

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 $C_6H_8O_2$. Its unsaturated nature was further confirmed by a positive fluorescein-bromine test on a chromatostrip (13).

Analysis. Calculated for $C_{20}H_{18}O_3$, 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 $C_6H_8O_2$. The acid was present to the extent of 2.9 mg. per kg. of juice.

Analysis. Calculated for $C_{20}H_{18}O_3$, 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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CITRUS FLAVORING

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-

The oil constituents of the volatile flavoring material from fresh, freshly canned, and stored canned grapefruit juice have been isolated, and separated into 21 compounds and three fractions of a complex nature. The following compounds were found in all three juices: *d*-limonene, β -caryophyllene, α -caryophyllene, another $C_{15}H_{24}$ hydrocarbon, α -pinene, traces of a hydrocarbon boiling lower than limonene, caryophyllene oxide, citral, carvone, $C_{15}H_{24}O$ or $C_{15}H_{26}O$, linalool, a ketone ($C_{15}H_{22}O$), carveol, α -terpineol, linalool monoxide, and an ester ($C_{12}H_{20}O_2$). The following were found only in the fresh and freshly canned juices: $C_{13}H_{15}N$, $C_{15}H_{28}$, and geraniol. The alcohol 3-hexene-1-ol was found only in the fresh juice, and furfural was found only in the freshly canned juice (traces) and the stored canned juice. The changes in the stored canned juice were the same as those in the freshly canned juice, but were greater quantitatively. The most significant changes were a decrease in limonene and an increase in linalool monoxide, α -terpineol, and furfural. Furfural undoubtedly arises from the sugars, while the remaining two oxygenated compounds would account for the decrease in limonene, although the mechanism of conversion to linalool monoxide is unknown. Taste tests showed these compounds produced undesirable flavors when added to fresh grapefruit juice.

cept as noted. The position of compounds on the completed chromatograms was most often detected by spraying with a 0.05% solution of fluorescein in water and then exposing to bromine vapors (9). Compounds containing unsaturated (ethylenic) linkages were revealed by this method. Other methods used as the occasion demanded were viewing of the fluorescent strips under ultraviolet light (9) and spraying with a 5% solution of concentrated nitric acid in concentrated sulfuric acid (9).

For the actual isolation of the constituents of the oils, adsorption and development on columns of adsorbent were used. The adsorbent was Merck's reagent grade silicic acid treated with starch and packed from a slurry of the developing solvent as described by Miller and Kirchner (17). Eluate fractions were collected from the columns with an automatic fraction collector and homogeneous zones combined after examination on chromatostrips (17).

Separation of Components

The oil layers collected from the various traps used in the distillation of the juice and from the ether extraction of the water layers (after removal of ether and alcohol by fractional distillation at low temperature) were combined where feasible (10). This material, consisting largely of *d*-limonene, other hydrocarbons, and nonhydrocarbons, was separated into a hydrocarbon and a nonhydrocarbon fraction by adsorption on a column of silicic acid with hexane as solvent as described by Kirchner and Miller (8).

As an example, a column containing 370 grams of starch-treated silicic acid was packed from a slurry with hexane, and 168 grams of oil from fresh grapefruit juice were then added. The column was developed with hexane until all the hydrocarbons had passed through the column and into the eluate as determined by chromatostrip tests. Ethyl acetate in

hexane in successive concentrations of 2, 4, and 5% was then used as the developer in order to give a crude separation of the nonhydrocarbon compounds. After suitable development, the developing solvent was removed and the column arbitrarily sectioned and eluted with ethyl acetate.

After removal of the solvent by low temperature evaporation, the hydrocarbon fraction had an optical rotation of $+81^\circ$. As this indicated the presence of material other than *d*-limonene, the hydrocarbons were fractionally distilled on an 8-mm. Podbielniak Hyper-Cal column, keeping the still-pot temperature below $85^\circ C$. Three fractions were obtained. Fraction 1 was mainly limonene with a trace of material boiling slightly lower than limonene and with an optical rotation less than that of limonene. Fraction 2 was pure *d*-limonene. Fraction 3 consisted of the still-pot residues

after complete removal of the limonene. Later this fraction was resolved into three sesquiterpenes by chromatographing with benzene-free hexane.

The separation of the hydrocarbons from the nonhydrocarbons on silicic acid by washing with hexane and subsequent development of the oxygenated compounds with successively stronger concentrations of ethyl acetate in hexane resulted in several fractions approximately separated into groups of compounds of similar R_f values. Each of these fractions was examined on silicic acid chromatostrips, using as developers the solvents listed in Table III. Each fraction usually consisted of two or more compounds. Using the solvent which the chromatostrips revealed as giving the best separation, the fractions were then separated on a silicic acid-starch column.

Table I. Summary of Volatile Oil Components of Grapefruit Juices

	Fresh	Freshly Canned	Stored Canned
	Mg. per kg. of juice		
Total oil (not including furfural)	20.92	26.00	27.59
Limonene	15.71	17.70	11.17
α -Pinene	Trace	Trace	Trace
α -Caryophyllene	0.10	0.10	0.12
β -Caryophyllene	1.40	1.40	0.87
$C_{15}H_{24}$	0.11	0.11	0.14
$C_{15}H_{28}$	Trace	Trace	None
Low boiling hydrocarbons	Trace	Trace	Trace
<i>N</i> -Methyl methyl anthranilate	Trace	Trace	None
$C_{13}H_{15}N$	Trace	Trace	None
Caryophyllene oxide	0.80	0.80	0.27
Citral	~ 0.1	~ 0.1	~ 0.1
Carvone	~ 0.1	~ 0.1	~ 0.1
$C_{15}H_{26}O$	0.19	0.23	0.54
Linalool	0.16	0.23	0.08
$C_{15}H_{22}O$	0.45	0.42	0.64
Carveol	0.30	0.30	0.27
α -Terpineol	0.03	0.88	2.02
3-Hexene-1-ol	0.12	None	None
Geraniol	~ 0.05	~ 0.05	None
Linalool monoxide	0.37	2.03	8.95
$C_{12}H_{20}O_2$	0.20	0.20	0.20
Polyoxygenated compounds	0.40	0.86	0.97
Oxides	0.32	0.32	0.32
Furfural	None	Trace	8.20

Table II. $R_f \times 100$ Values in Various Solvents for Hydrocarbons Found in Grapefruit Juices^a

	K	P	Q	R	S
Limonene	41	55	54	59	74
α -Caryophyllene	50	35	47	33	27
β -Caryophyllene	62	60	52	65	75
$C_{15}H_{24}$	80	82	87	90	83
α -Pinene	83	85	84	90	80

K. Hexane
P. 2,2-Dimethylbutane
Q. Cyclohexane
R. Methylcyclohexane
S. Isopentane

^a Compounds detected by fluorescein-bromine test.

The eluate from the column was arbitrarily divided into a large number of fractions by means of an automatic fraction collector. The fractions were then examined systematically by chromatographing small portions on chromatostrips, and those with like R_f values were combined. The solvent was removed from each fraction by evaporation under nitrogen at a pressure which kept the solution below 0° C. Each fraction was treated in this manner until all the original fractions had been separated into their individual components.

One of the samples from the stored grapefruit juice can be given as an example of the procedure outlined. One hundred thirty-four grams of the oil from stored canned grapefruit juice were adsorbed on a starch-silicic acid column of 320 grams packed from a hexane slurry. The column was developed with hexane until all the hydrocarbons had passed through the column. The column was then developed successively with 1680 ml. of 2% ethyl acetate in hexane, 1680 ml. of 4% ethyl acetate in hexane, and 2800 ml. of 10% ethyl acetate in hexane. The column was then eluted with ethyl acetate. From the development 300 eluate fractions were collected. Examination of these fractions on silicic acid chromatostrips with 15% ethyl acetate in hexane as the solvent resulted in their combination into seven fractions:

Fraction 1 (4.56 grams) was adsorbed on 360 grams of silicic acid with benzene as the developer and separated into unidentified oxides and caryophyllene oxide.

Fraction 2 (1.78 grams) was adsorbed on 120 grams of silicic acid with chloroform as the developer and separated into caryophyllene oxide, an ester ($C_{12}H_{20}O_2$), and carveol.

Fraction 3 (1.6 grams) was adsorbed on 120 grams of silicic acid and developed with 5% ethyl acetate in hexane. It consisted of the $C_{12}H_{20}O_2$ ester and carveol.

Fraction 4 (1.02 grams) was pure carveol.

Fraction 5 (1.00 gram) was adsorbed on 40 grams of silicic acid with 3% ethyl acetate in chloroform as the developer, and separated into a sesquiterpene ketone ($C_{15}H_{22}O$), carveol, and α -terpineol.

Fraction 6 (0.81 gram) was adsorbed on 40 grams of silicic acid and separated into carveol, α -terpineol, and polyoxygenated compounds with 5% ethyl acetate in chloroform as the solvent.

Fraction 7 (5.25 grams) was shown by chromatostrip tests and analysis to consist only of a series of polyoxygenated compounds which could not be separated further into individual components.

In this manner the various fractions from the three types of juices were separated into their individual components.

Establishing Purity of Compounds

After the individual compounds had been obtained, the question of their purity remained to be established. Each compound was chromatographed on silicic acid strips with each of the solvents listed in Tables II and III as developer. The completed chromatograms were examined under ultraviolet light, then sprayed with fluorescein, and exposed to bromine vapors. Separate chromatograms on plaster of Paris-silicic acid chromatostrips were developed with all the solvents, sprayed with 5% concentrated nitric acid in concentrated sulfuric acid, and then heated to 500° C. on a hot plate. If only one spot was revealed in all of these tests, the compound was chromatographed on 5 × 5 inch starch-silicic acid plates using one solvent in one direction, and after evaporation of the solvent, a second solvent was used at right angles to the first in the conventional two-dimensional chromatogram. These two-dimensional chromatograms were likewise examined with the various reagents for evidences of more than one compound.

Even though only one spot resulted from these chromatograms, the purity was not considered as established. A rough fractional distillation was performed as the final criterion of purity. A portion (200 mg.) of the sample was placed in the bottom of a microsublimation apparatus. With dry ice-alcohol in the condenser the pressure was lowered and the temperature of the fraction was raised until a drop appeared on the condenser. The vacuum was then released through a drying tube, the condenser

liquid replaced with room temperature alcohol, and the drop collected. [In some cases, the microstill of Gould *et al.* (6) was used.] In this way three to five fractions were collected. If chromatograms of these fractions showed no change in R_f value due to heating, the boiling point of each of the fractions was determined. If the boiling points were the same, the material isolated by the chromatographic procedure was considered to be a single pure compound.

Determination of Physical Properties

Boiling points at reduced pressures were determined on very small amounts of material (1 to 2 mg.) by the method of Natelson and Zuckerman (14). This method consists in slowly reducing the pressure in a heated chamber which contains the liquid to be tested in a capillary tube of very small bore with flared end. The pressure and temperature at which the liquid drops rapidly in the capillary are the boiling point of the liquid.

Refractive indices were determined on an Abbe refractometer at 25° C.

Optical rotations were measured on the liquids in either a 10-mm., 2.5-cm., 5.0-cm., or 10-cm. tube, depending on the amount of sample available. All measurements were made at room temperature (24° to 30° C.).

Taste Tests

Upon completion of the work of identifying the constituents of the volatile fractions, certain compounds were added singly and in combinations to freshly reamed grapefruit juice to test their effect on the flavor of the juice. These modified juices were compared with fresh juice using the triangular taste testing procedure (16). The members of the panel consisted of nine laboratory staff members trained in the tasting of citrus products.

Results and Discussion

The following compounds were isolated from the various juices:

Hydrocarbons *d*-Limonene. Boiling point, 10 mm., 57.2° C., $\alpha_D +103.8$, $n_D^{25} 1.4710$.

Derivatives. Tetrabromide, melting point 104–105° C.

β -Caryophyllene. Analysis. Calculated for $C_{15}H_{24}$, C 88.16; H 11.84. Molecular weight 204. Found, C 88.09; H 12.02. Molecular weight 216. $\alpha_D -7.36^\circ$, $n_D^{25} 1.4990$, boiling point at 2 mm. 100° C.

Derivatives. Caryophyllene alcohol was prepared by reaction of β -caryophyllene with sulfuric acid and water in acetic acid (19). A hexane extract of the reaction mixture was chromatographed on silicic acid with 10% ethyl acetate in hexane. The product was recrystallized from 70% alcohol. Melting point was 96° C. Caryophyllene oxide was pre-

pared by adding an equivalent amount of perbenzoic acid to β -caryophyllene and storing at 3° C. for 40 hours. The mixture was adsorbed on silicic acid and developed with 5% ethyl acetate in hexane. The product melted at 62–64° C. β -Caryophyllene isolated from clove oil by chromatography on silicic acid gave the same R_f values in all solvents and the same caryophyllene derivatives. The infrared spectrum of the grapefruit compound corresponded to that of the β -caryophyllene isolated from clove oil.

α -Caryophyllene. Analysis. Calculated for $C_{15}H_{24}$, C 88.16; H 11.84. Molecular weight 204. Found, C 87.94; H 11.76. Molecular weight 209. Boiling point at 10.4 mm. 132° C., α_D 0°, n_D^{25} 1.5094.

Derivatives. Because of the small amount available, no derivatives were prepared. α -Caryophyllene was isolated from clove oil by chromatography on silicic acid with hexane. The two compounds had identical R_f values in all solvents. The infrared spectrum of the grapefruit compound was identical with that of the clove oil α -caryophyllene.

Compound $C_{15}H_{24}$. Analysis. Calculated for $C_{15}H_{24}$, C 88.16; H 11.84. Molecular weight 204. Found, C 87.78; H 12.11. Molecular weight 241. α_D -31.4°, n_D^{25} 1.4906.

Derivatives. Because of the small amount available no derivatives were prepared. γ -Caryophyllene was isolated from clove oil by chromatography on silicic acid with hexane. This had identical R_f values with the compound from grapefruit juice; however, the infrared spectra of the two compounds were different.

Compound $C_{15}H_{28}$. Analysis. Calculated for $C_{15}H_{28}$, C 86.46; H 13.56. Found, C 85.63; H 14.06. This was a waxy material with high R_f values in oxygenated solvents. It gave no test with fluorescein-bromine, nor with concentrated nitric-sulfuric acid on the hot plate, and was not visible with ultraviolet light on fluorescein chromatostrips. It was only slightly soluble in acetone and after repeated recrystallizations from this solvent it melted at 54–63° C.

α -Pinene. Boiling point 20 mm., 52.9° C., α_D^{27} -49°, n_D^{25} 1.4580.

Derivatives. Quantities were too small to prepare a derivative. It was identified by constants, R_f values, and odor.

Other Hydrocarbons. A small amount of material appeared in the distillation of the hydrocarbons on the Podbielniak Hyper-Cal column before fractions analyzing as pure d -limonene were obtained. By combining this material from all three juices, α -pinene was isolated by chromatography and identified as indicated above. The remaining material, now free of α -pinene, had a rotation of +43° and was, without doubt,

contaminated with d -limonene. It had the same R_f values as limonene.

Oxygenated Compounds. Some material was isolated from the chromatographic columns of the oxygenated material in the first eluate portions. This material when examined on chromatostrips gave a streak rather than well defined spots. A portion of it, when distilled in the microapparatus described earlier, gave increasing boiling points for successive fractions. The R_f values for this material were high in all solvents. Analysis of some of the fractions indicated a compound approximating the empirical formula $C_{15}H_{26}O$. For these reasons this material was considered a mixture of compounds too complex and too small in amount to be separated into its components. Because of its high R_f values it has been termed "oxides," as this type of compound has been observed to be loosely adsorbed on silicic acid. The material had a very fragrant odor, floral in character.

Caryophyllene Oxide. Analysis. Calculated for $C_{15}H_{24}O$, C 81.76; H 10.98. Molecular weight 220. Found, C 81.60; H 11.31. Molecular weight 217. α_D -46.88°, n_D^{20} 1.4938, melting point 62–64° C. (from methanol).

Derivatives. No derivatives were made. The infrared spectrum of this sample showed a strong absorption in the region of 8 microns, which is characteristic of the ether or epoxy linkage. Caryophyllene oxide was synthesized from β -caryophyllene by reaction with

perbenzoic acid at 3° C. for 40 hours. After washing with 1 *N* sodium hydroxide and water, the mixture was adsorbed on silicic acid and developed with 5% ethyl acetate in hexane. The synthetic product melted at 62–64° C. A mixed melting point with the grapefruit compound did not show a depression.

Citral. The fractions which contained citral were too small to be identified by physical tests. Citral was identified by R_f values, by mixed chromatograms with known samples of citral, by its odor, and by reactions on chromatostrips. The material gave a positive aldehyde reaction when the chromatogram was sprayed with a saturated solution of *o*-dianisidine in glacial acetic acid. Both the grapefruit compound and citral after reaction with aluminum isopropoxide gave a chromatographic spot corresponding to geraniol (12).

Carvone. Carvone appeared in fractions containing citral and was identified by its R_f values and a very characteristic odor. When a small portion was reduced with aluminum isopropoxide and the reaction mixture chromatographed on chromatostrips, a spot corresponding to carveol was found.

Compound $C_{15}H_{24}O$ or $C_{15}H_{26}O$. Analysis. Calculated for $C_{15}H_{24}O$, C 81.76; H 10.98. Molecular weight 220.3. Calculated for $C_{15}H_{26}O$, C 81.02; H 11.79. Molecular weight 222.4. Found, C 80.64; H 10.93. Molecular weight 235. Boiling point at 1.6 mm., 120° C.; at 9 mm. 130.5° C.; at 14 mm. 150.5° C., α_D -14°, n_D^{25} 1.5079.

Derivatives. This compound reacted

Table III. $R_f \times 100$ Values in Various Solvents of Oxygen and Nitrogen Containing Constituents Found in Volatile Oil of Grapefruit Juices^a

	A	B	C	D	E	F	G	H	J	K	L
<i>N</i> -Methyl methyl anthranilate ^b	58	75	74	71	91	78	90	69	79	0	56
Caryophyllene oxide	55	70	66	52	79	77	84	65	74	0	27
$C_{15}H_{26}O$ (alcohol)	36	47	45	31	53	67	45	48	48	0	15
$C_{15}H_{22}O$ (ketone)	24	43	52	42	30	67	25	36	31	0	9
$C_{12}H_{20}O_2$ (ester)	44	45	64	46	72	75	41	62	75	0	19
$C_{13}H_{16}N^c$	55	77	86	72	94	80	91	69	86	0	63
Citral ^d	45	64	57	47	62	62	69	56	51	0	15
Carvone ^e	45	72	62	70	76	79	75	62	65	0	37
Linalool	36	47	45	31	53	67	45	48	48	0	15
Carveol	34	44	49	38	40	60	51	45	29	0	12
α -Terpineol	29	34	36	28	32	56	29	35	25	0	8
3-Hexen-1-ol	20	32	32	32	29	55	34	30	26	0	13
Geraniol	20	40	43	34	29	55	31	30	25	0	12
Linalool monoxide	21	18	20	10	8	42	13	18	16	0	3
Furfural ^d	21	44	41	41	44	41	38	37	17	0	6

A. 15% ethyl acetate in hexane (v./v.)

B. 10% ethyl acetate in chloroform (alcohol-free) (v./v.)

C. 15% ethyl acetate in benzene (v./v.)

D. 5% ethyl acetate in chloroform (alcohol-free) (v./v.)

E. 50% 1-nitropropane in hexane (v./v.)

F. 30% ethyl acetate in hexane (v./v.)

G. 15% ethyl carbonate in chloroform (alcohol-free) (v./v.)

H. 30% isopropyl formate in hexane (v./v.)

J. 50% isopropyl ether in hexane (v./v.)

K. Hexane

L. Chloroform (alcohol-free)

^a Compounds detected by fluorescein-bromine test unless otherwise indicated.

^b Detected by fluorescence of compound.

^c Detected by absorption of ultraviolet light on fluorescent strip.

^d Detected by *o*-dianisidine spray.

with phenyl isocyanate, but the product could not be crystallized from ethyl alcohol, ethyl acetate, acetone, or carbon tetrachloride. The derivative was chromatographed on silicic acid to rid it of unreacted compound and phenyl isocyanate. On standing with methanol a few crystals formed, melting point 115–120° C. No derivative could be formed with 3,5-dinitrobenzoyl chloride. When this compound was placed on a chromatostrip, covered with a drop of concentrated sulfuric acid, and chromatographed with hexane, no hydrocarbons were formed (12). The compound was suspected of being caryophyllene alcohol. However, it could not be crystallized from 70% alcohol. A sample which has been at 3° C. for several months is gradually crystallizing.

Linalool. Analysis. Calculated for $C_{10}H_{18}O$, C 77.87; H 11.76. Molecular weight 154. Found, C 77.76; H 11.50. Molecular weight 139. Boiling point at 9 mm., 87° C., $\alpha_D +2.06^\circ$, $n_D^{25} 1.4649$.

Derivatives. None made. Separation on the chromatographic column always left linalool mixed with the $C_{15}H_{26}O$ fraction. These two compounds could not be separated by any of the solvents used in this work. Linalool was fractionally distilled from this mixture on a semimicrocolumn. When chromatographed on a chromatostrip with a drop of chromic anhydride in glacial acetic acid (12), a spot corresponding in R_f values to citral was formed. This spot also gave a test for aldehyde when sprayed with *o*-dianisidine in glacial acetic acid. Chromatographed on a chromatostrip with a drop of concentrated sulfuric acid, the grapefruit compound showed the same hydrocarbons were formed as for a sample of known linalool.

Compound $C_{15}H_{22}O$. Analysis. Calculated for $C_{15}H_{22}O$, C 82.51; H 10.16. Molecular weight 218. Found, C 81.79; H 10.33. Molecular weight 191. Boiling point at 2.5 mm. 130° C., at 4.5 mm. 150° C., at 7.5 mm. 170° C., $\alpha_D +127.5^\circ$, $n_D^{25} 1.5205$.

Derivatives. A semicarbazone formed easily from semicarbazide hydrochloride in pyridine, melting point 193.5–194° C. (from alcohol). Analysis. Calculated for $C_{16}H_{25}N_3O$, C 69.79; H 9.15; N 15.25. Found, C 71.01; H 9.39; N 14.77. A 2,4-dinitrophenylhydrazone was prepared, melting point 133–136° C. (from alcohol). The compound gave a negative aldehyde test with fuchsin reagent. This compound is probably a sesquiterpene ketone. A search of the literature did not reveal any compound with these properties.

Carveol. Analysis. Calculated for $C_{10}H_{16}O$, C 78.89; H 10.59. Molecular weight 152. Found, C 79.38; H 10.84. Molecular weight 154. Boiling point at 5 mm. 89° C., at 9 mm. 100° C., at 15 mm. 110.5° C., at 22 mm. 120° C.,

at 33.5 mm. 130° C., $\alpha_D +22.2^\circ$, $n_D^{25} 1.4931$.

Derivatives. 3,5-Dinitrobenzoate, melting point 109–111° C. (from ethyl alcohol–ethyl acetate 3 to 1). The compound was oxidized on a chromatostrip to carvone with chromic anhydride in glacial acetic acid.

α -Terpineol. Analysis. Calculated for $C_{10}H_{18}O$, C 77.87; H 11.76. Molecular weight 154. Found, C 76.33; H 11.38. Molecular weight 144. Boiling point at 15 mm. 111.5° C., $\alpha_D +80.96^\circ$, $n_D^{25} 1.4809$.

Derivatives. The phenylurethane formed after standing 48 hours, melting point 108–110° C. (from methanol). Neither the sample nor α -terpineol was affected by chromic anhydride in glacial acetic acid on chromatostrips. The compound gave the same hydrocarbons as a known sample of α -terpineol with concentrated sulfuric acid on chromatostrips.

Linalool Monoxide. Analysis. Calculated for $C_{10}H_{18}O_2$, C 70.55; H 10.66. Molecular weight 170. Found, C 70.24; H 10.56. Molecular weight 162. Boiling point at 4 mm. 50° C., at 8.5 mm. 70° C., $\alpha_D -8.2^\circ$, $n_D^{25} 1.4507$.

Derivatives. None made. Linalool monoxide was synthesized from a known sample of linalool. Linalool (0.004 *M*) was added to perbenzoic acid (0.004 *M*) in ether and allowed to stand at 3° C. for 3 days. The ether was then evaporated and the mixture was adsorbed on 40 grams of silicic acid. The column was developed with 10% ethyl acetate in chloroform. Analysis. C 71.08; H 10.97. Molecular weight 166. The synthetic linalool monoxide was compared to the compound isolated from grapefruit juice. Neither compound was oxidized by chromic anhydride in glacial acetic acid on chromatostrips. Both compounds showed the same hydrocarbons when a small amount was dehydrated with phosphorus pentoxide and chromatographed on chromatostrips with hexane as solvent. The two compounds showed identical infrared spectra.

Geraniol. A trace of material whose R_f values agreed with those of geraniol was found in fresh and freshly canned grapefruit juice. This material was freed of extraneous material by shaking with calcium chloride in hexane for 2 hours; the addition product was filtered, washed with hexane, and decomposed with water. The free oil obtained had the same R_f values as a known sample of geraniol. The amount was too small for analysis or derivative preparation.

3-Hexen-1-ol. Analysis. Calculated for $C_6H_{12}O$, C 71.94; H 12.08. Molecular weight 100. Found, C 73.01; H 13.28. Molecular weight 120. Boiling point at 16 mm. 70° C., $n_D^{25} 1.4400$.

Derivatives. A 3,5-dinitrobenzoate was prepared, melting point 47–49° C. (from ethyl alcohol–ethyl acetate 3 to 1).

A mixed chromatogram of the alcohol with methyl heptenol proved the compound was not methyl heptenol.

Compound $C_{12}H_{20}O_2$. Analysis. Calculated for $C_{12}H_{20}O_2$, C 73.43; H 10.27. Molecular weight 196. Found, C 73.51; H 10.07. Molecular weight 194. Boiling point at 7 mm. 100° C., $n_D^{25} 1.4992$.

Derivatives. None. Linalyl acetate, terpinyl acetate, geranyl acetate, neryl acetate, and carvyl acetate were compared with this material on chromatostrips. It was none of these. The compound gave a positive ester test (4).

Polyoxygenated Compounds. In each of the oils from the grapefruit juices, some material was recovered that was very viscous and had R_f values very nearly zero in all solvents except 30% ethyl acetate in hexane. It was mostly soluble in ethyl acetate but insoluble in hexane. Some of this material was chromatographed on silicic acid with 30% ethyl acetate in hexane as the developer. Although no clear-cut compounds could be isolated, analyses of the high, middle, and low R_f value cuts showed compounds approximating:

High R_f value	$C_{15}H_{24}O_3$
Middle R_f value	$C_{12}H_{20}O_3$
Low R_f value	$C_{10}H_{18}O_4$

The material was classified as a complex mixture of polyoxygenated compounds.

Furfural. Furfural was isolated from canned grapefruit juice and proof of its identity has been given (10). It was isolated from the ether extract of the water condensate of stored canned grapefruit juice by extraction with sodium bisulfite and identified by means of R_f values. The semicarbazone melted at 190–192° C. As the semicarbazone derivative of hydroxymethyl furfural was found to be more soluble in ethyl acetate than that of furfural, the semicarbazone derivative was extracted with ethyl acetate. This solution was then compared to the semicarbazone of hydroxymethyl furfural on chromatostrips with ethyl acetate as developer. The R_f values showed that hydroxymethyl furfural was not present.

Nitrogen Compounds $C_{13}H_{15}N$. Analysis. Calculated for $C_{13}H_{15}N$, C 84.28; H 8.16; N 7.56. Molecular weight, 185.26. Found, C 84.70; H 7.90; N 7.40. Molecular weight, 179. Melting point, 45–47° C.

Derivatives. No derivatives were made. This compound was distinguished by the fact that upon being chromatographed on silicic acid chromatostrips it turned a bright blue color after exposure to ultraviolet light. It had an odor similar to skatole. A test for a tertiary ring nitrogen (5) was negative, as was a nitroprusside test. The compound was neutral to litmus paper. The Sasaki

test (18) for distinguishing indole from skatole gave a blue color with the compound. From the evidence it is possibly a substituted indole. This compound was not present in the stored juice.

N-Methyl Methyl Anthranilate.

This compound was identified by its characteristic fluorescence under ultra-violet light, by its R_f values, which are different from methyl and ethyl anthranilate (12), and by its odor. Pure N-methyl methyl anthranilate was prepared by chromatography from a commercial sample (17) and compared to the product obtained from grapefruit juice. It was comparable in all respects.

The R_f values of the compounds listed above are given in Tables II and III. In the case of identified compounds, they have been compared with known samples of the compounds.

The quantitative results shown in Table I are to be considered approximations only. These figures were obtained by weighing the oil before it was adsorbed on a column and weighing the individual fractions after they were recovered from the solvent. The over-all yield was then used to correct each fraction back to the original weight of the whole oil. It was determined experimentally that the major source of loss occurred during the evaporation of the solvent. For example, 10 grams of limonene in 150 ml. of hexane were evaporated until all the hexane was removed; 9.43 grams or 94% of the limonene was recovered. Again, 1 gram of limonene in 150 ml. of hexane was evaporated until all the hexane was removed; 0.697 gram or 69.7% was recovered. One gram of citral in 150 ml. of 15% ethyl acetate in hexane was evaporated until all the solvent was removed; 0.958 gram or 95.8% was recovered. The yield for the various separations in this work averaged between 80 and 90%. It was not possible to make corrections for differences of volatility of individual fractions. In some separations which left portions of the compounds as mixtures, the amounts of the individual compounds were estimated by comparison on chromatostrips.

The difference in total oil recovered from the fresh juice (20.92 mg. per kg.) and freshly canned juice (26.00 mg. per kg.) is due to the fact that the juice was extracted from the fresh fruit by holding the halved fruit with hand pressure on rotating reamers, while the juice from the freshly canned and the stored canned samples was extracted from the fruit on commercial citrus juice extractors. Machine extraction of the juice incorporated more peel oil in the juice than did the hand pressure procedure.

In Figure 1 the amounts of hydrocarbon and nonhydrocarbon compounds in the three types of grapefruit juices are

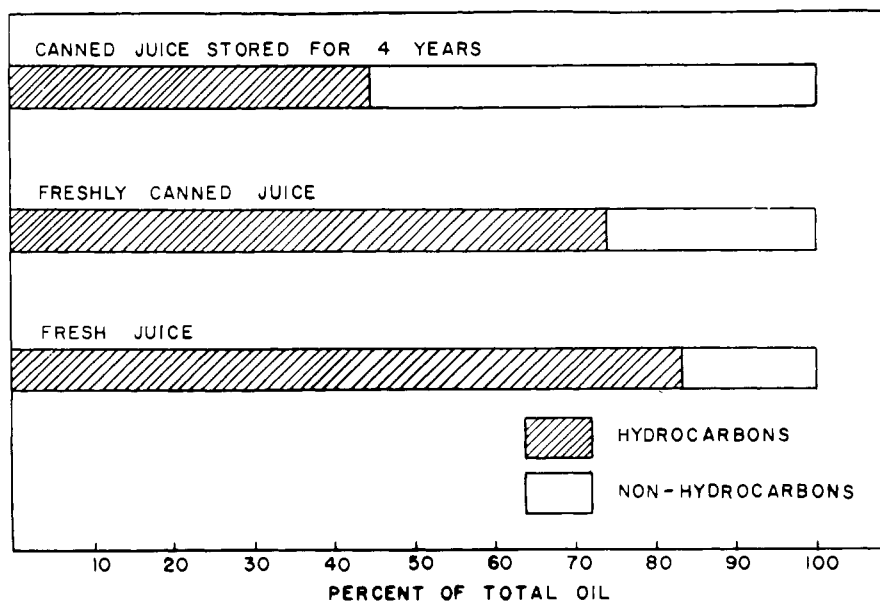


Figure 1. Volatile oils from grapefruit juice

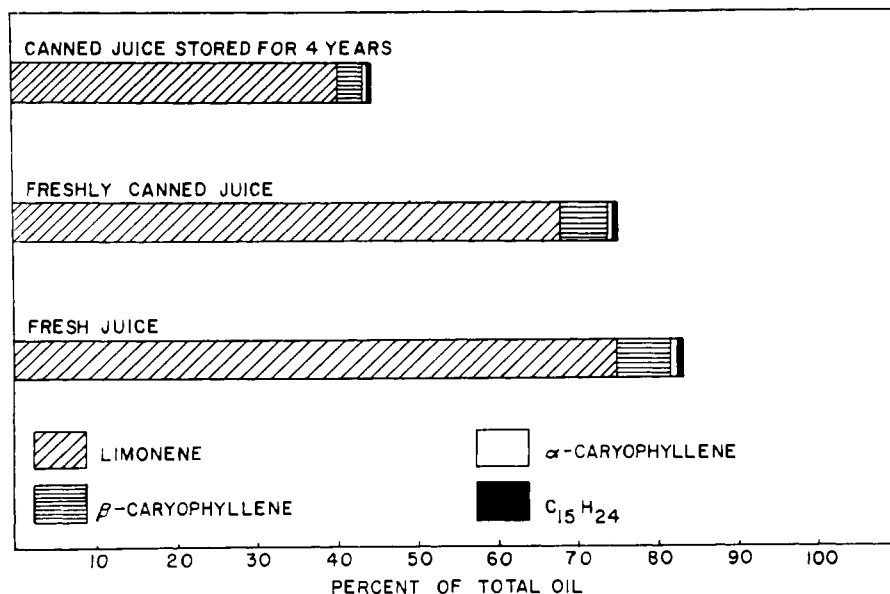


Figure 2. Changes in major hydrocarbon components of the volatile oil of grapefruit juice

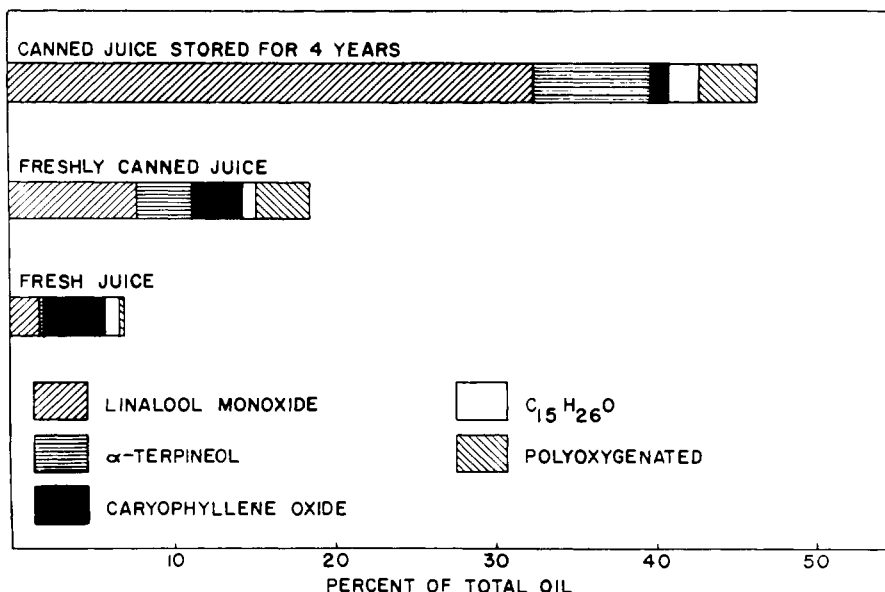


Figure 3. Significant changes in the oxygenated components of the volatile oil of grapefruit juice

Table IV. Taste Studies on Grapefruit Juice by Triangular Taste Test

Oil Added	Concentration, Mg./Kg. Juice	No. of Correct Judgments Out of 18	Comparison to Fresh Juice, +4 to -4	Panel Comments
Linaloöl monoxide	90	17	-1.5	Perfumy
α -Terpineol	20	18	-2	Musty
Furfural	90	13	-3	Burnt
Linaloöl monoxide	9)	15	-3	
α -Terpineol	2)			
Furfural	8)			

shown. It is evident that the major change in the volatile oils which occurred upon heating and storage has been an increase in the oxygenated compounds at the expense of the hydrocarbons. Figure 2 shows graphically the changes in the individual hydrocarbons. Aside from the traces of hydrocarbons boiling lower than limonene, five hydrocarbons were present in the oil from grapefruit juice. Of these hydrocarbons only the limonene and the sesquiterpene, β -caryophyllene, were significantly altered by heating or storage. Figure 3 shows the changes which have occurred in the oxygenated portion of the volatile oils. (As furfural arises from the sugars, it has been excluded from Figure 3 in order not to distort the changes occurring in the terpenes and oxygenated terpenes.) Noteworthy are the large increase in the linaloöl monoxide and the relatively smaller increases in α -terpineol and the $C_{15}H_{26}O$ fraction.

If the changes in the stored juice over the freshly canned juice are examined (as these are strictly comparable in method of reaming) it is seen that the limonene content has decreased by 6.53 mg. per kg. Linaloöl monoxide and α -terpineol together, if converted to a hydrocarbon basis, account for an increase of 6.55 mg. per kg. (as hydrocarbon) in the oxygenated constituents. The evidence, therefore, supports a conclusion that limonene has been converted to α -terpineol and linaloöl monoxide. The β -caryophyllene has decreased by 0.53 mg. per kg. The $C_{15}H_{26}O$ alcohol and the $C_{15}H_{22}O$ ketone, converted to hydrocarbon basis, account for an increase of 0.48 mg. per kg. It seems reasonable to postulate that the β -caryophyllene has been converted to this alcohol and ketone. The decrease in the caryophyllene oxide remains unexplained. The compound which would logically result from the oxidation or hydration of caryophyllene oxide—i.e., caryophyllene alcohol monoxide—was not found in the oils. The odor of caryophyllene oxide was most pleasing and, when mixed with small amounts of *N*-methyl methyl anthranilate, resembled the fragrance of fresh grapefruit.

The data show that the furfural, absent in the fresh juice and present in traces in the freshly canned juice and in considerable amounts in the stored

canned juice, could not have come from any changes in the volatile oil of the grapefruit juice. This material must have had its origin in the carbohydrate fraction of the juice.

The evidence indicates that extensive oxidation of the oils to glycols and the like did not take place. This type of compound would appear in the "poly-oxygenated" fraction. It would be expected that if appreciable amounts of highly oxygenated compounds were formed, substantial quantities would remain in the residue from the original distillation of the juice, with a consequent loss in total oil recovered. However, if the total oil in the freshly canned and stored canned juice is compared with allowance for the increase in weight of the stored oil sample due to adding water or oxygen to some of the molecules, the yield of oil in the stored sample is accounted for without assumption that some highly oxygenated compounds have been left in the residue from the evaporation of the juice.

Little experimental work has been done on the causes of off-flavors of citrus juices on heating and storage, and all this has been done on orange juice (7-3, 7, 13, 15, 17, 20); no work has been reported for grapefruit juice. Orange juice is currently being examined in this laboratory in the same manner as reported here for grapefruit juice. When this work is reported, the mechanism of off-flavor development will be considered.

The significant changes in the composition of the volatile oils from the three types of juices were studied in relation to their effect on the taste of the juice. It was not possible to study the effect of a decrease or disappearance of a particular constituent, but the cases where significant increases were encountered were examined. The results of the taste panel's examination of grapefruit juice with added linaloöl monoxide, α -terpineol, and furfural are shown in Table IV. The juices with oil added were compared to the same juice without added oil. All these additives gave highly significant taste differences that were considered very undesirable by the panel.

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