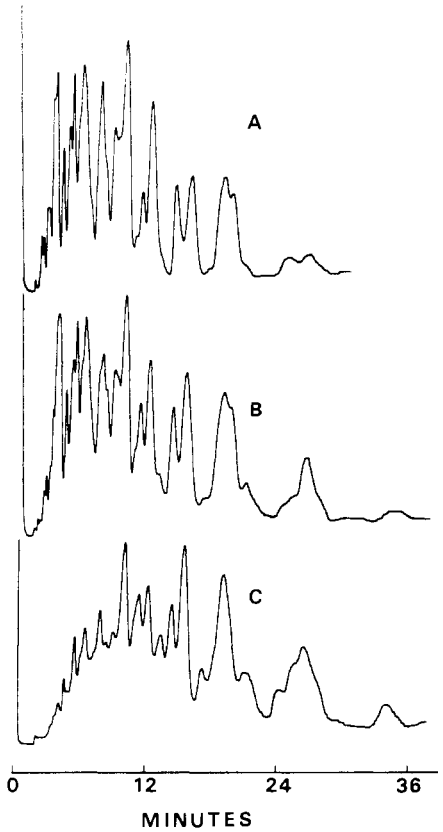

Figure 2. Gas chromatograms of toxaphene degraded in (A) sterilized salt marsh sediment (8 days after addition) and (B) sand-Fe(II)/Fe(III) system (14 days after addition). Chromatogram C is an undegraded toxaphene standard.

of other chlorinated pesticides (DDT, DDE), yet although we found parent toxaphene components in oysters, we could not identify toxaphene in the sediments. At the time of these analyses, however, we would not have recognized chromatograms such as Figure 1a as indicative of toxaphene degradation products and may have overlooked them. Thus, while toxaphene in anoxic marsh sediments appears to undergo changes in composition very quickly, the ultimate breakdown and toxicity of the alteration products has yet to be determined.

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Colorimetric Determination of Browning Precursors in Orange Juice Products

A colorimetric method for the determination of browning precursors in orange juice products is proposed, based on the thiobarbituric acid (TBA) color reaction with 5-hydroxymethylfurfural (HMF). Two clarification methods (Carrez solutions and saturated lead acetate) and several reaction conditions were evaluated for color complex intensity, stability, and repeatability. Pulp precipitation by lead acetate is recommended for the clarification step. A color complex, stable for up to 60 min, was formed in a reaction mixture of 2 mL of clear serum, 2 mL of trichloroacetic acid 40% w/v solution, and 1 mL of 0.05 M TBA, treated for 50 min at 40 °C. The proposed method offers some advantages in repeatability and convenience of HMF determination, in comparison to previously reported colorimetric or TLC procedures. Formation of HMF during storage in freeze-dried orange crystals, qualitatively evidenced by TLC and GLC methods, was measured by the suggested procedure.

Orange and other citrus juice products are susceptible to various deterioration reactions during processing and storage, resulting in off-flavor development and browning. The chemical changes have been extensively investigated during the last three decades, and the main deterioration mechanisms considered to cause off-flavor buildup include degradation of essential oil and aroma compounds (Blair et al., 1952; Bielig et al., 1972; Askar et al., 1973a,b),

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hydrolization and autoxidation of the lipid fraction (Huskins et al., 1952, 1953; Nagy and Nordby, 1970), and browning processes, involving ascorbic acid degradation (Joslyn, 1957) and Maillard reactions (Wolfrom et al., 1974).

In the initial and intermediate stages of browning reactions many compounds, having undesirable flavors, are formed (Hodge, 1967). However, most of these may occur at very low parts per million or parts per billion levels in deteriorated citrus products (Tatum et al., 1967; Shaw et al., 1970), making their isolation and identification very tedious and their application as deterioration indicators almost impractical. Two main chemicals which develop in the browning process of citrus products at levels easily detectable by chemical methods are furfural and 5-hydroxymethylfurfural (HMF), and they were proposed as browning indexes, although it was shown that they do not directly contribute to the perceptible off-flavor.

A colorimetric furfural measurement was developed by Dinsmore and Nagy (1972, 1974) and successfully applied for detection of storage temperature abuse in orange and grapefruit juices (Nagy et al., 1972, 1973, 1974). Berry and Tatum (1965) found significant development of HMF in stored orange powders and proposed a TLC method for its isolation and determination. International Federation of Fruit Juice Producers (IIFJP) (1964) published a colorimetric procedure for HMF determination based on its reaction with barbituric acid in the presence of *p*-toluidin. This reaction was also applied by Dhar and Roy (1972) for HMF in honey. However, this method offers certain disadvantages in procedure and accuracy, since the color complex stability is limited to 3 min. Another, much more stable color complex if formed by HMF and initial browning precursors with thiobarbituric acid under strong acid conditions, and it was used by Keeney and Bassette (1959) for the measurement of browning intermediates in milk products.

In course of freeze-dried citrus juice stability studies, undertaken by the authors, it was observed by TLC and GLC methods that HMF was developed in the dry product during storage. This investigation was undertaken with the aim of developing a rapid and reliable method for quantitative determination of HMF in citrus products based on the TBA color reaction. The method was subsequently applied for kinetic studies of browning deterioration in freeze-dried orange crystals.

EXPERIMENTAL SECTION

Materials. Fresh, hand-pressed orange juice and juice reconstituted from freshly dehydrated and dry-stored orange crystals were used for HMF determination. Pure 5-hydroxymethylfurfural was obtained from Fluka, AG, Switzerland. HMF solutions (0–15 ppm) were prepared in distilled water and fresh orange juice for determination of reactants absorption spectra and calibration curves. All other chemicals were of analytical grade.

Methods. *Clarification Procedures.* In the original method, used by Keeney and Bassette (1959) for milk products, clarification was obtained by addition of 2.5 N trichloroacetic acid (TCA) to a final concentration of 1 N, to coagulate and precipitate the milk proteins, followed by filtration through Whatman No. 1 filter paper. Thus, the TCA served for both clarification of the sample and acidification of the reaction medium. Direct addition of TCA solution to orange juice samples did not result with a fully clarified serum while using Whatman No. 1 paper and caused clogging and unpractically long filtration time (over 2 h for 20-mL samples) with Whatman No. 42 filter paper. Therefore, two other precipitation methods were tested: (1) by Carrez A and B solutions, as described in the IFFJP publication (1964); (2) by saturated neutral lead acetate solution (AOAC, 1970). The detailed lead acetate clarification procedure was as follows: 25 mL of single strength orange juice, followed by 4 mL of $Pb(OAc)_2$ solution, was pipetted into a 50-mL volumetric flask, the volume made up with distilled water, and shaken. The precipitated juice was vacuum filtered in a Buchner funnel through two layers of Whatman No. 1 filter paper. Excess $Pb(OAc)_2$ was precipitated from the clear filtrate by adding sodium or potassium oxalate crystals and filtered as above. This step was repeated once or twice for complete removal of the precipitating agent. It should be emphasized that thorough elimination of $Pb(OAc)_2$ from the filtrate was necessary to prevent haziness during the subsequent colorimetric reaction with TBA.

Table I. Effect of Clarification Method on Color Development in the TBA Test[a]

Clarification method	Absorbance: HMF solution (5 ppm)	Absorbance: Fresh orange crystals	Absorbance: Stored orange crystals[b]
None (1:2 dilution with distilled water)	0.296 (0.002)		
Carrez solutions (IFFJP, 1964)	*c*	0.166 (0.004)	0.199 (0.003)
Lead acetate (AOAC, 1970)	0.295 (0.002)	0.168 (0.005)	0.201 (0.005)

[a] Average and standard deviation of four clarification replicates, each duplicated during the TBA reaction (a total of eight measurements) are presented. [b] Orange crystals stored for 6 weeks at 25 °C. [c] Not tested.

The two clarification methods resulted with similar TBA readings in both fresh and stored orange crystals, and the lead acetate methods was selected due to its simplicity and quicker filtration rates. This method was further tested by subjecting pure HMF solutions to the clarification treatment. No adverse effect on the TBA color values was found in the $Pb(OAc)_2$-treated HMF solutions, and thus, its validity for this purpose has been confirmed (Table I).

TBA Reaction. Two milliliters of clarified orange serum were thoroughly mixed by a vortex mixer with 2 mL of 40 or 80% w/v TCA solution and 1 mL of 0.05 M thiobarbituric acid in screw-capped, 16 × 150 mm, culture tubes. The reaction was carried out at 40 ± 0.2 °C. Several reaction times from 25 to 60 min were tried. Then the test tubes were cooled for about 5 min in running tape water, and the colorimetric measurements were taken as soon as possible. Reference samples, in which 1 mL of distilled water was added instead of the TBA solution, were treated in the same manner. All reagent solutions were freshly prepared each day. The TBA was dissolved in water at 50–60 °C by vigorous shaking and immediately cooled with running tap water.

Colorimetric Measurements. Absorption spectra (Beckman D.B. Scanning Spectrophotometer) of TBA reaction products with pure HMF solutions and orange juice sera were compared, and the maximum absorption wavelength was determined. Consequently, the absorbance of all samples was measured at the 443-nm peak wavelength (Beckman D.U. with Gilford attachment for digital readout.)

RESULTS AND DISCUSSION

Absorbance spectra of TBA reactants with pure HMF solutions and with orange juice reconstituted from freeze-dried crystals showed a similar pattern, indicating that hydroxymethylfurfural or bicarbonyl browning precursors, which could have been converted to HMF at

Table II. Effect of Reaction Time and TCA Concentration on Color Formation and Stability in the TBA Test[a]

TCA concn, N	Time interval from reaction termination to color reading, min	Reaction time, min					
		25	30	35	40	50	60
		Aqueous HMF Solution (2.5 ppm)					
1	10	0.295	0.297	0.297	*b*	*b*	0.296
	30	0.290	0.291	0.290			0.288
2	10	0.285	0.290	0.303	*b*	*b*	0.298
	30	0.279	0.282	0.290			0.290
		Juice from Fresh Orange Crystals					
1	10	0.070	0.082	0.094	0.110	0.142	0.170
	30	0.090	0.096	0.105	0.120	0.145	0.172
	60	0.098	0.104	0.112	0.127	0.140	0.166
2	10	0.185	0.204	0.238	*c*		
	30	0.242	0.286	0.302			
		Juice from Stored Orange Crystals (11 weeks at 25 °C)[c]					
1	10	*b*	0.209	*b*	0.256	0.302	0.338
	30		0.248		0.268	0.293	0.334
	60		0.261		0.277	0.287	0.327

[a] Average reading of four replicates are presented for each treatment. The standard deviations were 0.002–0.003 and 0.003–0.006 absorbance units for HMF solutions and orange juice samples, respectively. [b] Not tested. [c] Measurements at 2 N TCA concentration in orange juice samples were discontinued due to the observed very high time dependence of color formation.

the elevated temperature and acidic test conditions (Eskin et al., 1971), were formed during storage in the dry orange crystals. The maximum absorbance at the wavelength of 443 nm, linear calibration curves of HMF solutions at concentrations up to 12 ppm, and the molar absorptivity of the TBA derivative (37 900, based on the molecular weight of HMF) were within very good agreement with the data previously reported by Keeney and Bassette (1959).

However, two substantial differences were observed while comparing the results for HMF in water and HMF added to fresh orange juice: (1) in freshly pressed orange juice with no HMF added, a slight color complex developed (equivalent to about 1.5 ppm of HMF), indicating that HMF, or its precursors, are formed in the juice to a certain extent during the TBA reaction. This may be expected at the reaction conditions, since formation of HMF, especially through sugar caramelization (Eskin et al., 1971), is strongly acid catalyzed (Pigmen and Goepp, 1948). (2) In aqueous HMF solutions, the range of absorbance measurements for six replicates did not exceed 1% of the mean values at all concentrations tested, whereas ranges of up to 6% were observed in orange juice solutions. In view of this finding, it has been deduced that the formation of TBA color reactants in orange juice products was affected by reaction conditions. Thus, various conditions were tested to select the most appropriate procedure for color complex intensity and stability.

The effect of two trichloroacetic acid concentrations (1 and 2 N in the reaction mixture) and several reaction times (from 25 to 60 min) on color formation and stability is given in Table II. These conditions were evaluated with aqueous HMF solutions (2.5 ppm) and with orange juice reconstitutes from fresh and stored freeze-dried crystals. The results show that with pure HMF solution the intensity of TBA color reactants is not dependent on reaction time and TCA concentration in the range tested. There seems to be a slight fading of the color with time after reaction termination, but even within 30 min this may introduce only a 2–3% relative error in the calculated HMF concentration. With orange juice samples, the reactants' color intensity is strongly affected by reaction conditions. At 2 N TCA concentration, an intense color developed even in fresh orange juice, indicating rapid HMF formation during the reaction. Moreover, the color increased substantially with time after the samples were cooled to room temperature, thus disabling consistent colorimetric measurements. Therefore, further studies at this acid concentration were discontinued.

Even at 1 N TCA, the color intensity increased consistently with reaction time. However, whereas at reaction times up to 40 min some more color developed in the cooled samples, relatively stable values were found in reaction mixtures treated for 50 or 60 min at 40 °C. This suggests that, during the TBA reaction in orange serum, color formation is affected both by HMF and browning precursors already found in the product and by those formed during the reaction. It may be assumed that at the higher reaction times most of the active precursors were complexed by thiobarbituric acid and therefore better color stability was obtained.

In conclusion, although very strict, empirically determined reaction conditions (2 mL of clear serum and 2 mL of 40% w/v TCA solution and 1 mL of 0.05 M TBA, treated for 50 min at 40 °F) should be observed for consistent and stable color readings, the suggested method enables quick and repeatable estimation of browning precursors in orange juice products. The relatively high stability of the color derivative (almost 60 min at room temperature) offers a distinctive advantage over the previously reported procedure (IFFJP, 1964). A simple formula has been derived for the content of browning precursors, expressed as milligram HMF/liter of single strength juice, based on the HMF molar absorptivity and the 1:2 serum dilution during the clarification step, as follows: HMF (mg/L) = 16.7 × absorbance at 443 nm.

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RH-6201 (Blazer): A New Broad Spectrum Herbicide for Postemergence Use in Soybeans

RH-6201 is a new herbicide with excellent potential for the selective control of most broadleaf weeds in soybeans. Current problem weeds such as cocklebur, velvetleaf, morningglory, and jimsonweed are susceptible at 0.5 lb/acre. Soybeans exhibit excellent tolerance to RH-6201. Chemically, RH-6201 is sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate.

The widespread use of preplant and preemergence herbicides in soybeans has resulted in effective annual grass control, but many broadleaf weeds are resistant and because of reduced competition now represent a major problem (Baldwin and Frans, 1972; Mahoney and Penner, 1975). This communication describes a new herbicide, RH-6201, which provides selective control of the major broadleaf weeds infesting soybean fields when applied as a postemergence treatment. The proposed common name for RH-6201 is acifluorfen-sodium.

EXPERIMENTAL SECTION

Synthesis of Compounds. *Synthesis of Sodium 5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate (RH-6201).* 3-[2-Chloro-4-(trifluoromethyl)phenoxy]-benzoic acid was prepared by the treatment of 3,4-dichlorobenzotrifluoride with the dipotassium salt of 3-hydroxybenzoic acid at 138–144 °C in dimethyl sulfoxide for 22 h. The cooled reaction mixture was poured into water, and the aqueous organic mixture was triturated with carbon tetrachloride to remove neutral organic materials. The aqueous layer was then decanted and acidified to pH 1 with concentrated hydrochloric acid to given an off-white solid, which was collected by filtration and vacuum dried at 60 °C overnight. The white powder (85% yield) melted at 124–125 °C and was used without further purification. Anal. Calcd for $C_{14}H_8ClF_3O_3$: C, 53.10; H, 2.55; Cl, 11.20; F, 18.00. Found: C, 53.18; H, 2.59; Cl, 11.16; F, 17.45.

5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid was prepared by nitration of 3-[2-chloro-4-(trifluoromethyl)phenoxy]benzoic acid in an ethylene dichloride/sulfuric acid cosolvent system at 0 °C with incremental addition of 1 equiv of potassium nitrate over a 0.5-h period. One-half hour after completion of addition, the reaction mixture was allowed to warm to room temperature, poured cautiously into an ice–water mixture, and extracted into chloroform. The insoluble solids were removed by filtration, the organic layer decanted and dried with anhydrous sodium sulfate, and the solvent removed in vacuo to give a solid product (82% yield) that melted at 137–150 °C. This was recrystallized from benzene–petroleum ether and melted at 151.5–157 °C. Anal. Calcd for $C_{14}H_7ClF_3NO_5$: C, 46.50; H, 1.95; N, 3.87; Cl, 9.80; F, 15.76. Found: C, 46.79; H, 1.91; N, 3.65; Cl, 9.46; F, 15.35.

This product was then titrated with 1 equiv of sodium hydroxide to give a quantitative yield of sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate (RH-6201).

Biological Testing. The results presented in Table I are from a field test in Newton, Pa., conducted in June 1976. The treatments were replicated three times and applied in 40 gal of water/acre. The sensitivity of the major soybean weeds to RH-6201 is indicated in Table II. These results were obtained from field trials throughout the major soybean areas of the U.S. in 1976. The weed height at application time was 0.25 to 3.0 in. Percent weed control and soybean injury was determined by visual estimation of percent plant growth reduction in treated compared to nontreated plots.

RESULTS AND DISCUSSION

The chemical structure of RH-6201 is shown in Figure 1.

The herbicidal results in Tables I and II show that RH-6201 is highly effective against many broadleaf weeds in soybeans including current problem weeds such as

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