Studies on the TBA Test for Rancidity Grading: II. TBA Reactivity of Different Aldehyde Classes[1]

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ABSTRACT

The TBA reactivities of several aldehydes, most of them known as ordinary products of lipid autoxidation, have been investigated systematically. Gas liquid chromatography-purified alkanals, 2-alkenals and 2,4-alkadienals were reacted with TBA in water solution. The formation of pigments with maximum absorbance at 450 and 530 nm was measured at optimum time-temperature conditions–different for readings at 450 and 530 nm– and values for absorbance per mole aldehyde were calculated. These values show that on reaction with TBA all studied aldehydes build a yellow 450 nm pigment, while only 2,4-alkadienals and, to a lesser extent, 2-alkenals produce the red 530 nm pigment. Consequently both pigments are measures of aldehydic products of lipid autoxidation: In the case of predominant unsaturated aldehyde formation, determination of the pigment with maximum absorbance at 530 nm is preferable. However, if alkanals are predominant, the determination of the yellow pigment at 450 nm is more appropriate, as it grants higher sensitivity.

In spite of an abundant literature, the thiobarbituric acid (TBA) test for the determination of fat rancidity is still debatable in respect to suitable conditions for the reaction, the appropriate areas of its application and the significance of the results for the determination of sensorial rancidity. In an earlier investigation at this laboratory (9), the dependence of color development on temperature was studied. Different pigments with different heat sensitivity were observed to develop: a red one with maximum absorbance at ca. 530 nm and a yellow one with maximum at ca. 450 nm. Only the former is generally regarded as a potential measure of sensorial rancidity. Occasionally its determination is disturbed, but such instances can be avoided by heating at a sufficiently high temperature, e.g., 95 C for 1 hr (9).

Various techniques have been proposed in the literature for separation of the products of lipid oxidation from other TBA-reactive material. Vacuum-distilled TBA-reactive substances appear to be representative of the state of oxidation of the sample (9). Vacuum distillable substances certainly represent only a part of the total TBA-reactive oxidation products, but due to their volatility they can be assumed particularly responsible for sensorial rancidity.

Further studies are in progress in order to identify the various TBA-reactive products of oxidation of different fatty materials by application of gas liquid chromatography-mass spectrometry (GLC-MS). The results will be reported elsewhere.

In this regard, a systematical investigation of the TBA reactivity of these substances appeared desirable. According to earlier papers (3,14-17) and our own observations (9), both the absorbances at 530 and 450 nm may be indicative

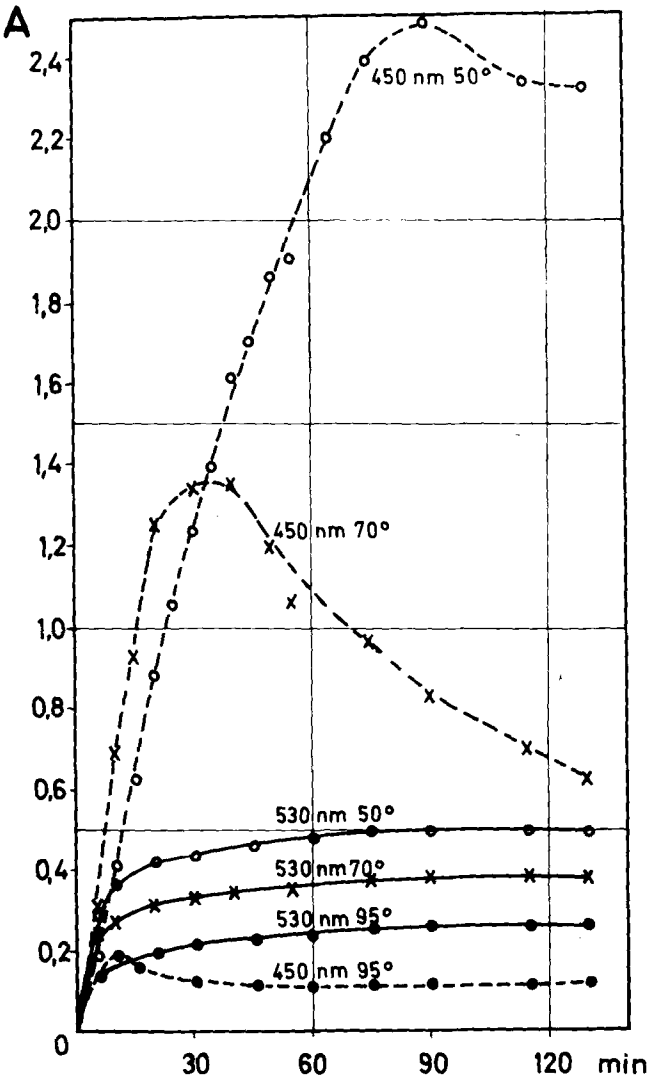

FIG. 1. A_{450} and A_{530} after reaction of vacuum-distilled components of oxidized linoleic acid with TBA at 50, 70 and 95 C as a function of time.

[1]The first paper in this series was published in *Fette, Seifen, Anstrichmittel* 72:635 (1970).

TABLE I

Vacuum-Distilled Products of Oxidation of Linoleic Acid[a] Separated by Gas Liquid Chromatography

Peak no.	A_{530}, 95 C	A_{450}, 50 C	Peak surface, cm^2	Relative TBA reactivity 530 nm, 95 C	Relative TBA reactivity 450 nm, 50 C	Identified substance
3	0.003	0.742	46.8	0.07	15.8	Hexanal
4	0	0.007	3.15	0	2.22	*trans*-2-Hexenal, *trans*-2-Heptenal
5	0.014	0.704	40.0	0.35	17.6	*trans*-2-Heptenal
6	0.025	1.182	61.6	0.41	19.2	2-Octenal (*cis* + *trans*)
10	0.092	0.096	3.50	26.2	27.5	*trans-cis*-2,4-Decadienal
11	0.065	0.138	2.80	23.2	49.5	*trans-trans*-2,4-Decadienal
13	0.014	0.020	1.35	10.4	14.8	Not identified

[a]Substrate: linoleic acid, Fluka, puriss; peroxide value: 725; oxidation: 200 hr, Warburg vessels (25 C); oxygen consumption: 61 ml O_2/ml linoleic acid (determined in automatic Warburg apparatus [8]).

of rancidity. Therefore the values of the absorbance per mole of aldehyde were determined at both these wavelengths.

EXPERIMENTAL PROCEDURES

Materials

Linoleic acid: Fluka, Buchs, Switzerland; saturated aldehydes: Fluka, Buchs, Switzerland; ethanolic solutions of unsaturated aldehydes: Compagnie Parento, Inc., New York and Chicago. These aldehydes were freshly purified by preparative gas chromatographic separation. The procedure was repeated until a sufficiently pure fraction was obtained, which was tested by analytical GLC. The aldehyde fraction was condensed, rapidly weighed under N_2 in a trap, and quantitatively dissolved in a small volume of diethyl ether. The solution was transferred to a volumetric flask and filled to volume with freshly distilled water. The concentration was generally ca. 5 mg/1000 ml.

Distillation of Oxidized Lipidic Material

1.0 ml samples were distilled for 30 min at 10^{-2} torr in a modified vacuum distillation apparatus according to Lea and Swoboda (6), and the distillates were condensed on a cold finger (9).

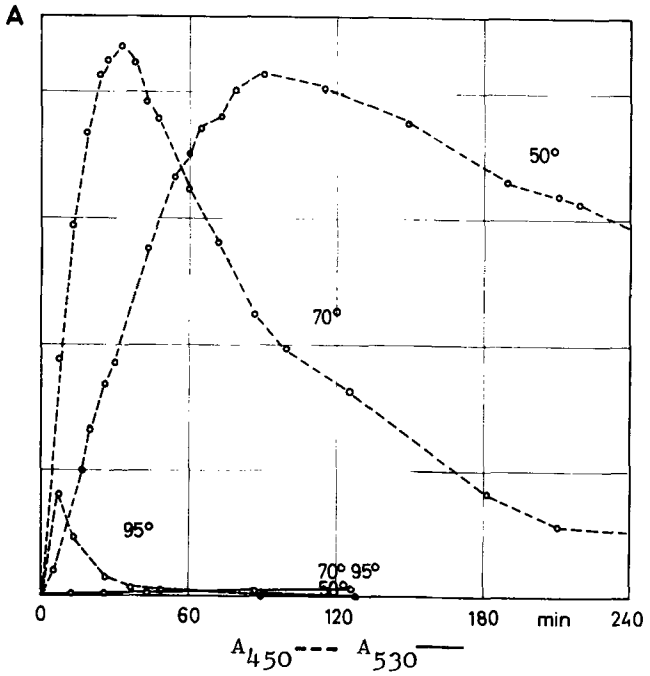

FIG. 2. A_{450} and A_{530} after reaction of hexanal with TBA at 50, 70 and 95 C as a function of time.

TBA Reaction

The condensate obtained after vacuum distillation of lipidic material or a certain amount of the known, pure aldehydes dissolved in a few milliliters of water, was allowed to react with the same volume of 0.020 M TBA in water at different temperatures (50, 70 and 95 C). The absorbance was read against a blank treated in the same manner as the sample, and the absorbance of the blank was checked by reading against distilled water. It averaged ca. 0.005 at 450 nm and ca. 0.000 at 530 nm.

When no other specifications are given, heating conditions for the absorbance were at 530 nm 1 hr at 95 C and at 450 nm 2 hr at 50 C.

Since the substances tested are known to be unstable towards oxidation (7,18), comparison was made with heating in a nitrogen atmosphere, e.g., bubbling nitrogen through the solution during heating. Practically the same results were obtained as when the determinations were carried out in the presence of air.

GLC Separation

For analytical or preparative GLC separation the condensate was dissolved in 300 µl diethyl ether. *Analytical GLC separation*: apparatus–Perkin-Elmer F 11; column–DC 550 on Chromosorb W DMCS 80/100 mesh (2.7 m x 1/8 in.); injection temperature–200 C; column temperature–50-130 C (program 4 C/min), 25 ml N_2/min; detection temperature–150 C. In certain cases the peaks were identified by coupled GLC-MS. *Preparative GLC separation*: apparatus–Aerograph 202/204 with FID (modified for preparative work); column–15% DC 550 on Chromosorb W AW DMCS 45/60 mesh (4.5 m x 3/8 in.); injection temperature–200 C; column temperature–50-130 C (4 C/min), 25 ml N_2/min; detection temperature–150 C; split–FID/outlet, ca. 1/60. The peaks obtained after separation were collected in cold traps immersed in liquid N_2 for the determination of TBA reactivity.

Mass Spectrometry

Apparatus: LKB 9000; electron energy: 70 eV. Same conditions as above for GLC separation.

DATA

The time-temperature dependence of the TBA reaction for a sample of volatiles obtained from oxidized linoleic acid is shown in Figure 1. The absorbance at 450 and 530 nm was read during 2 hr heating at 50, 70 and 95 C. Samples were taken out at certain time intervals and read after cooling to room temperature.

For the absorbance at 530 nm, heating for 1 hr at 95 C practically eliminates the risk of disturbance, as shown earlier (9). (The lower absorbance at 530 nm after heating

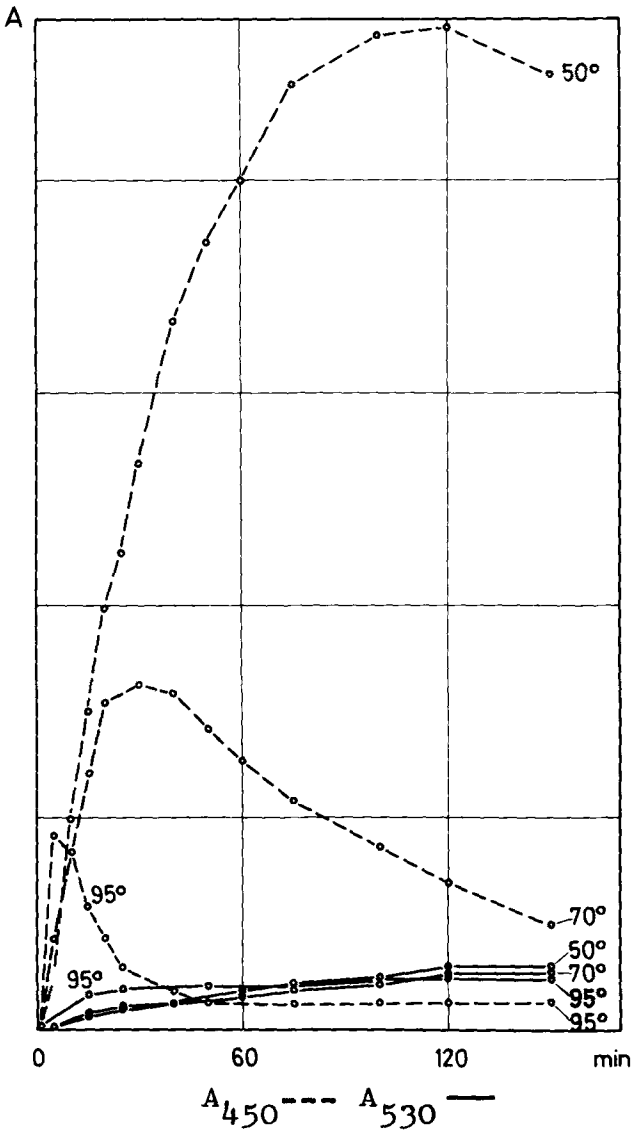

FIG. 3. A_{450} and A_{530} after reaction of 2-hexenal with TBA at 50, 70 and 95 C as a function of time.

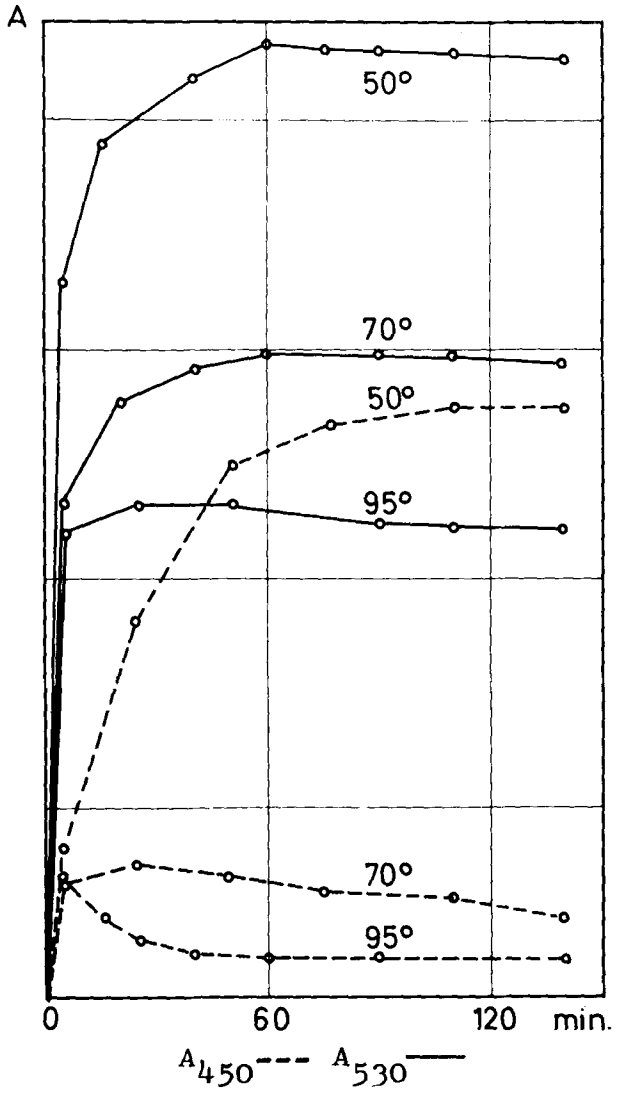

FIG. 4. A_{450} and A_{530} after reaction of 2,4-decadienal with TBA at 50, 70 and 95 C as a function of time.

at, e.g., 95 C for 1 hr, than, e.g., 50 C for 2 hr, may be at least in part due to the elimination of disturbance by other maxima and is therefore preferable.)

For measurement of the yellow pigment, heating for 2 hr at 50 C was found preferable. The readings at 50 C were considerably higher than those at 70 or 95 C.

The TBA reactivity of the distilled volatiles of oxidized linoleic acid (Fig. 1) is composed of a number of values for the individual components. These components were separated by preparative GLC and the fractions isolated, identified by MS and reacted with TBA. Results are shown in Table I and expressed as relative TBA reactivity, i.e., the absorbance of the separated fractions per unit of peak surface. The spectrophotometric behavior of the fractions when reacted with TBA differed considerably. Only 2,4-dienals gave rise to a considerable absorbance at 530 nm. The TBA reactivity of such dienals was high at both 450 and 530 nm. 2-Alkenals showed low TBA reactivity at 530 and high reactivity at 450 nm. Hexanal, finally, had practically no TBA reactivity at 530 nm, but high reactivity at 450 nm. The values obtained may be influenced by minor amounts of other TBA-reactive compounds present in the fractions.

The results shown in Table I indicate similarity of the TBA reactivity of aldehydes belonging to the same class, and differences among classes. It appeared desirable to elucidate these relationships by reacting known amounts of pure aldehydes with TBA. Commercial substances purified by preparative GLC were used for this purpose. It was confirmed that on reaction with TBA alkanals 2-alkenals and 2,4-alkadienals behave differently, while individual members of these classes behave similarly. Hexanal (Fig. 2), *trans*-2-hexenal (Fig. 3) and 2,4-decadienal (Fig. 4) were studied as representatives of the classes. Absorbance is shown in Figures 2-4 as a function of time of heating at 50, 70 and 95 C. Hexanal (Fig. 2) gave an absorbance at 450 nm and practically no absorbance at 530 nm. The formation of the yellow pigment with maximum absorbance at 450 nm was again found to be very heat-sensitive. The most appropriate procedure for its determination appears to be 2 hr heating at 50 C.

For hexenal (Fig. 3) the absorbance at 450 nm was similar to that observed for hexanal. However hexenal also gave a small absorbance at 530 nm.

2,4-Decadienal (Fig. 4) showed considerable absorbance at both 530 and 450 nm. In this case, too, absorbance at 450 nm showed the time-temperature dependence described above. The time-temperature dependence of the absorbance at 530 nm is similar to that observed in the case of oxidized linoleic acid (Fig. 1).

The appearance of the curves for the absorbance at 450 nm (Figs. 1-4) supports the conclusion that heating at 50 C for 2 hr should be the most appropriate procedure for the determination of the yellow pigment.

The visible light absorption of the products of TBA reaction with 2-hexenal and 2,4-decadienal is given in

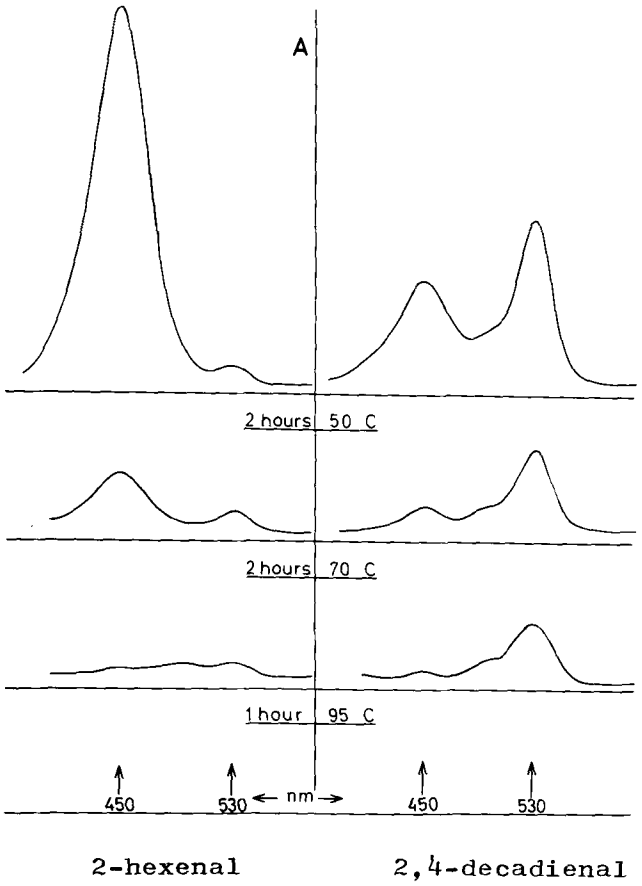

FIG. 5. Spectral composition of products of reaction of TBA with 2-hexenal and 2,4-decadienal with TBA after heating at different temperatures.

Figure 5 for heating for 2 hr at 50 C, 2 hr at 70 C and 1 hr at 95 C. Absorbance at 530 nm for 2,4-decadienal after heating at 50, 70 and 95 C was relatively high; for 2-hexenal, however, it was relatively small. The absorbance at 450 nm was, in both cases, large after heating at 50 C and considerably decreased after heating at 70 or 95 C.

To enable direct comparison of the TBA reactivities between classes and among individual aldehydes, the results were expressed in terms of absorbance per mole of aldehyde (Table II). In repeated determinations, each carried out with a freshly prepared GLC-fraction, this value showed certain variations, presumably due mainly to the difficulty of perfect purification and weighing of the fraction after GLC separation.

Mean values (MV) and standard deviations (SD) were therefore calculated for a certain number of experiments (n) (Table II). As expected, only the products of TBA reaction with 2,4-alkadienals have a high absorbance at 530 nm, while this value is small for 2-alkenals and practically zero for alkanals. As to the absorbance at 450 nm, all three aldehyde classes gave rise to the formation of products with an absorption maximum at this wavelength.

DISCUSSION

The results reported show that, on reaction with TBA, aldehydic products of lipid oxidation give rise to the formation of two pigments, which are differently dependent on heating conditions. One is the pigment with maximum absorbance at 530 nm, which in the conventional application of the TBA test generally is taken as an index of lipid oxidation; the other is a yellow pigment with a maximum at 450 nm. The formation of this yellow pigment is also mentioned in the literature (3,9,14-17), but is not generally used as a measure of lipid oxidation. Some authors have ascribed it to saturated aldehydes (3,15,16). Jacobson et al. (3) have investigated its applicability for grading of oxidative rancidity. These authors obtained, in part, results similar to ours for the TBA reactivity of some saturated and unsaturated aldehydes. Color, however, was developed by heating for 30 min at only 60 C, and the different heat sensitivity of the two pigments has not been considered. Some of their results are therefore different from those reported here. They found the absorbance at 452 nm to be of value in assessing flavor of beef fat and cottonseed oil, whereas the 532 nm wavelength was useful for the examination of soybean and pork fat.

Systematic studies on application of the TBA reaction to various fats may reveal the reason for this different behavior. Such studies are in progress in our laboratory.

The chemistry of the pigments formed by reaction with TBA is not exactly known. Early studies of the TBA pigments by Patton and Kurtz (10) and Sinnhuber and Yu (13) aimed mainly at identifying the pigments formed on reaction of TBA with malondialdehyde on one side and with oxidized lipids on the other. Later, systematic studies were carried out by Schmidt (12) and Täufel and Zimmermann (15), who suggested structures for the 450 and 530 nm pigments. The pigment measured at 530 nm was presumed to be composed of 2 molecules of TBA and 1 molecule of malondialdehyde (13). (No attempts were made in the present work to further elucidate the chemistry of the reaction of TBA with products of fat oxidation. The limited aim of this research was to study the TBA reactivity of authentic substances known to occur as products of oxidation.)

The formation of malondialdehyde as product of oxidation is often accepted as the basis of the TBA test as a measure of sensorial rancidity. This hypothesis has been widely discussed in the literature. Sometimes formation of the red pigment is even regarded as a proof of the presence of malondialdehyde or a measure of its content, which, however, does not appear justified, as shown by our results and those of other workers, e.g., Saslaw et al. (11): On reaction with TBA, alkadienals and, to a lower degree, alkenals, which are formed as products of lipid oxidation, can be supposed to behave like the pure substances studied by the present authors and to produce a pigment with absorbance at 530 nm.

The presence of malondialdehyde in oxidized lipid material has been demonstrated by the application of different methods, e.g., by Kwon and coworkers (4,5) and Hamberg et al. (2), and a theory for its formation during oxidation of polyunsaturated fatty acids has been developed by Dahle et al. (1).

Apparently both malondialdehyde as well as other aldehydes (especially alkadienals and, to a lesser degree, alkenals) are capable of producing red TBA pigments with maximum absorbance at ca. 530 nm. Certain alkanals (e.g., hexanal, known to contribute to sensorial rancidity in, e.g., potato and cereal products) do not, however, form the red pigment. Instead these aldehydes show the formation of the yellow pigment with maximum absorbance at 450 nm, permitting determination of their occurrence.

In summary, both the yellow 450 nm pigment and the red pigment with maximum absorbance at 530 nm should be measured for the grading of rancidity. Depending on the composition of the sample to be examined, one or the other absorbance may be of greater interest. Owing to the different heat sensitivity of the two pigments, the absorbance should be determined at 450 nm after 2 hr heating at 50 C and at 530 nm after 1 hr heating at 95 C. In this way the risk for interference of other absorbance with the readings at 530 nm is eliminated, and the determination of the 450 nm absorbance is performed under optimum conditions.

The application of these results may grant greater sensitivity and wider applicability of the TBA reaction for

TABLE II

Absorbance of Products of TBA Reaction with Different Types of Aldehydes per Mole of Aldehyde x b^{-3}[a]

Aldehyde	450 nm, 2 hr, 50 C			450 nm, 2 hr, 70 C			530 nm, 1 hr, 95 C		
	MV	SD	*n*	MV	SD	*n*	MV	SD	*n*
Alkanals									
Pentanal	6.9	1.0	(3)	1.1	0.1	(3)	0.0	0.0	(3)
Hexanal	4.2	0.8	(3)	0.7	0.1	(3)	0.0	0.0	(3)
Heptanal	5.5	1.0	(3)	0.7	0.0	(3)	0.0	0.0	(3)
Decanal	8.3	2.8	(3)	2.7	0.6	(3)	0.0	0.0	(3)
Alkenals									
2-Hexenal	9.8	1.6	(6)	0.7	0.0	(6)	0.1	0.0	(6)
2-Heptenal	11.3	1.0	(6)	0.7	0.2	(7)	0.1	0.0	(6)
2-Octenal	5.1	0.9	(5)	0.9	0.1	(5)	0.2	0.0	(5)
2-Nonenal	8.2	1.2	(3)	1.0	0.1	(3)	0.1	0.0	(3)
2-Decenal	9.5	2.1	(3)	1.0	0.2	(3)			
Alkadienals									
2,4-Hexadienal	2.0	0.3	(4)	0.6	0.1	(4)	2.1	0.8	(4)
2,4-Nonadienal	5.4	0.4	(2)	1.0	0.0	(2)	2.6	0.4	(2)
2,4-Decadienal	3.2	0.6	(3)	0.8	0.2	(3)	2.5	0.4	(2)
2,4-Undecadienal	5.0	0.6	(3)	0.6	0.1	(3)	1.9	0.1	(3)
2,4-Dodecadienal	3.5	0.7	(4)	0.5	0.1	(4)	1.1	0.2	(4)

[a]Mean values (MV) ± standard deviation (SD) and number of determinations (*n*).

rancidity grading.

ACKNOWLEDGMENTS

Experiments were carried out with the assistance of P. Brandt. Mass spectrometric analytical data were provided by G. Karlsson-Ekström.

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[Received December 10, 1971]