

CITRUS FLAVORING

Volatile Water-Soluble Constituents of Grapefruit Juice

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The nature of the chemical changes taking place in the volatile water-soluble flavoring constituents of grapefruit juice resulting from canning and storage has been investigated. There is only a small difference in the composition of the fresh and the freshly canned juices but these differ greatly from the stored juice. The freshly canned juice has a small amount of volatile acids and a trace of furfural not present in the fresh juice. The stored juice has a considerable increase in the volatile acids, methanol, and furfural. The lack of hydrogen sulfide in the freshly canned juice is taken to be due to deaeration before canning, and its reappearance in the stored juice as evidence of the breakdown of some sulfur compound in the juice. The volatile acids found in the freshly canned and stored juice were acetic and two unsaturated acids with the empirical formula $C_6H_8O_2$. A trace of a fourth acid was found in freshly canned juice in too small an amount to be identified.

THE FLAVORING CONSTITUENTS OF FRUITS have not been extensively investigated, and in only a few studies have the flavoring compounds been isolated and identified in an exhaustive manner (4, 5, 7-10, 17). Little work has been done on the chemistry of the flavoring constituents in canned fruit juices, and scarcely anything is known of the effect of the canning process on these constituents (1, 3, 11, 16).

The following investigations on grapefruit juice (*Citrus paradisi* Macfadyen) were carried out to determine the chemical nature of the volatile constituents in fresh juice, and particularly the effect of canning and storage on these constituents. Canned grapefruit juice develops an off-flavor on standing, which is distinct from the cooked flavor produced during the canning process. Although the exact nature of the constituents in the juice that produce flavor deteriorations is unknown, the flavor characteristics of this juice are due primarily to the volatile compounds which, if removed by vacuum distillation, leave the residue tasting merely sweet and sour. Therefore, it was desired to begin these studies by determination of the

volatile flavoring constituents in fresh, freshly canned, and stored canned juices.

This paper is a report on the isolation and identification of the volatile water-soluble constituents obtained from 2760 gallons of freshly reamed Marsh seedless grapefruit juice, 2470 gallons of freshly canned grapefruit juice, and 2470 gallons of stored canned juice.

In order to assure comparable juice throughout the experiment, each batch of fruit was divided into two lots, one for studies of fresh juice and the other for studies of the freshly canned and storage juices. The canned juice for the storage work was held at room temperature (27° to 32° C.; 80° to 90° F.) for 4 years to develop storage off-flavors. This long period was selected because it was suspected that the total amount of change in the flavoring constituents would be small. Table I gives the approximate amounts of the compounds found in the volatile water-soluble fractions of the three juices.

Experimental Work

Mature orchard-run Marsh seedless grapefruit [total soluble solids 11.74%, total acid (as citric acid) 1.30%] that had been hand sorted to remove soft

and damaged fruit was used in these studies. Two shipments of fruit were received per week, so that none of the fruit was stored for more than 2 days. The fresh fruit was stored at room temperature.

Juice from freshly hand-reamed grapefruit (2760 gallons) was charged into the evaporator in 10-gallon batches. The distillation was carried out at 40 mm. of mercury absolute, with the evaporator contents maintained below 45° C. (113° F.) at all times, until 50% of the juice had distilled over. The evaporator was heated by steam at 8 pounds' gage pressure (112° C., 233.6° F.). (It was found by a test run in which the distillate was collected in 10% lots, that essentially all of the volatile fractions were distilled off by the time the 50% point was reached.) The evaporator, a long vertical-tube, natural-circulation, vacuum evaporator with an external vapor-liquid separator, had a capacity under the above conditions of 10 gallons of distillate per hour. The vapors passed through two water-cooled stainless steel condensers into a flask at room temperature. This flask was connected to the vacuum pump through another condenser, refrigerated to 1° C. (34° F.) with an ethylene glycol system, and then through a series of traps main-

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Table I. Summary of Volatile Water-Soluble Constituents of Fresh, Freshly Canned, and Stored Canned Grapefruit Juice

(Mg. per kg. of juice)

	Fresh Juice	Freshly Canned Juice	Stored Canned Juice
Acetaldehyde	1.45	0.33	0.6
Acetone	None	None	0.1
Furfural	None	Trace	8.2
Ethyl alcohol	400	400	460
Methanol	0.2	0.2	23
Acetic acid	None	1.9	23.3
Acid A (C ₆ H ₈ O ₂)	None	4.8	2.9
Acid B (C ₆ H ₈ O ₂)	None	1.9	1.6
Acid C	None	Trace	None
Hydrogen sulfide	Trace	None	Trace

tained at -78°C . (-108°F .) with dry ice and alcohol. The efficiency of this trapping system was checked by adding liquid air traps between the vacuum pump and the rest of the system. As the liquid air traps yielded only a trace of acetaldehyde and carbon dioxide, and in the case of the fresh juice a trace of hydrogen sulfide (15), they were eliminated in the regular runs, and replaced by a trap containing 2,4-dinitrophenylhydrazine in sulfuric acid to trap the acetaldehyde.

The condensate from the room-temperature receiver was kept at 3°C . (37.4°F .) until material had been collected for several days. The combined condensate was then further concentrated by returning it to the evaporator and taking off half of it as distillate under the same conditions as the original juice. This operation was repeated until the total volume of condensate from the room temperature receiver had been reduced to 175 gallons. This material was then saturated with sodium chloride and extracted in a continuous extractor with diethyl ether. (All ether used in this work was anhydrous analytical grade ether containing not more than 0.01% alcohol. Prior to use it was distilled from alkaline ferrous sulfate to remove peroxides. A total of 15 gallons of ether was used for each type of juice.) The water layers in the traps were separated from the oils which they contained, saturated with salt, and then extracted with ether. All of the ether-extracted water fractions were then combined, freed of excess salt and ether, and concentrated in the vacuum evaporator to 8 gallons for the recovery of the alcohol fraction. The alcohol in this alcohol-water solution was recovered by fractionation on a Podbielniak Hyper-Cal column.

The canned juice for both the freshly canned and stored studies was extracted and canned commercially. It was deaerated at room temperature by flowing over a series of disks in a tank held at 97 mm. of mercury absolute. The juice was pasteurized at 88°C . (190.4°F .) by passage through a bank of 0.5-inch

tubes heated by steam and then filled directly into 46-ounce plain tin cans without previous cooling. After filling and sealing, the cans were cooled by a water spray to approximately 38°C . (100.4°F .) before being packed in cases. One half (2470 gallons) of this canned juice was then distilled in the same manner as the fresh juice.

The stored juice was distilled in the same manner as the fresh and freshly canned juices, except that the water-cooled condensate from the circulating evaporator was put through a stainless steel stripping column in order to concentrate the volatile water-soluble materials. In this manner the evaporator distillate was reduced to 140 gallons. This was then saturated with salt and extracted with ether. After removal of the salt and excess ether, the water layer was further concentrated to 4.3 gallons on the stripping column to recover the water-soluble material that was present. This volatile water-soluble concentrate was then carefully fractionated on a Podbielniak Hyper-Cal column, and the resulting fractions were systematically examined by microtests for carbonyl compounds, alcohols, esters, acids, and sulfur and nitrogen compounds.

The combined ether extracts of the water-layer condensates from the evaporation of the juice were freed of ether by distillation on the Podbielniak Hyper-Cal column. Any alcohols present in this fraction were fractionally distilled on this column. In the case of the stored-juice material, the oil remaining after the removal of ether and alcohol was extracted with sodium bisulfite to remove the large amount of furfural which was present.

Since the residue from the stripping of the evaporator distillate was acidic, 54.95 gallons (208 liters) of this residue were neutralized with sodium hydroxide. On evaporation of the neutralized solution, 14.27 grams of sodium salts were obtained. This material was converted to the *p*-phenylphenacyl esters (δ) for separation and identification of the acids (14).

Results and Discussion

Aldehydes Acetaldehyde was identified in the fresh juice fraction as a crystalline 2,4-dinitrophenylhydrazone melting at 167°C .; a mixed melting point with a known sample was not depressed.

Analysis. Calculated for C₈H₈N₂O₄, N 24.95. Found, N 24.84. Fresh juice contained 1.45 mg. of acetaldehyde per kg. based upon analysis with 2,4-dinitrophenylhydrazine.

Acetaldehyde was determined in the distillate from the freshly canned juice and the stored juice in the same manner. The values for these were 0.33 and 0.6 mg. per kg., respectively.

Furfural was not detected in the fresh-juice fraction by a test with *p*-toluidine acetate. A trace of this aldehyde was found in the water-soluble freshly canned juice fraction by means of the same test; a small amount of the purified aldehyde was isolated from one of the oil fractions of the same juice. It was positively identified by the formation of a semicarbazone melting at $190\text{--}191^{\circ}\text{C}$. (corrected). A mixed melting point with a known sample of furfural semicarbazone was not depressed.

Analysis. Calculated for C₆H₇N₃O₂, N 27.44. Found, N 27.01.

The preferred melting point for this derivative in the literature appears to be 203°C . (12). However, no analyses were found for a semicarbazone melting at this temperature, and Wolff (18) also reported a melting point of 190°C . for the semicarbazone of furfural, which yielded the theoretical values of carbon and hydrogen on analysis. A 2,4-dinitrophenylhydrazone was also prepared and melted at $224\text{--}226^{\circ}\text{C}$. The preferred melting point for this derivative is 229°C . (12).

The large quantity of furfural (8.2 mg. per kg. of juice) in the stored juice was isolated from the ether extract of the water distillate by means of the bisulfite addition product.

Acetone was found only in the stored juice in the amount of 0.1 mg. per kg. of juice. This was present in one of the alcohol fractions. It was identified as the 2,4-dinitrophenylhydrazone melting at $125\text{--}127^{\circ}\text{C}$. The melting point was not depressed when the derivative was mixed with a known sample of acetone 2,4-dinitrophenylhydrazone.

Alcohols Ethyl alcohol was identified in all three juice fractions as the crystalline 3,5-dinitrobenzoate melting at $92.3\text{--}93^{\circ}\text{C}$. Mixed melting points with a known sample did not show a depression. The fresh juice contained a minimum of 400 mg. of ethyl alcohol per kg., the freshly canned juice 400 mg. per kg., and the stored juice 460 mg. per kg.

Analysis. Calculated for C₉H₉N₂O₆, C 44.99; H 3.36. Found, C 44.92; H 3.31.

Methanol was detected in the distillate from all three juices. It was also identified by means of its 3,5-dinitrobenzoate, which melted at 107°C . Mixed melting points with a known sample were not depressed.

The methanol in the stored juice was separated by careful fractionation of the volatile fraction taken from the stripping column. The absolute amount of this methanol was determined by the refractometer method (19).

Because of the smaller quantities of methanol present in the fresh and the freshly canned juice, the actual amounts

were determined in these two by means of the sensitive chromatropic acid test (2). The fresh and freshly canned juices contained 0.2 mg. of methanol per kg. of juice and the stored juice, 23 mg. per kg.

Acids As acidic material was found in the residue from the stripping column run of the stored juice and a small amount in the freshly canned juice, a sample of fresh juice was rechecked for the presence of volatile acids. The distillate from fresh grapefruit juice was neutral.

The *p*-phenylphenacyl esters obtained from the acids in the stripper residue of the stored juice were separated by chromatographic adsorption on silicic acid (14). The major portion of this was found to be the derivative of acetic acid, representing 23.3 mg. of acetic acid per kg. of juice. The melting point of the ester was 110–112° C. and a mixed melting point with *p*-phenylphenacyl acetate gave no depression.

A *p*-phenylphenacyl ester melting at 76–77° C. was obtained. This analyzed for the derivative of an unsaturated acid with a formula of C₆H₈O₂. Its unsaturated nature was further confirmed by a positive fluorescein-bromine test on a chromatostrip (13).

Analysis. Calculated for C₂₀H₁₈O₃, C 78.40; H 5.92. Found, C 77.20; H 6.04. This acid was present to the extent of 1.6 mg. per kg. of juice.

The *p*-phenylphenacyl ester of a second unsaturated acid melted at 120–126° C. It, too, analyzed for the derivative of an unsaturated acid with a formula of C₆H₈O₂. The acid was present to the extent of 2.9 mg. per kg. of juice.

Analysis. Calculated for C₂₀H₁₈O₃, C 78.40; H 5.92. Found, C 78.57; H 5.92.

The unsaturated acids were not furoic nor sorbic acid, as evidenced by the melting points of the *p*-phenylphenacyl esters.

A small quantity of acetic acid was found in the ether extract of the freshly canned grapefruit distillate. As a further check on the acidic material, the distillate from a sample of freshly canned juice was neutralized and evaporated to dryness. The *p*-phenylphenacyl esters prepared from this were resolved into four derivatives. The acetic acid derivative represented 1.9 mg. of acid per kg. of juice. The two unsaturated acids found in the stored juice were also present in the freshly canned juice in the amounts of 1.4 mg. (76–77° C. melting derivative) and 4.8 mg. (120–126° C. melting derivative) per kg. of juice. A fourth acid was present in too small a quantity to be identified.

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Volatile Oil Constituents of Grapefruit Juice

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THE CONSTITUENTS of the volatile water-soluble flavoring fraction of grapefruit juice (*Citrus paradisi* Macfad-yen) and the effect of canning and storage on these constituents have been reported (70). The present paper deals with the volatile oil fractions from the same juices, and represents the oil from 2760 gallons of fresh grapefruit juice, 2470 gallons of freshly canned juice, and 2470 gallons of stored canned juice. The details of handling these juices and recovering the volatile material have been given (70). The volatile oils recovered

from these juices were derived from peel oil incorporated in the juice by the method of extraction and from the oil present in the juicesacs. The oils are those normally present in commercially prepared grapefruit juices.

Table I gives the approximate amounts of the various constituents found in the oil fractions of the three juices.

Chromatographic Methods

The chromatostrip technique of Kirchner, Miller, and Keller (9) was used

throughout this study. This technique consists of binding a thin layer of adsorbent with starch or plaster of Paris to a glass strip, and then using the microcolumn so obtained for diagnostic tests on oil mixtures, solutions, eluate fractions, and distilled fractions. The chromatostrips are also used for exact predictions of the performance of solvents and adsorbents on the larger columns, and for tentative identification by means of *R_f* values. The chromatostrips used in the work reported here were made with starch-silicic acid, ex-