A Rapid Gas Chromatographic Method for Studying Volatile Carbonyl Compounds from Orange Juice and Their Changes During Storage

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A method is described for gas chromatographic analysis of volatile aldehydes and ketones in citrus juice. Volatiles are stripped by a stream of nitrogen at reduced pressure and are passed through a solution of dinitrophenylhydrazine. The chemically trapped carbonyls are regenerated directly into the gas chromatograph. Mass spectra assisted in making identifications. Small sample size and a minimum of sample handling make the procedure convenient for observing chemical changes during storage.

The naturally pleasant flavor and aroma of orange juice deteriorate during storage at warm temperatures and prolonged storage periods. Juice stored at 30° C develops a very disagreeable aroma, when compared with the fresh aroma of juice stored at 5° C. Gas chromatographic analysis of juice volatiles should provide profiles indicative of changes in juice aroma which, in turn, could be related to autoxidative and underlying enzymic changes.

Techniques for analyzing fruit aromas have been explored and refined by many workers. Of most direct use in our studies has been the published work by Rhoades and Millar (1965), Attaway *et al.* (1962), and Wolford *et al.* (1962) on fruit aromas, and the work of Ralls (1960) and Dornseifer and Powers (1963) on volatile carbonyl determination. An ideal method must satisfy two requirements: efficient trapping of volatiles and convenient analysis. Often one of these objectives has been achieved at the expense of the other. Thus, physical methods for concentrating and trapping volatiles, *e.g.*, distillation, extraction, and freezing, all permit loss of very volatile components; however, the volatiles captured in this manner are readily subject to analysis by gas–liquid chromatography (glc). On the other hand, chemical trapping of volatiles is selective and efficient, but direct glc analysis of the trapped chemical derivatives is not always possible.

The present study was undertaken to investigate the possibility of coupling chemical trapping with glc analysis, and has been restricted to volatile carbonyl components, *i.e.*, aldehydes and ketones. Four salient features of the present work are: nitrogen stripping of volatiles at reduced pressure; selective trapping of carbonyls by dinitrophenylhydrazine (DNPH); glc analysis of regenerated carbonyls; and mass spectral (ms) verification of glc peaks. The first two techniques have been widely used, but usually have been followed by intricate and tedious chromatographic separations for isolation of individual DNPH fractions. By using an efficient carbonyl regeneration procedure, we have retained the same directness of glc analysis as though the free carbonyls themselves had been trapped. The procedure has been successfully tested on a model juice which was spiked with known carbonyls in the parts-per-million range.

The authors hope to develop a system which will function as an index in detecting the onset of off-flavor and off-odor development in commercially processed citrus juice. Study of other types of volatile components is now in progress.

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EXPERIMENTAL

Volatiles were stripped from juice by a stream of nitrogen under partial vacuum and passed directly into an ethanolic solution of dinitrophenylhydrazine sulfate, where aldehydes and ketones were retained as dinitrophenylhydrazones. Solid dinitrophenylhydrazones, isolated by precipitation or by solvent extraction, were characterized by infrared spectra (ir), thin-layer chromatography (tlc), and, most informatively, by combined glc–ms analysis of the regenerated carbonyls. The regeneration procedure of Jones and Monroe (1965) and the flash pyrolysis technique of Stephens and Teszler (1960) were employed.

Chemicals. DNPH Reagent. Three grams of dinitrophenylhydrazine were dissolved in 35 ml of 1:1 sulfuric acid:water and diluted by 70 ml of ethanol.

Regenerating Reagent. A mixture of equal weights of oxalic acid dihydrate and *p*-dimethylaminobenzaldehyde.

Apparatus and Techniques. Stripping Assembly. A three-neck 1-l. flask, which contained 500 ml of citrus juice, was connected to a fritted tube for nitrogen bubbler and a 30 cm Vigreux column for water condensation. The Vigreux column was, in turn, connected to a conventional cold-finger trap held at room temperature. The trap held the DNPH solution, received the volatiles, and led to a water aspirator. Nitrogen flow was controlled by a needle valve so that a dial gage at the aspirator read 3 to 5 in. (mercury) less than full aspirator vacuum. A hot plate and pan of water maintained the temperature around the 1-l. flask at 55 to 60° C.

Pyrolysis Unit. The unit was similar to the one described by Stephens and Teszler (1960). Application of 14 V brought the temperature to 150° C in 15 sec and to 280° C in 25 sec, the usual heating time. A 1 1/2 in. No. 23 hypodermic needle was used for entry into the glc.

Thin-Layer Chromatography. Separation of DNP derivatives was accomplished on 20 × 20 cm precoated silica gel G plates (250, Analtech, Inc., Wilmington, Del.). Plates were nonactivated and development was with benzene-ethyl acetate (97:3, v/v).

Infrared Spectroscopy. Micro KBr pellets were run on a Perkin-Elmer Infracord spectrophotometer equipped with a lens-type beam condenser.

Gas–Liquid Chromatography. Carbonyls were tentatively identified with an F&M Model 5750 gas chromatograph equipped with dual hydrogen flame detectors. For definitive identification of carbonyls the glc–ms combination consisting of a Loenco Model 160 gas chromatograph and a Bell &

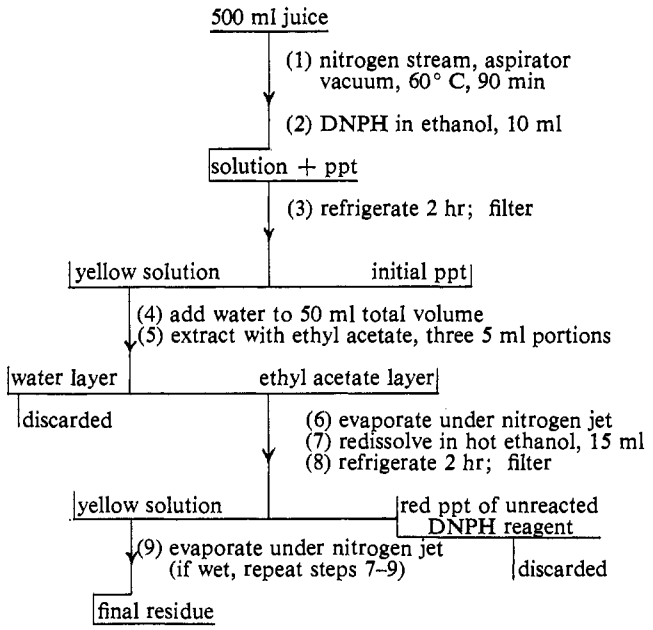

Figure 1. Flow chart of stripping and subsequent operations

Howell 21-490 mass spectrometer was employed. The glc–ms was interfaced with a jet separator and spectra were obtained employing the following conditions: molecular separator, 260° C; ion source, 250° C; and ionizing energy, 82 eV. Aluminum columns used were: 10% stabilized DEGS on 100/120 mesh, DMCS treated Chromosorb W, 1/4 in. × 6 ft, 10% Carbowax 20 M on 60/80 mesh Chromosorb W, 1/4 in. × 6 ft, and 10% UC W-98 on 70/80 mesh Chromosorb W, 1/8 in. × 20 ft. For DEGS and Carbowax 20 M columns, the temperature was programmed from 40 to 200° C at 10° C/min, and a helium flow rate of 80 ml/min was employed. The UCW-98 column was temperature programmed from 40 to 280° C at 10° C/min with a flow rate of 60 ml/min. The detector and injection port temperatures were 270 and 280° C, respectively.

Juice Samples and Preparation. JUICE SAMPLES. Fresh juice was prepared from Pineapple oranges by hand-reaming with a household juice extractor. Commercially prepared chilled orange juice samples in 1 qt glass jars were purchased from Adams Packing Co., Auburndale, Fla. The juice had been pasteurized at 105° C for 3 sec, then brought to 5° C in 3 sec and, finally, aseptically filled into glass containers. The samples were taken directly from the assembly line and placed in 5 and 30° C temperature-controlled storage rooms. Storage time was in excess of 20 months before our stripping studies were made.

MODEL JUICE. One microliter each of selected aldehydes was dissolved in 1 ml of ether, and this was added to 1 l. of a previously stripped fresh juice. Two such model samples were prepared, each containing hexanal, 2-hexenal, octanal, and decanal. One of them also contained *n*-butyraldehyde.

SAMPLE PREPARATION. Quantities and other pertinent information are given in the accompanying flow diagram, Figure 1. During 90 min nitrogen stripping of 500 ml juice, the 10 ml volume of ethanolic DNPH approximately doubled by water carried over. Dinitrophenylhydrazones which precipitated directly from the cold reagent were filtered with suction. Those still in the ethanol solution were extracted into ethyl acetate after addition of enough water to insure a separate ethyl acetate layer. Evaporation of ethyl acetate left a residue which was redissolved in ethanol; subsequent filtration and reevaporation gave the final residue. The ethanol solution and evaporation steps served to eliminate considerable unreacted DNPH reagent which precipitated from cold ethanol and gave a drier product than from ethyl acetate directly.

The initial precipitate and the final residue were the only DNPH products obtained from each stripping experiment. These were studied as separate fractions, but were not themselves further fractionated or reworked, inasmuch as mixtures of DNPH's presented no problem to glc analysis. The quantities of DNPH's thus isolated from 500 ml of juice were 5 to 10 mg of initial precipitate and 30 to 40 mg of final residue. These quantities of DNPH derivatives were far more than enough for ir study and for several glc runs. Each regeneration pyrolysis used about the quantity needed for a capillary melting point, the DNPH product being mixed with approximately five times as much regenerating reagent and packed into a normal melting point capillary to a length of 15 to 20 mm.

RESULTS

Figure 2 shows comparative glc profiles of aldehydes introduced directly into the gas chromatograph and aldehydes stripped from a model juice. The directly introduced free carbonyls were virtually indistinguishable from the trapped and regenerated carbonyls. Of the aldehydes captured, those C_6 and higher were all in the initial DNPH precipitate, while the final DNPH residue was entirely C_4. Outer C_2 to C_4 components manifest in these chromatograms come from incomplete previous stripping of the juice base used to make the model juice. From results like those in Figure 2 we would expect to detect easily 0.1 ppm of aldehydes as high as C_{10} from stripping a 500-ml juice sample

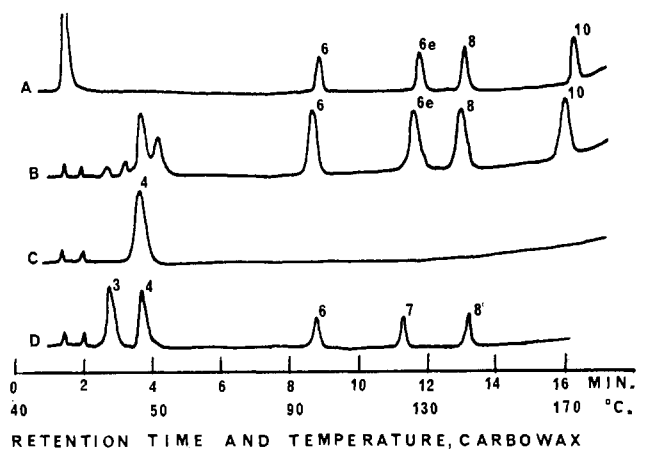

Figure 2. Gas chromatograms of recovered volatiles from model juice. 10% Carbowax 20M, 40–190° C at 10° C/min after initial 2 min hold. A. Ether solution of added C_6-C_{10} aldehydes. B. Pyrolysis of initial DNPH precipitate. C. Pyrolysis of final DNPH residue. D. Pyrolysis of reference DNPH aldehyde mixture. Carbon numbers are indicated; e(ene) indicates unsaturation

Gas chromatograms of volatiles from natural juices are shown in Figure 3. The most prominent captured volatile from fresh and 5° C juices was acetaldehyde, but from 30° C juice it was furfural. This distinctive difference between fresh flavor and bad flavor juices showed up in the initial DNPH precipitates and was clearly observed during the stripping process. After only a few minutes of stripping a 30° C juice red furfural-DNPH was conspicuous, whereas the original lemon color of the reagent persisted during the strip-

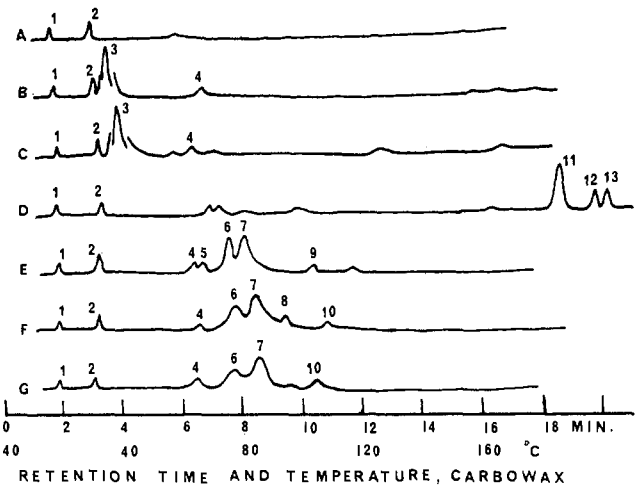

Figure 3. Gas chromatograms of volatile carbonyls from various juices. 10% Carbowax 20M, 40–200° C at 10° C min after initial 4 min hold. For peak identification see Table I. A. Pyrolysis reagent blank. B. Fresh juice, initial DNPH precipitate. C. 5° C juice, initial DNPH precipitate. D. 30° C juice, initial DNPH precipitate. E. Fresh juice, final DNPH residue. F. 5° C juice, final DNPH residue. G. 30° C juice, final DNPH residue

ping of fresh and 5° C juices. Capturing furfural from the 30° C juice supports a well-known observation of Rymal *et al.* (1968) and many others, but the absence of acetaldehyde seems noteworthy when one considers its prominence before storage. At this point we apparently contradict Rymal, who showed only a low initial acetaldehyde concentration which increased after storage. In favor of the present result is the fact that we are capturing this very volatile aldehyde as a nonvolatile derivative.

The final DNPH residues from all juices were similar in containing mainly acetone, butyraldehyde, and butanone. Still smaller glc peaks have been assigned to C_5 ketones and to an unknown compound characterized by *m/e* 84. The relative prominence of C_4 carbonyls in our chromatograms contrasts with an apparent absence of C_6 and higher carbonyls. Although Schultz *et al.* (1964) reported finding butyraldehyde and butanone, the majority of investigations of orange essence and peel oil (Attaway *et al.*, 1962; Moshonas and Lund, 1969; Senn, 1963; Wolford *et al.*, 1962) have placed no emphasis on C_4's but have emphasized C_6 and higher carbonyls, none of which were detected. Since the model juice results were entirely successful for carbonyls C_6 through C_{10}, their consistent absence seems valid for the comparatively small and dilute juice samples used in this study.

The combined use of several methods provided positive identifications. Mass spectral verifications (Table I) were obtained for each of the labeled glc peaks in Figure 3. Characteristic difference in ms cracking patterns clearly identified peak 4 to be acetone, rather than propionaldehyde, and likewise enabled butyraldehyde and butanone to be distinguished from one another. In a sense the ms results were only confirmatory, since glc retention times closely matched those of reference compounds, whether introduced as liquids or as DNPH derivatives. Thin-layer chromatograms of DNPH products supported glc findings and were useful in eliminating from consideration residues which were largely

Table I. Identities of Glc Peaks in Figure 3

Peak No.	Identity or Ms Data (Major Peaks)
1	CO and CO_2
2	unidentified (*m/e* 87, 59, 45–43)
3	acetaldehyde
4	acetone
5	unidentified (*m/e* 97, 44, 43, 15)
6	*n*-butyraldehyde
7	butanone
8	unidentified (preceding peak still in ms)
9	unidentified (*m/e* 84, 57, 44–41, 29)
10	C_5 ketones (*m/e* 86–84, 72–69, 57, 43–41, 29–27, 15)
11	furfural
12	unidentified (*m/e* 122, 121, 107, 106, 105)
13	unidentified (*m/e* 134, 133, 121, 120, 119, 91, 73)

unreacted DNPH reagent. Main constituents, such as acetaldehyde and furfural, were also readily identified in infrared spectra of the corresponding DNPH fractions, our spectra matching closely those published by Ross (1953).

The carbonyl regenerating procedure proved to be completely dependable. Modifications of the reagent mixture were explored but always with inferior results. Analysis of two small glc peaks from pyrolysis of just the regenerating compounds was of interest. Mass spectra showed CO and CO_2 at the first peak. The second peak had *m/e* values as high as 87, but has not been identified. Jones and Monroe (1965) identified this second peak as acetaldehyde on the basis of glc retention time; however, our ms data does not substantiate their identification.

In adapting the procedures of this report to other investigations, several modifications may be desirable. The DNPH reagent trap can be followed by other chemical traps or by traps at low temperatures for capturing other volatiles. In these cases an aqueous solution of DNPH (such as a saturated solution in 2 *M* HCl) should preferably be used in order to eliminate the otherwise large carryover of alcohol into later traps. Also extraction of DNPH's into ethyl acetate is easier from the aqueous reagent. Finally, we recommend the combining of all DNPH-carbonyl products into a single extract; thereby the entire carbonyl content of the sample will be displayed in a single gas chromatogram.

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Received for review November 4, 1970. Accepted January 4, 1971. References to specific products of commercial manufacture are for illustration only and do not constitute endorsement by the U.S. Department of Agriculture.