MODIFIED THIOBARBITURIC ACID-C_{18} (TBA-C_{18}) METHOD FOR MEASURING LIPID PEROXIDATION IN MEAT

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MMARY

e objective of extensive studies in our laboratory has been to modify the thiobarbituric acid (TBA) method so that it becomes ter, more specific and more sensitive in detecting malonaldehyde (MA) as an indicator of rancidity. The experiments were rformed with model systems or meats including beef, pork, lamb, turkey and chicken. The TBA-C_{18} method developed and sented here is a modification of the aqueous acid extraction TBA procedure involving use of a solid phase extraction Sep-Pak™ cartridge. In addition, 80 mM TBA was used, instead of 20 mM TBA, for the red color formation. The modified aqueous acid traction TBA-C_{18} method was more rapid (15-20 min) than other versions of the TBA test (40-60 min). Furthermore, the TBA- method was not only more specific, but also more sensitive for MA measurement in meat. In general, the TBA-C_{18} method s found effective for measuring MA content in meat from all species tested.

TRODUCTION

e thiobarbituric acid method, with its different variations, is the most widely used test for measuring the extent of lipid roxidation in muscle foods (Hoyland and Taylor, 1991). However, all versions of sample preparation for the TBA reaction have en criticized as being nonspecific and insensitive for the detection of low levels of MA in biological tissues (Draper and Hadley, 90; Squires, 1990). The objective of this paper is to summarize our extensive studies aimed at modifying the TBA method so at it becomes faster, more specific and sensitive than the existing variations of the TBA method for measuring MA as a marker lipid peroxidation in meat.

ATERIALS AND METHODS

eaction time of thiobarbituric acid reactive substances (TBARS): Ten grams of raw (18-22% fat) or cooked (12-15% fat) round beef samples, after 24 hr of aerobic storage at 4°C, were homogenized with 40 mL of 5% (w/v) aqueous trichloroacetic id (TCA) (Mallinckrodt, Paris, KY) solution in an Osterizer blender (Sunbeam Corp., Milwaukee, WI) for 1 min. The meat urry was centrifuged (Beckman Instruments Inc., Fullerton, CA) at 10,000x*g* (2-4°C) for 5 min. The supernatant was filtered hrough a Whatman GF/C filter (Whatman, Hillsboro, OR) and its volume was adjusted to 50 mL using the TCA solution. A 2 mL portion of the filtrate was reacted with 2 mL of 20 mM TBA (Sigma Chemicals Co., St. Louis, MO) or with 2 mL of 80 mM TBA for 0 to 40 min in a water bath of 94±1°C. The absorbance of the red pigment resulting from the reaction was measured pectrophotometrically at 525 nm.

hiobarbituric acid (TBA) methods: Raw (17.2-22.6% fat) and cooked (12.6-16.4% fat) ground beef samples were divided into liquots of 110 g each and aerobically stored in plastic cups at 4°C for 0, 2, 4, 6 and 8 days. Lipid peroxidation was measured by our versions of the TBA test including aqueous acid extraction-C_{18} (TBA-C_{18}) (FIGURE 1), direct heating (Uchiyama and Mihara, 1978), distillation, and unmodified aqueous acid extraction methods (Pikul et al., 1989). The TBA numbers, as mg of MA quivalents/kg meat, were calculated as described by Pikul et al. (1989). Prior to use the Sep-Pak™ C_{18} cartridge (Waters, Milford, MA) was washed with 10 mL of absolute methanol (Mallinckrodt) followed by 10 mL of distilled water at a flow rate of pproximately 20 mL/min.

Limit of determination: Limit of determination (LOD) of the TBA methods was obtained by adding graded levels of pure MA, derived from 1,1,3,3 tetraethoxypropane (Sigma Chemicals Co.), to the meat samples and subsequent analysis by the TBA methods. The LOD is defined as the smallest concentration of the MA added to the meat sample that satisfies the following requirements: a) LOD ≥ detection limit, (b) recovery value ≥ 70%, and (c) coefficient of variation ≤ 20% (Thier and Zeumer, 1987).

Applicability of the TBA-C_{18} method: Beef (7.8% fat), pork (12.8% fat), lamb (8.8% fat), chicken (5.6% fat), and turkey (6.1% fat) leg meat were ground through a 1.27 cm plate (Hobart Corp., Troy, OH). A portion of the meat from each species was cooked

in a water bath of 94±1°C for 20 min to reach an internal temperature of approximately 70°C. The raw and cooked meats were divided into 50 g aliquots and stored aerobically in plastic cups at 4°C. Lipid peroxidation was determined by two TBA methods including aqueous acid extraction (Salih et al., 1987) and aqueous acid extraction-C_{18} (TBA-C_{18}) methods (FIGURE 1) after 2, 4 and 6 days of storage.

Statistical analysis: Factorial experiments were used and all of the experiments were replicated four times. Analysis of variance and linear regression analysis were used for comparing the TBA numbers obtained by the TBA methods tested (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Reaction time of thiobarbituric acid reactive substances (TBARS): The use of 80 mM TBA, instead of 20 mM TBA, reduced the reaction time of TBARS from raw and cooked ground beef extracts to reach maximum absorbance (at 525 nm) from approximately 40 min to 5 min (TABLE 1). The most frequently used level of TBA for MA-TBA complex formation in TBA tests reported has been 20 mM (Tarladgis et al., 1960; Salih et al., 1987; Pikul et al., 1989). Most of these TBA reactions required at least 30 min, under boiling water bath temperature, to reach the maximum absorbance. Since the use of the higher level (80 mM) of TBA did not interfere with the analysis (TABLE 1), it is recommended that this concentration be used in order to increase the speed of the TBA test. This is important, especially when the results of the analysis are needed within a short period of time.

Thiobarbituric acid (TBA) methods: During storage for up to 8 days at 4°C, the direct heating TBA method resulted in significantly ($P<0.01$) higher slopes of TBA numbers in raw (3.7 times) and cooked (7.0 times) samples compared to those of the aqueous acid extraction TBA-C_{18} method (FIGURE 2). Higher slopes indicate larger increases of TBA numbers during product storage. In spite the presence of butylated hydroxytoluene (BHT) as an antioxidant during the analysis, heat treatment at 94±1°C for 40 min of the meat samples may result in degradation of fatty acid hydroperoxides into malonaldehyde precursors and other TBARS (Gutteridge and Quinlan, 1983). The slopes of the TBA numbers obtained by the distillation TBA method were also significantly ($P<0.01$) higher (2.4 to 3.4 times) than those of the aqueous acid extraction TBA-C_{18} method. The distillation TBA method uses heat treatment for a shorter time (15 min) than the direct heating TBA method (40 min) discussed above. The shorter heat treatment significantly ($P<0.05$) decreased the slopes of the TBA numbers obtained by the distillation TBA method. In addition, the unmodified aqueous acid extraction TBA method resulted in significantly ($P<0.01$) higher slopes of TBA numbers (2.2 to 2.8 times) than those of the modified aqueous acid extraction TBA-C_{18} method. Since no heat treatment was applied to the meat sample by either of these methods, the formation of additional malonaldehyde and other TBARS from their precursors under the assay condition was minimal. However, the unmodified aqueous acid extraction TBA procedure, as well as the direct heating and the distillation procedures, do not specifically measure malonaldehyde in meat samples (Draper and Hadley, 1990; Squires, 1990). Other aldehydes have been reported to interfere with the red MA-TBA complex during spectrophotometric measurement (Kosugi et al., 1989). The use of a Sep-Pak C_{18} cartridge in the TBA-C_{18} method was apparently capable of removing this interference problem. Thus, it made the TBA-C_{18} method more specific for MA-TBA complex detection than the other TBA methods tested in this study.

Limit of determination: The results indicated that the direct heating, distillation and aqueous acid extraction TBA methods had similar limits of determination, 2.00 nmol MA equivalents/mL meat extract (TABLE 2), when calculated using the procedure of Thier and Zeumer (1987). This is identical with a TBA number of approximately 0.72 mg MA equivalents/kg meat. The use of Sep-Pak™ C_{18} cartridge in the aqueous acid extraction TBA-C_{18} method improved the limit of determination from 2.00 to 0.10 nmol MA equivalents/mL meat extract. This is identical with a TBA number of approximately 0.036 mg MA equivalents/kg meat. This means that the TBA-C_{18} method had a limit of determination approximately 20 times lower than the other TBA methods tested. Results of the sample blank analyses were significantly ($P<0.01$) lower than its corresponding limits of determination. This means that whenever the results of the TBA analyses were not significantly ($P>0.01$) different from its blank, it should not be considered as a real value.

Applicability of TBA-C_{18} method: The rates of increase (slopes) and intercepts of the TBA numbers obtained by the aqueous

id extraction TBA method were significantly ($P<0.05$) higher than those determined by the modified aqueous acid extraction BA-C_{18} method in raw beef, lamb, turkey, chicken and pork (FIGURE 3). This means that the aqueous acid extraction TBA ethod resulted in significantly ($P<0.05$) higher TBA numbers than the TBA-C_{18} method. Similar results were also found in oked meats, except that the slopes of the TBA numbers obtained by these two methods were not significantly ($P>0.05$) different cooked beef, turkey and pork. All of the cooked meats had significantly ($P<0.05$) higher slopes of TBA numbers than their raw unterparts as determined by the TBA-C_{18} method. These results suggested that the TBA-C_{18} method can be used for measuring e extent of lipid peroxidation in beef, lamb, pork, chicken and turkey.

ONCLUSION

verall, the newly developed aqueous acid extraction TBA-C_{18} method had better specificity, lower limit of determination (20 es lower), and required shorter time (15-20 min) to do the analysis than other TBA methods tested. The TBA-C_{18} method was nd successful for measuring MA content in meat from all species tested.

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ABLE 1. Reaction time of thiobarbituric acid-reactive substances (TBARS) from raw and cooked ground beef extracts with 20 or 80 mM thiobarbituric acid (TBA) at 94±1°C to produce maximum absorbance of red colored TBARS complexes

		Reaction Time at 94 + 1°C (min)						
		0	3	5	10	20	30	40
TBA (mM)	Ground Beef	Absorbance at 525 nm						
20	RAW	0	0.05 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.15 ± 0.01*
80	RAW	0	0.09 ± 0.01	0.15 ± 0.01*	0.14 ± 0.01*	0.15 ± 0.01*	0.15 ± 0.01*	0.14 ± 0.02*
20	COOKED	0	0.20 ± 0.01	0.30 ± 0.02	0.33 ± 0.01	0.34 ± 0.01	0.35 ± 0.01*	0.36 ± 0.02*
80	COOKED	0	0.30 ± 0.01	0.36 ± 0.02*	0.37 ± 0.02*	0.36 ± 0.02*	0.36 ± 0.01*	0.36 ± 0.02*

(*) Indicates the maximum absorbance (mean ± standard deviation).

TABLE 2. Limit of determination (LOD) of malonaldehyde by four TBA methods and its sample blank measurement in ground beef (nmol MA equivalents/mL meat extract)

TBA Method	Raw Ground Beef		Cooked Ground Beef	
	Blank*	LOD**	Blank*	LOD**
Direct heating	1.03 ± 0.12	2.00	1.32 ± 0.12	2.00
Distillation	0.71 ± 0.14	2.00	0.90 ± 0.13	2.00
Aqueous acid extraction	0.61 ± 0.12	2.00	0.66 ± 0.14	2.00
Aqueous acid extraction-C_{18}	0.04 ± 0.01	0.10	0.05 ± 0.01	0.10

(*) Mean ± standard deviation. (**) Calculated according to the procedure of Thier and Zeumer (1987).

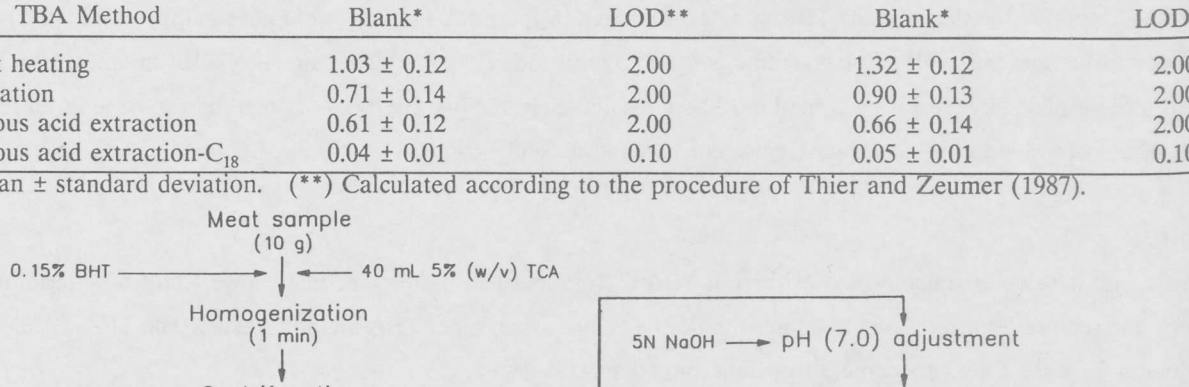

FIGURE 1. Diagram indicating the steps involved in the aqueous acid extraction thiobarbituric acid-C_{18} (TBA-C_{18}) method. BHT: butylated hydroxytoluene, TCA: trichloroacetic acid, TBARS: thiobarbituric acid-reactive substances, MA: malonaldehyde.

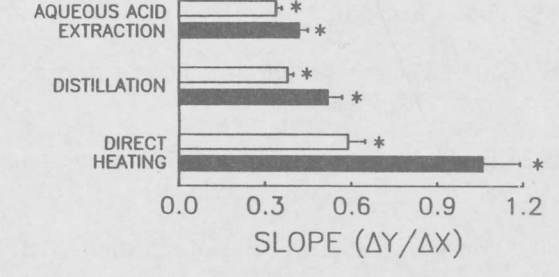

FIGURE 2. Slopes of linear regression of thiobarbituric acid (TBA) numbers of raw and cooked ground beef during aerobic storage at 4°C for 8 days determined by four TBA methods. (*) Slope is significantly (P<0.05) different from the slope of the aqueous acid extraction-C_{18} method within each group of meat (raw or cooked). Y = TBA numbers, X = days of aerobic storage (0 to 8) at 4°C.

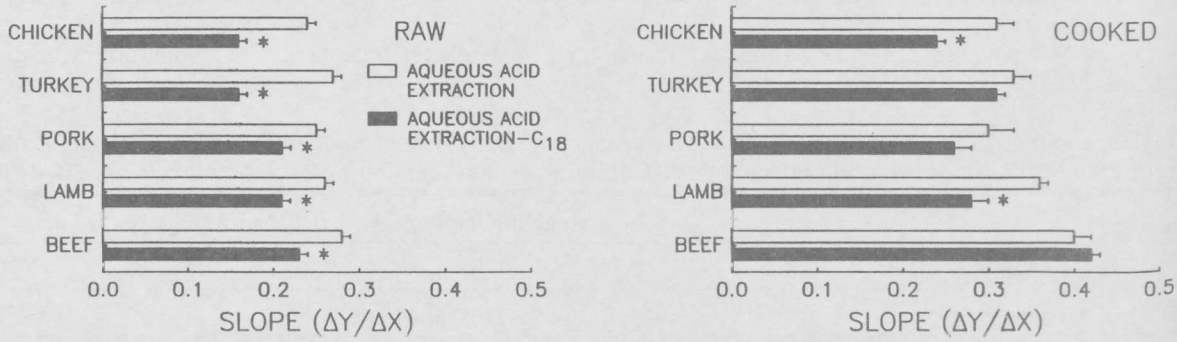

FIGURE 3. Application of unmodified aqueous acid extraction TBA and aqueous acid extraction-C_{18} (TBA-C_{18}) methods for measuring lipid peroxidation of ground beef, pork, lamb, chicken and turkey during aerobic storage at 4°C for 6 days. (*) Indicates significant difference (P<0.05) between the slopes of the two TBA methods within each type of meat. Y = TBA numbers, X = days of aerobic storage (0 to 6) at 4°C.