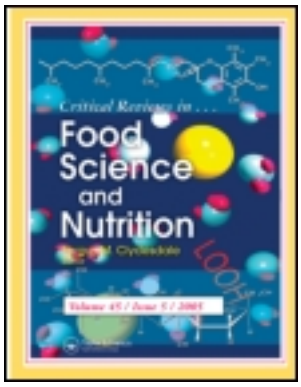

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The Thiobarbituric Acid (TBA) Reaction in Foods: A Review

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The Thiobarbituric Acid (TBA) Reaction in Foods: A Review

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ABSTRACT: The wide diffusion of 2-thiobarbituric acid (TBA) in the scientific literature is due to the TBA assay, or TBA test, which has been employed in the determination of autoxidative alterations of fats and oils. Two processes occur in autoxidation, generally: the free radical and the photo-oxidation mechanisms. The better studied is the free radical mechanism. The hydroperoxiepidioxides and bicycloendoperoxides are malonaldehyde (MDA) precursors. The absorption spectrum obtained with oxidized fatty foods is like the spectrum obtained when TBA and MDA react. However, during the secondary phase of the autoxidation process other aldehydes (alkanals, 2-alkenals, dienals) are formed which react with TBA, and they are responsible for off-flavors. Three kinds of pigments (yellow, orange, red adducts) are involved. Also, aromatic aldehydes, which constitute the flavor profile of diverse fruits and essential oils, form with TBA the characteristic arylidene-2-TBA acids. Other substances, such as ketones, ketosteroids, acids, esters, sugars, imides and amides, amino acids, oxidized proteins, pyridines, pyrimidines, and vitamins can react with TBA; they are named TBARS (substances that react with TBA), and form principally in meats and meat derivatives. Several organic or bio-organic acids, as shikimic and sorbic acids, react photometrically with TBA if a Malaprade reaction takes place before. A structural study of the red adduct TBA-MDA has been carried out.

KEY WORDS: 2-Thiobarbituric acid (TBA), autoxidation of fats, malonaldehyde (MDA), aldehydes, off-flavors, arylidene-2-thiobarbituric acids, TBARS.

I. INTRODUCTION

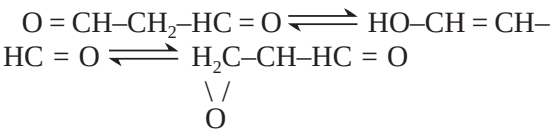
2-Thiobarbituric acid (TBA) is a widely used compound owing to its reactivity, generally with carbonyl substances (aldehydes, ketones), although acids, esters, amides, sugars, and pyrimidine compounds can also display reactivity with TBA.[1] The wide diffusion of TBA in the scientific literature is due to the TBA assay, which has been used in the determination of the autoxidative spoilage of fats and oils.[2] This special facility of its reaction with the C=O group arises as a consequence of the lability of the methylene group in the C-5 position on the molecule. The reagent produces an addition reaction.[3] The products formed show a polar double linkage, and they can be considered as Lewis' organic acids.[4]

Although, like dimedone, TBA could be considered a general reagent for aldehydes, its main utilization has been in the recognition of the fat autoxidation level, or the rancidity of fats. Rancidity is the usual term to describe unsaturated fatty acid oxidation. It is a sensorial term. Originally "rancid" meant fetid, smelly. The oxidation kinetics for unsaturated fatty acids increases by geometric progression. The reaction process is similar in free acids and in esterified fatty ones (glycerides, phospholipids). The TBA assay has become nearly as common as the peroxide values, as previously proposed. It must be taken into account that the TBA value represents the aldehyde level in the lipidic fraction of foods which has been autoxidized. From this point of view, the TBA value is a representative parameter for the

secondary oxidation step, as is the anisidine value. TBA assay is applied in any type of food, although the assay is nearly worthless with fried foods.[5] However, it is a valuable method to measure the first steps of autoxidation in vegetable oils, lard, and cooking fats.[6]

The TBA assay was first described by Kohn and Liversedge[7] in a paper on the oxidation products of animal tissues. These authors suggested that the reactant substance could be a carbonyl compound. Bernheim et al.[8] studied the reaction between TBA and the oxidation products from certain lipidic substances. According to these pioneer experiments, the colored reaction products were alkylidenethiobarbituric acids. Patton and Kurtz[9] were the first authors who employed the assay in foods (milk fat). They verified that the absorption spectrum obtained when oxidized milk fat reacted with TBA was similar to the red pigment which was obtained in the reaction of malonaldehyde (MDA) and TBA. From this behavior, the authors inferred that MDA was the essential compound in the rancidity process and in the biological oxidation of unsaturated fatty acids. Moreover, they demonstrated that the TBA assay was more sensitive than the Kreis test and peroxide value. Glavind and Hartman[10] showed that both TBA and the Kreis assays were appropriate for the measurement of the products of the second step in the autoxidative process (dialdehydes with three carbons, such as epihydrinaldehyde and MDA). To reach this conclusion, they carried out comparative studies with the TBA assay, Kreis test, and peroxide values, using samples of lard and codfish liver oil, and also model substances (hydroperoxides, benzoylperoxide, among others). Furthermore, they stated that the Kreis and TBA assays could be used for other compounds and not only for linolenic acid.

It seems that the results obtained with the TBA assay are highly correlated with the oxidized flavor of animal foods, such as milk. The similar results found with the Kreis and TBA assays occur because epihydrinaldehyde and MDA are in isomeric equilibrium,



However, the TBA assay proved to be more sensitive than the Kreis test.[11] In fact, the Kreis test is obsolete. Dunkley and Jennings[12] proposed the TBA assay and offered certain information about its reproducibility and the influence of diverse factors (pH, heating time, contamination by copper). Also, other studies about the cited factors were carried out with butter, milk powder, and cheddar cheese.[13]

Turner et al.[14] applied, for the first time, the TBA assay in meats (frozen pork). They studied the reproducibility of the method as well as the following factors: carbohydrate presence, optimum heating time, and the size of the sample. In the 1950s, Sidwell et al.[15] applied the TBA assay in vegetable oils for the first time; soybean and cotton oils were thermally oxidized, submitted to the active oxygen method (AOM), and exposed to ultraviolet light. The authors employed the singlet extraction procedure, the solvent extraction of the oxidized substances. Then the removed organic phase was placed with the TBA reagent in an acid medium. The two phases were then shaken, the colored compound was developed in a waterbath, and then it was measured at 530 nm vs. distilled water.[16] A series of partial reviews of the TBA assay have been published in recent years.[17–24] In earlier TBA testing, results were exposed as an absorption or absorbance index. Sinnhuber and Yu[25] introduced the TBA number magnitude.

II. PRODUCTS ORIGINATED IN THE LIPIDIC OXIDATION OF FOODS AND IN *IN VIVO* PEROXIDATION

Reviews of the autoxidation mechanism have been carried out, like that of Ragnarsson and Labuza,[26] which includes the influence of diverse factors, such as temperature, metals, and others. However, undoubtedly Frankel's studies[27–29] have been the most systematic and thorough. He carried out comparative studies between the free radical and photoxidation theories, and he considered everything from the first steps of the mechanism to the final products (volatile, secondary oxidation products). Generally, it is accepted that the first product formed during the mechanism is

an odorless intermediary called hydroperoxide. Two kinds of mechanism operate:

- The classic free radical mechanism, which can act in the dark
- The photo-oxidation mechanism, which is initiated by exposition to light

A third mechanism is still not thoroughly studied: the lipoxygenase mechanism.

The best studied has been the free radical mechanism.[30,31] Within this mechanism, a primary (primary oxidation) and a secondary phase (secondary oxidation) can be distinguished. The primary phase includes the initiation, propagation, and termination steps, in this order. The initiation steps can be presented as:

$$RH + O_2 \rightarrow R\cdot + HOO\cdot$$
$$RH \rightarrow R\cdot + H\cdot$$

A free radical, R·, is formed from the triglyceride or free fatty acid molecule. This occurs by interaction with O_2 in the presence of an initiator. These initiators can be external energy sources: heat, light, and high-energy radiation. But metal ions or metal-proteins can also act as initiators. The propagation step is summarized as:

$$R\cdot + O_2 \rightarrow ROO\cdot$$
$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

The free radical, R·, can react with oxygen to form a peroxide radical. This new radical reacts in turn with a triglyceride or a free fatty acid to produce hydroperoxides, and a free radical is again formed, which can reinitiate the chain process. These considerations are not limited exclusively to molecular oxygen, but also to other species, like O_2^-, HO·, H_2O_2.[32] During the propagation step, a lot of hydroperoxide molecules are formed, as has been supported by Sunderman and William.[33] The termination step is summarized as:

$$R\cdot + R\cdot \rightarrow R\text{–}R$$
$$ROO\cdot + R\cdot \rightarrow ROOR$$

The reaction chain, which propagates itself, stops when the termination reactions appear (e.g., when two radicals react and an inactive substance is formed). During the primary phase, oxidation proceeds slowly and uniformly. This is the induction period. The cyclical behavior of the process can be observed.[34]

A good understanding of the oxidation mechanisms of unsaturated fatty acids, whose incidence is very important in the edible vegetable oils (oleic, linoleic, linolenic acids), could lead to an improvement of the methods to avoid spoilage and an off-flavor presence in vegetable oils.[35] During the autoxidative process of methyl oleate and linoleate, a blend of 9-, 12-, 13-, and 16-hydroperoxides is originated. In the 1960s, the hydroperoxides of methyl linolenate were isolated[36] for the first time. In the presence of initiators or sensitizers, a hydrogen transfer with an adequate acceptor radical, R·, from the doubly activated allylic methylene group, takes place; this occurs at the C-11 and C-14 positions to form two pentadienyl radicals. The reaction with oxygen at the terminal carbon positions produces a blend of four peroxide radicals. From here, the correspondent 9-, 12-, 13-, and 16-hydroperoxides, conjugated dienoic, which have an isolated double bond, are formed. The peroxide radicals of 12- and 13-hydroperoxides undergo a rapid 1,3-cyclation. Peers et al.[37] demonstrated that if 5% α-tocopherol (hydrogen donor) was added, cyclation was inhibited. Because linolenic acid hydroperoxides are unstable, the free radicals of the antioxidant are less effective in oils containing linolenic acid than in oils containing linoleic acid.[38]

The intermediary free radical, formed during the cyclation process, can act in two ways:

- To form hydroperoxiepidioxides with five links.[39]
- To form again a cyclic, as bicyclo-endoperoxides, structurally related to prostaglandins; abundant formation of these substances (conjugated carbonyls with a ring of hydroxipentanone) is observed.

Hydroperoxiepidioxides and bicyclo-endoperoxides are MDA precursors.[40–42]

As model substances in the triglyceride oxidation study of vegetable oils, trilinolein and trilinolenin were purchased.[43] The major autoxidation products obtained from trilinolein were identified as mono-, *bis*- and trihydroperoxides,

which are formed by sequential addition of oxygen. Later, the monohydroperoxides oxidized to give a blend of 1,3- and 1,2-*bis*-hydroperoxides that oxidized later to trihydroperoxides. Trilinolenin autoxidation gives 1(3)- and 2-monohydroperoxides, 1,3- and 1,2-*bis*-hydroperoxides and trihydroperoxides, through sequential oxidation.[43] Thermal oxidation (40°C) of synthetic triglycerides, which contained linoleate and linolenate located at the three possible positions of triglyceride, originated monohydroperoxides and hydroperoxiepidioxides as the major products.[44] Synthetic triglycerides oxidized in the following decreasing order of relative rate:[38]

$$LnLnL > LnLLn > LLnL > LLLn$$

where L = linoleic rest and Ln = linolenic rest.

During the secondary phase, the oxidative process goes quickly. Hydroperoxide fragmentation can take place through a homolytic or heterolytic break.[45] The homolytic β-scission gives alkoxy radicals as intermediaries, which later undergo a scission in the –C–C– bond. Homolytic breaking of a part of the alkoxy carbon gives pentane and methyl 13-oxo-9,11-tridecadienoate from the 13-hydroperoxide of methyl linoleate; and 2,4-decadienal and methyl octanoate are formed from the 9-hydroperoxide of methyl linoleate. The homolytic break gives hexanal and methyl 9-oxo-nonanoate from 13- and 9-hydroperoxides of methyl linoleate, respectively.

In an acid medium, heterolysis gives ethers of carbonium ions as intermediaries, which in turn undergo a selective breaking to reach the same compound formed in the homolytic break, that is, hexanal and methyl 9-oxononanoate.[46] If the reaction starts from methyl linolenate hydroperoxides, outstanding differences are observed; a thermal decomposition of hydroperoxide at 150°C or the use of $FeCl_3$/ascorbic acid as a catalyst is necessary.[47] Thermal decomposition produces a greater quantity of methyl octanoate and 2,4,7-decatrienal and a lesser quantity of 2,4-heptadienal, methyl 9-oxononanoate, and propanal than catalytic decomposition. These formed aldehydes are greatly involved in off-flavors and the biological effects of lipidic oxidation.[45,48]

The influence of lipid oxidation on prooxidants, antioxidants, oxygen content, and the protection against such oxidation has been considered.[49] The decrease of tocopherols was accompanied by the increase of MDA, but 2-TBA acid-reactive substances (TBARS) did not correlate with the change of tocopherols.[50,51] An effective antioxidant product was prepared with ascorbic acid in a solution of propylene glycol or with a nonionic surface-active agent.[52] The effectiveness of natural antioxidants has been reviewed.[53] Butylated hydroxyanisole (BHA) is often added to food packaging materials with the aim of stabilizing against oxidation of lipid foods.[54] The detection and inhibition of singlet oxygen oxidation in vegetable oils and other foods have been reviewed.[55] Vitamin C can interact with vitamin E radicals in the membrane-water interphase, and regenerate vitamin E, which is very active in the biological autoxidation repression.[56] Maillard reaction products inhibited autoxidation by acting as peroxide destroyers.[57]

Some of the MDA detected in the TBA assay is formed during the peroxidation process itself; however, most is generated by decomposition of lipid peroxides during the acid-heating treatment of the assay.[19,58] In the 1970s, the distillation technique was modified through the addition of antioxidants and chelating agents (propyl galate, EDTA), with the aim to avoid the autoxidation of other lipids of the sample.[59] Antioxidants (AH) act through a chain reaction with the peroxy radicals (ROO·) formed during the acid-heating treatment as follows:

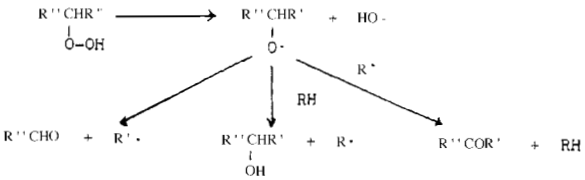
$$ROO\cdot + AH \rightarrow ROOH + A\cdot$$
$$RO\cdot + AH \rightarrow ROH + A\cdot$$
$$R\cdot + AH \rightarrow RH + A\cdot$$
$$OH\cdot + AH \rightarrow H_2O + A\cdot$$

The first among these reactions is considered to be the most important in the overall protection process. Inhibitory potency for several antioxidants has been tested with this scope, and quantified with the TBA assay.[60]

In the photo-oxidation mechanism, in the presence of light, unsaturated fatty acids can form hydroperoxides, by reacting with singlet oxygen, which originates through a sensitized photo-oxidation. When light and some photosensitizer molecules are present, hydroperoxides are formed. Oxygen goes into an excited state (singlet oxy-

gen) through an energy transference mechanism, in the presence of a photosensitizer exposed to light energy.[61] Singlet oxygen appears in the presence of sensitizers, such as chlorophyll, myoglobin, erythrosin and other pigments, riboflavin, and metal ions.[62,63] Tocopherol, as well as carotenoids, inhibits photosensitized oxidation of vegetable oils.[38] Singlet oxygen reacts with methyl linoleate 1500 times faster than triplet oxygen (normal oxygen) to form hydroperoxides.[64] Singlet oxygen reacts directly with the double bonds (addition reaction). An allyl hydroperoxide is formed where the double bond shifted.

Lipid hydroperoxides are very unstable and they undergo cleavage to give free alkoxy radicals, which decompose to give mainly aldehydes, but also ketones and hydrocarbons. Alcohols are formed to a lesser extent, but these do not contribute appreciably to the oxidized flavor profile:



III. ALDEHYDES

During the secondary oxidation process, mainly aldehydes are formed. These are chiefly responsible for off-flavors, either directly or indirectly through their enol or tautomer forms. Gaddis et al.[65] used model substances (methyl oleate, linoleate, and linolenate) to confirm the formation of carbonyl compounds during secondary oxidation. They concluded:

- From linolenate, mainly propanal and 2,4-heptadienal are obtained (as we have already seen, these aldehydes come from 12- and 16-conjugated dienoic hydroperoxides, respectively).
- The amount of minority aldehydes increases as the unsaturation degree of fatty acid increases.
- The amount of carbonyl compounds in the fats correlated with the promedium number of fatty acids from form the fat.
- As thermal oxidation temperature increases, the unsaturated aldehydes formed are higher.

Alkanals give potency, ardour, resonance, profundity, roundness, and freshness during organoleptic evaluation. Generally, when TBA and alkanals react, a colored system that absorbs at 450 nm appears.[66,67] The proposed structure for this chromophore suggests that a single condensation reaction always occurs[68–70]. Patton[71] stated that the utility of the absorbance at 450 nm to measure lipid oxidation was limited because this absorbance occurs in the general reaction between TBA and the aldehydes. However, the absorption at 450 nm correlates with organoleptic evaluation in beef fat, hydrogenated vegetable fat, and cottonseed oil.[72,73] Nevertheless, when alkanals react with TBA, a predominant maximum wavelength at 450 nm is observed, unstable in an aqueous medium.[74] There is no absorption at 530 nm. So, if in the aldehyde blend the alkanals predominate, the adequate wavelength for the measurement is 450 nm.

Aliphatic aldehydes in the presence of catalytic Fe(III) salts degrade to short-chain aldehydes, which can be detected at 450 nm in the TBA assay; these aldehydes are involved in toxicity and mutagenicity problems.[75] In autoxidized chicken fat by the AOM, unstable aldehydes are released, which react with TBA in trichloroacetic acid to give a yellow chromophore (λ_{max} = 455 nm).[76] This chromophore is not formed in an inert N_2 atmosphere and it is unstable in an aqueous medium, but is stable in a glacial acetic acid medium; the colored compound correlates well with peroxide value.[77] Sensory evaluation in cooked beef also correlates with TBA number and hexanal content.[78] When TBA and some TBA derivatives react with alkanals in a 1:1 aqueous-acetic acid medium at 70°C during 30 min, diverse chromophores appear[79] as:

- TBA-heptanal (λ_{max} = 452 nm)
- 1,3-diethyl-TBA-heptanal (λ_{max} = 464 nm)
- 1,3-diphenyl-TBA-heptanal (λ_{max} = 458 nm)

If the reaction takes place in the same aqueous-acid medium, but is heated at 100°C, an orange chromophore TBA-acetaldehyde (λ_{max} = 496 nm) is obtained. The reaction between TBA and heptanal is catalyzed by traces of metal ions (Fe, Cu, Mn). In a model system that contains linoleate and disrupted peroxidase, pentanal and hexanal are recognized by the TBA assay.[80]

Alkenals contribute to more concrete sensory descriptors, e.g., sweetness, fruitiness, oiliness. The absorption intensity of alkenals (λ_{max} = 452 nm) is higher than that of alkanals, although the areas of their absorption bands are similar. Absorption by monoenals depends on the double-bond position.[73] Generally, monoenals (e.g., *trans*-2-hexenal) show maximum absorption at 450 nm and low absorption at 530 nm.[74] α,β-Unsaturated aldehydes, which were recently described, do not show in the assay; but when they are thermally oxidized in the presence of copper (II), they give a positive reaction.[81] 2-Alkenals having from four to eight carbon atoms react with TBA[82] as follows:

- If the aldehyde is placed with TBA in a 1:1 ratio in a 15% acetic acid-aqueous medium at 100°C, after 15 min a yellow chromophore (λ_{max} = 455 nm) appears, and after 6 h have elapsed, a red chromophore appears at 532 nm.
- If, in the same acid-aqueous medium, there is an excess of TBA at 100°C, after 15 min a yellow chromophore is formed, between 2 and 6 h an orange chromophore predominates (λ_{max} = 495 nm), and lastly, after 6 h, the red chromophore predominates.

Autoxidized methyl linoleate and soybean oil, in the presence of initiators (hydroperoxides, iron), show characteristics of reaction with TBA similar to those of alkenals and alkadienals.[83] Reactivity of TBA with alkenals and alkadienals is favored in the presence of Fe(III), and is inhibited in the presence of EDTA. It seems that Fe(III) increases the reactivity of released aldehydes from vegetable oils, instead of producing an increase in the MDA released by the same oils. The formed adduct is suppressed in the presence of copper, probably due to a specific capacity of copper to destroy the adduct.[84] The TBA assay has shown the existence of *trans*-4-hydroxy-2-hexenal and other alkenals that result from the cellular peroxidation of isolated rat hepatocytes.[85] If arachidonic acid is submitted to Fe/ascorbic acid catalysis during 1 d, hexanal and 4-hydroxy-nonenal, among others, are detected by the TBA assay.[86]

Like alkenals, the alkadienals exhibit concrete sensory descriptors, such as sweetness, fruitiness, oiliness. The chromophores obtained with dienals and TBA absorb principally at 532 nm.[66,74] Only 2,4-alkadienals, like 2,4-hexadienal and 2,4-heptadienal, show considerable absorption at 530 nm, together with a little absorption at 450 nm, and an absorption peak at 400 to 410 nm.[73] But the dienals do not absorb in the UV region.[87] The specific extinction of the red pigment is similar to that of MDA when it reacts with TBA (27.5 absorbance units/μmol).[77] Kosugi and Kikugawa[77] state that in the case of dienals, a low absorption peak appears at 495 nm, with a specific extinction of 2 absorbance units/μmol. Fats and oils with a high percentage of linoleic acid or linoleates give 2,4-alkadienals after prolonged thermal oxidation, as is demonstrated by the TBA assay.[88,89]
Peroxides and hydroperoxides of linoleates and linolenates decompose to give alkanals (e.g., hexanal and others). Through aldolic condensation and subsequent dehydration, two alkanal molecules became substituted 2-alkenals. These alkenals reacted with TBA to give an adduct in the C-5 location. This adduct is rather labile, and it autoxidized to give a new 2-propenal adduct, which in turn, underwent a Michael addition of a second TBA molecule,[3] and the 2:1 TBA-MDA red pigment is formed.[83,90]

Disfunctional derivatives (dicarbonyl compounds) can be off-flavor components in rancid foods. One example is glyoxal, with adduct wavelengths at 525 and 550 nm, which is present in irradiated meats.[91] Smith[92] stated that a red pigment glyoxal-TBA is formed after development at 100°C during 10 min.[9] Most authors[73] attribute the absorption of the red chromophore at 532 nm to the MDA produced in the oxidative cleavage of unsaturated lipids.[25,93] It has been shown that very different autoxidized foods with TBA give the same absorption spectrum (λ_{max} = 530 nm) as pure MDA with TBA present.[87]

The red pigment formed in the TBA assay was attributed for a long time to a dialdehyde with three carbon atoms, which appears in the last phase of the oxidation process in unsaturated fatty acids.[8] The red pigment was identified with the polymethinic pigment obtained in the reaction of TBA and tetraethoxypropane (TEP) or tetramethoxypropane (TMP); both of these release MDA by hydrolysis.[2] Several authors[73,94] have

correlated the TBA index or TBA number with the organoleptic scores. Täufel and Zimmerman[79] carried out a comparative spectral analysis of the MDA reaction with:

- Barbituric acid-MDA: λ_{max} = 485 nm
- 2-TBA-MDA: λ_{max} = 530 nm
- 1,3-diethyl-2-thiobarbituric acid-MDA: λ_{max} = 540 nm
- 1,3-diphenyl-2-thiobarbituric acid-MDA: λ_{max} = 537 nm

The authors obtained the best recovery by heating at 70°C during 30 min in a 50% aqueous-acetic acid medium. This reaction has been revised, and the mechanism and potential interferences discussed.[95,96]

A polymethinic dye (λ = 533 nm) has been extracted from chloroplasts (vegetable) with ethyl acetate-benzene-buthanol as the extractant, then separated by high-performance liquid chromatography (HPLC).[97] If unsaturated fatty acids are treated by the AOM, and are then placed with TBA in a trichloroacetic medium, the absorbance at 532 nm increases more rapidly than at 455 nm. The explanation supposes that during the autoxidation process, MDA is released slowly in the early steps, and rather rapidly in the last steps.[76] The red pigment shows good development when stoichiometry was MDA-TBA 1:1; in an excess of TBA there is less development.[82] The formation of the red adduct is favored in the presence of Fe(III) and is inhibited in the presence of EDTA.[84] When linoleic, linolenic, and arachidonic acids are submitted to UV radiation, a complex blend which reacts with TBA is obtained: alkanals (formaldehyde, acetaldehyde, hexanal), alkenals (4-hydroxy-2-nonenal), β-dicarbonyl compounds (acrolein, MDA).[98]

Other aldehydes can also react with a TBA reagent. For example, if acetaldehyde, crotonaldehyde, and succinylaldehyde are placed with TBA in a 50% aqueous-acetic acid medium at 100°C during 30 min, an outstanding absorption peak appears at 496 nm.[79] By hydrolysis in an acid medium, epihydrinaldehyde becomes glyceraldehyde, which reacts with TBA.[99] Likewise, a reaction between glycilaldehyde and TBA has been shown.[100] Furfural and its derivatives give yellow compounds with TBA; nonenzymic browning can be inferred from the reaction between hydroxymethylfurfural and TBA.[101]

Aromatic aldehydes react easily with TBA to produce diverse colored pigments, the arylidene-2-thiobarbituric acids.[1] These aldehydes contribute to the flavor profile of fruits and essential oils. Anisaldehyde and salicylaldehyde are present in anise and vanilla extracts.[102,103] Physicochemical properties and other features of these compounds have been studied in various papers.[103–113] Also, Akiyama et al.[114] have studied the synthesis and properties of some of these aromatic aldehydes. Absorption of arylidene-2-thiobarbituric acids is less uniform and, consequently, they are more difficult to systematize than aliphatic aldehydes; aromatic ketones react with more difficulty than aldehydes.[115] Several aliphatic aldehydes (formaldehyde, acetaldehyde, and citral) do not react under the same reaction conditions as those of aromatic aldehydes.[69] The structural composition of these arylidenethiobarbituric acids shows a larger variety. Many of these derivatives melt and decompose above 300°C and dissolve easily in alkali or protophilic solvents.

Benzaldehyde derivatives with different substituents in the aromatic ring showed absorption spectra that are rather dependent on the type and location of the substituent in the aromatic ring (*orto-*, *meta-*, *para-*), being able to give rise to structures with a 2:1 TBA-to-aldehyde ratio, in certain cases, as the salicylaldehyde.[109] Cinnamaldehyde offers with TBA a strong absorption maximum at 450 nm, and other maximum at 530 nm of very low absorptivity. Nevertheless, in organic solvents, such as chloroform, the maximum at 530 nm can undergo simultaneously batochromic and hyperchromic effects.[116]

IV. MDA

Patton[71] comments on the increasing utility of the TBA test, and he concludes that a large number of aldehydes and other carbonyl compounds, as a result of secondary oxidation, produce a three-carbon dialdehyde, as MDA or similar. It has long been known that unsaturated oils and fats produced MDA in the last steps of the autoxidation

process. In the case of oleic acid and oleates, the presence of Cu(II) as a catalyst is necessary. Even saturated organic acids, through a controlled oxidative process, give MDA.[117] Kosugi and Kikugawa[76] state that MDA is released slowly in the earlier stages and rapidly in the later ones. It seems that a low pH value is necessary for MDA to be released.[67] Although MDA contributes partially, it is certain that MDA and rancidity are closely related.[91] A low pH, although not excessively acid, is essential for the release of MDA as well as for the development of the colored adduct with TBA (Figure 1).[83,96] A low pH value and a heat source release MDA from its adducts in food (e.g., amino acids, proteins).[118]

A practically anhydride medium (glacial acetic acid) is unfavorable for the reaction development. In the presence of concentrated mineral acids or oxyacids, alteration of the TBA molecule has been shown.[96] On the other hand, all the procedures which develop the TBA assay employ invariably TBA dissolved in an acidic medium.[24] It has been proved that the TBA structure does not change if it is submitted to heating treatment in a diluted acidic medium.[96,119,120] For high-acid concentrations, especially with oxyacids, an oxidative-hydrolytic effect could be produced.[1,121] Trichloroacetic acid must only be used if the TBA assay would be applied to protein foods, at a concentration ≤0.5%, whenever an optimum pH ca. 2 was reached.[122] A high concentration of acetic acid affects negatively the sensitivity of the TBA assay.[76] TBA derivatives are more stable in acidic and neutral media than in an alkaline medium; in the latter medium, hydrolysis takes place.[1,123–125] The 2:1 TBA-MDA red adduct presents the better stability in an optimum pH of 2.9.[16] MDA is not a stable product.[126] Sinnhuber and Yu[25] were the pioneers in expressing the results of the TBA assay as milligrams of MDA per unit of weight of food.

Dahle et al.[40] describe a mechanism which was valid until Frankel's research. They studied fatty acids with a diverse unsaturation degree (dienes, ..., hexaenes). In the first stages of oxidation, the results of the TBA assay change linearly with dienoic conjugation, peroxide value, and oxygen absorption. This research definitively demonstrated the presence of a cyclic peroxide with five links which, through a chain propagation (intramolecular redistribution), leads to MDA by a degradative process. Pryor et al.[41] support the Dahle et al.[40] theory: MDA appears in thermal or acid-catalyzed decomposition of endoperoxides with three or more double bonds formed during polyunsaturated fatty acid (PUFA) autoxidation. By using combined mass spectrometry-gas chromatography techniques, Frankel and Neff[127] studied MDA formation. These authors conclude that the most outstanding MDA precursors are cyclic hydroperoxides (with five links), epidioxides, and 1,3-dihydroperoxides.

When equimolar quantities of TBA and MDA react, an intermediary product (colorless adduct) is first formed, which is only stable in an inert (N_2) atmosphere. In aerobic conditions, this colorless adduct gives a mixture of the yellow (450 nm) and red pigments, with an outstanding prevalence of the red pigment. With excess TBA, the colorless adduct decomposes to give a mixture of the yellow, orange, and red pigments.[82] It is evident that the presence of iron increases TBA-MDA reactivity. Some of the proposed techniques include the presence of iron in the reaction medium.[128] A distinction can also be made, especially with biological samples such as microsomal incubations, between free and labile MDA, which proceeds from lipid peroxidation.[129] Free MDA is present in a small percentage in foods and is scarcely detectable in edible vegetable oils.[130]

V. OTHER SUBSTANCES THAT REACT WITH TBA

Besides the aldehydes, MDA included, other substances such as ketones, ketosteroids, acids, esters, sugars, imides and amides (urea), amino acids, oxidized proteins, pyridines, and pyrimidines can react with TBA, with the result that they are called TBARS (substances that react with TBA). This fact could concede a new attractiveness to the TBA assay, inasmuch as it would be applicable to nearly all foods, and could make it useful in detecting oxidative spoilage in material distinct from the triacylglycerides or fatty acids.

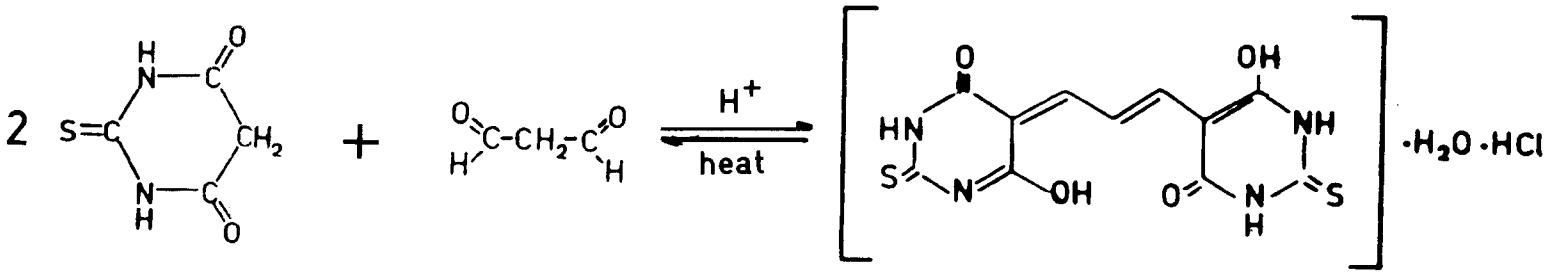


FIGURE 1. TBA-MDA reaction in the TBA assay.

Kwon et al.[118] inferred the formation of these adducts.

Substances formed especially in meats and meat derivatives are usually designated as TBARS. In cooked poultry meat, Salih et al.[131] used perchloric acid to extract TBARS. In ground beef, the TBARS (at 24 h "post-mortem") showed a strong correlation with the percentage of PUFAs (%).[132] TBARS were determined in autoxidized meats, and the recovery of TBA by single-extraction and distillation techniques was compared.[133,134] By using gas chromatography, hexanal and 2,3-octanedione were determined in cooked beef; these carbonyl compounds exhibit a strong correlation with sensory appreciation.[78]

In the original TBA distillation technique, it was necessary to heat at 100°C for 1 h, with the aim of releasing TBARs completely; but if autoclave heating is used, only 15 min is necessary.[135] In a routine control of freezing sardines, the threshold limit for consumption was fixed at 1.1 mg/kg TBARS.[136] In the disrupted protein of egg yolks, TBARS were measured at room temperature.[137] Using the TBA assay, several amine-MDA adducts were recognized by Buttkus and Bose;[138] with compounds such as – RN=CH–CH=CHOH and – RN=CH–CH=CHNHR, MDA recovery (from a molar basis) was 100%. However, in derivatives from pyrazole and pyrazolines, the recovery was 4 to 0%.

In thermal oxidation of methyl linoleate, MDA was not detected, but several types of lipophile TBARS were recognized by HPLC.[139] During thermal oxidation (170°C) of soybean oil in the presence of tocopherol, MDA amounts increased as the temperature increased; nevertheless, TBARS amounts were constant during the heating process.[140]

TBA reagent reacts directly with formic,[141–143] glyoxylic,[144] glycolic, and glyceric acids.[141] Shikimic,[145] sialic,[146] saccharinic,[147] and sorbic acids[148] react photometrically with TBA, if oxidation with potassium peryodate has previously occurred (Malaprade reaction). The TBA-sorbic acid reaction proves that TBA can react with conjugated double-bond compounds.[149]

Glyceraldehyde reacts with TBA to form a colored compound (λ_{max} = 440 to 460 nm).[99] When carbohydrates are heated at 100°C in the presence of TBA, a deep absorption band (λ_{max} = 454 nm) appears.[150] The best sensitivity is obtained with fructose.[144] So, the presence of sugars causes considerable interference in the TBA assay, with an absorption band located between 450 and 460 nm.[141]

Pyridinic and pyrimidinic derivatives, through an oxidative process, undergo the cleavage of the heterocyclic ring and MDA is formed.[151] Shepherd[70] states that TBA is a specific reagent for the pyrimidine ring, whenever the four-, five-, or six-ring locations are not replaced. The reaction products absorb at 532 nm (pH = 1) and at 540 nm (pH = 7 to 11); but the reaction time changes in accordance with the kind of pyrimidine derivative. Although the formation kinetics are variable, molar absorption is the same. Thus, a sole compound, MDA, is the reacting substance.[2,25]

The presence of orotic acid in multivitaminic preparations has been detected with the TBA assay.[152] TBA is also an appropriate reagent for pyridoxine[153] and for the ketonic compounds formed during an extended storage of ascorbic acid.[154]

VI. FORMATION OF COLORED PIGMENTS

When linoleic acid and methyl linoleate are oxidized by UV light during 2 weeks, and their oxidation products and TBA are heated together, a blend of three pigments is obtained. These pigments, once separated by HPLC, are yellow (450 nm), orange (495 nm), and red (532 nm). If the solution is submitted to heat during 10 to 50 min, the absorption of orange and red pigments increases, whereas the absorption of the yellow pigment decreases and disappears at 60 min.[155]

If sodium nitrite is added to the TBA reagent, the yellow pigment does not appear and the sensitivity of the red adduct considerably increases.[156] Marcuse[143] emphasized that red pigment offers an optimum development at about 100°C (waterbath); in this manner the formation of the interferent yellow pigment is avoided. Although in the first steps the yellow and red pigments could be useful in revealing lipid oxidation, the yellow pigment is more thermolabile.[157] The yellow pigment discol-

ors if sodium hydroxide is added, but reverts to yellow when placed in an acid medium.[79] In the case of equimolar quantities of TBA and MDA, uncolored adducts are formed at the beginning; then, in aerobic conditions, a blend of the yellow and red pigment is obtained. Nevertheless, if there is an excess of TBA with respect to MDA, at the end a blend of yellow, orange, and red pigments is obtained.[82] α,β-Unsaturated aldehydes with biological activity, such as acrolein, crotoaldehyde, *trans, trans*-muconaldehyde, and other short-chain alkenals react with TBA to give an orange pigment (λ_{max} = 495 nm).[77,158]

Red pigment was first obtained from three different sources: rancid salmon oil, sulfadiazine, and an acid hydrolyzate of tetraethoxypropane (TEP). The adduct was a crystalline compound (deep violet needless) with an absorption maximum at 530 to 535 nm, and others at 305 and at 245 nm. By paper chromatography the authors concluded that the red pigment was the same from the three sources.[25] By hydrolysis at 100°C during 5 to 10 min, new absorption maxima were formed: 262 to 265 nm (TBA) and 240 nm (MDA). TBA hydrolyzes in an acid medium to give malonic acid and thiourea.[1,67] A structural study of the red pigment has been carried out.[159,160]

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