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A Review of the Methodology of the 2-Thiobarbituric Acid Test

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ABSTRACT

The 2-thiobarbituric acid (TBA) test for the measurement of lipid oxidation was discovered over 40 years ago. Since then it has been widely used for the measurement of the oxidative state of biological and food materials. Despite copious literature references and its widespread usage there are still uncertainties over the exact chemistry of the reaction and its applicability. This review attempts to draw together the relevant publications and discuss the merits of the TBA test, particularly in the analysis of foods.

HISTORY AND CHEMISTRY OF THE TBA TEST

In 1944, Kohn & Liversedge observed that animal tissues which had been incubated aerobically with 2-thiobarbituric acid (TBA) produced a pink colour. Bernheim *et al.* (1948) found the colour was the result of a complex formed from oxidation products of unsaturated fatty compounds and TBA. This reaction forms the basis for the most widely used test for measuring the extent of oxidative deterioration of lipids—the TBA test (Melton, 1983; Igene *et al.*, 1985).

The chemistry of the reaction is still not fully understood but it is thought that malondialdehyde (MA), a product of lipid oxidation, is the major TBA reactive substance (TBARS) (Yu & Sinnhuber, 1957; Sinnhuber & Yu, 1958; Sinnhuber *et al.*, 1958; Tarladgis *et al.*, 1960, 1962). Other workers (Patton &

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Kurtz, 1951, 1955; Saslaw *et al.*, 1963; Kwon *et al.*, 1965; Patton, 1974; Baumgartner *et al.*, 1975; Esterbauer *et al.*, 1982; Igene *et al.*, 1985; Kosugi & Kikugawa, 1985, 1986; Kosugi *et al.*, 1988) have reported that other oxidation products may also be involved including α,β-unsaturated aldehydes (e.g. 4-hydroxyalkenals) and several unidentified non-volatile precursors of these substances.

Esterbauer *et al.* (1982) reported that α,β-unsaturated aldehydes formed a major portion of the carbonyl products of liver microsome peroxidation. These compounds, however, gave molar absorption coefficients at 535 nm which were significantly lower (1000 times) than MA when reacted with TBA. It has also been reported that alkanals (Kosugi & Kikugawa, 1986), 2-alkenals (Patton & Kurtz, 1955; Kosugi *et al.*, 1987) and 2,4-alkadienals (Marcuse & Johansson, 1973; Esterbauer *et al.*, 1982; Kosugi *et al.*, 1988) can all produce chromagens absorbing at around 532 nm when reacted with TBA under specific reaction conditions. In a detailed study of the reaction of TBA with aldehydes Kosugi *et al.* (1988) reported that effective complex formation from 2,4-alkadienals required pretreatment of the reaction mixture at 5°C before heating at 100°C, whereas formation from other aldehydes (alkanals, 2-alkenals) did not. They found that for the reaction of TBA with 2,4-alkadienals, the ratio of reactants, presence of water, oxygen, other aldehydes and hydroperoxides, and time and temperature of pretreatment all influenced pigment formation and proposed a two step reaction TBA test to measure lipid oxidation products such as 2,4-alkadienals. They concluded that a large part of the pink pigment produced in the TBA test may be due to monofunctional aldehydes. Saslaw & Waravdekar (1965) presented evidence from TLC studies of extracts of irradiated fatty acids that none of the TBARS was MA.

Yu & Sinnhuber (1964) stated that decomposition of hydroperoxides or MA derivatives/precursors by acidic TBA reagent gives rise to free MA. Dahle *et al.* (1962) postulated a mechanism of MA formation and concluded that only peroxides which possessed unsaturation β,τ to the peroxide group were capable of undergoing cyclisation to form MA and that such peroxides formed only from fatty acids with three or more double bonds. More recently, Frankel & Neff (1983) investigated formation of MA from a wide assortment of primary and secondary lipid oxidation products and reported five-membered hydroperoxy epidioxides and 1,3-dihydroperoxides to be the most important precursors of MA.

The theory that TBARS is formed in substantial amounts only from polyunsaturated fatty acids containing three or more double bonds is supported by other workers (Pryor *et al.*, 1976; Sinnhuber & Yu, 1977; Rhee, 1978; Bird & Draper, 1984). However, Tarladgis & Watts (1960) claimed that fatty acids with less than three double bonds also appear to give rise to

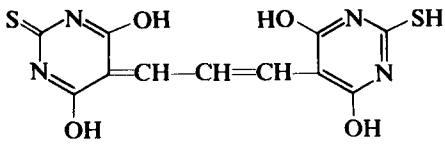
smaller amounts of MA. Lillard & Day (1964) reported that secondary oxidation of 2-nonenal and 2,4-heptadienal derived from the oxidation of methyl linoleate yielded small amounts of TBARS. They postulated formation of three isomeric hydroperoxides from 2-nonenal, the oxidative degradation of one of which would yield MA. Sinnhuber & Yu (1977) claimed that the TBA reaction that does occur with polyunsaturated fatty acids containing less than three double bonds, such as that reported by Tarladgis & Watts (1960), is partially due to secondary oxidation of primary carbonyl compounds (e.g. 2-nonenal).

Pryor *et al.* (1976) studied endoperoxides (2,3-dioxanobornane compounds) related to prostaglandin biosynthetic pathways and showed them to be non-volatile precursors of MA capable of yielding MA with heat or acid. Such endoperoxides are thought to give rise to at least some MA in meat samples. MA has also been identified among the products of the oxidative decomposition of amino acids, complex carbohydrates, pentoses, and hexoses formed in the presence of a metal catalyst and as a product of free radicals generated by ionising radiation *in vivo* (Bird & Draper, 1984).

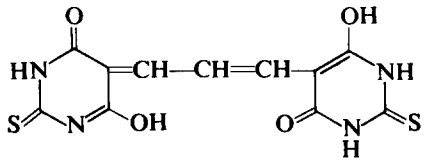
MA is thought to be a carcinogenic initiator and mutagen and therefore can affect the safety of food (Shamberger *et al.*, 1977; Newburg & Concon, 1980; Shahidi *et al.*, 1987*a*). It has been found that the type of cooking (e.g. microwave, roasting) (Newburg & Concon, 1980) and cooking time and temperature (Huang & Greene, 1978) both affected MA content. In the pH range of most fatty foods, in the presence of water, MA exists as the dissociated, non-volatile, enolate anion (Kwon & Watts, 1964; Kwon *et al.*, 1965; Igene *et al.*, 1985). Because of its reactivity, MA can complex with amino acids, proteins, glycogen and other food constituents to form products in which MA is in a bound form (Kwon *et al.*, 1965; Kakuda *et al.*, 1981; Bird & Draper, 1984; Negbenebor & Chen, 1985). Sinnhuber *et al.* (1958) reported that less than 2% of total MA measured in a highly oxidised sample of salmon oil was in the free form.

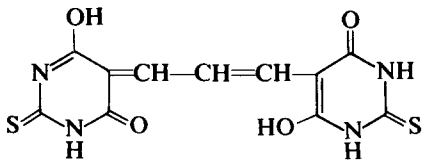
The TBA reaction measures the total MA present in free form under the conditions of the TBA reaction (Bird & Draper, 1984). Sinnhuber *et al.* (1958) reported that the reaction is thought to involve one molecule of MA which reacts with two molecules of TBA with the elimination of two molecules of water (Fig. 1(A)) to yield a pink crystalline pigment with an absorbance maximum at 532–535 nm and secondary maxima at 245 nm and 305 nm. Yu *et al.* (1986) used modern analytical techniques (e.g. mass spectrometry, Fourier transform infrared (FTIR) and high pressure liquid chromatography (HPLC)) to elucidate the TBA–MA structure and reported the adduct to be unequivocally established as that previously proposed by Sinnhuber *et al.* (1958) (Fig. 1(A)).

In a separate study using modern analytical techniques (FTIR and NMR)



(A)





(B)

Fig. 1. (A) TBA–MA adduct proposed by Sinnhuber *et al.* (1958). (B) Two tautomeric forms of TBA–MA adduct proposed by Nair & Turner (1984).

Nair & Turner (1984) claimed that the adduct exists as two spectrally equivalent tautomeric structures (Fig. 1(B)) and that formation of the 2:1 adduct of TBA and MA is probably initiated by nucleophilic attack involving C–5 of TBA onto C–1 of MA. This is thought to be followed by dehydration and similar subsequent reaction of the intermediate 1:1 adduct with a second molecule of TBA. Kosugi *et al.* (1988) reported that water and oxygen were necessary in the formation of the pink pigment with absorption maximum at 535 nm in the reaction of TBA with alkanals, 2-alkenals and 2,4-alkadienals and that hydrolytic and oxidative mechanisms may therefore be involved in the complex formation. Nair & Turner (1984) also noted that variation in concentration of solutions and presence of contaminants might cause prototropic shifts to favour equilibrating structures similar to those proposed (Fig. 1(B)) but bearing 3-hydroxyl and 2-amide hydrogens. Sinnhuber & Yu (1958) proposed that the amount of MA in a sample could be expressed as TBA number (mg of MA per 1000 g of sample).

Heat and strong acid are thought to be essential for liberation of MA from a precursor, for condensation with TBA and for maximal colour development (Patton & Kurtz, 1951; Sidwell *et al.*, 1955; Sinnhuber *et al.*, 1958; Tarladgis *et al.*, 1960; Kwon & Watts, 1964; Kwon *et al.*, 1965; Pikul *et al.*,, 1983). Tarladgis *et al.* (1964), however, reported that the acid–heat treatment only accelerates the procedure and showed that maximum colour

development could be obtained after 15 h using an unacidified aqueous extract. Kwon *et al.* (1965) claimed that this method may only be useful when insoluble TBARS–protein products are absent from the sample. Salih *et al.* (1987) reported no significant difference in colour formed or TBA number between incubation at room temperature for 15–17 h and boiling for 30 min when using an aqueous extraction method. They recommended incubation at room temperature to avoid potential interference reported when using the boiling method. Contrary to this report, Pikul *et al.* (1989) tested the same aqueous extraction method with 1 h boiling and 15 h incubation at room temperature and found boiling to give significantly higher (1·3–1·4 times) results. Tarladgis *et al.* (1962) reported that the structure of TBA is altered upon acid–heat treatment leading to degradation products which absorb at around 530 nm. In a later investigation into this report Yu & Sinnhuber (1964) noted the absorbance was due to impurities in the acetic acid reagent and therefore stressed the need for pure reagents.

Colour development during the TBA test is usually assessed by measuring the absorbance of the pink chromagen at 530–537 nm (Melton, 1983). It has been reported, however, that reaction with TBA can produce yellow (455 nm), orange (495 nm) and pink (532 nm) absorbing pigments depending on the conditions of the reaction and the TBARS present (Kosugi & Kikugawa, 1985; Kosugi *et al.*, 1987). Jacobson *et al.* (1964) used a modified one-phase system to monitor a yellow pigment with absorbance maximum at 452 nm and reported satisfactory results. The formation of the yellow colour has been attributed to the acidic degradation of TBA (Tarladgis *et al.*, 1964) or the TBA method employed (Schwartz & Watts, 1957). Fioriti *et al.* (1974) reported that monounsaturated aldehydes are responsible for the yellow chromagen whereas the pink chromagen was due mainly to diunsaturated compounds.

Earlier work on the TBA reaction described the formation of a yellow chromagen on reaction with a number of aldehydes including epihydrin aldehyde and glyceraldehyde (Patton, 1960), hydroxymethylfurfural (Keeney & Bassette, 1959), various aromatic aldehydes (Dox & Plaisance, 1961) and 2,4-decadienal (Patton *et al.*, 1959). Marcuse & Johansson (1973) found that all the aldehydes they studied formed the yellow chromagen but only alka-2,4-dienals, and to a lesser extent alk-2-enals, formed the pink chromagen. They concluded that both the yellow and pink chromagens should be measured separately for the grading of rancidity. Patton (1974) agreed with these results and reported that the value of measuring absorbance at 452 nm is limited as it is produced by aldehydes in general which therefore may not be products of lipid oxidation. Kosugi & Kikugawa (1985) found that the yellow pigment formed with a variety of aldehydes was unstable in the TBA reaction. There are also reports that the

yellow chromagen can be formed with a number of non-aldehyde compounds and has been found to interfere with the determination of the pink chromagen. This subject is discussed in more detail in a later section of this paper.

TBA TEST PROCEDURES

The various TBA test procedures which have been used on food products can be divided into four major types:

1. Test on the whole sample (Biggs & Bryant, 1953; Turner *et al.*, 1954; Schwartz & Watts, 1957; Yu & Sinnhuber, 1957; Sinnhuber & Yu, 1958; Younathan & Watts, 1960; Tsoukalas & Grosch, 1977; Ohkawa *et al.*, 1978; Uchiyama & Mihara, 1978; Williams *et al.*, 1983; Pokorny *et al.*, 1985).
2. Test on an aqueous or acid extract of the sample (Dunkley & Jennings, 1951; Patton & Kurtz, 1951; Caldwell & Grogg, 1955; Younathan & Watts, 1960; Tarladgis *et al.*, 1964; Witte *et al.*, 1970; Fioriti *et al.*, 1974; Vyncke, 1975; Siu & Draper, 1978; Newburg & Concon, 1980; Hung & Slinger, 1981; Pikul *et al.*, 1983, 1989; Kosugi & Kikugawa, 1985; Poste *et al.*, 1986; Salih *et al.*, 1987).
3. Test on a steam distillate (Sidwell *et al.*, 1955; Tarladgis *et al.*, 1960; Keskinel *et al.*, 1964; Rhee & Watts, 1966*a*; Witte *et al.*, 1970; Seo, 1976; Shamberger *et al.*, 1977; Gokalp *et al.*, 1978, 1983; Huang & Greene, 1978; Rhee, 1978; Siu & Draper, 1978; Chen & Waimaleongora, 1981; Rhee & Ziprin, 1981; Yamauchi *et al.*, 1982; Pikul *et al.*, 1983, 1989; Williams *et al.*, 1983; Igene *et al.*, 1985; Negbenebor & Chen, 1985; Ang, 1986; Salih *et al.*, 1987; Shahidi *et al.*, 1987*a*; King & Earl, 1988; Hoyland & Taylor, 1989).
4. Test on extracted lipid from sample (Younathan & Watts, 1960; Pikul *et al.*, 1983, 1989).

The whole sample method is reported to be quantitative (Sinnhuber & Yu, 1958), but is very time consuming and involves many solvent extractions (Yu & Sinnhuber, 1957; Almandos *et al.*, 1986). It is also thought that oxidation is brought on by the nature of the whole sample test itself (Tarladgis *et al.*, 1960). Witte *et al.* (1970) claimed that the solvent extraction method is easier to use than the distillation method, uses less equipment and heating is not essential. Salih *et al.* (1987) and Pikul *et al.* (1989) also found solvent extraction methods to be faster and easier to perform than distillation and Pikul *et al.* (1989) recommended solvent extraction procedures for use where a large number of samples need to be analysed rapidly. However, Hoyland & Taylor (1989) proposed a distillation method which employed a rapid

distillation apparatus capable of significantly reducing the analysis time when compared to other distillation procedures.

The distillation method has been found to give lower recoveries compared to the solvent extraction method (Siu & Draper, 1978; Williams *et al.*, 1983; Salih *et al.*, 1987) but is considered more sensitive and also more suitable for high fat samples (> 10%) where turbidity may occur in extracted samples (Siu & Draper, 1978; Williams *et al.*, 1983; Salih *et al.*, 1987). Turbidity may also pose a problem in the solvent extraction method in samples high in carbohydrate or microbial contamination (Salih *et al.*, 1987). Although Salih *et al.* (1987) and Pikul *et al.* (1989) reported high correlation between solvent extraction and distillation methods it has been noted by several workers that the solvent extraction method gives lower TBA numbers than the distillation method for duplicate samples (Witte *et al.*, 1970; Vyncke, 1975; Salih *et al.*, 1987). Pikul *et al.* (1983) suggested that the lower value is due to reduced sample autoxidation during the extraction method. This hypothesis was supported by Siu & Draper (1978) who reported no differences between TBA values determined by distillation and solvent extraction methods when antioxidants were added to meat samples prior to analysis. Rhee (1978), however, attributed the higher value from the distillation method to thermal decomposition of the MA precursor and its liberation by heat from its bound state with proteins.

Pikul *et al.* (1989) were of the opinion that the extracted fat method gave TBA numbers higher than the distillation method for replicate chicken meat samples. They recommended the lipid extraction procedure was particularly appropriate when the susceptibility to oxidation of different kinds of lipids or individual lipid components (e.g. phospholipids) was studied. The lipid extraction method is suitable because it expresses lipid oxidation in mg of MA per unit of lipid as opposed to the more usual mg MA per kg sample.

Sinnhuber & Yu (1977) provided a comprehensive listing of food applications on which the various TBA test procedures have been applied. Although tests on the whole sample or on the extracted fat may be appropriate for some samples, the distillation method has the advantage that it can be applied to any foodstuff and is both rapid and reproducible (Sidwell *et al.*, 1955). The fact that the TBARS is obtained in clear aqueous solution, so that the pink reaction product can be measured accurately, is a major advantage (Tarladgis *et al.*, 1960; Bird & Draper, 1984). Distillation also reduces interference, noted in the whole sample and extracted fat procedures, by compounds which may be present in a food sample. Sinnhuber & Yu (1977), however, note that the main disadvantage of the distillation method is that distillation is an empirical procedure requiring the collection of a specified volume of distillate.

INTERFERENCE IN THE TBA TEST

Sinnhuber & Yu (1977) found interference due to the yellow chromagen (λmax 450–460 nm) overlapping the pink peak (λmax 530–537 nm) causing erroneously high values if it was of sufficient intensity. Crackel *et al.* (1988), working with fresh products, noted interference from the yellow chromagen but found the interference became less significant as TBA numbers increased. It has already been noted that the yellow chromagen may be formed by a variety of aldehydic compounds reacting with TBA. Wertheim & Procter (1956) noted that the yellow chromagen interference could be ascribed to sugars and their degradation products. Earlier, Wilbur *et al.* (1949) demonstrated the formation of yellow colours on the reaction of TBA with sugars (galactose, maltose, dextrose, fructose, sucrose). Biggs & Bryant (1953) noted interference in the TBA test caused by lactose and both Turner *et al.* (1954) and Baumgartner *et al.* (1975) found sucrose formed a yellow complex when reacted with TBA.

More recently, Salih *et al.* (1987) demonstrated the occurrence of an interfering yellow pigment when poultry meat samples containing added sugar were subjected to TBA analysis. They suggested that solvent extraction methods should only be used if compounds producing the yellow pigment are absent, or present in small quantities that do not interfere. Caldwell & Grogg (1955) and Yu & Sinnhuber (1962) proposed modifications of the whole sample method that permit chromatographic separation of the interfering yellow colour from the pink chromagen prior to analysis, and Asakawa *et al.* (1975) claim that addition of sodium sulphite to the TBA reaction mixture prevents production of the yellow chromagen and enhances development of the pink chromagen.

It has been reported that a product of the pyrolysis of sucrose appears to react with acetaldehyde and TBA to form a pink chromagen absorbing at 532 nm (Baumgartner *et al.*, 1975). This may be evidence for the formation of a 532 nm chromagen in the total absence of lipid peroxidation. Other substances which have been reported to produce interference in the various TBA test procedures include proteins (Chio & Tappel, 1969; Buttkus & Bose, 1972; Shamberger *et al.*, 1977; Gardner, 1979; Pietrzyk & Stodola, 1981; Negbenebor & Chen, 1985), plant pigments (Shamberger *et al.*, 1977; Bird & Draper, 1984), seasonings (Siu & Draper, 1978) and formaldehyde (Almandos *et al.*, 1986; Careche & Tejada, 1988).

Besides organic compounds, interference has been noted with various metal ions (Dugan, 1955; Castell & Boyce, 1966) including copper (Dunkley & Jennings, 1951; Patton & Kurtz, 1955; Patton *et al.*, 1959) and both ferric and ferrous iron (Jacobson *et al.*, 1964; Wills, 1964; Castell & Boyce, 1966). Jacobson *et al.* (1964) suggested that ferric iron may cause the breakdown of

TBA itself. Bird & Draper (1984) state that iron salts catalyse the breakdown of hydroperoxides to MA and catalyse degradation of amino acids, sugars (deoxyribose, hexoses, pentoses) and DNA in the presence of air to yield MA.

Ke *et al.* (1984) assessed the interference of many compounds including short chain fatty acids, sulphur-containing compounds, transition metals and antioxidants and found none to interfere in the distillation method. Smith & Alford (1968) found that certain bacteria (e.g. *Pseudomonas*) completely destroyed alka-2,4-dienals and Gray (1978) therefore noted that variations in TBA values in poultry and other meat products might be due to varying levels of micro-organisms present, or changes in microflora. In a study of antibiotic effects Moerck & Ball (1974) found that aureomycin inhibited bacterial growth and subsequently increased TBA number drastically for chicken meat.

Some interference, however, is not removed by distillation including that from woodsmoke components (Turner *et al.*, 1954) and nitrite (Zipser & Watts, 1962). Zipser & Watts (1962) modified the distillation method by adding sulphanilamide prior to distillation in order to prevent nitrite interference. Shahidi *et al.* (1985) and Shahidi (1989) reported that, in the absence of residual nitrite in a sample, sulphanilamide may react with MA resulting in underestimation of the TBA values, however, no statistical evidence for these claims was presented in either paper. Shahidi *et al.* (1985) suggested that sulphanilamide should only be added to cured meats when residual nitrite is present.

MODIFICATIONS TO THE TBA TEST

Several modifications to the original TBA procedures have been proposed, the most common of which is the addition of antioxidants to the sample in an attempt to prevent oxidation during the test (Sinnhuber & Yu, 1958). Various antioxidants have been added during the solvent extraction method (Yu & Sinnhuber, 1967; Pikul *et al.*, 1983, 1989) and the distillation method prior to sample blending (Yamauchi *et al.*, 1982; Ang, 1986; Crackel *et al.*, 1988; Pikul *et al.*, 1989), or after blending (Moerck & Ball, 1974; Rhee, 1978; Ke *et al.*, 1984; Rhee & Ziprin, 1981). Pikul *et al.* (1983) reported that the antioxidant butylated hydroxytoluene (BHT) has no effect on the binding of MA to TBA at levels appropriate to the test but at higher levels a slight effect was noted. Rhee (1978), however, noted that phenolic antioxidants, including BHT, could increase TBA number as they increase the decomposition of lipid peroxides. Rhee (1978) also found that antioxidants had no significant effect on pork, beef or chicken samples but did on fish

samples. This was attributed to the differing fatty acid composition of the fish samples.

Other modifications to the original TBA procedures include the use of chilled blending (Rhee, 1978) and flushing of sample flasks with nitrogen (Ke *et al.*, 1984), both of which are further attempts to prevent oxidation during the test. Salih *et al.* (1987) found that blending can accelerate lipid oxidation of poultry meat samples unless an antioxidant is used. Yu & Sinnhuber (1967) used silicone coated tubes in the extraction method to prevent a thin oil film forming on the tube sides which was believed to be a major cause of inconsistent results possibly because the oil sample was not in contact with the reagents or because, as a thin film, the oil would be subject to further oxidation.

Rhee & Watts (1966*a*) modified the distillation method of Tarladgis *et al.* (1960) for use with raw plant tissues by adding acid during blending in order to inactivate lipoxidase to prevent its 'lipid oxidation potential'. This method has been applied successfully to frozen vegetables (Rhee & Watts, 1966*b*) and defatted soya flours (Melton *et al.*, 1981). Hoyland & Taylor (1989) developed a rapid distillation apparatus which greatly reduced distillation time compared to conventional distillation methods. They reported satisfactory results for both standard solutions and a variety of food samples although recoveries were lower than the conventional distillation methods.

STANDARDISATION AND RECOVERY FROM TBA TESTS

Williams *et al.* (1983) suggest that a 'standard' sample should be devised so that laboratories using any of the TBA methods could report results in terms of a standard. Several workers recommend that recovery values for MA should be determined and standard curves prepared for calculation of the appropriate TBA conversion factor (Siu & Draper, 1978; Crackel *et al.*, 1988). Kwon & Watts (1964) studied the formation of MA from the acid hydrolysis of 1,1,3,3-tetraethoxypropane (TEP), its stability and reactivity, and found one mole of TEP to yield one mole of MA. TEP and the similar compound 1,1,3,3-tetramethoxypropane (TMP) have therefore both been used as standards to assess recoveries in the TBA test (Sinnhuber & Yu, 1958; Asakawa *et al.*, 1975; Siu & Draper, 1978; Ke *et al.*, 1984; Salih *et al.*, 1987; Hoyland & Taylor, 1989; Shahidi, 1989).

LIMITATIONS OF THE TBA TEST

TBA studies frequently show a fall of TBA number from earlier higher values with time (Tarladgis & Watts, 1960; Dugan, 1961; Seo, 1976; Gokalp *et al.*, 1978; Kosugi & Kikugawa, 1985; Almandos *et al.*, 1986). Tarladgis &

Watts (1960) and Dugan (1961) stated that MA and other short chain carbon products of lipid oxidation are not stable for a long period of time. Further oxidation of these products yields organic alcohols and acids which are not determined by the TBA test. This secondary oxidation is therefore thought to be responsible for the decline in TBA values. Another explanation is that the TBARS react with food constituents or polymerise (Seo, 1976).

Despite reports that the TBA test is one of the best available chemical methods for the assessment of rancidity (Kwon *et al.*, 1965; Siu & Draper, 1978; Almandos *et al.*, 1986), de Konig & Silk (1963) were unable to apply the TBA test successfully in any form for fish oils. They blamed this on the two-phase system of the extraction method and the inefficient extraction of the distillation method. Fioriti *et al.* (1974) applied the modified solvent extraction method of Jacobson *et al.* (1964) in the determination of oxidation in a variety of fat samples. They found that TBA values correlated well with flavour scores in the case of lard but rather poorly with other fats. They concluded by saying that the modified solvent extraction procedure is of limited value in measuring the extent of oxidised flavours in fats. Witte *et al.* (1970) reported that the TBA test was of limited use for frozen samples and Shahidi (1989) claimed that the TBA test was also of limited use for cured meat samples. Kenaston *et al.* (1955) demonstrated that the TBA test was the most sensitive of all chemical methods used for the detection of the oxidation products of linolenic and linoleic acids but was relatively insensitive to oleic acid.

Lea & Swoboda (1962) noted that MA does not contribute to any considerable degree to off-flavours and therefore the TBA test must suffer from showing a variable relationship to flavour depending on the nature of the fat and the conditions under which it is oxidising. Kwon & Watts (1964) noted that in dehydrated foods (e.g. flour) advanced lipid oxidation still gave low TBA values. They suggested that this was due to the MA produced being in 'volatile chelated' form, because of the absence of water, and therefore not being held in the food. It was found that the longer a raw meat sample is stored frozen, the lower its TBA value is after cooking and that this was related to MA reactions with proteins during storage (Melton, 1983). Dahle *et al.* (1962) showed that meaningful TBA results could only be obtained by comparison of samples of a single material and Patton (1974) concluded that the test conditions themselves contribute in varying degrees to the results obtained in any TBA test.

CORRELATION OF TBA TEST WITH CHEMICAL AND SENSORY ANALYSIS

When the TBA test is compared to other chemical methods used for the determination of lipid oxidation, several contradictory reports are found.

Kenaston *et al.* (1955) reported that TBA values paralleled peroxide value (PV), Kreis test and conjugated diene results for linolenic and linoleic acids but were much lower for oleic acid. Ohkawa *et al.* (1978) found that, in the early stages of oxidation of linoleic acid, TBA, conjugated diene, oxygen absorption and peroxide values all paralleled one another and Yu & Sinnhuber (1967) reported peroxide and TBA value to have a linear relationship up to a PV of 800 for fish oil. Sidwell *et al.* (1955) showed that higher TBA values were obtained for soybean oil than for cottonseed oil at comparable PV; however, Dahle *et al.* (1962) reported a linear relationship between TBA value and PV for polyunsaturated fatty acids. Gray (1978) concluded that TBA value correlates well with PV only in oils containing fatty acids with three or more double bonds.

Hung & Slinger (1981) assessed a variety of chemical methods, including a solvent extraction TBA method, to measure the oxidative quality of salmon oil, soybean oil, canola oil and canola soap stocks which had been oxidised in different ways. For oils oxidised at room temperature, PV was the most sensitive method for measuring the onset of oxidation. Measurement of oxidation in oils which were highly oxidised could be achieved by any of the methods tested while anisidine value was the most sensitive method for canola oil oxidised at 100°C for 240 h. The lack of sensitivity of the TBA test in parts of this experiment was related to the fact that TBA was more suitable for measuring oxidation in oils with fatty acids containing three or more double bonds (Gray, 1978). Contradictory to these reports Turner *et al.* (1954) found that PV showed considerably more variation and was much less reliable than TBA value. Zipser *et al.* (1964) showed that rancidity of animal feeds correlated better with the TBA test than peroxide determinations. Tsoukalas & Grosch (1977), however, found the TBA test to be less sensitive than both the ferrous isothiocyanate test and diene absorption method.

Shahidi *et al.* (1987*b*) studied the relationship between TBA value and hexanal content in cooked pork during storage. They used a distillation TBA method and measured hexanal content of a steam distillate by a purge-and-trap technique and subsequent gas chromatographic analysis. They found, in general, that the amount of hexanal present in the distillates showed linear correlation with the corresponding TBA values after 35 days' storage but concluded that hexanal content was a better measure of the oxidative state of cooked meats in the early stages of storage.

Biggs & Bryant (1953) reported that the TBA test was capable of detecting levels of oxidation below the organoleptic thresholds for off flavours in butter, cheese and whole milk powder. Greene & Cumuze (1981) claimed that, in muscle foods, TBA values were highly correlated with sensory scores of oxidised flavour but Fioriti *et al.* (1974) found that a good correlation was only obtained for lard and not other fats. Melton (1983) stated that fatty

acids with three or more double bonds must be present for TBA number to be correlated with oxidised flavour and Gray (1978) added that TBA values and change in flavours would have to be established for a given oil before the TBA value could be used as an index of flavour.

Despite these claims, a number of workers have reported good correlation between TBA values and sensory scores. Such reports include work on oxidised flavour in milk (Dunkley & Jennings, 1951; Patton & Kurtz, 1951), whole milk powder (Sidwell *et al.*, 1955), wieners and pork patties (Turner *et al.*, 1954), poultry (Salih *et al.*, 1987), and warmed-over flavour in ground lean pork (Poste *et al.*, 1986). Patton (1974), however, concluded that in light of the complex factors leading to pigment production in the TBA reaction, test results need to be considered with caution and should be compared with organoleptic evaluation or with findings by other suitable tests.

OTHER TBA PROCEDURES

Other chemical and physical methods have been used occasionally to determine MA contents. Kwon & Watts (1963) measured MA content in steam distillates of oxidised food samples using a method based on the pH–dependence of the UV absorption spectrum of MA. They reported the sensitivity of the method was only 40% of the TBA test; however, the method was reported to be simpler, more rapid, and more specific for MA than the TBA test.

Sawicki *et al.* (1963) carried out a detailed study of ten diverse spectrophotometric and spectrophotofluorometric methods for the determination of MA using standard TMP solutions. They found the whole sample TBA method was the most sensitive spectrophotometric method studied but also susceptible to interference. They reported that the spectrophotofluorimetric methods studied were far more sensitive and selective than any of the spectrophotometric methods used. More recently, a spectrophotofluorimetric method was used to evaluate oxidation of ground meat and TBA numbers calculated from this method agreed well with those obtained from standard TBA procedures (Williams *et al.*, 1983). Yagi (1984) used the fluorescent properties of the TBA–MA complex to measure trace levels of MA in blood samples.

Several high pressure liquid chromatographic (HPLC) methods have been developed for the determination of trace levels of MA, mainly in biological samples. Kakuda *et al.* (1981) used one such method to determine MA in a steam distillate and reported a linear relationship between TBA number and HPLC results. Williams *et al.* (1983) used this method successfully for determination of oxidation in meat; however, Melton (1983) was unable to

use the HPLC method accurately and Fletcher (1983) found MA eluted with the solvent front. Other HPLC methods have also been developed which do not require prior isolation by distillation (Esterbauer & Slater, 1981; Bird *et al.*, 1983; Csallany *et al.*, 1984). Csallany *et al.* (1984) found the HPLC method was more sensitive, accurate and specific for the detection of free MA than a solvent extraction TBA method for rat liver, beef, pork and chicken samples.

Esterbauer *et al.* (1984) modified the HPLC method to prevent MA eluting with, or near, the solvent front; however, this method was still susceptible to interference. Bull & Marnett (1985) therefore developed an ion-pair HPLC method which reportedly avoids interference found in other HPLC methods. Hirayama *et al.* (1984) determined total MA (free MA and that existing as precursors/derivatives) in vegetable oils by reaction with dansyl hydrazine and analysis of the derivative formed using HPLC. The results correlated well with TBA values for methyl linoleate, although HPLC results were only 30% of the TBA value. The HPLC methods, however, only detect MA and therefore may not relate to oxidised flavours (Kakuda *et al.*, 1981).

In a study of pure lipid oxidation products related to biological systems, Frankel & Neff (1983) analysed MA by gas chromatography–mass spectrometry of the tetramethylacetal derivative. The methodology permitted more reliable evaluation of the potential of lipid oxidation products to form MA but no correlation between TBA values and MA content was found by this method.

MA has also been isolated as its non-volatile, chemically stable 2-hydroxy-pyrimidine derivative and analysed using a variety of chemical and physical methods (UV spectroscopy, gas chromatography, NMR spectroscopy, mass spectrometry, reductive ozonolysis) (Hamberg *et al.*, 1968). This method has been applied to the identification of MA in biological samples. Bond *et al.* (1980) developed a method using differential pulse polarography for the determination of MA in standard TMP solutions and biological samples and reported the method was sensitive and free from interference.

CONCLUSIONS

Despite its limitations, the TBA test is frequently used to measure lipid oxidation in foods, especially on a comparative basis. Some form of standardisation is desirable using TMP or TEP. When used as an index of rancidity, a positive correlation between TBA number and sensory analysis should be established. The large number of interfering compounds makes distillation the preferred test procedure although even this is not universally

applicable (for example samples containing nitrite give misleading values without suitable correction factors). Samples where the products of lipid oxidation are irreversibly bound to macromolecules cannot be analysed adequately by the TBA test. Similarly, the TBA test is unsuitable for following lipid oxidation in some shelf life studies where TBA values increase and then decrease while sensory analysis shows a steady increase. Providing it is used wisely, the TBA test can provide useful data on the state of lipid oxidation in foods.

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