

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

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**Özet: Balıklarda lipit oksidasyonunu belirlemek için iki tiyobarbiturik asit (TBA) metodunun 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 spektrofotometrik 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 sarda* (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:** Malonaldehide, balık, tiyobarbiturik asit metodu, distilasyon metodu, ekstraksiyon 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 sarda* (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 significant 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-thiobarbituric 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 sarda* (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 distilled 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 thiobarbituric 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<sup>1,2</sup>

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 <sup>a</sup>	2.474±0.112 <sup>b</sup>	1.967±0.069 <sup>a</sup>
large	0.97±0.04	1.119±0.171 <sup>a</sup>	0.785±0.086 <sup>b</sup>	0.624±0.030 <sup>c</sup>
Sardine				
small	1.59±0.04	2.177±0.586 <sup>a</sup>	2.574±0.313 <sup>a</sup>	2.046±0.249 <sup>a</sup>
large	2.18±0.07	6.159±0.221 <sup>a</sup>	8.210±0.327 <sup>b</sup>	6.526±0.260 <sup>a</sup>
Atlantic bonito	6.49±0.34	1.779±0.203 <sup>a</sup>	1.119±0.117 <sup>b</sup>	0.953±0.093 <sup>b</sup>
Bluefish	1.19±0.06	0.397±0.116 <sup>a</sup>	0.413±0.024 <sup>a</sup>	0.329±0.019 <sup>a</sup>

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 significant 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.

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