Spectrophotometric Profiles of Off-Flavor Aldehydes by Using Their Reactions with 2-Thiobarbituric Acid

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The reaction of flavor aldehydes with 2-thiobarbituric acid (TBA) and the factors affecting this reaction have been studied. The formation rate of the 530−532 nm absorption band for detecting food rancidity through the TBA assay was dependent on the type of aldehyde and the reaction conditions. TBA reacted slowly with alkanals, and the yellow pigment (450 nm) was formed. Only after 1 h of heating at 100 °C was the red pigment formed. With 2-alkenals the reaction rate was more favorable for the red adduct (530−532 nm) formation, especially in excess TBA reagent and by employing an adequate heating time at 100 °C (water bath). For 2,4-dienals the orange pigment (494 nm) formation prevailed; the formation of the red adduct underwent a slow development (5 h at least). The development of the red adduct was favorable in an acetic acid concentration of 15−25% v/v. The yellow pigment was the only one that developed (although slowly) in glacial acetic acid medium.

Keywords: *Aldehydes; flavor; rancidity; 2-thiobarbituric acid (TBA)−aldehyde pigments; 2-thiobarbituric acid (TBA) assay*

INTRODUCTION

Aldehydes have been detected as flavor and off-flavor components in foods. Peas contain traces of acetaldehyde, and cinnamon contains cinnamaldehyde. Benzaldehyde is the characteristic impact substance in almonds and cherries. Anisaldehyde and salicylaldehyde appear in anise and vanilla extracts (Schwartz, 1934; Guillén-Sans and Guzmán-Chozas, 1988a). Hexanal and 2-hexenal are responsible for the "unripe odors" in fruits and vegetables. During the ripening of fruits, lipoxygenases originate aldehydes by oxidation of the lipid membrane fatty acids. Likewise, enzymatic transamination and subsequent decarboxylation of amino acids produce aldehydes. Pyridinic and pyrimidinic derivatives, whenever the 4-, 5-, or 6-locations of the heterocyclic ring are not substituted, undergo a ring cleavage yielding malonaldehyde (MDA) (Shepherd, 1948; Guzmán-Chozas et al., 1984). Nevertheless, aldehydes were mainly detected as off-flavors from the secondary autoxidation step of lipids in foods, by means of the 2-thiobarbituric acid reagent (TBA) (Frankel et al., 1981; Frankel, 1991; Guillén-Sans and Guzmán-Chozas, 1995).

The TBA assay has been widely applied for the measurement of lipid oxidation in food and biological tissues (Esterbauer and Cheeseman, 1990). The red pigment absorbance (532 nm) [attributable to the malondialdehyde (MDA)−TBA adduct] is the most commonly used index for this purpose. Since MDA is not the only aldehyde present in oxidized lipids, it is of interest to know to what extent other food aldehydes could contribute to the TBA test for oxidized lipids in foods (Kosugi et al., 1987; Guillén-Sans and Guzmán-Chozas, 1995).

The aim of this paper was to study the profile and evolution of the absorption spectra of the pigments formed by reaction between TBA and several food aldehydes. Also, the influence of several factors (heating time, acid concentration, and TBA−aldehyde ratio) on this reaction was assessed in three representative off-flavor aldehydes with the same hydrocarbon chain length but different unsaturation levels (hexanal, *trans*-2-hexenal, and 2,4-hexadienal).

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EXPERIMENTAL PROCEDURES

Apparatus. A UV−vis Spectronic 3000 Milton Roy spectrophotometer and a thermostated bath Selecta Unitronic-320 were used.

Reagents. Acetaldehyde, hexanal, heptanal, *trans*-2-hexenal, crotonaldehyde, benzaldehyde, 3,5-hydroxymethylfuran-2-carbaldehyde, glycolaldehyde, and acetic acid were purchased from Merck (Darmstadt, Germany); 2-undecenal, 2,4-hexadienal, 2,4-heptadienal, 2,4-decadienal, and citral were purchased from Aldrich (Milwaukee, WI); and glyoxal, 1,1,3,3-tetramethoxypropane (TMP), furan-2-aldehyde, and glyceraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO).

Procedures. *TBA−Aldehyde.* Solutions [1.0×10^{-3} M (1.0 mM)] of TBA (Merck) and aldehydes were prepared in 50% v/v aqueous acetic acid. Standard aqueous TBA and MDA solutions were prepared as previously described (Guillén-Sans et al., 1985; Vicario et al., 1988). Five milliliters of both the aldehyde and TBA solutions at the indicated concentration (1.0 mM) were mixed in a 10 mL volumetric flask, with the final concentration of each at 0.5 mM. These solutions were transferred to Pyrex tubes with screw-caps and heated at 100 °C in a water bath for 30 min, then removed and cooled for 2 min under tap water. Once room temperature was reached (22−25 °C), the absorption spectra of the colored systems were registered in a UV−vis spectrophotometer versus an appropiate blank. This blank consisted of an aqueous acetic acid solution (at the concentration of the assay) without TBA or aldehyde and submitted to the same procedure as the sample. Spectra were recorded immediately and at 15 and 30 min and 1, 5, 24, and 48 h.

Heating Time Effect. Pigment solutions of hexanal−TBA, *trans*-2-hexenal−TBA, and 2,4-hexadienal−TBA were prepared as described above. Heating times (100 °C) employed were 10 and 30 min and 1, 3, and 5 h. The samples were

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Table 1. Evolution (A_λ/mmol of Aldehyde) of the Reaction of TBA−Aldehydes in Equimolar Concentration

	$\lambda_{450/445}$[a]						$\lambda_{494/490}$[a]						$\lambda_{530/520/515}$[a]					
	15 min	30 min	60 min	5 h	24 h	48 h	15 min	30 min	60 min	5 h	24 h	48 h	15 min	30 min	60 min	5 h	24 h	48 h
acetaldehyde	0.04	0.04	0.04	0.09	0.09	0.09							0.04	0.04	0.04	0.07	0.08	0.09
hexanal	0.10	0.12	0.15	0.18	0.20	0.26												
heptanal	0.16	0.20	0.24	0.28	0.30	0.34												
crotonaldehyde	0.44	0.42	0.40	0.40			0.30	0.30	0.30	0.30	0.36	0.42	0.16	0.16	0.16	0.26	0.26	0.30
trans-2-hexenal	0.48	0.61	0.74	0.80	0.54	0.54							0.24	0.24	0.23	0.24	0.28	0.30
2-undecenal	0.20	0.19	0.25	0.47	0.39	0.22							0.08	0.08	0.08	0.09	0.09	0.08
2,4-hexadienal							1.28	1.26	1.22	1.36	1.56	1.66						
2,4-heptadienal							1.26	1.26	1.27	1.27	1.33	1.04						
2,4-decadienal							0.98	1.00	0.98	0.97	1.04	1.05						
citral	0.07	0.08	0.08	0.11	0.13	0.10							0.07	0.07	0.07	0.07	0.07	0.08
benzaldehyde	0.33	0.33	0.34	0.40	0.38	0.36							0.25	0.26	0.29	0.31	0.28	0.26
malonaldehyde													3.96	4.05	4.10	4.49	4.94	4.63
glyceraldehyde							0.17	0.17	0.17	0.14	0.11	0.15						
furan-2-aldehyde													0.24	0.24	0.24	0.23	0.26	0.28
3,5-HMF-2-carbaldehyde													0.21	0.21	0.21	0.22	0.25	0.29
glyoxal													1.88	1.87	1.87	1.94	2.45	2.17
glycolaldehyde	1.72	1.72	1.72	1.73	1.77	1.40							0.69	0.69	0.77	0.99	0.99	1.31

[a] As specified in the text for each aldehyde.

cooled under tap water for 2 min, and absorption spectra were recorded versus an appropiate blank (prepared as previously described) 15 min after the samples were taken out of the water bath.

Acid Concentration Effect. The general technique described above was followed, but 15, 25, 35, 50, 70, and 85% v/v aqueous acetic acid solutions and glacial acetic acid were employed. When the acetic acid concentration was >50%, solutions were prepared by dissolving TBA reagent in the acid directly. The samples were cooled under tap water for 2 min, and absorption spectra were recorded versus an appropiate blank (prepared as previously described) 15 min after the samples were taken out of the water bath.

TBA−Aldehyde Ratio Effect. The general technique described above was followed. Aqueous acetic acid−TBA solutions (50% v/v) of several concentrations (0.3, 0.5, 1.0, 2.0, and 3.0 mM) were reacted each one with 5.0 mL of 50% v/v aqueous acetic acid solutions of the aldehydes (hexanal, *trans*-2-hexenal, and 2,4-hexadienal, respectively). The concentration of aldehydes solutions was 1.0 mM. The samples were cooled under tap water for 2 min, and absorption spectra were recorded versus an appropiate blank (prepared as previously described) 15 min after the samples were taken out of the water bath.

RESULTS AND DISCUSSION

Profile and Evolution of the Reaction of TBA with Aldehydes in Equimolar Concentration. Several authors have assigned the yellow pigment formation to saturated aldehydes (Tarladgis et al., 1960; Jacobson et al., 1964; Kwon et al., 1965). When hexanal and heptanal were reacted with an equivalent amount of TBA reagent, only the yellow absorbing pigment (450 nm) was formed, showing a great unstability at the concentration of medium used (50% v/v aqueous acetic acid). This behavior is in agreement with previous findings by Kosugi et al. (1987). Hexanal is an outstanding aldehyde of rancidity in low-fat food. Hexanal, formed by scission of 9- and 13-hydroperoxides of linoleic acid, can be recognized by its reaction with TBA reagent (Frankel, 1985). Data obtained for the evolution of absorbance per millimoles of aldehydes at representative wavelengths are shown in Table 1.

The reaction with acetaldehyde yielded a pigment with two absorption bands at 450 and 530 nm. Both of them showed similar absorptivities, as previously reported by Taüfel and Zimmerman (1960).

Yellow pigment formation could be favored by lower heating temperatures and shorter heating periods (e.g. no more than 1 h), avoiding the orange pigment formation (496 nm) (Marcuse and Johansson, 1973; Bigwood et al., 1990).

The mechanism proposed for the reaction of alkanals with TBA implies an aldolic condensation and subsequent dehydratation of two molecules of alkanals yielding substituted 2-alkenals. These alkenals react with TBA to form a labile adduct at the C-5 location which autoxidizes to give an intermediate compound (alkenal−TBA adduct). This adduct then undergoes a Michael addition of a second TBA molecule (Sykes, 1985), and the 2:1 red pigment is formed.

Reaction of TBA reagent with alkenals (crotonaldehyde, *trans*-2-hexenal, and 2-undecenal) showed a characteristic absorption maxima at 450 nm, as reported previously by other authors (Marcuse and Johansson, 1973; Patton, 1974; Kosugi and Kikugawa, 1985; Dobarganes et al., 1986), and also at 532 nm. *trans*-2-Hexenal and 2-undecenal reacted slowly, yielding an unstable yellow pigment (450 nm) with a maximum absorption band at 450 nm after 5 h. It was a very unstable peak, disappearing rapidly, in contrast to the 532 nm absorption band that increased slowly and remained even after 2 days.

When TBA and crotonaldehyde were reacted, a band at 494 nm (that could be assigned to the orange pigment) was observed, besides the 450 and 532 nm absorption bands. As time elapsed the 450 nm band disappeared (after 1 day), and only the 494 and 530 nm bands remained, absorptivity being higher for 494 nm band. These results are in agreement with those found by other authors for crotonaldehyde and *trans*,*trans*-muconaldehyde (Taüfel and Zimmerman, 1960; Kosugi and Kikugawa, 1985), both of great interest for being biologically reactive aldehydes. Molar absorptivity at 494 nm (orange pigment) has been found to vary according to the type of α,β-unsaturated aldehyde (Witz et al., 1986).

2,4-Hexadienal−TBA and 2,4-heptadienal−TBA pigments had a strong absorption band at 494 nm (orange pigment) after 2 days. Color evolved from yellow (at start) to greenish yellow, and finally to orange hues after 5 h. 2,4-Decadienal content is an important criteria for sensory quality in oils (Pokorný et al., 1985). In the reaction with TBA it yielded an orange pigment (494 nm), which developed slowly, reaching a maximum intensity after 48 h. Citral reacted slowly with TBA,

originating the yellow pigment (450 nm) that was noticeable after 5 h; the red pigment (532 nm) did not develop.

Aromatic aldehydes stand out in the flavor profile of fruit and essential oils. Their reaction with TBA led to arylidene-2-thiobarbituric acids (Guillén-Sans et al., 1985; Guillén-Sans, 1986; Akiyama et al., 1987; Guillén-Sans and Guzmán-Chozas, 1988b–d). Many of these derivatives melt with decomposition above 300 °C, and they dissolve easily in alkali and protophilic solvents. In the TBA–benzaldehyde reaction two absorption zones were detected at 452 and at 532 nm, which increased slowly for 5 h and decreased afterward. Absorption spectra of benzaldehyde derivatives, which are substituted in the aromatic ring, are dependent on the type and location of substituents in the aromatic ring (ortho, meta, or para). Under certain conditions, such as recrystallization of the red TBA–salicylaldehyde adduct, a 2:1 TBA–aldehyde structure is obtained (Guillén-Sans and Guzmán-Chozas, 1988d).

The reaction between MDA and TBA produces a deep pink color and an intense narrow absorption band at 532 nm that was adequately stable. When equimolecular amounts of MDA and TBA were reacted, an intermediary product (colorless adduct) was first formed that was only stable in an inert atmosphere (N_2). In aerobic conditions this colorless adduct yields a mixture of yellow and red pigments, the red one being predominant. The orange pigment also appeared, in excess of TBA and aerobic conditions, as described by Kosugi et al. (1987).

When furan-2-aldehyde and 3,5-hydroxymethylfuran-2-carbaldehyde (heterocyclic aldehydes) were reacted with TBA, an absorption peak at 532 nm (red pigment) developed, which increased slightly after 24 h. Keeney and Bassette (1959) observed the formation of yellow compounds when furfural and its derivatives were reacted with TBA. In the case of hydroxymethylfurfural (HMF), some confusion could be drawn due to the incidence of the nonenzymatic browning reaction. In the reaction of glyoxal and TBA a stable narrow absorption band at 520 nm was found. In addition, a characteristic and stable band at 550 nm, as reported by other authors (Patton and Kurtz, 1951; Smith, 1958), was also observed. In the reaction of glycolaldehyde and TBA, absorption peaks at 445 and 515 nm were obtained, which were quite stable (5 h and 30 min, respectively) (Yu and Sinnhuber, 1962; Amer et al., 1975; Guillén-Sans and Guzmán-Chozas, 1995).

Table 2. Heating Time Effect on the Yellow and Red Pigment Development in the TBA–Hexanal Reaction

heating time	absorbance at 450 nm	absorbance at 530 nm	color
10 min	0.078	0.019	very pale yellow
30 min	0.037	0.028	colorless
1 h	0.030	0.031	colorless
3 h	0.036	0.041	very pale pink
5 h	0.058	0.086	pale pink

Table 3. Heating Time Effect on the Yellow and Red Pigment Development in the TBA–*trans*-2-Hexenal Reaction

heating time	absorbance at 450 nm	absorbance at 530 nm	color
10 min	0.092	0.039	pale yellow
30 min	0.186	0.116	orange-yellow
1 h	0.270	0.300	orange
3 h	0.273	0.978	pale pink
5 h	0.250	1.125	deep pink

The reaction of TBA with glyceraldehyde is important for the carbohydrates assay, because when these substances were hydrolyzed at 100 °C in the presence of TBA reagent, the colored TBA–glyceraldehyde system was formed (Caldwell and Grogg, 1955; Patton, 1960). Just as we have proved, the spectrophotometric behavior of the TBA–glyceraldehyde pigment was simple. We observed only one stable (for 1 h, at least) absorption peak at 490 nm, which then underwent a hypochromic effect. It has already been reported that epihydrinaldehyde, in an acid medium, yields glyceraldehyde, which can be detected by the TBA test (Patton, 1960).

Heating Time Effect. For hexanal (saturated aldehyde) only a 450 nm absorption band appeared at the initial stage of the reaction and evolved to the colorless adduct, which was clearly observed (Table 2). After 1 h of heating, the red adduct (532 nm) was developed. In the case of monoenals (*trans*-2-hexenal) yellow and red pigments were formed at the beginning. Increasing the heating time induced an intensification of the 532 nm peak, which was stabilized after heating for 3 h (Table 3). When TBA was reacted with dienals, the absorption band at 494 nm (orange pigment) was predominant at room (22–25 °C) temperature, but if a longer heating time (5 h) was employed, the red pigment (532 nm; $A = 0.945$) stood out against the orange pigment (494 nm; $A = 0.720$) (Figure 1).

Acid Concentration Effect. For alkanals the yellow pigment (450 nm) is favored either at high acetic

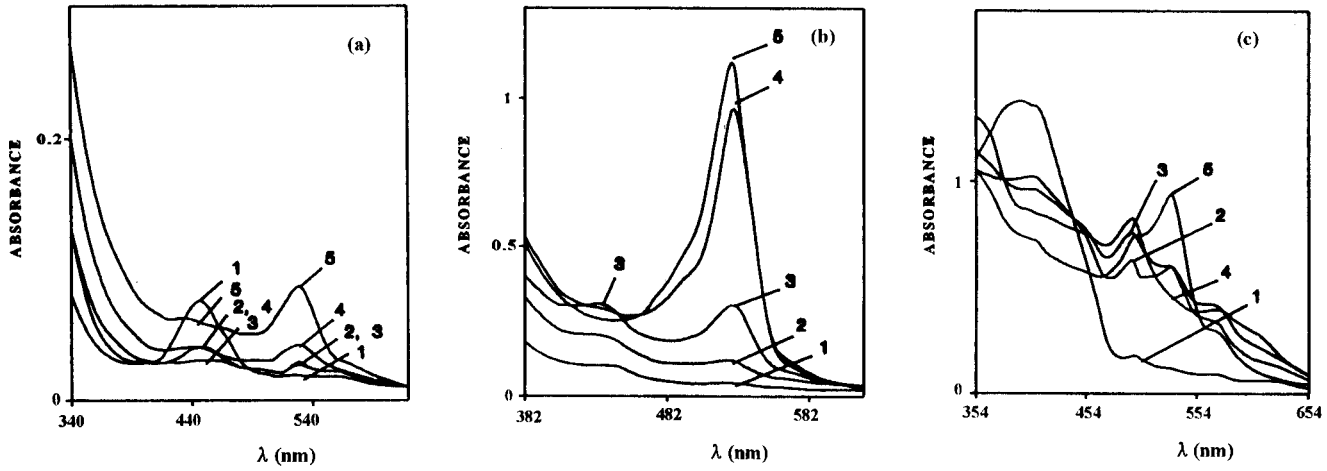

Figure 1. Influence of heating time on the development of the reaction: (a) TBA–hexanal; (b) TBA–*trans*-2-hexenal; and (c) TBA–2,4-hexadienal. (1) 10 min; (2) 30 min; (3) 1 h; (4) 3 h; (5) 5 h.

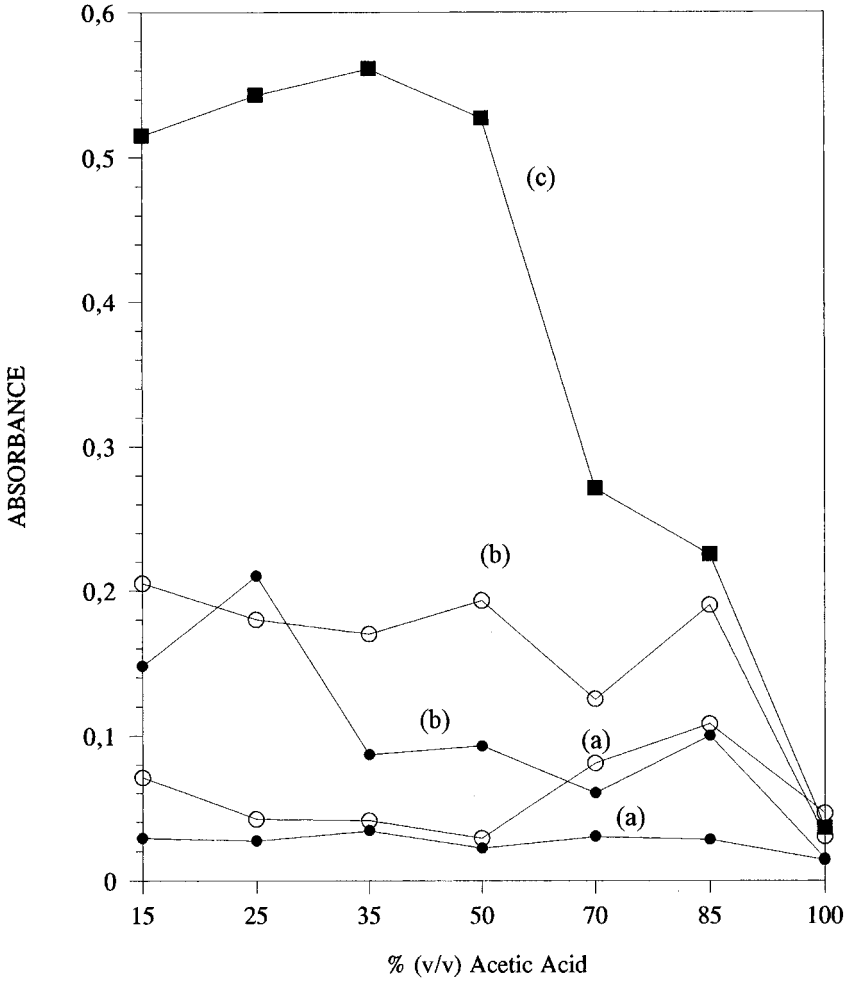

Figure 2. Influence of the percentage (v/v) of acetic acid on the development of the red (●) (532 nm), yellow (○) (450 nm), and orange (■) (494 nm) pigments in the reaction of (a) TBA−hexanal, (b) TBA−*trans*-2-hexenal, and (c) TBA−2,4-hexadienal.

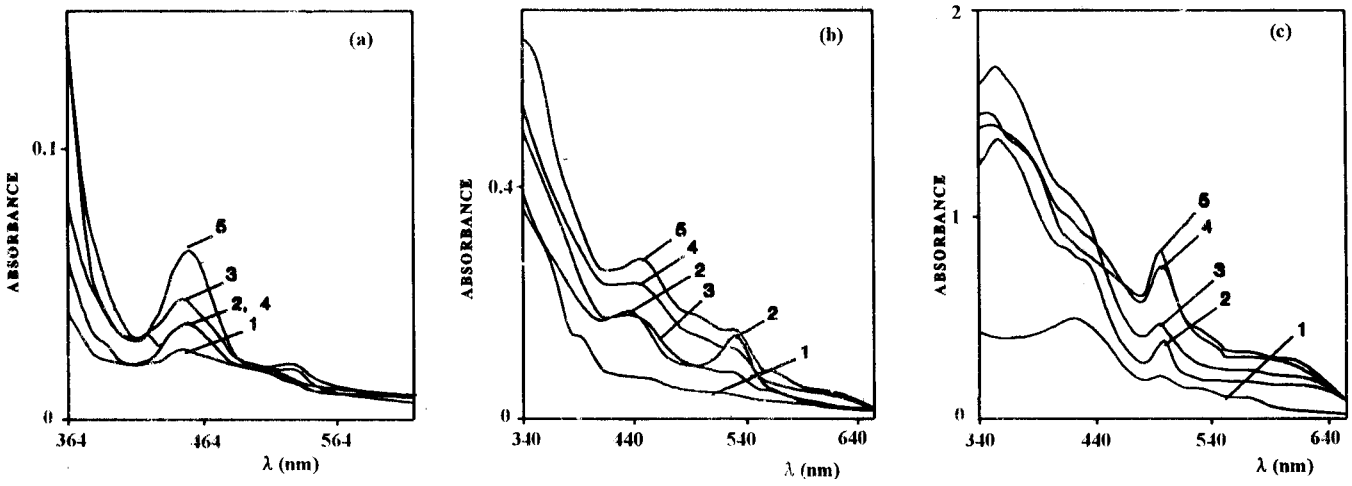

Figure 3. Influence of the reactants ratio on the development of the reaction (a) TBA−hexanal, (b) TBA−*trans*-2-hexenal, and (c) TBA−2,4-hexadienal. (1) 1:3 TBA−aldehyde; (2) 1:2 TBA−aldehyde; (3) 1:1 TBA−aldehyde; (4) 2:1 TBA−aldehyde; (5) 3:1 TBA−aldehyde.

acid concentrations (70 and 85% v/v) or at low acetic acid concentrations (15% v/v). The latter is the lowest concentration percentage (v/v) of acetic acid for dissolving the reagents involved in the TBA assay. The red adduct is favored in the case of monoenals within an acetic acid concentration interval of 15−25% v/v. For higher acetic acid concentrations (70% v/v) only the yellow pigment appeared. When pure acetic acid (glacial acetic acid) medium was used, a slight development of the yellow pigment was observed (Figure 2). Due to this behavior the optimum wavelength of 455 nm had been selected in glacial acetic acid medium for the TBA evaluation of rancidity in most saturated fats (beef and pork fats) (Kosugi and Kikugawa, 1985). The prevalence of the orange pigment (494 nm) in an acetic acid medium becomes evident in the case of dienals

Table 4. Reactants Ratio Effects on the Yellow, Red, and Orange Pigment Development in the TBA−Hexanal, TBA−*trans*-2-Hexenal, and TBA−2,4-Hexadienal Reaction

TBA−aldehyde ratio	absorbance at 450/494 nm[a]	absorbance at 530 nm	color
hexanal			
1:3	0.025	0.016	colorless
1:2	0.035	0.018	colorless
1:1	0.040	0.022	very pale yellow
2:1	0.036	0.026	very pale yellow
3:1	0.064	0.014	pale yellow
trans-2 hexenal			
1:3	0.066	0.035	pale yellow
1:2	0.155	0.140	pale orange
1:1	0.172	0.074	orange-yellow
2:1	0.234	0.116	orange
3:1	0.285	0.152	orange
2,4-hexadienal			
1:3	0.209	0.148	orange
1:2	0.303	0.186	greenish yellow
1:1	0.475	0.266	greenish yellow
2:1	0.755	0.434	orange
3:1	0.818	0.396	dark orange

[a] $A_{450\,nm}$ for hexanal and *trans*-2 hexenal; $A_{494\,nm}$ for 2,4-hexadienal.

within 15 and 35% v/v concentrations of acetic acid (Figure 2). A glacial acetic acid medium was unfavorable for the pigments development.

TBA−Aldehyde Ratio Effect. The formation of the yellow pigment (450 nm) is evident when alkanals react with excess TBA reagent (Table 4). It is necessary to use at least a 1:1 TBA−alkanal ratio. With monoenals the yellow and red pigments are preferentially formed in the lack of TBA. With excess of TBA the orange pigment is stabilized (Kosugi et al., 1987). In the case of dienals, if an excess of TBA reagent is not present, several absorption zones are detected, which include those of the three colored pigments (Figure 3). In the presence of an excess of TBA reagent the orange pigment clearly predominates, at the same time that an absorption band at 620 nm is pointed out.

LITERATURE CITED

Akiyama, S.; Nakashima, K.; Nakatsuji, S.; Suzuki, M.; Umekawa, C. H. Synthesis and properties of 5-substituted 2-thiobarbituric acid dyes. *Dyes Pigm*. **1987**, *8*, 459−463.

Amer, M. M.; Ahmad, A. K. S.; El-Tarras, M. F. M. Studies on the rancidity of oils and fats. X. A contribution to the study of thiobarbituric acid value and Kreis test as measures of the degree of oxidative deterioration of fats. *Egypt. J. Food Sci*. **1975**, *3*, 37−48.

Bigwood, T.; Delve, R.; Read, G. A novel iron (III) catalyzed degradation of aliphatic aldehydes to their lower homologs with implications for lipid peroxidation chemistry. *J. Chem. Soc., Chem. Commun*. **1990**, *10*, 776−778.

Caldwell, E. F.; Grogg, B. Application of the thiobarbituric acid test to cereal and baked products. *Food Technol*. **1955**, *9*, 185−186.

Dobarganes, M. C.; Ríos, J. J.; Pérez-Camino, M. C. Relaciones entre la composición de aceites vegetales y los componentes volátiles producidos durante su termooxidación. *Grasas Aceites* **1986**, *37*, 61−67.

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

Frankel, E. N. Chemistry of free radical and singlet oxidation of lipids. *Prog. Lipid Res*. **1985**, *23*, 197−221.

Frankel, E. N. Recent advances in lipid oxidation. *J. Sci. Food Agric*. **1991**, *54*, 495−511.

Frankel, E. N.; Neff, W. E.; Selke, E. Volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. *Lipids* **1981**, *16*, 279−285.

Guillén-Sans, R. Caracterización y estudio de ácidos arilidentiobarbitúricos formados por reacción entre el acido 2-tiobarbitúrico y aldehídos aromaticos. Ph.D. Dissertation, The University of Sevilla, Spain, 1986.

Guillén-Sans, R.; Guzmán-Chozas, M. Study of the extraction behaviour in various solvent systems of 5-anisylidene-2-thiobarbituric acid and 5-(salicylidene)bis-2-thiobarbituric acid. *Pharmazie* **1988a**, *43*, 48.

Guillén-Sans, R.; Guzmán-Chozas, M. Spectroscopic data of some arylidenethiobarbituric acids. *Pharmazie* **1988b**, *43*, 415−417.

Guillén-Sans, R.; Guzmán-Chozas, M. Spectrophotometric assay of 5-(salicylidene)bis-2- thiobarbituric acid. *Arch. Pharm. (Weinheim)* **1988c**, *321*, 65−68.

Guillén-Sans, R.; Guzmán-Chozas, M. Thiobarbituric acid condensation reaction. Spectral and stability features of the chromogens obtained with flavour aldehydes. *Belg. J. Food Chem. Biotechnol*. **1988d**, *43*, 147−149.

Guillén-Sans, R.; Guzmán-Chozas, M. Aldehydes in Food and its Relation with the TBA Test for Rancidity. *Fat Sci. Technol*. **1995**, *7*, 285−286.

Guillén-Sans, R.; Bautista, J.; Guzmán-Chozas, M. Synthesis, properties and preliminary analytical evaluation of 5-anisylidene-2-thiobarbituric acid. *Boll. Chim. Farm*. **1985**, *124*, 115−123.

Guzmán-Chozas, M.; Troncoso, A. M.; Guillén-Sans, R.; Benítez, M. Isonicotinic acid: properties and analytical methods. A review. *Boll. Chim. Farm*. **1984**, *123*, 200−209.

Jacobson, G. A.; Kirkpatrick, J. A.; Goff, H. E. J. A study of the applicability of a modified thiobarbituric acid test to flavor evaluation of fats and oils. *J. Am. Oil Chem. Soc*. **1964**, *41*, 124−128.

Keeney, M.; Bassette, R. Detection of intermediate compounds in the early stages of browning reaction in milk products. *J. Dairy Sci*. **1959**, *42*, 945−960.

Kosugi, H.; Kikugawa, K. Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid. *Lipids* **1985**, *20*, 915−921.

Kosugi, H.; Kato, T.; Kikugawa, K. Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem*. **1987**, *165*, 456−464.

Kwon, T. W.; Menzel, D. B.; Olcott, H. S. Reactivity of malonaldehyde with food constituents. *J. Food Sci*. **1965**, *30*, 808−813.

Marcuse, R.; Johansson, L. TBA (thiobarbituric acid) test for rancidity grading. II. TBA reactivity of different aldehyde classes. *J. Am. Oil Chem. Soc*. **1973**, *50*, 387−391.

Patton, S. Response of epihydrinaldehyde and glyceraldehyde in the thiobarbituric acid test for fat oxidation. *Food Res*. **1960**, *25*, 554−556.

Patton, S. Malonaldehyde, lipid oxidation, and the thiobarbituric acid test. *J. Am. Oil Chem. Soc*. **1974**, *51*, 114.

Patton, S.; Kurtz, G. W. 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. *J. Dairy Sci*. **1951**, *34*, 669−674.

Pokorný, J.; Davidek, J.; Valentová-Alexova, H.; Velisek, J.; Marcin, A. Correlation between the oxidation degree and the sensory quality of refined vegetable oils. *Sb. Vys. Sk. Chem.-Technol. Praze, E: Potraviny* **1985**, *E59*, 147−166.

Schwartz, M. A. Application of several methods for the determination of aldehydes in essential oils. *Ann. Chim. Appl*. **1934**, *24*, 352−355.

Shepherd, R. G. A specific analytical method for certain pyrimidines. *Anal. Chem*. **1948**, *20*, 1150−1153.

Smith, N. L. The thiobarbituric acid test in irradiation-sterilized beef. M.S. Thesis, Oregon State College, Oregon, 1958.

Sykes, P. A. *Mecanismos de Reacción en Química Orgánica*; Reverté: Barcelona, 1985; pp 199−200.

Tarladgis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. R., Jr. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44−48.

Taüfel, K.; Zimmerman, R. Reaction of 2-thiobarbituric acid with aldehydes. *Naturwissenschaften* **1960**, *47*, 133.

Vicario, I.; Guillén-Sans, R.; Guzmán-Chozas, M. Optimization of single-extraction TBA assay for olive oils. *Rev. Fr. Corps Gras* **1988**, *35*, 443−446.

Witz, G.; Lawrie, N. J.; Zaccaria, A.; Ferran, H.; Goldstein, B. The reaction of 2-thiobarbituric acid with biologically active α,β-unsaturated aldehydes. *Free Radicals Biol. Med.* **1986**, 33−39.

Yu, T. C.; Sinnhuber, R. O. Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction. *Food Technol.* **1962**, *16*, 115−116.

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