were also performed on distillates of beef developed with both acidic and aqueous TBA reagents using a Bausch and Lomb Spectronic 2000 spectrophotometer.

The usefulness of TBHQ in preventing artifactual formation of TBARS during the TBA assay was also evaluated. Antioxidant solutions were prepared by dissolving Tenox TBHQ (Eastman Chemical Products, Kingsport, TN) in absolute ethanol. Prior to sample homogenization, 1·0 ml of various ethanolic TBHQ solutions was added to yield a final antioxidant concentration of 0·01% based on the fat content of the meat. Literature values of lipid content were used for the chicken and fish samples, whereas the lipid content of the beef samples had been previously determined (Crackel *et al.*, 1988). Samples were then distilled in the usual manner and developed with aqueous TBA reagent.

RESULTS AND DISCUSSION

Aqueous TBA assay

Malonaldehyde calibration curves were linear with r values of 0·99 (Fig. 1). Recovery of malonaldehyde ranged from 68·6% to 77·2% with an average of 73·1%. Using the equations of Tarladgis *et al.* (1960) and the appropriate calibration curves, the TBA multiplicative factors for acidic and aqueous TBA assays were calculated to be 7·1 and 6·2, respectively.

The conversion factor for the acidic TBA method was slightly less than the 7·8 factor determined by Tarladgis *et al.* (1960), presumably due to a higher recovery of malonaldehyde in the present study and slight differences in absorbance values between the standard curves. On the basis of these

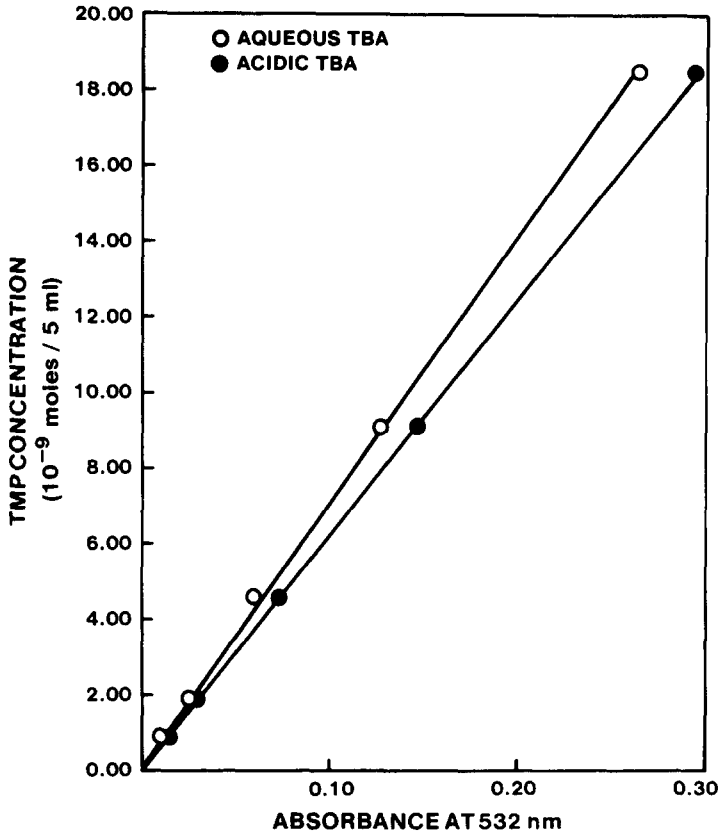

Fig. 1. Calibration curves for malonaldehyde–TBA complexes developed under aqueous and acidic conditions.

results, it is recommended that researchers determine recovery values and prepare standard curves for calculating the appropriate TBA conversion factor.

The appearance of yellow pigment was not observed in any reactions utilizing TMP as the source of malonaldehyde. However, distillates from meat samples consistently produced the yellow pigment when developed with acetic acid–TBA reagent. Analysis of distillates from all muscle samples except cooked chicken thigh showed that absorbance at 450 nm was greater in samples developed with acidic rather than aqueous TBA reagent while absorbance at 532 nm was consistently higher in samples developed with aqueous reagent. These trends were apparent in both raw and cooked samples (Tables 1 and 2).

Figure 2 shows the spectral scans of a distillate from beef developed with both types of TBA reagent. In the sample developed with aqueous reagent, a sharp absorption peak was noted at 532 nm while only a small peak was

TABLE 1
Absorbance Values and TBA Numbers[a] of Distillates from Various Raw Muscle Products Developed with Acidic and Aqueous TBA Reagents

Sample	*TBA reagent*	*Absorbance*		*TBA[b] number*
		450 nm	*532 nm*	
Chicken breast	H_2O	0·107	0·071	0·44
Chicken breast	acid	0·222	0·024	0·17
Chicken thigh	H_2O	0·064	0·061	0·38
Chicken thigh	acid	0·120	0·021	0·15
Beef	H_2O	0·082	0·108	0·67
Beef	acid	0·401	0·052	0·37
Fish	H_2O	0·048	0·112	0·69
Fish	acid	0·055	0·099	0·70

[a] Analyses were performed in duplicate.
[b] Malonaldehyde in mg/1000 g of sample.

present near 450 nm. However, in the sample developed with acidic TBA reagent, a substantial peak appeared at 450 nm and absorbance at 532 nm was suppressed.

Suppression of the 532 nm peak was also evident when TBA numbers from identical samples developed with both types of TBA reagents were compared (Tables 1 and 2). TBA numbers were consistently lower for samples developed with acidic, rather than aqueous, TBA reagent with the

TABLE 2
Absorbance Values and TBA Numbers[a] of Distillates from Various Cooked Muscle Products Developed with Acidic and Aqueous TBA Reagents

Sample	*TBA reagent*	*Absorbance*		*TBA[b] number*
		450 nm	*532 nm*	
Chicken breast	H_2O	0·109	0·138	0·86
Chicken breast	acid	0·227	0·073	0·52
Chicken thigh	H_2O	0·133	0·386	2·39
Chicken thigh	acid	0·051	0·196	1·39
Beef	H_2O	0·075	0·082	0·50
Beef	acid	0·228	0·050	0·36

[a] Analyses were performed in duplicate.
[b] See Table 1.

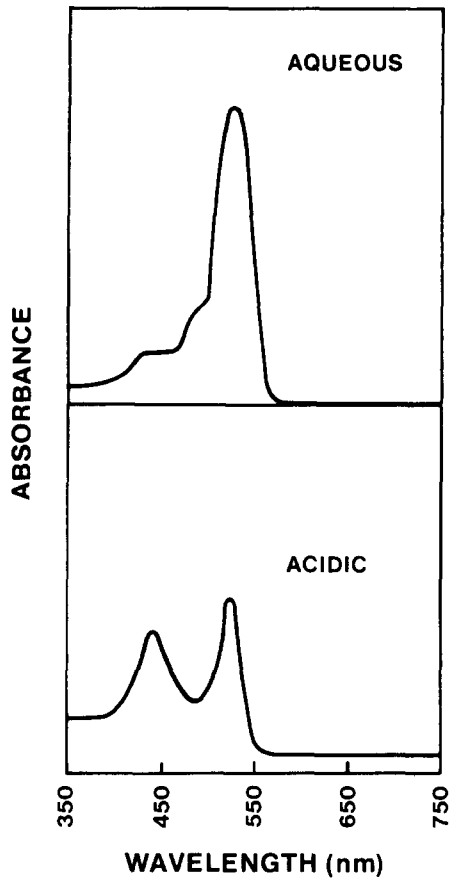

Fig. 2. Spectra of distillates from beef developed with aqueous and acidic TBA reagents.

exception of raw fish samples. In this instance, absorbance values at 450 nm were low and similar in magnitude to the TBA numbers. However, in other sample pairs when absorbance at 450 nm differed substantially, so did TBA numbers, with lower numbers generally being associated with samples possessing the large 450 nm peak. This provides further evidence that the large absorption peak near 450 nm interferes with the TBA reading at 532 nm.

The appearance of the yellow pigment has been noted by several other researchers. Tarladgis & Watts (1960) reported the formation of a pigment absorbing at 450 nm during the TBA assay of oxidized oleic, linoleic and arachidonic acids. Igene *et al.* (1985) reported the formation of a yellow pigment when distillates from cooked chicken were developed with acetic acid TBA reagent. Tarladgis *et al.* (1962) noted increasing absorbance at 450 nm as acetic acid TBA reagent was heated from 5 to 35 min. These

TABLE 3
Absorbance Values and TBA Numbers[a] of Distillates from Fresh Raw Beef Developed with Acetic Acid TBA Reagent

Sample[b]	*Visual appearance*	*Absorbance at 532 nm*	*TBA*[c] *number*
1*a*	Orange	0·010	0·07
b	Pink	0·015	0·11
2*a*	Orange	0·045	0·32
b	Pink	0·060	0·43
3*a*	Orange	0·073	0·52
b	Pink	0·091	0·65

[a] Values represent the mean of three measurements.
[b] Samples from a single source were analyzed in quadruplicate on successive days. Values from two replications were chosen for illustrative purposes.
[c] See Table 1.

researchers postulated that TBA may undergo partial hydrolysis or be oxidized at the C-SH or CH_2 groups and suggested that TBA reagent should not be heated in the presence of acid.

Further analysis of data from the current study indicates that, while the yellow pigment interfered with TBA assays of fresh products, interference was not as substantial as TBA number increased (Tables 3 and 4). Distillates from fresh beef which produced the yellow pigment yielded lower

TABLE 4
Absorbance Values and TBA Numbers[a] of Distillates from Various Muscle Products developed with Acetic Acid TBA Reagent

Sample	*Absorbance*		*TBA*[b] *number*
	450 nm	*532 nm*	
Chicken breast	0·032	0·075	0·53
Chicken breast	0·227	0·073	0·52
Chicken thigh	0·051	0·196	1·39
Chicken thigh	0·301	0·200	1·42
Fish	0·023	0·150	1·06
Fish	0·111	0·149	1·06

[a] Values represent the mean of three measurements.
[b] See Table 1.

absorption values at 532 nm than those possessing only minor absorption peaks at 450 nm (Table 3). In oxidized samples, absorbance at 532 nm changed little despite large differences in absorbance at 450 nm (Table 4). This demonstrates that, at high concentrations of TBARS, absorbance at 532 nm was not suppressed by the 450 nm pigment which, in turn, suggests that results of the acetic acid TBA assay would be less reproducible for fresh products than for more highly oxidized samples. In the light of the findings of previous research and results of the current study, which indicated that the formation of the yellow pigment was prevented by the use of aqueous TBA reagent regardless of the degree of sample oxidation, all subsequent TBA assays utilized aqueous TBA reagent.

Effect of TBHQ on TBARS formation

Table 5 shows the effect of TBHQ on the formation of TBARS in various raw and cooked muscle samples during the TBA assay. The addition of 0·01% TBHQ (fat basis) to distillation mixtures had no significant effect ($p < 0·05$) on the TBA numbers of beef or cooked chicken thigh. However, TBA numbers of fish and chicken breast were significantly lower when

TABLE 5
TBA Numbers[a] of Distillates from Various Muscle Samples Distilled With and Without Added TBHQ

Sample	*TBA Number*	
	− TBHQ	*+ TBHQ*
Beef		
Raw	$0·51 \pm 0·06^b$	$0·56 \pm 0·13^b$
Cooked	$0·51 \pm 0·02^b$	$0·52 \pm 0·10^b$
Chicken breast		
Raw	$0·60 \pm 0·04^b$	$0·45 \pm 0·02^c$
Cooked	$1·14 \pm 0·02^b$	$0·96 \pm 0·10^c$
Chicken thigh		
Raw	$0·42 \pm 0·03^b$	$0·28 \pm 0·02^c$
Cooked	$2·08 \pm 0·30^b$	$1·86 \pm 0·10^b$
Fish		
Raw	$0·86 \pm 0·17^b$	$0·55 \pm 0·07^c$
Cooked	$1·97 \pm 0·20^b$	$1·67 \pm 0·06^c$

[a] Analyses were performed in triplicate.
[b,c] Values in the same row bearing the same superscript are not significantly different from each other at $p < 0·05$.

TBHQ was added prior to blending and distillation of raw and cooked samples. TBHQ also significantly lowered the TBA number of raw chicken thigh meat. The TBA number for cooked chicken thigh was substantially lower when TBHQ was added, but was not significantly lower as previously stated, possibly due to the fairly large standard deviations shown in Table 5.

The data from this study confirm the findings of Rhee (1978) and Pikul *et al.* (1983) that chicken and fish are susceptible to oxidative changes during the TBA distillation procedure. Rhee (1978) reported that addition of PG and EDTA prior to sample distillation significantly reduced the TBA numbers of both raw and cooked catfish but not those of beef, chicken or pork. Addition of PG and EDTA during sample homogenization was beneficial for ground beef samples although the effect was much less substantial than for fish. Chilled blending reduced further lipid oxidation in fish but was less effective than the addition of PG and EDTA. Based on these findings, Rhee (1978) recommended the addition of PG and EDTA during sample homogenization as the most effective one-step method for minimizing further lipid oxidation during the TBA test.

Pikul *et al* (1983) found that 125 μg of BHT/mg of fat was necessary to prevent autoxidation of chicken lipids during the heating step of the TBA assay. This concentration of antioxidant was achieved by the addition of 0·01% BHT to the solvent used for lipid extraction together with the addition of 75 μg BHT/mg fat before the heating step.

The protective effect of antioxidants noted by Rhee (1978) and Pikul *et al.* (1983) is significant for fish and chicken, most likely because of the lability of their component lipids which are known to be richer in phospholipids (Igene *et al.*, 1979) and more highly unsaturated than those of red meats (Pearson *et al.*, 1977).

CONCLUSIONS

The modified aqueous TBA method yielded reproducible results for both fresh and oxidized muscle samples without promoting the formation of an interfering yellow pigment. The conversion factor for this method was calculated to be 6·2. It is recommended that this factor be recalculated by individual researchers to account for variability in TBA assay procedures among various laboratories.

The addition of 0·01% TBHQ (fat basis) to samples prior to homogenization significantly reduced the TBA numbers of fish, chicken breast and thigh meat but not for beef. Based on these findings, antioxidants should be added to samples, especially chicken and fish, prior to blending to further minimize lipid oxidation during the TBA test.

ACKNOWLEDGEMENT

This study was supported in part by a research grant from the California Beef Council and in part by a grant from the National Live Stock and Meat Board.

REFERENCES

Crackel, R. L., Gray, J. L., Booren, A. M. & Pearson, A. M. (1988). Effect of antioxidants on lipid stability in restructured beef steaks. *J. Food Sci.* **53** (in press).

Gray, J. I. (1978). Measurement of lipid oxidation. *J. Am. Oil Chem. Soc.*, **55**, 39.

Igene, J. O., Pearson, A. M., Merkel, R. A. & Coleman, T. H. (1979). Effect of frozen storage time, cooking and holding temperature upon extractable lipids and TBA values of beef and chicken. *J. Anim. Sci.*, **49**, 701.

Igene, J. O., Yamauchi, K., Pearson, A. M., Gray, J. I. & Aust, S. D. (1985). Evaluation of 2-thiobarbituric acid reactive substances (TBARS) in relation to warmed-over flavor (WOF) development in cooked chicken. *J. Agric. Food Chem.*, **33**, 364.

Marcuse, R. & Johansson, L. (1973). Studies on the TBA test for rancidity grading. 1. TBA reactivity of different aldehyde classes. *J. Am. Oil Chem. Soc.*, **50**, 387.

Melton, S. L. (1983). Methodology for following lipid oxidation in muscle foods. *Food Technol.*, **37**(7), 105.

Pearson, A. M., Love, J. D. & Shorland, F. B. (1977). Warmed-over flavor in meat, poultry and fish. *Adv. Food Res.*, **23**, 1.

Pikul, J., Leszcynski, D. E. & Kummerow, F. A. (1983). The elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *J. Agric. Food Chem.*, **31**, 1338.

Rhee, K. S. (1978). Minimization of further lipid peroxidation in the meat distillation 2-thiobarbituric acid test of fish and meat. *J. Food Sci.*, **43**, 1776.

Siu, G. M. & Draper, H. H. (1978). A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.*, **43**, 1147.

Tarladgis, B. G. & Watts, B. M. (1960). Malonaldehyde production during controlled oxidation of pure, unsaturated fatty acids. *J. Am. Oil Chem. Soc.*, **37**, 403.

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

Tarladgis, B. G., Pearson, A. M. & Dugan, L. R., Jr. (1962). The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. *J. Am. Oil Chem. Soc.*, **39**, 34.

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