Measurement of 2-Thiobarbituric Acid Values in Dark Chicken Meat through Derivative Spectrophotometry: Influence of Various Parameters

Anna Grau,[†] Francesc Guardiola,*[,†] Josep Boatella,[†] Ana Barroeta,[‡] and Rafael Codony[†]

Nutrition and Food Science Department-CeRTA, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain, and U. D. Nutrició i Alimentació Animal, Facultat de Veterinària, UAB, 08193 Bellaterra, Spain

Several variables (kind of filter paper, amount of sample, antioxidant addition, stability of the spectrophotometric measurement, and handling and storage of samples) were found to influence 2-thiobarbituric acid (TBA) values in dark chicken meat when an acid aqueous extraction method with derivative spectrophotometry was used. Filter papers with larger pore diameter or increasing sample weight led to lower TBA values. After incubation of reaction mixtures at 70 °C and ice-cooling, tempering for 45 min at room temperature was necessary to stabilize the spectrophotometric measurement. Furthermore, addition of butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid disodium salt (EDTA) in the early steps of the method prevented artifactual formation of TBA reactive substances during analysis. Vacuum packaging and storage of samples at −20 °C were useful to avoid sample oxidation. The method finally proposed has a coefficient of variation of 3.81 or 4.13% for raw or cooked samples, respectively.

Keywords: *Aqueous acid extraction method; dark chicken meat; derivative spectrophotometry; malondialdehyde; thiobarbituric acid*

INTRODUCTION

Many methods have been proposed for assessing malondialdehyde (MDA) content in meat as a marker of lipid oxidation. The advantages and disadvantages of these methods were extensively reviewed by Fernández et al. (1997) and Raharjo and Sofos (1993). As they tend to be more simple and rapid, methods based on quantification of the red complex formed between 2-thiobarbituric acid (TBA) and MDA are generally preferred to those that quantify MDA selectively by HPLC or GC. Among these former procedures, the distillation method (reaction of a distillate of the sample with TBA) has been the most widely used in meat samples (Fernández et al., 1997; Raharjo et al., 1992; Raharjo and Sofos, 1993). However, despite the fact that in this method interference due to nonlipid materials is almost eliminated (Raharjo and Sofos, 1993; Raharjo et al., 1993), heating of the sample during distillation promotes further oxidation, leading to the formation of additional MDA and other TBA reactive substances (Raharjo and Sofos, 1993; Raharjo et al., 1993; Witte et al., 1970). Because of this, the reaction of TBA with an aqueous acid extract of the sample seems to be a more appropriate method for meat samples (Raharjo and Sofos, 1993; Witte et al., 1970) because it uses milder conditions than other TBA tests (Raharjo et al., 1993). As well, this method is faster and easier to perform (Pikul et al., 1989; Salih et al., 1987), and its results correlate closely with results from distillation methods and with sensory evaluation (Pikul et al., 1989; Raharjo et al., 1993; Salih et al., 1987; Witte et al., 1970). However, this method has been criticized for its lack of specificity (Cecconi et al., 1991; Raharjo et al., 1992, 1993). One of the main problems using this method to calculate MDA in meat samples is that compounds other than MDA that may be present in the sample (e.g., carbohydrates or their degradation products, glyceraldehyde, furfural, alkanals, alkenals, alkadienals, and other aldehydes and ketones) can also react with TBA, resulting in a yellow chromophore with an absorption band between 450 and 460 nm (Botsuglou et al., 1994; Guillén and Guzmán, 1988; Kosugui et al., 1987; Marcuse and Johansson, 1973; Sinnhuber and Yu, 1977). This peak may overlap the MDA−TBA peak, which absorbs between 530 and 537 nm. To overcome this interference, Botsoglou et al. (1994) proposed a method using derivative spectrophotometry. Recording the third-derivative absorption spectrum of the reaction mixture avoids interference from other complexes absorbing at surrounding wavelengths and also minimizes interference due to turbidity that may be present in samples with a high fat content. In addition, the limit of determination with this method was comparable to the limit with HPLC methods and much lower than the limits of nonderivative spectrophotometric methods.

A common drawback of this and other methods for measuring TBA values (e.g., distillation methods) is that interference from other aldehydes (alkanals, alkenals, and alkadienals) that also react with TBA to give a red complex absorbing at 530−537 nm is not eliminated by derivative spectrophotometry. However, these compounds do not occur in sufficiently high concentrations in tissue extracts under normal conditions (Slater, 1984), and the color yielded by them is much lower than the color from MDA (Esterbauer et al., 1991; Kosugui et al., 1987, 1988). In addition, some of the most

* To whom correspondence should be addressed. Tel.: 34-934024511. Fax: 34-934021896 or 34-934035931. E-mail: fibarz@farmacia.far.ub.es.

[†] University of Barcelona.

[‡] UAB.

10.1021/jf990518q CCC: $19.00
Published on Web 03/07/2000

abundant of these compounds, alka-2,4-dienals (Marcuse and Johansson, 1973), are completely destroyed by bacteria of the genus *Pseudomonas*, which are abundant in muscle foods (Smith and Alford, 1968). Furthermore, alkenals only give the red complex when the aldehyde/TBA ratio is 1:1 (Kosugui et al., 1987), which is unusual when TBA determinations are performed, since TBA is added in excess.

The objective of this paper is to highlight some analytical questions that may seriously affect the results of analysis of chicken leg meat with the method of Botsoglou et al. (1994).

MATERIALS AND METHODS

Reagents and Standards. Trichloroacetic acid (TCA) and disodium tetraborate 10-hydrate (both ACS grade) were from Panreac (Barcelona, Spain). Butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid disodium salt (EDTA), 1,1,3,3-tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), and 5% (w/v) aqueous 2,4,6-trinitrobenzene-1-sulfonic acid (TNBSA) were supplied by Sigma (St. Louis, MO). Distilled deionized water was used throughout the procedure.

Sample Preparation. Chicken legs were purchased from local supermarkets. These legs were hand-deboned, ground (muscle plus skin), and vacuum-packed in 13.5 × 16 cm polyamide/polyethylene bags containing approximately 15 g of meat. They were kept at −20 °C (1 month maximum) and thawed for 1 h at room temperature before analysis. Cooked legs were obtained from various experimental designs directed to study the influence of the diet (fat source and α-tocopheryl acetate supplementation) on meat quality. These legs were hand-deboned, vacuum-packed in 20 × 40 cm polyamide/polyethylene bags, and cooked at 80 °C for 35 min in a pressure cooker or in a hot air oven to an internal sample temperature of 80 °C. Afterward, samples were cooled and ground in a refrigerated room (14 °C), vacuum-repacked in 13.5 × 16 cm polyamide/polyethylene bags (∼15 g of meat/bag), and stored for different periods (7 days and 3.5 and 7 months) at −20 °C until analysis.

Study of the Influence of Various Factors on TBA Value Determination. Some modifications to the method proposed by Botsoglou et al. (1994) were sequentially assayed in order to adapt it to dark chicken meat samples. In all tests, reaction mixtures were cooled (after incubation at 70 °C) in an ice bath instead of under tap water, as Botsoglou et al. (1994) did.

Influence of Kind of Filter Paper. The influence of different filter papers (Whatman Nos. 1, 4, and 42) used to clean the acid aqueous sample extract after centrifugation was studied. First, 12 aliquots of a raw meat sample were analyzed using alternately filter paper Nos. 4 and 42 ($n = 6$). Next, 12 aliquots were analyzed in the same way, using filter papers Nos. 4 and 1 ($n = 6$).

Influence of the Amount of Sample. Samples of raw meat (1.5, 2.0, 2.5 and 3.0 g) were alternately weighed and analyzed using No. 1 Whatman filter papers ($n = 3$). Another experiment was performed alternately weighing 1.5 or 2.0 g of another raw meat sample ($n = 8$).

In addition, fat from 30 g of a raw chicken meat sample was extracted three times with 300, 200, and 100 mL of chloroform/methanol (2:1) using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). After addition of 125 mL of water, the mixture was centrifuged (20 min at 400*g*), and the chloroformic phase was filtered through a Whatman No. 42 filter paper to eliminate remainders of proteins. Anhydrous sodium sulfate was added, the extract was filtered (Whatman No. 1), and solvent was evaporated using a vacuum rotatory evaporator at 40 °C. After 24 h in a desiccator, extracted fat was left for 60 min at 85 °C in order to promote MDA formation from lipid hydroperoxides. Then, fat was redissolved in 30 mL of hexane. Aliquots of 1, 2, and 3 mL ($n = 4$) were pipetted into 50 mL centrifuge tubes, and 4, 3, and 2 mL of hexane with BHT (40 mg) were respectively added. From this point, the TBA method was performed as described next.

Influence of BHT Addition during Analysis. Two runs of eight aliquots of a vacuum-packed, ground, raw sample (1.5 g, No. 1 Whatman filter paper) were carried out. In both runs, all the aliquots were first weighed from a bag containing approximately 15 g of sample. Next, in the first run, TCA and BHT solutions were sequentially added to each aliquot just before its homogenization while, in the second run, BHT was added immediately after all aliquots were weighed, and the TCA was added just before homogenization of each aliquot. Consequently, in the first run, the time spent by each aliquot without BHT greatly depended on its processing order number. In the second run, however, as adding BHT to all the aliquots meant only 2 min (approximately), the time elapsed without the antioxidant was similar for all of them. It has to be born in mind that once the bag containing the sample is opened, the vacuum is lost, and it has very little relevance, in terms of sample oxidability, whether the meat stays in the bag or is transferred to the tube (weighed).

Stabilization of the Spectrophotometric Measurement (Peak Height at 521.5 nm). Reaction mixtures from 3 aliquots of a raw meat sample (1.5 g of raw meat, No. 1 Whatman filter paper) were cooled in an ice bath and submitted to third-derivative spectrophotometry every 15 or 30 min.

Influence of Handling and Storage of Samples. To study the influence of thawing and refreezing of samples on TBA values, a vacuum-packed, ground, cooked sample was thawed for 2 min in a water bath (22 °C), four aliquots were weighed (1.5 g), and the remaining meat was placed in a plastic zip-bag and refrozen. The TBA test was performed on these aliquots using No. 1 Whatman filter papers. In the following 2 days, the same sample was rethawed in the same way, another four aliquots were weighed (1.5 g), and the remaining meat was frozen again in the same way. Thus, the TBA test was carried out on the same sample for three consecutive days. In addition, to study storage condition effects on TBA results, another three cooked samples were analyzed before and after a period of 24 h at 4 °C in a plastic zip-bag. Six vacuum-packaged ground and cooked samples (from another experimental design) were also analyzed for TBA values after different periods of storage at −20 °C (7 days and 3.5 and 7 months).

Final Method Proposed. A prethawed sample (raw or cooked) of 1.5 g was weighed in a 50 mL centrifuge tube, and 1 mL of 0.3% aqueous EDTA was added immediately. After gentle agitation, 5 mL of 0.8% BHT in hexane was also added, and the tube was gently shaked again. Then, just before homogenization, 8 mL of 5% aqueous TCA was added to the tube, and homogenization was carried out for 30 s at 19 000 rpm using a Polytron PT 3000. After centrifugation (5 min at 1400*g*), the top hexane layer was discarded and the bottom layer was filtered through a No. 1 Whatman filter paper into a 10 mL (raw or cooked samples) or 50 mL (very oxidized cooked samples) volumetric flask. TCA (5% aqueous) was used to make up the volume. A 3 mL aliquot was pipetted into a screw-capped tube (13 × 100 mm), and 2 mL of 0.8% aqueous TBA was added (pH of reaction mixture = 0.9). The reaction mixture was incubated for exactly 30 min at 70 °C in a water bath under gentle agitation and then cooled in an ice bath for 5 min. After the tube was tempered for 45 min at room temperature, the reaction mixture was submitted to third-derivative spectrophotometry against a blank reaction mixture, containing 3 mL of 5% aqueous TCA and 2 mL of 0.8% aqueous TBA. A Shimadzu UV-160A double-beam spectrophotometer was used to measure the peak height at 521.5 nm in the third-derivative spectrum (Figure 1). Spectrophotometric conditions were as follows: spectrum range, 400−650 nm; scan speed, 480 nm/min; and derivative difference setting ($\Delta\lambda$), 21 nm. If the peak height value exceeded 1.0 (linearity of the method was only checked up to this value), the reaction mixture was diluted with water. The TBA value (expressed as μg of MDA/kg of sample) was calculated on the basis of our calibration curve ($Y = -4.11 \times 10^{-3} + 6.68 \times 10^{-3}X$, where Y is the peak height at 521.5 nm expressed in arbitrary units, as printed on the instrument chart, and X the concentration in ng of

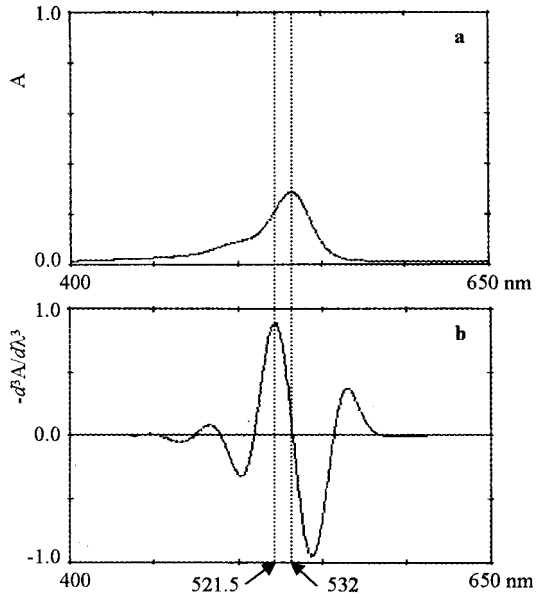

Figure 1. Zero-order (a) and negative third-derivative absorption spectrum (b) of a reaction mixture from a cooked dark chicken meat sample (the spectrophotometer used gave the negative derivative spectra).

MDA/mL of reaction mixture), taking into account the sample weight and the dilutions made during the analytical procedure. The calibration curve was constructed as described by Botsoglou et al. (1994), with TEP used as MDA precursor. The method was performed under attenuated light conditions.

Precision. Precision of the modified method was assessed on 16 aliquots of both a raw and a cooked chicken meat sample. To compare our coefficient of variation to the value reported by Botsoglou et al. (1994), which had been calculated with raw cow muscle samples ($n = 5$) with a TBA value similar to ours, we also calculated the coefficient of variation with the first five and the last five TBA values taken from the 16 above-mentioned raw sample aliquots, and then we took the average among them.

Determination of Primary Amino Groups in Filtered Sample Extracts. Eight extracts from 1.5 g of raw dark chicken meat were filtered through Whatman No. 1 or No. 42 filter papers ($n = 4$) and made up to 10 mL with TCA. Following the method proposed by Cayot and Tainturier (1997), slightly modified, TCA solutions were neutralized with 15% aqueous NaOH, buffered with 0.1 M aqueous borate (pH 9.18 ± 0.04), and diluted to 50 mL with water. Next, 2 mL of the diluted solution was mixed with 2 mL of 0.01 M aqueous TNBSA, and after 15 min, absorbance at the absorption maximum (432 nm) was measured.

Statistics. Normality of variables studied was checked using the Saphiro-Wilks test. To determine whether any significant effect was produced by the kind of filter paper or the amount of sample used on TBA results, a Student−Fisher's *t* test was performed. ANOVA was performed to check the influence of fat amount on TBA values. Coefficient of correlation of Spearman was used to study the influence of the time that samples were left without BHT addition on TBA values. In this case, a number from 1 to 8 was assigned to each aliquot depending on its processing order number. In all cases, *P* values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Influence of Kind of Filter Paper. As shown in Table 1, TBA values obtained using No. 42 Whatman filter papers were higher than those obtained using filter papers with a larger pore diameter (Whatman No. 4 or No. 1). In addition, no statistically significant differences were found between filter papers No. 4 and

Table 1. Influence of the Type of Filter Paper on TBA Values

	filter paper No.	
	4 ($n = 6$)	42 ($n = 6$)
avg wt (g)	2.0489	2.0534
(cv, %)	(3.22)	(1.49)
peak height at 521.5 nm[a]	0.029	0.049
(cv, %)	(3.04)	(7.69)
TBA value (μg MDA/kg)	40.48	63.63[b]
(cv, %)	(3.53)	(6.34)

[a] Peak height is expressed in arbitrary units. [b] Mean values are significantly different ($P < 0.0001$).

No. 1 (data not shown). As proteins can bind MDA (especially through their free amino groups) and thus prevent its reaction with TBA (Aubourg, 1993; Buttkus, 1967; Esterbauer et al., 1991; Schmedes and Hölmer, 1989), our findings may be due to the fact that No. 42 filter papers retain smaller protein particles and so give filtrates with lower protein concentration than filtrates from No. 1 and No. 4 filter papers. This was confirmed when primary amino groups were quantified in the filtrates by the TNBSA colorimetry, and it was observed that filtrates from No. 1 filter papers had a 4-fold higher absorbance at 432 nm than those from No. 42. In addition, Botsoglou et al. (1994) found that heating may cause further MDA binding to proteins, which could occur to a greater extent during the incubation step in filtrates from Nos. 1 and 4 filter papers. However, No. 42 filters paper showed lower precision than No. 1 or No. 4 filter papers (Table 1). This may be related to its much lower filtration speed and wider range of filtration times (20−30 min vs 5−6 min), causing greater variability in the time available for side reactions in the meat extract. Possibly involved side reactions include: (i) little MDA or MDA-like substances formation from hydroperoxides and soluble aldehydes present in the meat extract (Capella, 1989; Esterbauer et al., 1991; Frankel, 1987; Sinnhuber and Yu, 1977), (ii) MDA reaction with water-soluble nitrogenated molecules also present in the extract (Aubourg, 1993; Esterbauer et al., 1991), and (iii) aldol-type self-condensation of MDA to give dimers and trimers (Esterbauer et al., 1991). As a result, No. 42 filters were discarded and No. 1 filters were preferred to No. 4 filters, since they showed a similar filtration speed, gave similar TBA values, and were cheaper.

Influence of Amount of Sample. Greater sample weight led to lower TBA values (Table 2). As this was observed in chicken meat samples (filtered through No. 1 filter papers) but also in chicken fat filtered through No. 42 filters, which are able to retain almost all the protein particles (Table 3), and in different oils (data not shown), this could be mainly due to a more incomplete extraction of MDA when higher amounts of sample are used.

Influence of BHT Addition during Analysis. The time elapsed from the moment that the bag containing the sample is opened (vacuum is lost) to the BHT addition has great influence on sample oxidation. When BHT and TCA solutions were both added just before homogenization (first run), the last aliquots of the run gave higher TBA values. In fact, there was a statistically significant correlation between the processing order number of the aliquot (which is related to the time that the aliquot is left without BHT since the moment that the bag is opened) and its TBA value ($r_s = 0.8095$; $P = 0.0322$). However, when BHT was added to all the

Table 2. Influence of Sample Weight on TBA Values

	wt of sample[a] (g)				wt of sample[b] (g)	
	1.5 ($n = 3$)	2.0 ($n = 3$)	2.5 ($n = 3$)	3.0 ($n = 3$)	1.5 ($n = 8$)	2.0 ($n = 8$)
avg wt (g)	1.5020	1.9980	2.5030	3.0040	1.5213	2.0277
(cv, %)[c]					(1.44)	(1.56)
peak height at 521.5 nm[d]	0.027	0.037	0.040	0.044	0.075	0.094
(cv, %)					(4.54)	(4.34)
TBA value (μg MDA/kg)	51.7	50.7	43.3	40.3	130.32	120.46[e]
(cv, %)					(3.85)	(5.01)

[a,b] Data from two different raw meat samples (A and B, respectively). [c] cv and statistical analysis are not stated for sample A as only $n = 3$ repetitions were performed. [d] Peak height is expressed in arbitrary units. [e] For sample B, mean values are significantly different ($P = 0.0032$).

Table 3. Influence of Fat Amount on TBA Values

	mL of fat solution[a]		
	1 ($n = 4$)	2 ($n = 4$)	3 ($n = 4$)
peak height at 521.5 nm[b]	0.061	0.078	0.093
(cv, %)	(9.08)	(7.85)	(5.98)
TBA value (μg MDA/L)	163.07[c]	102.22[d]	80.49[d]
(cv, %)	(9.01)	(7.33)	(6.05)

[a] Fat was extracted from 30 g of raw dark chicken meat and dissolved in 30 mL of hexane. [b] Peak height is expressed in arbitrary units. [c,d] Mean values with different superscripts are significantly different ($P = 0.0001$).

aliquots just after they were weighed, and TCA just before homogenization (second run), this correlation was not significant ($r_s = 0.5476$; $P = 0.1474$). In this case, all the aliquots spent similar time without BHT from the moment that the bag was opened. Thus, addition of the BHT/hexane solution immediately after weighing is useful to protect ground meat from oxidation during analysis and to reduce variability of the method. Moreover, it has been demonstrated that the addition of an antioxidant such as BHT (Botsoglou et al., 1994; Pikul et al., 1989) or BHA (Salih et al., 1987) prior to the blending step is essential to prevent meat from oxidation. Furthermore, in cooked samples, addition of BHT was not enough to avoid sample oxidation. It was necessary to add EDTA immediately after weighing in order to chelate metal ions, especially free iron, which is released during the cooking procedure and plays a marked role in lipid oxidation (Decker and Xu, 1998; Rhee et al., 1996). This is in disagreement with Botsoglou et al. (1994), who found that EDTA was not necessary. However, their study was carried out with only raw fish samples.

Stabilization of the Spectrophotometric Measurement (Peak Height at 521.5 nm). Figure 2 shows that the main decrease in TBA value occurred during the first 45 min after the TBA reaction mixture was cooled, and from that time on, the value became almost stable. Therefore, it is advisable to temper the reaction mixture for 45 min at room temperature before submitting it to spectrophotometry. This modification of the method is important because, when working with a series of samples, submitting all of them to third-derivative spectrophotometry may involve a large time difference between the reading of the first and last samples of a run. For example, a series of 16 samples was processed without letting them temper for these suggested 45 min, and the first three samples were reread immediately after the last one had been read (55 min later). It was observed that the TBA value had

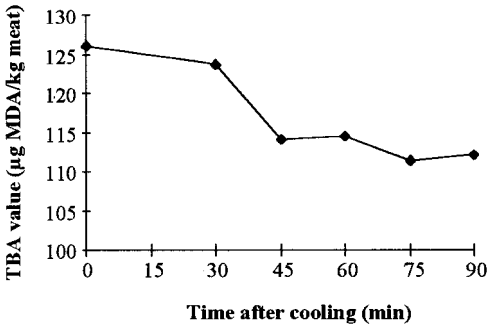

Figure 2. Evolution of TBA values depending on the time elapsed between cooling of the TBA reaction mixture and before spectrophotometric measurement of the peak height at 521.5 nm ($n = 3$).

decreased by 8.30% on average. However, the same experiment with the suggested tempering step resulted in only a 2.97% decrease.

Influence of Handling and Storage of Samples. The thawing and refreezing of a cooked sample for three consecutive days led to a great increase in TBA values (from 592.76 to 1107.32 and from this value to 2295.51 μg MDA/kg). Storage of three cooked samples for 24 h at 4 °C in a plastic zip-bag also led to a dramatic increase in TBA values (from 1328.02 to 2708.67). However, storage of vacuum-packed cooked samples for 7 months at −20 °C did not lead to an increase in TBA values. TBA values after 7 days and 3.5 and 7 months of storage were, respectively: 3682.79, 3508.10, and 3417.58 μg of MDA/kg. Samples used in these three experiments were from chickens fed different diets and processed in different ways, what accounts for the different initial oxidation level of the samples. The small drop in TBA values found in frozen chicken is consistent with the findings of Witte et al. (1970) and Igene et al. (1979) in various frozen meats (pork, beef, and chicken) and could be attributed to the fact that MDA binding to myosin takes place even at −20 °C (Buttkus, 1967). Indeed, this author described that the rate of this reaction is greater at −20 °C than at 0 °C and was almost equal to that at 20 °C. From these results, it can be concluded that proper handling and storage of meat samples before TBA determination is very important and that vacuum-packaging and storage at −20 °C are good conditions to avoid lipid oxidation in chicken meat samples.

Precision. The coefficient of variation ($n = 16$) was 3.81% for raw meat and 4.13% for cooked meat (mean TBA value = 113.63 and 1201.11 μg of MDA/kg, respectively). Comparison of the precision of our method to the value reported by Botsoglou et al. (1994) for raw cow muscle samples [the coefficient of variation ($n = 5$) was calculated from our results as previously described] revealed substantial improvement. Our method produced a cv of 2.6% ($n = 5$) as compared to the 4.5% (mean TBA value = 88 μg of MDA/kg) reported by Botsoglou et al. (1994). Hence, we are certain that these modifications will lead to more reliable results when using this method for assessing MDA in meat samples.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid disodium salt; MDA, malondialdehyde; TEP, 1,1,3,3-tetraethoxypropane; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; TNBSA, 2,4,6-trinitrobenzene-1-sulfonic acid.

ACKNOWLEDGMENT

S. Grimpa, R. Bou, S. Constantí, and D. Alonso provided assistance with the laboratory work.

LITERATURE CITED

Aubourg, S. P. Review: interaction of malondialdehyde with biological molecules − new trends about reactivity and significance. *Int. J. Food Sci. Technol.* **1993**, *28*, 323−335.

Botsoglou, N. A.; Fletouris, D. J.; Papageorgiou, G. E.; Vassilopoulos, V. N.; Mantis, A. J.; Trakatellis, A. G. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *J. Agric. Food Sci.* **1994**, *42*, 1931−1937.

Buttkus, H. The reaction of myosine with malonaldehyde. *J. Food Sci.* **1967**, *32*, 432−434.

Capella, P. Les produits de l'évolution des hydroperoxydes (I) (Products resulting from hydroperoxide evolution (I)). *Rev. Fr. Corps Gras* **1989**, *36*, 313−323.

Cayot, P.; Tainturier, G. The quantification of protein amino groups by the trinitrobenzenesulfonic acid method: a reexamination. *Anal. Biochem.* **1997**, *249*, 184−200.

Cecconi, C.; Cargoni, A.; Pasini, E.; Condorelli, S.; Curello, S.; Ferrari, R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. *Am. J. Physiol.* 260 *(Heart Circ. Physiol.* 29*)* **1991**, H1057−H1061.

Decker, E. A.; Xu, Z. Minimizing rancidity in muscle foods. *Food Technol.* **1988**, *52*, 54−59.

Esterbauer, H.; Schaur., R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *11*, 81−128.

Fernández, J.; Pérez-Álvarez, J. A.; Fernández-López, J. A. Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chem.* **1997**, *59*, 345−353.

Frankel, E. N. Secondary products of lipid oxidation. *Chem. Phys. Lipids* **1987**, *44*, 73−85.

Guillén Sans, R.; Guzmán Chozas, M. Reactividad del ácido 2-tiobarbitúrico (ATB) con compuestos carbonílicos. Su importancia en la rancidez oxidativa y en el perfil del flavor de los alimentos (Reactivity of 2-thiobarbituric acid (TBA) with carbonyl compounds. Its importance in oxidative rancidity and the flavor profile of foodstuff). *Grasas Aceites* **1988**, *39*, 185−189.

Igene, J. O.; Pearson, A. M.; Merkerel R. A.; Coleman, T. H. Effect of frozen storage time, cooking and holding temperature upon extractable lipids and TBA value of beef and chicken. *J. Anim. Sci.* **1979**, *49*, 701−707.

Kosugi, H.; Kato, T.; Kikugawa, K. Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem.* **1987**, *165*, 456−464.

Kosugi, H.; Kato, T.; Kikugawa, K. Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid oxidation. *Lipids* **1988**, *23*, 1024−1031.

Marcuse R.; Johansson, L. Studies on the TBA test for rancidity grading. II. TBA reactivity of different aldehyde classes. *J. Am. Oil Chem. Soc.* **1973**, *50*, 387−391.

Pikul, J.; Leszczynski, D. E.; Kummerow, F. A. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* **1989**, *37*, 1309−1313.

Raharjo, S.; Sofos J. N. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: a review. *Meat Sci.* **1993**, *35*, 145−169.

Raharjo, S.; Sofos, J. N.; Schmidt, G. R. Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C_{18} method for measuring lipid peroxidation in beef. *J. Agric. Food Chem.* **1992**, *40*, 2182−2185.

Raharjo, S.; Sofos, J. N.; Schmidt, G. R. Solid-phase acid extraction improves thiobarbituric acid method to determine lipid oxidation. *J. Food Sci.* **1993**, *58*, 921−924.

Rhee, K. S.; Anderson, L. M.; Sams, A. R. Lipid oxidation potential of beef, chicken and pork. *J. Food Sci.* **1996**, *61*, 8−12.

Salih, A. M.; Smith, D. M.; Price, J. F.; Dawson, L. E. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poult. Sci.* **1987**, *66*, 1483−1488.

Schmedes, A.; Hölmer, G. A New thiobarbituric acid (TBA) method for determinig free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. *J. Am. Oil Chem. Soc.* **1989**, *71*, 267−276.

Sinnhuber, R. O.; Yu, T. C. The 2-thiobarbituric acid reaction, an objective measure of the oxidative deterioration occurring in fats and oils. *J. Jpn. Oil. Chem. Soc.* **1977**, *26*, 259−267.

Slater, T. F. Overview of methods used for detecting lipid peroxidation. *Methods Enzymol.* **1984**, *105*, 283−293.

Smith, J. L.; Alford, J. A. Action of microorganisms on the peroxide and carbonyls of rancid fat. *J. Food Sci.* **1968**, *33*, 93−97.

Witte, V. C.; Krause, G. F.; Bailey, M. E. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* **1970**, *35*, 582−585.

Received for review May 17, 1999. Revised manuscript received December 14, 1999. Accepted December 30, 1999. This work was supported in part by research grants from the Comissió Interdepartamental de Recerca i Innovació Tecnològica (CIRIT), the Comisión Interministerial de Ciencia y Tecnología (CICYT), and the Instituto Danone.

JF990518Q