E.Ü. Su Ürünleri Dergisi 2006
E.U. Journal of Fisheries & Aquatic Sciences 2006
Cilt/Volume 23, Sayı/Issue (3-4): 331–334

ISSN 1300 - 1590
http://jfas.ege.edu.tr/

Comparison of Two Thiobarbituric Acid (TBA) Method for Monitoring Lipid Oxidation in Fish

*Bahar Tokur[1], Koray Korkmaz[1], Deniz Ayas[2]

[1]*Çukurova Üniversitesi, Su Ürünleri Fakültesi, Avlama ve İşleme Teknolojisi Bölümü, Adana, Türkiye*
[2]*Mersin Üniversitesi, Su Ürünleri Fakültesi, Avlama ve İşleme Teknolojisi Bölümü, Mersin, Türkiye*
**E mail: b_tokur@yahoo.com*

Özet: ***Balıklarda lipit oksidasyonunu belirlemek için iki tiyobarbiturik asit (TBA) metotunun karşılaştırılması.*** Bu çalışmada, yağlı balıklarda önemli bir kalite kriteri olan tiyobarbiturik asit (TBA) analizi, standart bir distilasyon metodu olan Tarladgis ve diğ. (1960) ve bir sıvı asit ekstraksiyon metodu olan Lynch and Frei (1993) olmak üzere iki farklı yöntemle karşılaştırılmıştır. Tarladgis ve diğ. (1960)' nın yönteminde 7.8 sabiti ile çarpılan specktrofotometrik değerler, ayrıca Crackel (1986)' in önerdiği üzere 6.2 sabiti ile de çarpılmış ve elde edilen TBA değerleri karşılaştırılmıştır. Araştırmada kullanılan küçük boylu sardalyaların (*Sardina pilchardus* (W., 1972), 12.7±0.15 cm) lipit içeriği %1.59, büyük boylu sardalyaların (*S. pilchardus* (W., 1972),16.5±0.98 cm) lipit içeriği %2.18, küçük boylu kefallerin (*Mugil cephalus* (L., 1758) 21.0±0.68 cm) lipit içeriği %2.08, büyük boylu kefallerin (*M.* cephalus (L., 1758), 33.2 ±1.23 cm) lipit içeriği %0.97, palamutun (*Sarda sadra* (B., 1793), 32.1±3.25 cm) lipit içeriği %6.49 ve lüferin (*Pomatomus saltator* (L., 1766), 23 ± 1.01cm) lipit içeriği %1.19 olarak bulunmuştur. Küçük boylu kefallerde ve büyük boylu sardalyalarda ekstraksiyon metoduna göre elde edilen TBA değerleri, 6.2 sabiti ile hesaplanarak bulunan distilasyon metoduna ile elde edilen TBA değerlerine göre önemli bir farklılık göstermezken ($p>0.05$), 7.8 sabiti ile hesaplanan TBA değerleri 1.3 kat daha yüksek bulunmuştur ($p<0.05$). Küçük boylu sardalyalarda ve lüferlerde ise her bir üç TBA değeri arasında önemli bir fark bulunmamıştır ($p<0.05$). Küçük boylu kefallerde ve palamutta ise, ekstraksiyon metoduna göre elde edilen TBA değerleri, 6.2 ve 7.8 sabitine göre hesaplanan TBA değerlerinden daha yüksek bulunmuştur. Ayrıca, sardalyalarda her iki yöntemle saptanan TBA değerlerinin lipit içeriğine göre arttığı saptanmıştır. Bu çalışmanın sonucunda, balık kasında lipit oksidasyonu sonucu ortaya çıkan malonaldehit miktarını belirlemek için kullanılan Lynch and Frei (1993) metodunun, Tarladgis ve diğ. (1960) metoduna alternatif olarak kullanılabileceği saptanmıştır.

Anahtar Kelimeler: Malonaldehyde, balık, tiyobarbiturik asit metodu, distilasyon metodu, ekstarksiyon metodu.

Abstract: In this study, the thiobarbituric acid (TBA) analysis that is an important quality index for fatty fish was compared with the method of Tarladgis *et al*. (1960) which is a commonly used distillation method and aqueous acid extraction method according to Lynch and Frei (1993). The results of spectrophotometric values multiplying by 7.8 constant in the method of Tarladgis *et al*. (1960) were also multiplied by 6.2 constant according to Crackel (1986) and the results of TBA value were compared. The lipid contents of fish used in the experiment were found to be %1.59 for small sardine (*Sardina pilchardus* (W., 1972), 12.7±0.15 cm), %2.18 for large sardine (*S. pilchardus* (W., 1972),16.5±0.98 cm), %2.08 for small grey mullet (*Mugil cephalus* (L., 1758), 21.0±0.68 cm), %0.97 for large grey mullet (*M. cephalus* (L. 1758), 33.2 ±1.23 cm), %6.49 for Atlantic bonito (*Sarda sadra* (B., 1793), 32.1±3.25 cm) and %1.19 for bluefish (*Pomatomus saltator* (L. 1766), 23 ± 1.01cm). TBA values obtained by extraction method in small grey mullet and large sardine weren't significantly different from those of the distillation method when calculated by 6.2 constant ($p>0.05$), while TBA values of them obtained by distillation method calculated by 7.8 constant were found to be significantly 1.3 times higher than those of extraction method ($p<0.05$). No significantly differences were found between the each three TBA values for small sardine and bluefish ($p<0.05$). In small grey mullet and Atlantic bonito, TBA values obtained by extraction method were found higher than those of distillation method calculated by both 7.8 and 6.2. Moreover, the higher TBA value was obtained with the higher lipid content in small and large sardine. As a result of this study, it is suggested that the extraction method according to Lynch and Frei (1993) can alternatively be used to determine lipid oxidation in fish muscle in stead of the distillation method according to Tarladgis *et al*. (1960).

Key Words: Malonaldehyde, fish, thiobarbituric acid methods, distillation method, extraction method.

Introduction

The 3-carbon compound malonaldehyde (MDA) is a major carbonyl decomposition products of autoxidized, polyunsaturated lipid materials (Tamura *et al*. 1991, Crawford *et al*. 1966, Pegg *et al*. 1992). Spectrophotometric detection of the malonaldehyde-thiobarbutiric acid (TBA) complex has been widely used for measuring lipid oxidation in food and biological tissues (Kwon and Veen 1968, Esterbauer and Cheseeman 1990). The basic principle of the method is the reaction of 1 molecule malonaldehyde and 2 molecules TBA to form a pink pigment malonaldehyde-TBA complex, which can be quantitated spectrophotometrically (Gutteridge 1981).

The tissue distillation-TBA assay method of Tarladgis *et al*. (1960) is most commonly used and may be regarded as the standard method for MDA analysis. However, the main disadvantage of the distillation method is that distillation is an experimental procedure requiring the collection of a specified volume of the distillate. So it needs more time than the aqueous acid extraction method (Botsoglu *et al*. 1994) and the

method requires distillation units, which can place a limitation on the number of samples that can be analyzed in one day. The foaming of distillate according to some fish species during distillation is also other disadvantage in distillation methods. As consequences of this, the aqueous acid extraction method is preferred by many workers because it is simple and gives results that are highly correlated with those of distillation (Pikul *et al*. 1989) Although many procedures have been developed to measure the aqueous acid extraction method for MDA, all variations are similar in that they each have a critical heating step with an acid to liberate MDA from its precursors or bound forms as well as to separate the TBA-reactive substances (TBARS) from food matrix (Pikul *et al*. 1983, Peg *et al*. 1992). TBA reagent may be added with an acid to food directly and the mixture be added with an acid to food directly and the mixture then heated for a sufficient period of time to obtain maximum color development. The pink pigment formed during heating may be extracted into butanol or a butanol-pyridine mixture after cooling and then quantified (Peg *et al*. 1992).

Among the aqueous acid extraction, the methods described by Tarladgis *et al*. (1960) is the most extensively used to estimate rancidity of fish and fishery products. The purposed of this study was to evaluate the aqueous acid extraction according to Lynch and Frei (1993) for monitoring lipid oxidation in fish.

Material and Methods

Small sardine (*Sardina pilchardus* (W., 1972), 12.7±0.15 cm), large sardine (*S. pilchardus* (W., 1972),16.5±0.98 cm), small grey mullet (*Mugil cephalus* (L. 1758), 21.0±0.68 cm), large grey mullet (*M. cephalus* (L. 1758), 33.2 ±1.23 cm), Atlantic bonito (*Sarda sadra* (B., 1793), 32.1±3.25 cm) and bluefish (*Pomatomus saltator* (L. 1766), 23 ± 1.01cm) compared with distillation and extraction methods were obtained from a local grocery fish store in Adana and immediately transferred to laboratory in iced.

The lipid contents of fish samples were analyzed according to Bligh and Dyer (1951) lipid extraction method.

The distillation TBA method was performed as described by Tarladgis *et al*. (1960). The homogenized 10 g of fish sample was transferred to a Kjeldahl flask and 97.5 ml of distillated water and 2.5 ml of 6 N HCl. The mixture was heated with steam distillation until 200 ml of distillate was collected. Five ml of distillate was added to 5 ml of thiobarbutiric reactive reagent containing 0.02 M TBA in 90 % glacial acetic acid and incubated for boiling water for 35 min. After cooling with tap water, the absorbance of the pink solution was read at 538 nm. The constant 7.8 and 6.2 were used to calculate the distillation TBA number as recommended by Tarladgis *et al*. (1960) and Crackel (1986), respectively.

The acid extraction TBA method was performed as describe as Lynch and Frei (1993). Muscle samples (1 g) were homogenized in 10 ml KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed). Samples of 0.5 ml of homogenate were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 ml) and 2.8% (w/v) trichloroacetic acid (0.25 ml) in a boiling water bath for 10 min. After cooling at room temperature for 20 min, the pink chromogen was extracted with n-butanol (2 ml) and its absorbance measured at 535 nm against a blank of n-butanol. TBA-RS concentrations were calculated using 1,1,3,3 tetraethoxypropane (0–0.8 µM) as standard. Results were expressed as mg MDA per kg of meat (TBA units).

The effects of methods on TBARs value were analysed by one-way analysis of variance (ANOVA). The comparison of the TBA numbers in different fish species calculated according to Tarladgis *et al*. (1960), Crackel (1986) and Lynch and Frei (1993) were performed for significance by Duncan's multiple range tests. Three replications were made and six readings were made for each replication. The SPSS statistical package version 7.5 was used for statistical analysis.

Results and Discussion

The results of lipid contents and TBA numbers in different fish species obtained by distillation and extraction methods are given in Table 1.

Table1. The TBARs values (mg malonaldehyde/kg meat) of different fish species obtained by distillation and extraction method[1,2]

Fish species	Total Lipids	Extraction method	Distillation Calculated by constant 7.8	Method Calculated by constant 6.2
Grey mullet				
small	2.08±0.87	1.974 ±0.163[a]	2.474± 0.112[b]	1.967± 0.069[a]
large	0.97±0.04	1.119±0.171[a]	0.785±0.086[b]	0.624±0.030[c]
Sardine				
small	1.59±0.04	2.177±0.586[a]	2.574±0.313[a]	2.046±0.249[a]
large	2.18±0.07	6.159±0.221[a]	8.210±0.327[b]	6.526±0.260[a]
Atlantic bonito	6.49±0.34	1.779±0.203[a]	1.119±0.117[b]	0.953±0.093[b]
Bluefish	1.19±0.06	0.397±0.116[a]	0.413±0.024[a]	0.329±0.019[a]

The lipid contents of fish used in the experiment were found to be 1.59% for small sardine (*Sardina pilchardus*, 12.7±0.15 cm), 2.18% for large sardine (*S. pilchardus*, 16.5±0.98 cm), 2.08% for small grey mullet (*Mugil cephalus*, 21.0±0.68 cm), 0.97% for large grey mullet (*M. cephalus*, 33.2 ±1.23 cm), 6.49% for Atlantic bonito (*Sarda sarda*, 32.1±3.25 cm) and 1.19% for bluefish (*P. saltator*, 23 ± 1.01cm). In the present study, the low lipid contents in sardines were not in agreement with the common classification of sardines as the fat species. It is known that the season, stage of sexual

maturity and body size can affect the lipid contents of sardines with values ranging from 0.5 % in winter and 20% in summer (Hardy and Keay 1972, Christie 1987, Nunes *et al*. 1991, Bandarra *et al*. 1997). Additionally, the total lipid contents of sardines were found the higher in longer species than in the shorter ones. Similar results were obtained in sardines by Caponia *et al*. (2004). The lipid contents of grey mullet were found to be higher in shorter species than in the longer ones. Perera and De Silva (1978) found that total lipid content with length increased for reared young mullet (*M. cephalus*) but not for wild mullet. They explained that the reason of these results may be due to the high calorific value of the food presented to the reared fish and the lack of activity in the confined space of the rearing tanks. In this study, the low lipid content found in Atlantic bonito may be attributed to its diet, which consists mainly of small pelagic fish species like sardines (*S. pilchardus*) (Zaboukas *et al*. 2006). The lipid contents of sardines were also found to be poor in this study.

TBA values obtained by extraction method in small grey mullet and large sardine weren't significantly different from those of the distillation method when calculated by 6.2 ($p>0.05$), while TBA values of them obtained by distillation method calculated by 7.8 were found to be significantly 1.3 times higher than those of extraction method ($p<0.05$). Similar results have been found by Pikul *et al*. (1989), who found that TBA numbers obtained by the distillation method were higher than 1.3-1.4 times higher than those of extraction method in the absence of BHT. However, Witte *et al*. (1970), Vycnke (1975) and Salih *et al*. (1987) found that the TBA values of pork, fish, beef and poultry meat obtained by distillation method were 2.0 and 2.6 times higher than those of extraction method in the presence of BHT. The researchers suggested that when the higher TBA value were obtained by distillation method, the heating during distillation can cause to increase quantities of aldehydes and disrupt certain carbonyl products formed by reactions between malonaldehyde and amino acids, pyridines, or protein (Buttkus 1967, Girón-Calle *et al*. 2003) and/ or to speed up the oxidation process. (Salih *et al*. 1987,Siu and Draper 1978). Salih *et al*. (1987) demonstrated that the addition of BHT as an antioxidant during blending of sample in both extraction and distillation methods resulted in lower value of TBA, indicating blending could accelerate lipid oxidation without addition of BHT. On the other hand, Siu and Draper (1978) found no differences between TBA values determined by distillation and TCA extraction method in the presence of antioxidant or EDTA. There were no significantly differences in TBA values of small sardine and bluefish between the three results ($p<0.05$). In small grey mullet and Atlantic bonito, TBA values obtained by extraction method were higher than those of distillation method calculated by both 7.8 and 6.2. Similar results have been found by Pikul *et al*. (1983). Heras *et al*. (2003) explained that when the higher values of TBA obtained by different methods than those of distillation method could result in the possible reactivity of the TBA reagent with compounds present in oxidized lipids other than MDA. In addition, heating and acidic condition may cause overestimation of thiobarbituric acid reactive substances (TBARS) (Jo and Ahn 1998). As can be seen in Table1, the TBA values in sardine and grey mullet increased with increasing lipid content, which generally agrees with the fact that the higher lipid content gives the higher TBA values in fish sample.

In conclusion, the results of this study suggest that the extraction method according to Lynch and Frei (1993) can alternatively be used to determine the amount of lipid oxidation in fish muscle in stead of the distillation method according to Tarladgis *et al*. (1960). This procedure also makes it possible to determine lipid oxidation products in large numbers and small quantities of samples.

References

Bandarra, N. M., I. Batista, M. L.Nunes, J. M. Empis, and W. W. Christie. 1997. Seasonal changes in lipid composition of sardine (Sardina pilchardus). J. Food Sci., 62: 40–42.

Bligh, E., and W. V. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–91

Botsoglu, N.A., D.J. Fletouris, G.E. Papageorgiou, V.N. Vassilopoulos, A.J. Mantis, and A.G. Trackatellis. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedsstuff samples. J. Agric. Food Chem., 42: 1931-1937.

Buttkus, H. J. 1967. The reaction of myosin with malonaldehyde. J. Food Sci., 32:432–434

Christie, W. W. 1987. The lipid composition of animal tissue, pp. 55–79. In: HPLC and lipids: a practical guide. W. W. Christie (Ed.). Pergamon Press, Oxford, UK

Crackel, R.L. 1986. Stability of lipids in restructured beef steaks. M.S. Thesis, Michigan State University, East Lansing, MI.

Crawford, D. L., T. C. Yu, and R. O. Sinnhuber. 1966. Reaction mechanism, reaction of malonaldehyde with glycine. J.Agric. Food Chem, 14: 184.

Esterbauer, H., and K.H. Cheeseman. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol, 186: 407-421

Caponio, F., A. Lestingi, C. Summo, M. T. Bilancia, and V. Laudadio. 2004. Chemical characteristics and lipid fraction quality of sardines *(Sardina pilchardus W.)*: influence of sex and length. J. Appl. Ichthyol., 20: 530–535.

Girón-Calle, J., M. Alaiz, F. Millán, V. Ruiz-Gutiérrez, and E. Vioque. 2003. Bound malondialdehyde in foods: Bioavailability of the N,N'-di-(4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde)lysine. Journal of Agricultural and Food Chemistry, 51: 4799–4803

Gutteridge, J.M. 1981. Thiobarbituric acid-reactivity following iron- dependent free-radical damage to amino acids and carbohydrates. FEBS Lett., 128: 343-346

Hardy R, and J.N. Keay. 1972.Seasonal variations in the chemical compositions of Cornish mackerel (*Scomber scombrus* L.) with detailed reference to the lipids. J Fd Tech , 7: 125–237.

Heras, A., A. Schoch, M.Gibis, and A. Fischer. 2003. Comporison of methods for determining malonaldehyde in dry sausage by hplcl and the classic tba test. Eur. Food Res. Technol., 217: 180-184.

Hultin, HO. 1992. Lipid oxidation in fish muscle, pp. 99–116. In: Flick Jr, GJ and Martin RE (eds). Advances in Seafood Biochemistry. Technomic Publishing Co., PA.

Jo, C., D.U. Ahn. 1998. Flourometric analysis of 2-thiobarbutiric acid reactive substances in turkey. Poultry Science, 77: 575-480.

Kwon, T., J.V. Veen. 1968. Ultraviolet and infrared absorbtion of spectra of malonaldehyde in organic solvents. J. Agric. Food Chem., 16: 639-642.

Lynch, S.M., and B. Frei. 1993. Mechanism of copper-and iron-dependent oxidative modification of human low density lipoprotein. J. Lipid Research, 34: 1745-1753.

Masengi, S., Y. Sato, J. Shindo, and H. Miki. 2001.Quality improvement by vacuum treatment of boiled skipjack loins for the production of dried

skipjack. International Journal of Food Scie. And Technology, 67(4):730

Nunes, M. L., M. Cardinali, R. Mendes, R. M.Campos, N. M. Bandarra, H. M. Lourenco, and M. Jerome. 1991. Effect of season and ice storage on proteins and lipids of sardine (*Sardina pilchardus*) minces and surimi, pp. 63–72. In: Quality assurance in the fish industry. H. Huss, M. Jakobsen and J. Liston (Eds). Elsevier, Amsterdam.

Pegg, R.B., F. Shahidi, and C.R. Jablonski. 1992. Interaction of sulfanilamide and 2-thiobarbutiric acid with malonaldehyde:structure of adducts and ımplications in determination of oxidative state of nitrite-cured meats. J. Agric. Food Chem., 40: 1826-1832.

Perera P. A. B. and S. S. De Silva. 1978. Studies on the chemical biology of young grey mullet Mugil cephalus L. Journal of Fish Biology., 13: 297 – 304

Pikul, J., D.E. Leszczynski, and F.A. Kummerow. 1989. Evaluation of three modified tba methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem., 37: 1309-1313.

Pikul, J., D.E. Leszczynski, and F.A. Kummerow. 1983. Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobabutiric acid assay for malonaldehyde in fat from chicken meat. J. Agric. Food Chem., 31: 1338-1342.

Salih, A.M., D.M. Smith, J.F. Price, and E. Dawson. 1987. Modified extraction 2_thiobarbutiric acid method for measuring lipid oxidation in poultry. Poultry Science, 66: 1483-1488.

Siu, G.M., and H.H Draper. 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci., 43: 1147-1149.

Tamura, H., K. Kitta, and T.Shibamoto.1991. Formation of reactive aldehydes from fatty acids in a Fe^{+2}/H_2O_2 oxidation system. J. Agric. Food Chem., 39: 439-442.

Tarladgis, B.G., B.M. Watts, and M. Yonathan. 1960. Distillation method for the determination of malonaldehyde ın rancid foods. J. of American Oil Chemistry Society, 37(1): 44–48

Vyncke, W. 1975. Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombrus* L.). Fette Seifen Anstrichm. 77:239–240.

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

Zaboukas, N., H. Mılıou, P. Megalofonou, M. Moraıtou-Apostolopoulou, 2006. Biochemical composition of the Atlantic bonito *Sadra sarda* from the Aegean Sea (Eastern Mediterranean Sea) in different stages of sexual maturity. Journal of Fish Biology, 69: 347–362