Malondialdehyde Measurement in Oxidized Foods: Evaluation of the Spectrophotometric Thiobarbituric Acid Reactive Substances (TBARS) Test in Various Foods

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ABSTRACT: The ability of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test to determine malondialdehyde (MDA) in various food matrices was evaluated. MDA was extracted from the foods; the extract reacted with thiobarbituric acid (TBA); and the formed TBA−MDA adduct was measured spectrophotometricaly at 532 nm. In parallel, the TBA−MDA adduct was analyzed with high-performance liquid chromatography (HPLC) coupled with fluorescence detection. Oils and unprocessed and uncooked meat and fish products did not exhibit any significant difference in the amount of MDA measured by the two methods, indicating that the major substance reacting with TBA and forming an adduct that absorbs at 532 nm was MDA. However, in products such as dry nuts, pork sausages, cooked fish, and gouda cheese, an overestimation of MDA was observed, indicating that TBARS test was unsuitable for accurate determination of MDA. Furthermore, the results in the present work suggest that the overestimation of MDA by the TBARS test as it was applied is related to the interference of other than secondary lipid oxidation products.

KEYWORDS: *Lipid oxidation, malondialdehyde, thiobarbituric acid reactive substances*

■ INTRODUCTION

Lipid oxidation is one of the major causes of deterioration of fat-containing foods, especially those containing polyunsaturated fatty acids (PUFAs).[1] Unsaturated fatty acids are oxidized to form odorless, tasteless hydroperoxides. The formed hydroperoxides are further decomposed to flavorful secondary oxidation products, which are mainly aldehydes, such as hexanal, 4-hydroxynonenal (HNE), and malondialdehyde (MDA).[2] MDA is a three-carbon dialdehyde with carbonyl groups at the C-1 and C-3 positions and is known to be mutagenic to humans because it can form adducts with proteins and DNA.[3−5] In products such as fish and meat, MDA determination along with peroxide values is often used in assessment of lipid oxidation.[6−8] The most common method to determine MDA in foods is the spectrophotometric measurement of the pink-colored adduct of MDA with 2-thiobarbituric acid (TBA), which gives a maximum absorbance at 532−535 nm.[9] TBA can react directly with the food sample, and the TBA−MDA adduct is extracted prior to the analysis. Alternatively, MDA can be extracted from the samples by either distillation[10] or solvent extraction.[11] All of the spectrophotometric methods have been criticized because of their unspecificity toward MDA.[12] It is well-accepted that components present in food matrices, such as browning reaction products and protein and sugar degradation products, participate in the formation of the TBA color complex;[13,14] thus, more specific analysis of the TBA−MDA complex is required. Although the distillation method is minimizing the interferences with TBA, there are limitations because of the long analysis time and the possible artifactual formation of MDA during heating.[15] Application of high-performance liquid chromatography (HPLC) analytical techniques has offered better specificity and sensitivity towards MDA determination in foods and biological systems based on the analysis of the MDA−TBA complex.[16−18] Furthermore, there are available HPLC methods based on the analysis of MDA derivatives with hydrazine compounds, such as 2,4- dinitrophenylhydrazine (DNPH),[19,20] or on the direct measurement of MDA.[21,22] However, the spectrophotometric method is preferred for routine analysis of a large amount of samples because of its simplicity and low cost;[23] thus, an evaluation of the ability of the thiobarbituric acid reactive substances (TBARS) test to measure MDA within a broad range of foods is necessary. There is no study to our concern evaluating the efficiency of TBARS test in measuring MDA in fatty foods as well as different type of oils.

The aim of the present work was to evaluate the potential of the spectrophotometric TBARS test to measure MDA in a broad variety of foods and oils. The amount of MDA measured with the TBARS test was compared to the results obtained from the HPLC method. Furthermore, a novel enzyme-based extraction technique for MDA was developed for food samples that have a high starch content.

■ MATERIALS AND METHODS

Chemicals and Materials. TBA, α-amylase, propyl gallate, and 1,1,3,3-tetraethoxypropane (TEP) were purchased from Sigma-Aldrich (St. Louis, MO). KH_2PO_4, ethylenediaminetetraacetic acid (EDTA), and glacial acetic acid of analytical grade were purchased from Chem-Lab (Zedelgem, Belgium). Trichloroacetic acid (TCA) was purchased from Acros Organics (Geel, Belgium). Methanol HPLC grade was provided by Fisher Scientific (Leicestershire, U.K.).

Food Samples. Various food samples (Table 1), with limited knowledge of storage history, with developed rancid odor at collection

Table 1. Short Description of the Oxidized Products Used in the Experiment

product	description
pork meat sausages	stored at 4 °C
pork	uncooked, minced meat stored in the freezer (−18 °C)
beef	uncooked, stored for several days at 4 °C
salmon	cooked and stored in the freezer (−18 °C)
herring	uncooked, stored in the freezer (−18 °C)
gouda cheese	stored at 4 °C for an unknown period
peanut paste	stored at room temperature, bought in bulk from Uganda
peanuts	opened packaging for an unknown period
walnuts	opened packaging for an unknown period
potato crisps	opened packaging for an unknown period
soybeans	stored in bulk, provided by the industry
soy−corn mixture A	fresh preparation before extrusion
soy−corn mixture B	fresh preparation after extrusion
vegetable oil A	disposed frying oil from a restaurant
vegetable oil B	stored at room temperature and exposed to air

time were analyzed in the lab with both spectrophotometric (TBARS test) and HPLC methods. Furthermore, a storage experiment with controlled conditions was conducted with products supplied from the local supermarket to further investigate the ability of the TBARS test to measure the MDA formation during storage. This group included peanuts, almonds in sliced and powder form, walnuts, two different brand names of potato crisps (crisps A and B), tortilla crisps, sweet cookies with cinnamon, rainbow trout (*Oncorhynchus mykiss*), and herring (*Clupea* sp.). Beheaded, frozen, vacuum-packed herring was provided by the industry and was unpacked and stored at −27 °C. Rainbow trout was bought fresh from the local supermarket, filleted, and stored at −27 °C. All of the rest samples were packed in transparent polyethylene (PE) bags, sealed, and stored under light with an average illuminance of 1577 lx. The temperature measured throughout storage varied between 20 and 26 °C. To further evaluate the ability of TBARS test to measure MDA in oil samples, different types of oils and mixtures of them were stored at 75 °C in the dark for 6 days to induce oxidation.

MDA Extraction. Dependent upon the nature of the product, different extraction techniques were applied, as described below.

Meat Products, Cheese, and Dry Nuts. Approximately 7 g of sample was weighted in a 50 mL falcon tube. A total of 15 mL of 7.5% TCA (w/v) with 0.1% (w/v) of EDTA and 0.1% (w/v) of propyl gallate was added. The mixture was homogenized with an Ultraturax (Janke and Kunkel, IKA-Werk, Staufeb, Germany) for 1 min at 18 000 rpm, and the volume was adjusted to 30 mL with the addition of TCA. The homogenate was filtered through 150 mm filter paper, and a specific volume reacted with the TBA reagent, as described later.

Oil Samples. A total of 1 g of oil was weighted in a 50 mL falcon tube. A total of 5 mL of water was added. The mixture was vortexed (VWR, Leuven, Belgium) for 2 min and centrifuged (Sigma 4K15, Sartorius, Goettingen, Germany) at 5000g for 5 min. The aqueous layer was collected, and the procedure was repeated 2 times. The collected extract reacted with the TBA reagent, as described later.

Products Rich in Starch. Food products having a high starch content (potato crisps) were analyzed using an enzyme-based

analytical technique. This is the first time such a method for MDA extraction is reported. The necessity of the enzymatic hydrolysis of the starch was pointed by the fact that, during the homogenization of the potato crisps with TCA, a very viscous paste was formed, because of the absorption of water by the starch. Approximately 7 g of sample was weighted in a 50 mL falcon tube and homogenized with 15 mL of water. A total of 1 mL of 3000 units of α-amylase preparation was added, and the mixture was incubated in a water bath at 30 °C for 40 min. A total of 1 unit corresponds to the amount of enzyme that liberates 1 μmol of maltose per minute at pH 6.0 and 25 °C. The enzymatic reaction was stopped by the addition of TCA to a fixed volume, followed by centrifugation at 5000g for 10 min. The supernatant was filtered through a 150 mm filter paper, and a specific volume reacted with TBA. All of the aforementioned extractions were performed in triplicate.

MDA Determination. The reaction of MDA with TBA and the determination of the formed adduct with the two different methods was set as follows: For the HPLC determination of MDA, the procedure described by Mendes et al.[18] was followed, with slight modifications. A total of 1 mL of extract and 3 mL of TBA reagent (40 mM dissolved in 2 M acetate buffer at pH 2.0) were mixed in a test tube and heated in a boiling water bath for 35 min. The reaction mixture was chilled prior to the addition of 1 mL of methanol, and 20 μL of the sample were injected into a Varian C18 HPLC column (5 μm, 150 × 4.6 mm) and held at 30 °C. The mobile phase consisting of 50 mM KH_2PO_4 buffer solution, methanol, and acetonitrile (72:17:11, v/v/v, pH 5.3) was pumped isocratically at 1 mL min^{-1}. Fluorometric detector excitation and emission wavelengths were set at 525 and 560 nm, respectively. For the spectrophotometric determination of MDA,[11] 2.5 mL of extract and 2.5 mL of TBA reagent (46 mM in 99% glacial acetic acid) were mixed in a test tube and heated in a boiling water bath for 35 min. The reaction mixture was chilled, and the absorbance was measured at 532 nm using a Cary 50 UV−vis spectrophotometer from Varian (Sint-Katelijne-Waver, Belgium). For quantification with both methods, standard solutions of MDA in 7.5% TCA were prepared from TEP and calibration curves were prepared at a concentration ranging from 0.6 to 10 μM.

Fat Content Determination. The fat content of the samples was determined according to the Weibull method.[24]

Fatty Acid Composition. The fatty acid profile was determined after the preparation of fatty acid methyl esters (FAMEs) and analysis with gas chromatography according to the American Oil Chemists' Society (AOCS) official method.[25] Extraction of the oil was carried out according to the method described by Blight and Dyer,[26] with some modifications. In brief, approximately 20 g of sample was weighted in a centrifuge tube, 20 and 40 mL of dichloromethane and methanol were added, respectively, and the mixture was homogenized with the Ultraturax at 18 000 rpm for 2 min. Subsequently, 20 mL of dichloromethane were added, followed by homogenization for 30 s. Finally, 20 mL of water was added and mixed for 30 s. The mixture was centrifuged at 2800g for 10 min; the lower layer was collected; and the oil was recovered after evaporation of the solvent using a rotary evaporator (Heidolph Instruments GmbH and Co., Schwabach, Germany) operating at low temperatures.

Statistical Analyses. One-way analysis of variance (ANOVA) was applied to detect the differences between samples and to evaluate the significance of the correlation. Whether the differences were detected, multiple comparisons were performed by the Student−Newman−Keuls (S−N−K) test. All of the analysis were carried out with SPSS 18 statistics package (IBM, SPSS, Inc.)

■ RESULTS AND DISCUSSION

To evaluate the efficiency of the spectrophotometric TBARS test to determine MDA compared to the HPLC separation method of the TBA−MDA complex, 15 food samples with developed rancidity were collected, and the results are shown in Table 2. The TBA test resulted in higher or similar amounts of MDA compared to the results obtained by the HPLC method. Oils and unprocessed and uncooked meat and fish products did

Table 2. MDA Values Measured with the TBARS Test and the HPLC Method and the Total Lipid Content[a]

	MDA (μg g^{-1} of sample)		
sample	TBARS test	HPLC	total lipids (%)
pork meat sausages	0.62 ± 0.02 a	0.40 ± 0.03 b	41.15 ± 1.70
pork	1.02 ± 0.11 a	0.87 ± 0.08 b	18.96 ± 2.44
salmon	2.69 ± 0.26 a	1.53 ± 0.04 b	9.96 ± 0.49
gouda cheese	0.25 ± 0.01 a	0.11 ± 0.02 b	30.26 ± 1.27
peanut paste	5.00 ± 0.08 a	0.36 ± 0.06 b	48.83 ± 1.76
peanuts	5.50 ± 0.83 a	0.86 ± 0.10 b	51.87 ± 0.03
walnuts	11.59 ± 1.21 a	3.24 ± 0.03 b	67.12 ± 0.04
potato crisps	7.27 ± 1.38 a	0.51 ± 0.03 b	37.87 ± 1.77
soybeans	18.22 ± 0.58 a	2.18 ± 0.33 b	17.88 ± 0.55
soy–corn mixture A[b]	7.08 ± 0.28 a	1.06 ± 0.10 b	6.89 ± 0.16
soy–corn mixture B[b]	6.09 ± 0.06 a	0.51 ± 0.01 b	8.07 ± 0.07
beef	3.56 ± 0.13 a	3.64 ± 0.01 a	N/A
herring	2.49 ± 0.71 a	2.33 ± 0.70 a	8.76 ± 0.38
vegetable oil A	0.79 ± 0.09 a	0.71 ± 0.11 a	
vegetable oil B	1.07 ± 0.11 a	0.94 ± 0.02 a	

[a] Values are the average of three and two replications for MDA and total lipids determination, respectively, ± the standard deviation. Different letters within the rows indicate statistically significant differences ($p < 0.05$) in the MDA content. [b] Results are expressed on a dry matter basis.

not exhibit any significant difference in the amount of MDA measured by the two methods, indicating that the major substance reacting with TBA was MDA. Mendes et al.[18] were able to detect the same amounts of MDA with both methods in stored sardines, while others[22] have reported a slight overestimation of MDA with the TBARS test in oxidized vegetable oil. Hirayama et al.,[27] however, detected significantly higher MDA levels with the TBARS test compared to HPLC analysis of MDA–dansyl hydrazine derivatives in vegetable oils. In cooked fish, processed meat, potato crisps, and dried nuts, spectrophotometric measurements demonstrated an overestimation of MDA up to a factor of more than 10. The observed overestimation of MDA by the classic TBARS test in meat products has also been reported by other researchers.[28] In the case of the soy–corn mixture, extrusion resulted in a considerable decrease in the MDA content that the TBA test failed to detect.

Specificity of the TBARS method to measure accurately MDA has always been contested. It is well-accepted that components present in food matrices, such as browning reaction products and protein and sugar degradation products, participate in the formation of the TBA color complex,[2] resulting in false estimation of the MDA content. Reduction of the reaction temperature along with the usage of higher concentrations of the TBA reagent has been suggested as potential solutions to increase the specificity of TBA for MDA.[29] However, even though the results suggest that, in some matrices, MDA can be accurately measured by the TBARS test, the presence or absence of interfering compounds cannot be assumed prior to the analysis. Furthermore, statistical analysis did not reveal any significant correlation between the MDA content ($p > 0.05$; $R^2 = 0.123$) measured by the two methods when all of the samples were taken into account.

To further investigate the ability of the TBARS test to measure the MDA formation during storage, a controlled storage experiment was carried out, and the results are shown in Table 3. The correlation coefficients along with correlation

Table 3. Changes in the MDA Content (μg g^{-1} of Sample) as Measured by the Two Methods[a]

storage time (days)	peanuts TBARS test	peanuts HPLC	almond slices TBARS test	almond slices HPLC	almond powder TBARS test	almond powder HPLC	walnuts TBARS test	walnuts HPLC	storage time (days)	herring TBARS test	herring HPLC
0	5.01 ± 0.43 a	0.44 ± 0.12 a	3.16 ± 0.08 a	0.32 ± 0.02 a	5.21 ± 0.29 a,b	0.44 ± 0.04 a	7.21 ± 0.41 a	0.72 ± 0.07 a	0	0.46 ± 0.15 a	0.30 ± 0.04 a
14	5.45 ± 1.86 a	0.52 ± 0.06 a	4.15 ± 0.42 b	0.33 ± 0.02 a	5.38 ± 0.77 a,b	0.32 ± 0.01 b	9.08 ± 0.67 b	1.52 ± 0.11 b	21	2.65 ± 1.34 a	2.72 ± 1.13 a
37	7.52 ± 0.16 b	0.81 ± 0.05 b	6.37 ± 0.54 c	0.48 ± 0.06 b	6.06 ± 0.35 b,c	0.52 ± 0.01 c	14.15 ± 0.65 c	3.23 ± 0.17 c	103	12.64 ± 1.71 b	12.87 ± 2.51 b
69	8.75 ± 0.66 c	0.38 ± 0.03 a	4.99 ± 0.34 d	0.38 ± 0.06 a	6.90 ± 0.72 c	0.33 ± 0.08 b	15.95 ± 0.82 c,d	4.32 ± 0.24 d	159	15.03 ± 1.43 b	14.42 ± 1.46 b
92	6.16 ± 0.50 a	1.00 ± 0.13 c	4.45 ± 0.09 b	0.48 ± 0.04 b	4.46 ± 0.38 a	0.38 ± 0.02 a,b	14.84 ± 0.60 d	4.46 ± 0.38 d			

storage time (days)	cookies TBARS test	cookies HPLC	crisps A TBARS test	crisps A HPLC	crisps B TBARS test	crisps B HPLC	tortilla crisps TBARS test	tortilla crisps HPLC	storage time (days)	rainbow trout TBARS test	rainbow trout HPLC
0	3.13 ± 0.00 a	1.60 ± 0.01 a	2.48 ± 0.28 a	0.33 ± 0.07 a	3.99 ± 0.31 a	0.62 ± 0.24 a	3.56 ± 0.51 a	0.52 ± 0.04 a	0	0.19 ± 0.04 a	0.09 ± 0.02 a
14	28.18 ± 0.65 b	1.06 ± 0.13 b,c	2.03 ± 0.48 a	0.57 ± 0.11 a	3.58 ± 0.17 a	0.84 ± 0.04 a	1.66 ± 0.07 b	0.28 ± 0.02 b	15	0.31 ± 0.07 a	0.15 ± 0.06 a
37	28.98 ± 2.26 b	1.41 ± 0.15 a	6.40 ± 0.00	1.46 ± 0.00 b	4.06 ± 0.00 b	1.34 ± 0.35 b	2.44 ± 0.21 c	0.41 ± 0.01 c	36	0.27 ± 0.01 a	0.15 ± 0.02 a
69	27.16 ± 1.47 b	1.15 ± 0.03 b	5.87 ± 0.70 b	0.99 ± 0.08 c	15.04 ± 3.06 b	3.07 ± 0.23 c	3.93 ± 0.45 a	0.41 ± 0.03 c	118	0.43 ± 0.03 a	0.33 ± 0.01 b
92	24.07 ± 4.38 b	0.87 ± 0.11 c	3.59 ± 0.16 c	1.21 ± 0.11 c	8.49 ± 1.13 c	2.94 ± 0.10 c	2.70 ± 0.21 c	0.60 ± 0.05 d	176	2.48 ± 0.21 b	1.53 ± 0.05 c

[a] Values are the average of three replications ± the standard deviation. Different letters within the columns indicate statistical significant differences ($p < 0.05$) in the MDA content.

equations are shown in Table 4. For peanuts and both types of almonds, no significant correlation between MDA measured

Table 4. Correlation Coefficients and Correlation Equations between MDA Values Measured with the Two Methods in a Control Storage Experiment Involving Various Matrices

product	correlation significance	correlation equation
peanuts	$p = 0.830$	
almond slices	$p = 0.185$	
almond powder	$p = 0.767$	
walnuts	$p < 0.05$	$y = 2.23x + 5.89$
cookies	$p = 0.365$	
crisps A	$p = 0.014$	$y = 2.94x + 1.33$
crisps B	$p = 0.001$	$y = 3.64x + 0.80$
tortilla crisps	$p = 0.088$	
herring	$p < 0.05$	$y = 1.00x + 0.12$
rainbow trout	$p < 0.05$	$y = 1.59x + 0.02$

with the two methods ($p > 0.05$) was observed. The results suggest that MDA is not the major compound that reacts with TBA and forms a complex that absorbs at 532 nm. Thus, the TBARS test cannot be considered reliable for MDA determination. It should also be mentioned that the applied extraction technique mainly focuses on the free MDA. Aldehydes, especially MDA, have been found to react with ε-amino and sulfhydryl groups of proteins, resulting in the alteration of their functionality.[30,31] In peanut samples, the decrease in the MDA content as measured with the HPLC method at the 69th day of storage could possibly be explained by MDA−protein interactions. The results from walnut samples indicate a significant correlation between the two methods ($p < 0.05$). This correlation could be explained by the high levels of MDA present in the samples. Analysis of walnut oil found 11.5% to be PUFAs with more than two double bonds, the peroxides of which are known to be the main precursors of MDA. However, 10-fold overestimation of the MDA level at day 0 indicated that, also in this case, other than secondary lipid oxidation products are significantly interfering with the TBARS test. Similar results were obtained for two types of potato crisps (A and B). Statistical analysis indicated significant correlation between the two methods ($p < 0.05$) for both samples. In contrast, no correlation was found in tortilla crisps. With regard to the results obtained from two different types of frozen fish, both demonstrated significant correlation between the two methods of analysis ($p < 0.05$). A similar

strong correlation was found by other researchers that analyzed fresh and oxidized fatty fish species.[32] For herring samples, the results suggest that the TBARS test can give a precise estimation of MDA, while in rainbow trout samples, MDA is overestimated when measured with the TBARS test. Alghazeer et al.[33] reported similar results from frozen Atlantic mackerel, and the LC−MS analysis indicated the presence of a gluteraldehyde−TBA adduct that can be considered interference in the TBARS test.

To further evaluate the ability of the TBARS test to measure MDA in oil samples, different types of oils with diverse fatty acid composition and mixtures of them were stored at 75 °C in the dark for 6 days to induce oxidation. The levels of MDA measured using the two selected methods were similar (Table 5), and furthermore, a strong correlation was observed (Figure 1) ($p < 0.05$; $R^2 = 0.999$) between these two methods. The
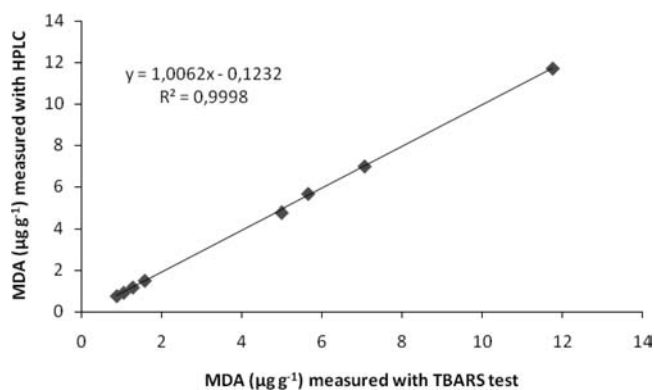
Figure 1. Linear correlation between the MDA amounts determined with both methods in oxidized oil samples.

main secondary oxidation products of monounsaturated fatty acids subjected to autoxidation, for example, C18:1, include volatiles, such as decanal, 2-undecanal, nonanal, and octanal.[2] Autoxidation of linoleate (C18:2, ω-6) leads to the formation of 2,4-decadienal, 3-nonenal, hexanal, and pentanal. Moreover, the formation of HNE from oxidized linoleate has attracted a lot of interest in recent years.[34] As with oleate and linolate, decomposition of linolenate (C18:3, ω-3) hydroperoxides derived from autoxidation lead to the formation of numerous volatiles. Furthermore, it is well-accepted that oxidized PUFAs with more than two double bonds are the major source of MDA because of the degradation of hydroperoxides.[3,35] It has

Table 5. Fatty Acid Composition (%) and Changes in MDA Content (μg g^{-1} of Sample) as Measured by the Two Methods[a]

oil samples	saturated fatty acids	monounsaturated fatty acids	polyunsaturated fatty acids	ω-6	ω-3	MDA (μg g^{-1}) TBARS test	HPLC
corn oil	13.7	28.8	56.7	55.8	0.9	1.28 ± 0.17 a	1.17 ± 0.14 a
sunflower oil	10.6	34.7	53.8	53.6	0.2	0.88 ± 0.15 b	0.76 ± 0.11 b
arachid oil	15.7	64.3	19.0	18.9	0.1	1.05 ± 0.10 a	0.92 ± 0.08 a
colza oil	7.1	63.1	29.0	20.0	9.0	11.75 ± 0.44 c	11.70 ± 0.30 c
olive oil	15.4	73.5	10.1	9.5	0.7	1.58 ± 0.59 a	1.50 ± 0.54 a
mix 1 (50:50 colza oil/olive oil)	11.3	68.3	19.6	14.8	4.9	4.51 ± 1.17 d	4.28 ± 1.47 d
mix 2 (50:50 colza oil/arachid oil)	11.4	63.7	24.0	19.4	4.6	4.99 ± 2.49 d	4.77 ± 2.52 d
mix 3 (80:20 mix 1/mix 2)	11.1	63.8	24.2	19.3	4.9	5.65 ± 2.94 d	5.66 ± 3.023 d

[a]Values are the average of three replications ± the standard deviation. Different letters indicate statistically significant differences ($p < 0.05$) in the MDA content.

also been proposed that linoleic acid is a weak precursor of MDA.[35] All of the above-mentioned secondary oxidation products can potentially react with TBA. With the fatty acid composition of the oil samples taken into account, the presence of aldehydes other than MDA can be expected. However, the TBARS test appeared to measure exclusively MDA, and the results suggest that other than secondary lipid oxidation products are responsible for the overestimation of MDA in complex food matrices.

The results show that the TBARS test is reliable when applied for the determination of MDA in vegetable oils and in unprocessed meat and fish products. In processed beef, pork, and fish, dry nuts, cheese, and potato crisps, the TBARS test is overestimating the content of MDA because of the interference of other compounds with TBA. Furthermore, the results also indicated that other than secondary lipid oxidation products interfere with the TBARS test.

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Notes
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