Taste Evaluation of Nonenzymic Browning Compounds from Orange Powder and Use of Inhibitors

Philip E. Shaw, James H. Tatum, Theodore J. Kew, Charles J. Wagner, Jr., and Robert E. Berry

Taste threshold levels are reported for 18 products identified from stored, dehydrated orange crystals. Two of these, 3-hydroxy-2-pyrone and tiglic acid, were not previously reported as storage products from orange crystals. Methods of synthesis are given for those compounds not readily available. Quantitative estimates were obtained for nine of the storage products. Storage stability of orange crystals at 70° F was improved by addition of sulfur dioxide or certain carboxymethylcellulose types. These browning inhibitors were not effective at 85° F.

Flavor changes in dehydrated orange juice crystals (instant orange juice–IOJ) which develop during prolonged storage at ambient temperatures have been ascribed to the formation of nonenzymic browning products (Berry and Tatum, 1965). Tatum *et al.* (1967) identified 16 storage products from IOJ and model studies showed that D-fructose (Shaw *et al.*, 1967) and ascorbic acid (Tatum *et al.*, 1969) could be precursors for most of these products.

Information on taste thresholds of these storage decomposition products was needed to help assess the relative importance of individual compounds on the flavor of IOJ. Such information would help provide leads as to which type of browning reaction is contributing most to off-flavor development as well as a basis for establishing a product quality index. Information was also needed on the effects of nonenzymic browning inhibitors which might be expected to retard off-flavor development.

This paper reports the taste evaluation of 18 storage decomposition products from IOJ, a method of synthesis for those compounds not readily available, quantitative estimates for nine of the storage decomposition products, and the effect of some browning inhibitors on storage stability of IOJ.

PROCEDURE

Infrared spectra were obtained on a Perkin-Elmer 137 Infracord. Mass spectra were determined at 70 eV with a Bendix Model 3012 Time-of-Flight mass spectrometer equipped with an Electronics Associates Industries' Varian plotter 1110 recorder.

Chromatography. Glc-analytical gas-liquid chromatography was carried out as described by Tatum *et al.* (1967).

Preparative gas-liquid chromatography was performed on a Nester Faust 850 Prepkromatic instrument using a 12-ft × 2-in. Carbowax 20M packed column (20% on 30 to 60 mesh Chromosorb WAW) with a helium flow rate of 2 l. per min. The temperature was 100° C initially, and was raised to 230° at 4° C per min, then held isothermally to the end of the run.

Fruit and Vegetable Products Laboratory, Winter Haven, Fla. 33880

Tlc. Thin-layer chromatography was carried out as described by Tatum *et al.* (1967).

Taste Evaluation Samples. The compounds from Table I obtained from the commercial sources indicated in footnotes were checked for purity by glc and tlc prior to their use in taste evaluation. Compound 8 was distilled prior to use. Compounds 3 (R.T. 41 min) and 13 (R.T. 29 min) were purified by preparative glc prior to use.

3-Hydroxy-2-pyrone (5) was synthesized starting with 100 g of mucic acid by a modification of the procedure of Wiley and Jarboe (1956), which was followed through collection of

Table I. Taste Thresholds of Instant Orange Juice Storage Products

Compound	Estimated Threshold (ppm)	Estimated Am't. Pres. (ppm)
(1) N-Ethylpyrrole-2-carboxaldehyde[a]	2	0.5
(2) Methylcyclopentenolone[b]	5	1
(3) 5-Methyl-2-furfural[b]	10	
(4) Acetylformoin (4-hydroxy-2,3,5-hexanetrione)[c]	18	
(5) 3-Hydroxy-2-pyrone	30	1
(6) Furfuryl alcohol[d]	30	
(7) Tiglic acid[b]	60	2
(8) Furfural[e]	80	
(9) Benzoic acid[e]	85	12
(10) Acetic acid[e]	110	5
(11) Levulinic acid[f]	110	
(12) 5-Methylpyrrole-2-carboxaldehyde	110	
(13) 2-Acetylfuran[d]	110	
(14) 2-Acetylpyrrole[g]	200	
(15) 2-Hydroxyacetylfuran[h]	>200	
(16) 5-Hydroxymethylfurfural[f]	>200	14
(17) γ-Butyrolactone[d]	>200	0.5
(18) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	>200	17

[a] Procedure of Tatum *et al.* (1967), b.p. 48–52° C (1 mm). [b] K & K Laboratories, Inc., N.Y. [c] Procedure of Goto *et al.* (1963), m.p. 72–76° (sublimes). [d] Eastman Organic Chemicals, Rochester, N.Y. [e] Fisher Scientific Co., Fair Lawn, N.J. [f] Aldrich Chemical Co., Inc., Milwaukee, Wis. [g] Procedure of Berlin (1944), m.p. 88–88.5° C. [h] Procedure of Kipnis *et al.* (1948) and Miller and Cantor (1952), m.p. 79–80° C.

Table II. Extended Storage Life of Instant Orange Juice with Added SO_2 and CMC at 70° F

SO_2 Added		SO_2 Recovered	Moisture content (%)	Time for detect. diff. (weeks)
220		13.	1.1	19
440		19.5	1.0	16
880		48.7	1.1	>25
9693		629.	1.2	22
	Control	1.2		12
	CMC, %			
1.0	7AP		1.4	>22
0.75	7HF		1.2	>21
0.75	7L2P		1.1	12
1.50	7L2P		1.2	>18
	Control			12

the distillate. At that point, the distillate (~50 ml) was quite acidic (pH < 2) rather than alkaline, as Wiley and Jarboe found. The pH was adjusted to about 8 by the addition of solid sodium bicarbonate, then extracted with six 50-ml portions of ether. The combined extracts were dried over sodium sulfate and concentrated to about 20 ml volume, cooled, and the precipitate collected to give 3.0 g of yellow prisms, m.p. 83–84° C. Recrystallization from methylene dichloride-ether-pentane gave 2.1 g of 3-hydroxy-2-pyrone, m.p. 85–86° C.

5-Methylpyrrole-2-carboxaldehyde (12) was synthesized as described by Tatum *et al.* (1967) starting with 3.0 g of 2-methylpyrrole. Preparative glc of crude compound 12 afforded, after recrystallization from ether-hexane, 0.23 g of material, m.p. 67–68.5° C, which was recrystallized from the same solvent mixture to give the sample, m.p. 67–68° C, used for taste evaluation.

Compound 18, m.p. 69–71° C, was synthesized by the procedure of Severin and Seilmeier (1968). This compound to which they assigned the structure 2-acetyl-4,5-dihydro-3,4-dihydroxy-furan is identical to a compound assigned the structure 4-hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone (Shaw *et al.*, 1967). Hodge *et al.* (1970) have shown that the correct structure for this compound is 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

Taste Tests. All threshold levels were determined using IOJ reconstituted to single strength juice. The triangular comparison tests discussed by Boggs and Hanson (1949) were employed, using 12 experienced tasters being given one presentation each. The number of presentations had to be limited due to the limited amount of available material. At the beginning of each series of tests, tasters were presented an easily detectable concentration in order to help familiarize them with the flavor being studied. All thresholds should be considered approximate as established by this particular panel, but should serve as a rough estimate of the relative degree of flavor influence imparted by the compounds studied. Each compound was tested initially at 100 ppm. Then the concentration was halved or doubled, and in succeeding tests the difference was halved until the compound could no longer be detected. Following this procedure, threshold values were determined within 10 ppm or less, except that of compound 14, which was within 50 ppm. No compound was tested at a level above 200 ppm because actual amounts present in stored instant orange juice were considerably below that level. Some compounds were dissolved in a minimum amount of 95% ethanol (3 ml or less) prior to their addition to 1200 ml of reconstituted IOJ (Berry *et al.*, 1967). In these cases the same small amount of ethanol was also added to the control sample.

Storage stability flavor evaluations were carried out using triangular comparison with 12 experienced tasters, each being given two presentations. Storage test values in Table II are at a confidence level of 95% or greater.

Quantitative Analysis Using Tlc. A 1-g sample of IOJ crystals that had been stored at 100° F for 4 weeks was placed in a plastic-capped vial (10 ml capacity). After 1 ml of water was added, the mixture was stirred briefly and allowed to stand 10 min. Adding 2 ml of acetone, shaking, and allowing the layers to separate gave a clear, upper layer (about 3 ml) and a thick gelatinous lower layer containing the pectins, sugars, and some water. Aliquots of the upper layer were spotted on thin-layer chromatoplates with known amounts of compounds 16 or 18 (See Table I) which were spotted on both sides of the sample to be analyzed. For both compounds, the intensity of the spot developed with anisaldehyde spray reagent (Tatum *et al.*, 1967) could be determined on 15 μl samples within 0.05 μg when compared to standards spotted on the same plate. The other storage decomposition products were either not present in sufficient quantity or not separated adequately from interfering material for this method of analysis to be applicable.

Quantitative Estimates Using Glc. A 168-g sample of IOJ that had been stored at 100° F for 32 days was slurried with 300 ml of 1:1 acetone-water for 10 min using a Brookfield counterrotating mixer, Model L998, and then allowed to settle for 20 min at room temperature. This mixture was extracted with 12 250-ml portions of ether in an open beaker, mixing for 4 min for each extraction, and decanting the ether layer each time. The ether extracts were combined, 100 ml of water was added, and the mixture was evaporated under reduced pressure with tap water as a heat source until most of the ether and acetone were removed. The remaining aqueous mixture was filtered through a glass wool plug and the clear filtrate saturated with salt and extracted 12 times with 50-ml portions of ether. The ether extracts were combined, dried over sodium sulfate, and concentrated to small volume under reduced pressure. Acetone was added to give about 2 ml total volume and 400 μl of this was injected into the analytical gas chromatograph. Peak areas for nine compounds were estimated using height × width at half-height. From the concentration of one of these (hydroxymethylfurfural, 14 ppm) as determined previously by tlc, the others were calculated by proportionate peak areas. The results are listed in Table I. The concentrations of the other nine compounds could not be accurately estimated by this procedure because of the very small quantities isolated and because of interfering peaks. Most of them were present at less than 0.5 ppm.

RESULTS AND DISCUSSION

Table I shows taste threshold levels determined in orange juice for each of 18 storage decomposition products isolated and identified from IOJ. Two of these products, 3-hydroxy-2-pyrone (5) and tiglic acid (7), are being reported here for the first time as IOJ storage decomposition products, whereas the other 16 were reported previously by Tatum *et al.* (1967).

Tiglic acid at retention time 43 min and 3-hydroxy-2-pyrone at retention time 47 min were both isolated under conditions described by Tatum *et al.* (1967) and glc-separated samples were identified by comparison of glc retention times, and by infrared, mass spectral, and tlc comparison with authentic samples.

The wide range of taste threshold levels found in this study indicates that some components are more likely than others to be important contributors to the off-flavor. Compounds 1, 2, and 3 could be highly influential on flavor in very small quantities. Others, such as 5-hydroxymethylfurfural (16), are probably present in quantities sufficiently below their flavor thresholds so that they do not significantly influence flavor (Berry and Tatum, 1965).

Threshold levels on individual components do not take into account the influence of one component on another at below threshold levels, *i.e.*, possible synergistic flavor effects. In one taste-test designed to test this possibility, compounds 1, 2, and 3 of Table I were added in combination at half their threshold levels (at 1, 2.5, and 5 ppm, respectively). The taste panel was able to detect the presence of this mixture at these levels, indicating synergistic effects were active.

Attempts have been made to analyze quantitatively IOJ for some of these storage decomposition products at the point where off-flavor first develops. 5-Hydroxymethylfurfural (16) was not present in sufficient concentration to apply to reconstituted IOJ the analytical procedure that Winkler (1955) had used to detect this compound in honey. Extraction of an acetone-water slurry of IOJ crystals with ether to concentrate the 5-hydroxymethylfurfural present did not give reproducible quantitative results (Berry and Tatum, 1965).

The tlc procedure described for quantitative analysis (See Procedure) has been found to give the best reproducibility and the highest quantities to date for 5-hydroxymethylfurfural (compound 16) and compound 18. The 5-hydroxymethylfurfural concentration was 0.5 μg in 15 μl, and for compound 18 it was 0.6 μg in 15 μl. This represents a concentration in IOJ reconstituted to single strength of 14 ppm for 5-hydroxymethylfurfural and 17 ppm for compound 18. Each of these compounds was virtually completely extracted into the acetone layer in a single extraction. In one experiment, seven successive portions of acetone were added to the aqueous phase, decanting the acetone fraction each time. The seven extractions combined showed no greater quantity of either compound than when a single acetone treatment was used.

Since the quantities of 5-hydroxymethylfurfural (14 ppm) and compound 18 (17 ppm) present in IOJ at a point where a flavor change is first detected are much less than the threshold level of either compound (which is >200 ppm), neither of these is likely to be an important contributor to the flavor change at the point when it is first detected unless strong synergestic effects are acting. However, this analysis does serve to indicate the amounts of some of the other compounds with lower threshold levels that might be present.

Quantitative estimates of seven other storage decomposition products were made by relating their glc peak areas to that for hydroxymethylfurfural. Using 14 ppm as the amount of hydroxymethylfurfural present, concentrations of eight other products were estimated as listed in Table I. The quantity of one of these (compound 18) calculated by this method was the same as had been found above by tlc. Peak areas of the remaining nine compounds were either obscured by interfering peaks or were too small and broad to be integrated. In most cases, the concentration was less than 0.5 ppm based on the peak areas observed for compounds 1 and 17.

Model studies have previously shown that hexoses and ascorbic acid can be the precursors for most of the nonenzymic browning products found in stored IOJ. For example, of the five most potent compounds, numbers 3 and 4 can originate from acid-catalyzed degradation of D-fructose (Shaw *et al.*, 1967), numbers 2 and 5 from acid-catalyzed ascorbic acid degradation (Tatum *et al.*, 1969), and number 1 presumably from degradation of hexoses by amino acids (Spark, 1969).

One effective method of retarding browning in foods has been to add carbonyl binding agents such as sulfur dioxide. Table II shows the results of added sulfur dioxide on the storage stability of IOJ crystals stored at 70° F when compared to control samples stored at −5° F. When bisulfite was added to orange concentrate (the IOJ made from this concentrate was analyzed for sulfur dioxide), it extended the storage life, and at 48.7 ppm SO_2 it was more than doubled. Use of > indicates that the supply of sample was exhausted at the indicated time (25 weeks) with still no detectable difference noted. At 85° F, sulfur dioxide addition did not prolong storage life; a difference was detected at 6 weeks when compared to control samples stored at −5° C whether or not sulfur dioxide was added.

An unexpected observation led to the use of different carboxymethylcellulose (CMC) types as potential browning inhibitors. During experiments on double drum drying of orange concentrate, different CMC types were added to enhance the viscosity of the concentrate. Orange concentrate accumulating in the nip of the hot rollers became brown as it was heated except when CMC had been added. This observation suggested CMC as a potential browning inhibitor in IOJ.

Table II also shows the effectiveness of CMC in extending the storage life of IOJ crystals. Both 7AP and 7HP are high viscosity types, while 7L2P is a low viscosity type (Hercules Powder Company, Wilmington, Del.). All CMC samples except the one with 0.75% 7L2P showed extended storage times at 70° F before a detectable difference occurred. However, at 85° F, the added CMC did not prolong storage life of IOJ. Thus, these "browning inhibitors" appeared to have some effects, but they are not sufficient to counteract the higher browning reaction rates which occurred at the higher temperature.

LITERATURE CITED

Berlin, A. A., *J. Gen. Chem.* (USSR) **14,** 438 (1944).
Berry, R. E., Tatum, J. H., J. AGR. FOOD CHEM. **13,** 588 (1965).
Berry, R. E., Wagner, C. J., Jr., Moshonas, M. G., *J. Food Sci.* **32,** 75 (1967).
Boggs, M. M., Hanson, H. L., *Advan. Food Res.* **2,** 219 (1949).
Goto, R., Miyagi, Y., Inokawa, H., *Bull. Chem. Soc. Jap.* **36,** 147 (1963).
Hodge, J. E., Mills, F. D., Fisher, B. E., *Cereal Sci. Today*, in press.
Kipnis, F., Soloway, H., Ornfelt, J., *J. Amer. Chem. Soc.* **70,** 142 (1948).
Miller, R. E., Cantor, S. M., *J. Amer. Chem. Soc.* **74,** 5236 (1952).
Severin, Th., Seilmeier, W., *Z. Lebensm. Unters. Forsch.* **137,** 4 (1968).
Shaw, P. E., Tatum, J. H., Berry, R. E., *Carbohydrate Res.* **5,** 266 (1967).
Spark, A. A., *J. Sci. Food Agr.* **20,** 308 (1969).
Tatum, J. H., Shaw, P. E., Berry, R. E., J. AGR. FOOD CHEM. **15,** 773 (1967).
Tatum, J. H., Shaw, P. E., Berry, R. E., J. AGR. FOOD CHEM. **17,** 38 (1969).
Wiley, R. W., Jarboe, C. H., *J. Amer. Chem. Soc.* **78,** 2398 (1956).
Winkler, O., *Z. Lebensm. Unters. Forsch.* **102,** 161 (1955); Chem. Abstr. **49,** 16259*b* (1955).

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