

given wash system. While these experiments have shown that the Vaughn concept is not valid for the detergent systems here studied, this testing approach involving multiple washes appears to have considerable merit as an evaluation tool. The procedure used here provides for a measure of soil removal which is free from the confounding effects of soil redeposition. The early washing periods, during which soils removal is rapid, must be brief so that negligible redeposition can occur. Results obtained from the multiple wash runs are then solely a measure of soil removal. The linear log time plot is useful in reducing the required number of data points.

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A Study of the Applicability of a Modified Thiobarbituric Acid Test to Flavor Evaluation of Fats and Oils¹

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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 60C, with no added acid, was used to minimize breakdown of hydroperoxides and TBA. Under certain reaction conditions, temperature, sunlight, ferric ion, and oxygen can influence results. Of the aldehydes examined, the TBA reaction products of dienals showed an absorption peak at 532 m μ only, while the TBA reaction products of the saturated aldehydes showed a peak at 452 m μ only. The spectral behavior of TBA reaction products of monoals was found to vary with the location of the double bond. Fat samples, aged at either 60C or 37.8C, were examined organoleptically and by the modified TBA test. The 452 m μ peak was of value in assessing the flavor of beef fat, cottonseed oil, and used frying fat, whereas the 532 m μ 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 Heniek et al., and gas chromatography (1-4). Perhaps the most widely used test in recent times for measuring unfractionated 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 m μ . 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 carbonyls 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 m μ . However, several have noted the appearance of a peak in the 452 m μ region, denoting yellow color (11,12). Recently, it has been shown that the absorption peak at ca. 452 m μ can be attributed to the reaction product of TBA with aldehydes (10,13). Tafel and Zimmerman reported that the presence of ferric ion greatly increased the intensity of yellow formed by the reaction of TBA with aldehydes at 70C instead of the customary 100C (13). These workers also reported that the reaction products of dienals with TBA absorb at 532 m μ (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 m μ to measure undesirable aldehyde flavors. No acid was added, and a moderate 60C temp was used to minimize changes in fat or reagent from excess acidity or heat. It was found that at 60C the yellow or 452 m μ peak became prominent. The pink color, representing absorption at 532 m μ , 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 60C in glass beakers to accelerate off-flavor development from oxidation and to break down any

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off-flavor precursors present. The cottonseed oil was aged in glass beakers at 37.8C.

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 60C 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 60C 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 $m\mu$.

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 unstoppered 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 60C 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 this 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 soy-

bean oil aged 2 days at 60C. 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 150C 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 125C 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 60C, the absorption peak at 452 $m\mu$ 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, Taufel'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

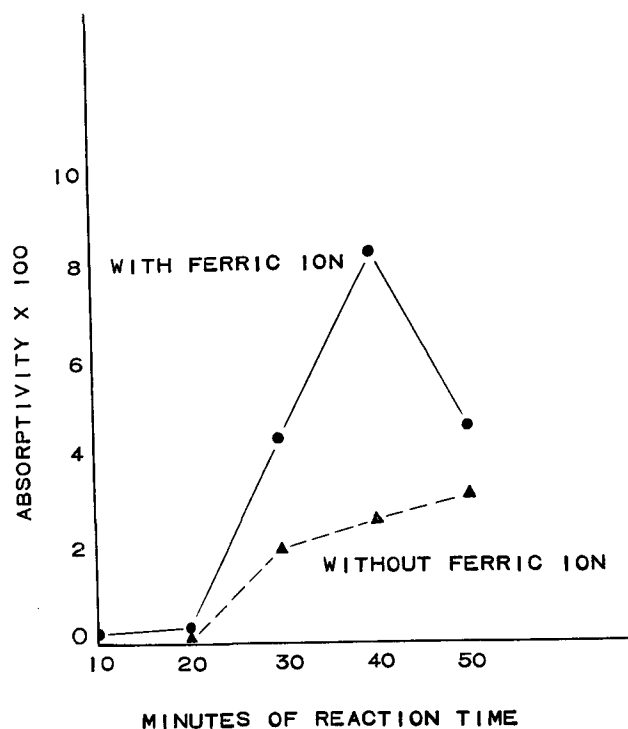


FIG. 1. The effect of ferric ion on the development of yellow in the reaction of TBA with beef fat.

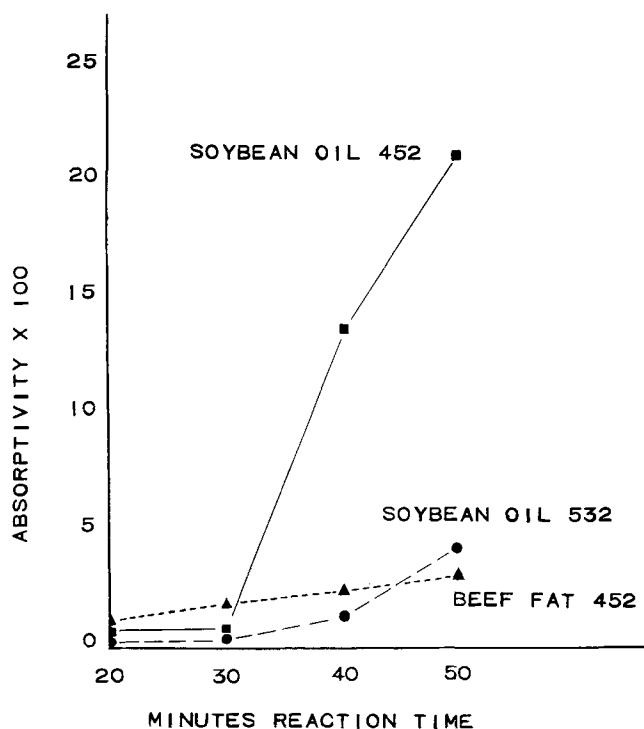


Fig. 2. Soybean oil and beef fat: rate of color development at 452 and 532 $m\mu$.

and solvents in order to measure the aldehydes of the fat with a minimum of background interference.

Light was considered another possible source of variation in the modified TBA reaction. Solutions of TBA in the solvent were held 4.5 hr under nitrogen, either under sunlight or daylight type fluorescent lamps. The OD at 452 $m\mu$ of the samples were 0.580 and 0.107, respectively. No unusual precautions were necessary when samples were handled under fluorescent lamps.

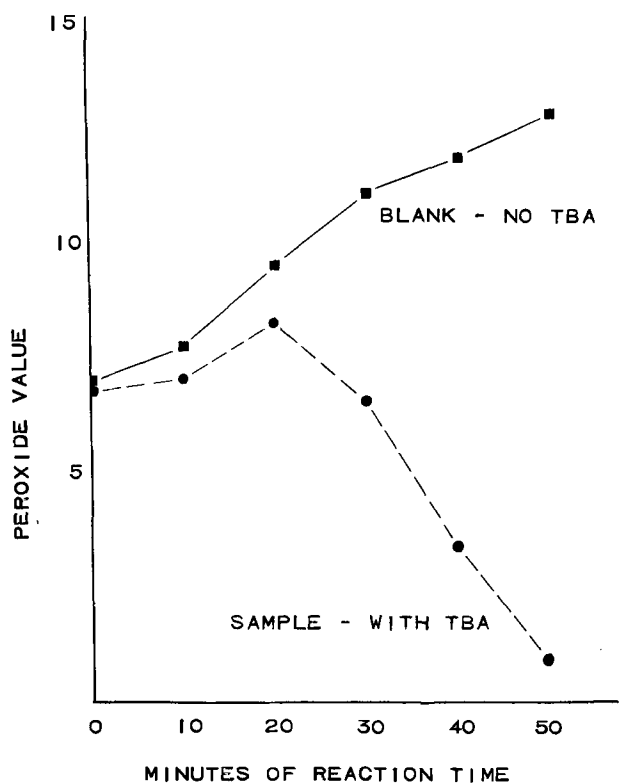


Fig. 3. Soybean oil: destruction of peroxides during the modified TBA test.

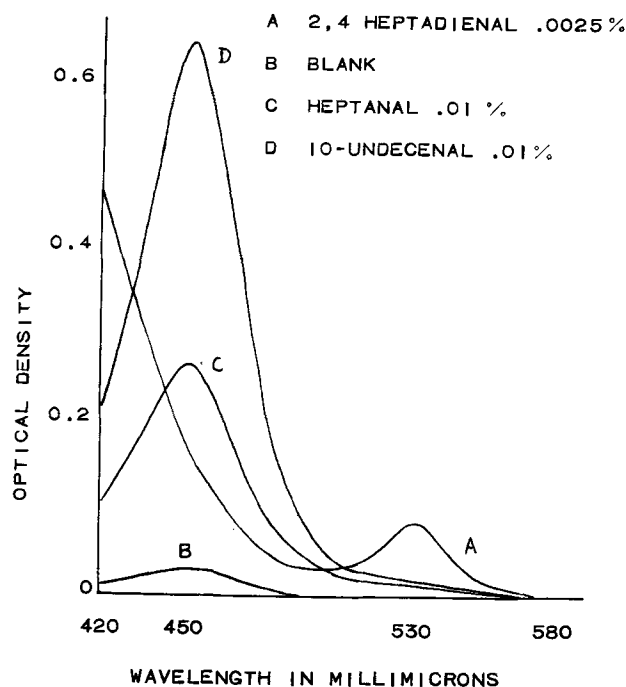


Fig. 4. Comparison of absorption spectra of several TBA reaction products.

Several fats were tested in solvent containing 0.036% ferric chloride. The ferric ion greatly increased the OD of the solutions as well as influencing the pattern of color development (Fig. 1). Whereas the maximum color development occurred at 40 min reaction time when ferric ion was present, only a slight decrease in rate of color development occurred at 30 min when it was absent. The increased sensitivity with iron might appear advantageous, but the resultant high blanks suggested a possible breakdown of the TBA itself in the presence of ferric chloride. Furthermore, reproducibility was poor with ferric ion present, and the usefulness of this modification was limited.

Different fats developed color at different rates, as would be expected because of their varied aldehyde composition and possible content of aldehyde precursors (Fig. 2). For example, the yellow developed gradually and uniformly with beef fat, but the 452 $m\mu$ peak with soybean oil became strong after 30 min. This difference may reflect the breakdown of aldehyde precursors under the test conditions, as well as the presence of more of the strongly absorbing mono-unsaturated aldehydes. The 532 $m\mu$ peak showed more gradual development and less prominence when reaction was at 60C.

The fate of peroxides under the reaction conditions was determined, since it is known that aldehydes can be formed on decomposition of peroxides. Soybean oil which had been aged 2 days at 60C showed a steady increase in peroxide value when heated in the reaction solvent without TBA (Fig. 3). When TBA was present, peroxide destruction seemed to occur significantly after 20 min heating, and the peroxide level dropped to near that of unheated fat after 30 min at 60C.

To gain insight into the relative absorptivities and positions of absorption peaks of the different aldehydes, several typical aldehydes were subjected to the modified TBA test (Fig. 4). As expected, unsaturation in the 10-undecenal molecule greatly increased its absorptivity as compared to that of the saturated aldehyde, heptanal. No difference in the

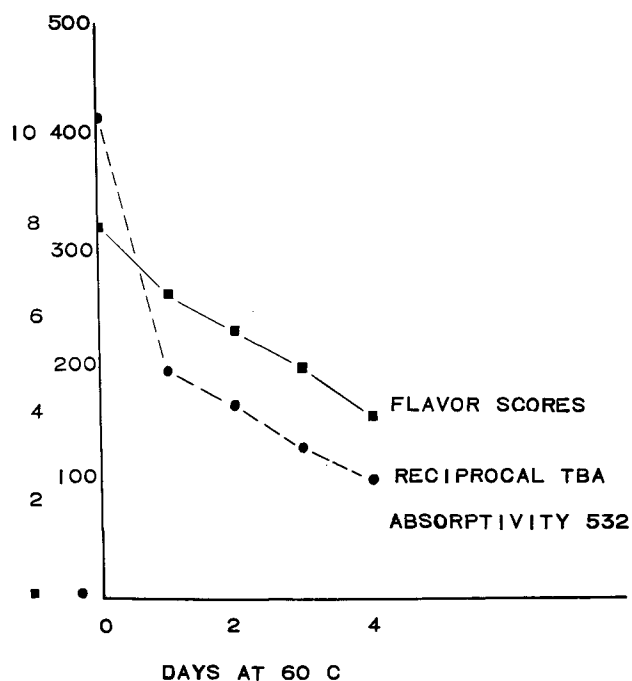


FIG. 5. Soybean oil: comparison of flavor scores with reciprocal TBA absorptivities at 532 m μ .

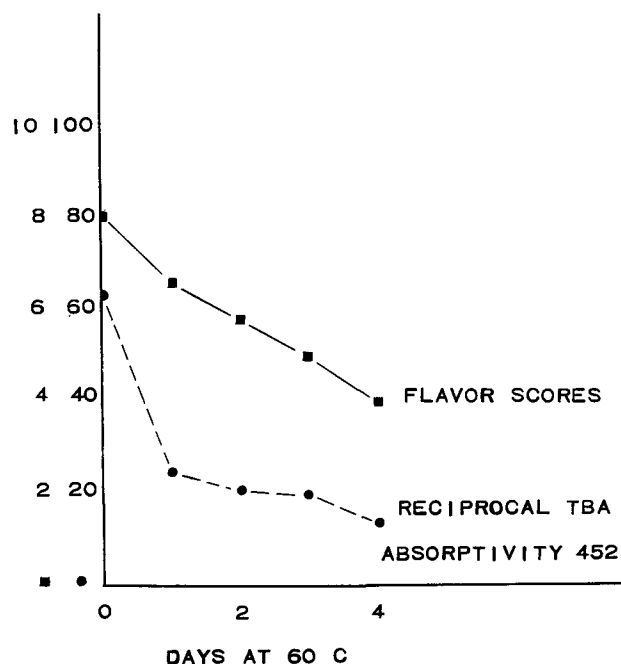


FIG. 6. Soybean oil: comparison of flavor scores with reciprocal TBA absorptivities at 452 m μ .

area of absorption was evident since both aldehydes produced reaction products absorbing at 452 m μ . When either crotonaldehyde or trans-2-hexenal was reacted with TBA, slight absorption was found ca. 532 m μ in addition to the major peak at 452 m μ . Although each of these aldehydes represented a single peak collected from the gas chromatography apparatus, the possibility that the 532 m μ absorption resulted from breakdown of the aldehyde or trace impurities cannot be completely ruled out. Further work is anticipated on this point.

The TBA reaction products of the dienals examined, 2,4-hexadienal and 2,4-heptadienal, showed a definite maximum at 532 m μ but not at 452 m μ . Although the 532 m μ peak is often interpreted as malonaldehyde content, malonaldehyde as prepared by hydrolysis of tetraethoxypropane does not have a particularly offensive odor. Certain dienals, such as 2,4-hexadienal, definitely contribute to the rancid odor and flavor of certain fats. Under the conditions of the modified TBA test, the reaction products of TBA and the dienals examined showed an absorption maximum only at 532 m μ . Since the TBA reaction products of some monoenes such as 2-hexenal and crotonaldehyde may also absorb at 532 m μ , it is evident that a reinterpretation should be made of some previous TBA studies attributing pink color development primarily to malonaldehyde or closely related compounds.

Comparison of flavor scores of the soybean oil series and reaction product reciprocal absorptivities at 532 m μ suggests that compounds of the 2-hexenal and dienal type might be important in the drop in flavor score of soybean oil aged at 60C (Fig. 5). This view is supported by Martin et al., who reported the presence of 2-heptenal in mixtures isolated from reverted soybean oil, and of Hoffmann who reported that certain dienals, such as 2,4-heptadienal, are present in the volatile decomposition products of soybean oil (14,4). It should be noted that 532 m μ absorptivities increase considerably after the first day of aging, but seem to be less prominent in unaged samples. This increased absorptivity perhaps indi-

cates a change in the relative importance of off-flavor components, brought about by the 60C aging temp.

The 452 m μ reciprocal absorptivity curve of aged soybean oils did not exhibit a well-defined relationship to the flavor scores, presenting the possibility that dienals were the major factor of the soybean oil off-flavor, rather than saturated aldehydes or mono-enals (Fig. 6). The pronounced increase in 452 m μ absorptivity after one day of aging was similar to that observed at 532 m μ .

Absorption at 452 m μ for the beef fat series appeared a good indication of the progressive flavor deterioration of the aged samples (Fig. 7). The type of curve illustrated might be of value for chemically screening beef fats for carbonyl type off-flavors.

The fried chicken frying fat represented a complicated system for both organoleptic and chemical

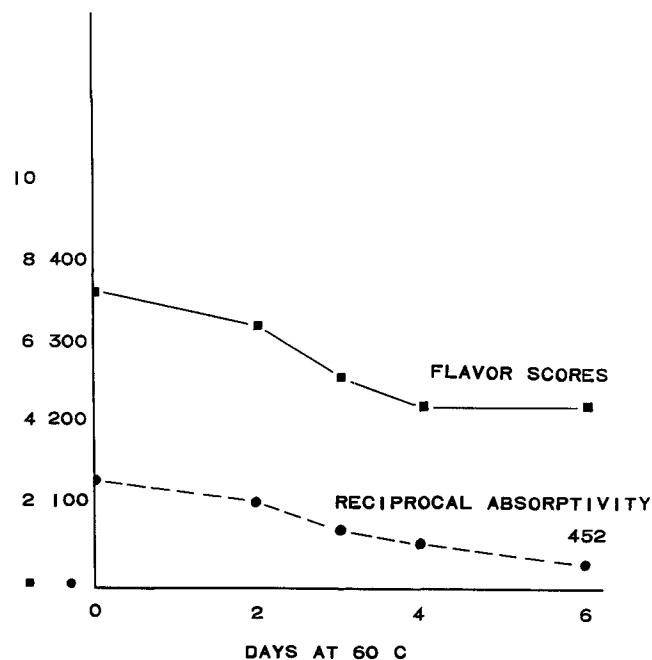


FIG. 7. Beef fat: comparison of flavor scores with reciprocal TBA absorptivities at 452 m μ .

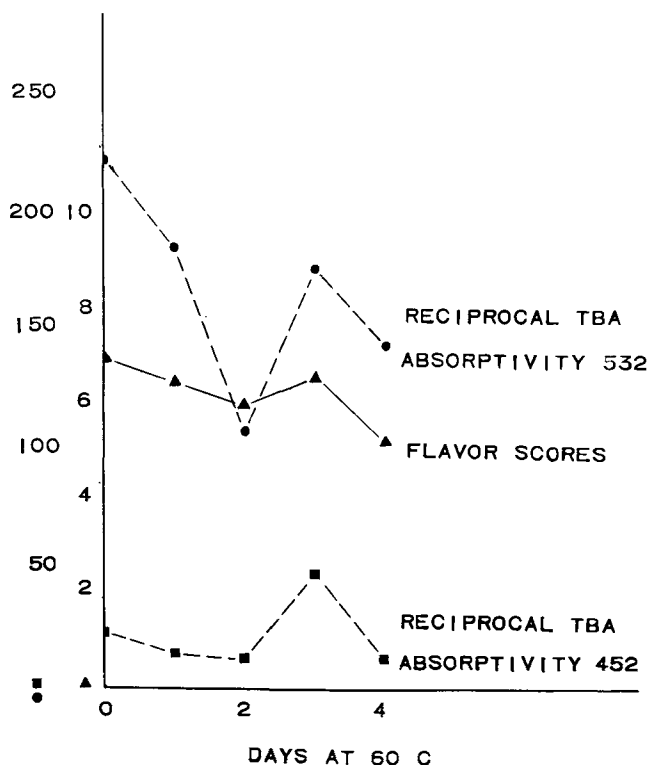


Fig. 8. Fried chicken frying fat: comparison of flavor scores with reciprocal TBA absorptivities at 532 and 452 $m\mu$.

evaluation because of the complex reactions taking place at frying temperatures, and the high level of pleasant flavor in the fat (Fig. 8). It has been shown that frying promotes the development of certain aldehydes, such as 2,4-decadienal, which are sometimes associated with the pleasant flavor of fried foods (15). The overall similarity of the 452 $m\mu$ reciprocal absorptivity and flavor score curves indicated that the saturated and monoenoic aldehyde content undoubtedly strongly influenced the overall flavor score. The possible role of the dienals cannot be overlooked, however, since increases in 532 as well as 452 $m\mu$ reciprocal absorptivity were followed by improvement

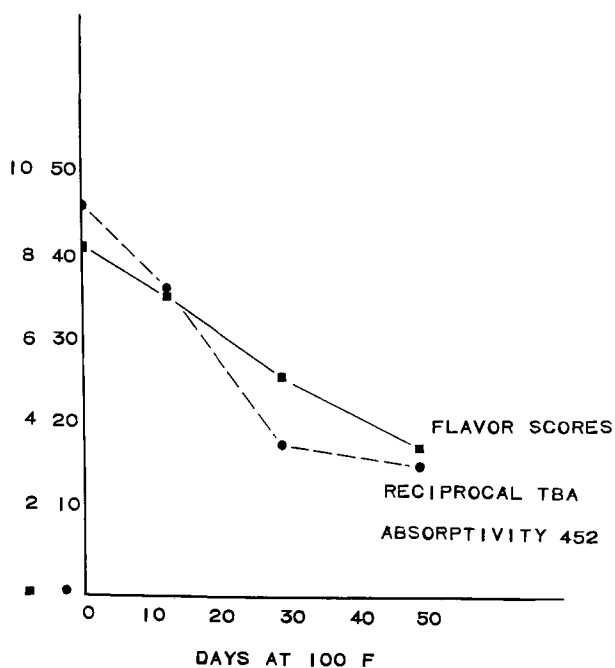


Fig. 9. Cottonseed oil: comparison of flavor scores with reciprocal TBA absorptivities at 452 $m\mu$.

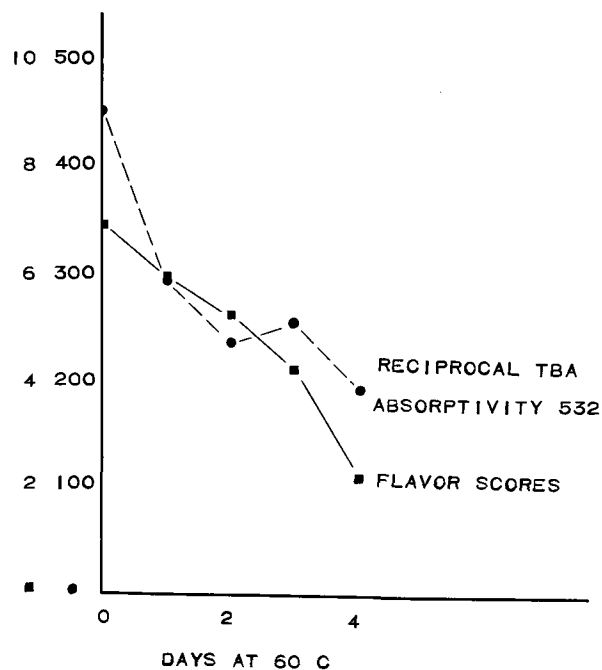


Fig. 10. Pork fat: comparison of flavor scores with reciprocal TBA absorptivities at 532 $m\mu$.

in flavor of the frying fat aged 3 days. A drop in aldehydes occurred between 2 and 3 days aging.

The 452 $m\mu$ reciprocal absorptivity values for the cottonseed oil series provided a good index of oxidative off-flavors of cottonseed oil in the edible range (Fig. 9). When flavor was well below acceptable range, the test did not provide an accurate test of flavor, as may be seen by the values of the 28 and 48 day samples.

The 532 $m\mu$ peak has been previously used for estimating flavor deterioration in pork (5). The specific role of the fat decomposition products in pork off-flavors has not been completely defined, however. Hornstein and Crowe have shown that alkanals, monoaldehydes, and dienals are present in pork fat heated in air at 100C, although amounts varied with the different class of aldehyde (16). In the present study the TBA-aldehyde reaction products absorbing at 452 $m\mu$ did not follow a pattern which correlated with the decline in flavor acceptability. The reaction products with absorption at 532 $m\mu$ did give a general indication of fat flavor (Fig. 10). It appears possible that flavor components not measured by this test played a significant part in the flavor of this sample of rendered pork fat.

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