A Study of the Applicability of a Modified Thiobarbituric Acid Test to Flavor Evaluation of Fats and Oils

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Abstract

A modified thiobarbituric acid test has been devised for the determination of certain components of oxidized fat. The reaction was carried out in a single phase solvent designed to eliminate the color extraction or fat removal steps of other TBA tests. A moderate temperature of 60°C, with no added acid, was used to minimize breakdown of hydroperoxides and TBA. Under certain reaction conditions, temperature, sunlight, ferrie ion, and oxygen can influence results. Of the aldehydes examined, the TBA reaction products of dienals showed an absorption peak at 532 mμ only, while the TBA reaction products of the saturated aldehydes showed a peak at 452 mμ only. The spectral behavior of TBA reaction products of monoenoals was found to vary with the location of the double bond. Fat samples, aged at either 60°C or 37.8°C, were examined organoleptically and by the modified TBA test. The 452 mμ peak was of value in assessing the flavor of beef fat, cottonseed oil, and used frying fat, whereas the 532 mμ wavelength was of value in assessing the flavor of soybean oil, used frying fat, and pork fat.

Introduction

A number of different chemical methods have been used as aids in evaluating the flavor of fats and fat oxidation products. Among these are the benzidine test for aldehyde value, the carbonyl index of Chang and Kummerow, the carbonyl test of Henick et al., and gas chromatography (1–4). Perhaps the most widely used test in recent times for measuring unfractinated lipid off-flavors, however, has been the thiobarbituric acid (TBA) test. The TBA reaction has been of value in measuring off-flavor components of various fat-bearing foods such as pork, milk, cereals and baked goods, and oysters, as well as fats and oils (5–9). The basis of these tests has been the reaction of TBA with a fat oxidation product or products to form a red pigment absorbing at 532 mμ. Most of the TBA tests mentioned involve strongly acid conditions with some exposure to air. However, Tarladgis et al. have shown that acidity, heat, and the presence of peroxides in the fat sometimes can strongly influence the test results because of decomposition of the TBA reagent (10). Therefore, it appeared important to minimize these factors in any attempt to measure accurately the more subtle changes in carboxyls that occur in fats and fat-bearing products when held at moderate temperatures.

Most workers who have correlated TBA values with organoleptic scores have used the pink color developed at 532 mμ. However, several have noted the appearance of a peak in the 452 mμ region, denoting yellow color (11,12). Recently, it has been shown that the absorption peak at ca. 452 mμ can be attributed to the reaction product of TBA with aldehydes (10,13). Taufel and Zimmerman reported that the presence of ferric ion greatly increased the intensity of yellow formed by the reaction of TBA with aldehydes at 70°C instead of the customary 100°C (13). These workers also reported that the reaction products of dienals with TBA absorb at 532 mμ (13).

In this study a single-phase solvent of iso-octane, n-propanol, and water was developed to evaluate the usefulness of absorption peaks at 452 and 532 mμ to measure undesirable aldehyde flavors. No acid was added, and a moderate 60°C temp was used to minimize changes in fat or reagent from excess acidity or heat. It was found that at 60°C the yellow or 452 mμ peak became prominent. The pink color, representing absorption at 532 mμ, is less intense at this temp. Of considerable importance was the effect of oxygen, sunlight, temp, and ferric ion on results obtained. Of further importance was the spectral behavior of TBA reaction products of various aldehydes known to be flavor constituents of oxidized fats.

Procedures

The following fats were examined: hydrogenated vegetable oil used for frying chicken, beef fat, soybean oil, cottonseed oil, and rendered pork fat. The various fats and oils, except the cottonseed oil, were aged at 60°C in glass beakers to accelerate off-flavor development from oxidation and to break down any

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[Received April 8, 1963—Accepted September 10, 1963]
off-flavor precursors present. The cottonseed oil was aged in glass beakers at 37.8°C.

Flavor evaluation was made by a panel of five or six members. The scoring scale was 1-10 using the following rating: 10 excellent, 8 good, 6 fair, 4 poor, and 2 very poor. Each series of samples was evaluated at three or four sessions. Samples were heated to 60°C and served promptly to the panelists after a warmup with a bland hydrogenated vegetable oil. Next, the unaged sample of the test series was presented and identified as a reference standard. During the first session of a particular series, samples were usually identified and presented in order of increased aging at 60°C to familiarize the panelists with the development of the off-flavors characteristic of the fat under study. In the remaining sessions, the samples were unidentified.

Solvents and reagents were purified as follows: iso-octane and n-propanol were refluxed over zinc and KOH, distilled, and redistilled over 2,4-dinitrophenylhydrazine, using only the fraction from the respective bp. Water was deionized, then distilled, to eliminate trace impurities which would react with the TBA. Thiobarbituric acid was recrystallized two to three times from distilled water, using the minimum heat to effect solution. The crystals were dried in vacuo at room temp. It was found that the recrystallized TBA must have no odor to be acceptable. When solvents and reagents were properly purified, the heated blank showed no absorption at either 452 or 532 mµ.

The single phase solvent used was a mixture of 50 parts of iso-octane, 27 of n-propanol, and 3 of water. The reaction was carried out in 125 ml iodine flasks with solid glass stoppers. Joints were covered with high-vacuum silicone stopcock grease (Dow-Corning). This grease did not affect the reaction, although some other types did. Fat (0.1 g) was added to 10 ml of solvent, followed by 10 mg crystalline TBA, and the unstopped flasks were shaken 10 sec on a reciprocal shaker at 180 cycle/min. Shaking had two purposes: 1) to minimize variation in oxygen content as the reaction product of saturated aldehydes and TBA must have oxygen to produce the yellow color (13); 2) to ensure uniform mixing. After shaking, the flasks were stoppered and heated to 60°C in sand in an oven 30 min. After heating, the flasks were cooled under tap water before unstoppering, then scanned immediately in the visible spectrum with a Cary Model 14 recording spectrophotometer. The blank consisted of the solvent mixture and TBA. No correction for fat color was necessary in the samples investigated. In this study, an oven was used for heating as a water bath caused the stoppers to become dislodged. It is permissible to add the TBA to 90% propanol as the solvent is being prepared, although the solution was found not stable for more than 2 hr.

Cleanliness of glassware is of prime importance as traces of impurities such as iron or organic material can cause poor reproducibility and high blanks. Some of the TBA analyses reported here were made in triplicate, but duplicate tests are satisfactory if the solvents and TBA are carefully purified.

The time the samples were shaken affected the intensity of yellow produced when TBA was reacted with heptanal. For example, by increasing shaking from 10-30 sec, a more intense color was obtained. However, the stoppers would not always remain seated during heating when samples were shaken longer than 10 sec.

A modified peroxide value test was made on soybean oil aged 2 days at 60°C. Fat samples (0.4 g) were dissolved in 20 ml of the single-phase solvent in an iodine flask, 40 mg TBA was added, samples were aerated by mechanical shaking for 30 sec, and heated for various periods. At the end of the heating period, 1 ml of water was added which produced a two-phase system. Ten ml of the upper (iso-octane) phase was mixed with 5 ml of 2:1 acetic acid-chloroform solution for the peroxide determination. A blank determination was made similarly, but without the TBA. The determination, as made on various fat samples, yielded peroxide values of ca. 25% of those found by the AOCS method Cd. 8-53.

The GLC separations of 2,4-heptadienal and 2,4-hexadienal were made using a 150°C column temp, helium flow at 22 cc/min, and a column of succinate polyester of diethylene glycol. The crotonaldehyde and 2-hexenal were separated under the same conditions except that a 125°C column temp was used. Each fraction under examination was collected directly in the single-phase test solution. The remaining aldehydes were purified by distillation.

Results and Discussion

Early in this study it became obvious that a number of factors would affect the development of yellow color when TBA was reacted with aldehydes. In one experiment a 10-mg portion of unrecrystallized TBA was dissolved in unpurified single-phase solvent. After heating 30 min at 60°C, the absorption peak at 452 mµ had an OD of 1.62, compared to 0.06 for an identical solution blanketed with nitrogen. It was apparent that the presence of oxygen markedly influenced the production of yellow from any impurities in the solvent or TBA. However, Tauler's observation on the development of yellow color indicated that it might be desirable to have oxygen present in TBA reactions involving fats that contain saturated aldehydes as important flavor constituents (13). It also was found necessary to purify carefully the TBA

![Graph](https://via.placeholder.com/150.png?text=Graph)

**Fig. 1.** The effect of ferric ion on the development of yellow in the reaction of TBA with beef fat.