Volatile Water-Soluble and Oil Constituents of Valencia Orange Juice

J. G. KIRCHNER[1] and
JOHN M. MILLER[1]

Fruit and Vegetable Chemistry Laboratory, Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Pasadena, Calif.

The nature of the chemical changes in the water and oil constituents of the volatile flavoring material of fresh, freshly canned, and stored canned California Valencia orange juices has been investigated. Acetic, propionic, isovaleric, and traces of butyric acid were found in the stored juice. There was a decreased acetaldehyde content and traces of diacetyl. A $C_6H_8O_2$ unsaturated acid previously found in grapefruit juice was present in all three orange juices. Twenty-nine compounds from the oil fraction were identified and their amounts determined in each of the three juices. Analyses were made by chromatographic techniques when possible. The major changes in the oil fraction of orange juice on canning and storage were loss in total volatile oil, conversion of hydrocarbons to alcohols, and loss in esters, aldehydes, and terpene aliphatic alcohols. The predominant off-flavor of stored canned orange juices appears to arise from the non-volatile precursors.

THE VOLATILE FLAVORING CONSTITUENTS of orange juice have been considered in this study to be materials that can be removed from the juice by distillation methods. These include low-boiling organic constituents and high-boiling oils, the latter being removed as aqueous azeotropes.

If these volatile constituents are removed from the juice by distillation at atmospheric pressure, the recovered fraction has an odor strongly suggesting that its composition has been considerably altered in the process. If they are removed at reduced pressures and correspondingly lower temperatures, the aroma of the recovered fraction resembles more closely the aroma of the original juice. The lower the temperature of distillation the more closely the volatiles resemble the original fruity aroma.

A citrus juice from which a portion of the water has been removed by distillation shows a definite change in flavor, and it was early recognized that the aroma of orange juice was associated with the volatile fraction (10). It was also shown that the flavor of orange juice is largely lost by evaporation, but that this loss may be partly overcome by the addition of fresh juice to the concentrate to restore flavor and aroma (22).

The flavor of a concentrated orange juice which has been restored to its original strength by the addition of pure water tastes insipid and is lacking in orange character (27). It is generally recognized that the flavor and aroma of fresh orange juice, as well as certain off-flavors of the processed juice, are associated with the volatile fractions (2, 4, 11). These fractions consist of constituents soluble and insoluble in water; whereas some of the aroma resides in the water-soluble fraction (10, 16, 23), most of the characteristic flavor and aroma of citrus juices is considered to be in the volatile, sparingly water-soluble, oil fractions (14, 16, 23, 27).

The method of extraction of the juice from the fruit results in the incorporation of some peel oil into the juice, but the juice itself contains some oil present in the juice sacs. In experiments in which fruit was carefully peeled and washed, appreciable amounts of oil were recovered from the juice sacs (2, 4, 23, 27). Globules of oil have been demonstrated by histological studies on the juice sacs of various citrus fruits (6, 26).

Orange juice has been investigated extensively, particularly as regards changes in the composition of the volatile fractions resulting from heating and storage. Hall and Wilson (10) investigated the volatile materials obtained from 10,000 gallons of fresh California Valencia orange juice.

They reported the following constituents: acetone, acetaldehyde, ethyl alcohol (0.018% on separate sample), formic acid, citronellal, caprylic acid as ester, acetic acid as ester, formic acid as ester, isoamyl alcohol, phenyl ethyl alcohol, possibly geraniol, α-terpineol, and a $C_{10}H_{18}O$ alcohol similar to linalool.

Hydrogen sulfide has been reported in green oranges and in orange juice (7, 18). Furfural has been detected in orange juice stored in the presence of oxygen (24), and seasonal acetaldehyde and alcohol contents of orange juice have been determined (32).

This paper reports the isolation and identification of the constituents in the volatile water-soluble and oil fractions from freshly reamed, canned, and stored canned California Valencia orange juices, and represents a continuation of long-term studies on citrus flavoring (14, 16).

Water-Soluble Constituents

Experimental

In the preparation of the canned single-strength orange juice used in this study, processing methods followed standard commercial practices. Arrangements were made to purchase the orange juice from a commercial processor, where the oranges were carefully selected to prevent the inclusion of soft and rotten fruit, and thoroughly washed. Total soluble solids in the juice was 13.8%, and total acid (as citric acid) was 1.26%.

The juice, obtained from an automatic citrus juice extractor, was screened and run into a large stainless steel mixing tank. One third of the juice was quick-frozen for delivery to the laboratory for immediate analysis, while the remaining juice was pasteurized at 92° C. (198° F.) and filled directly into 46-ounce citrus enamel-lined cans. After being filled and sealed, the cans were cooled to approximately 38° C. (100.4° F.) by passage through a cold water tank before being packed in cartons. The following volumes of orange juice were distilled and studied individually: 3000 gallons of freshly reamed juice, 2500 gallons of freshly canned juice, and 1520 gallons

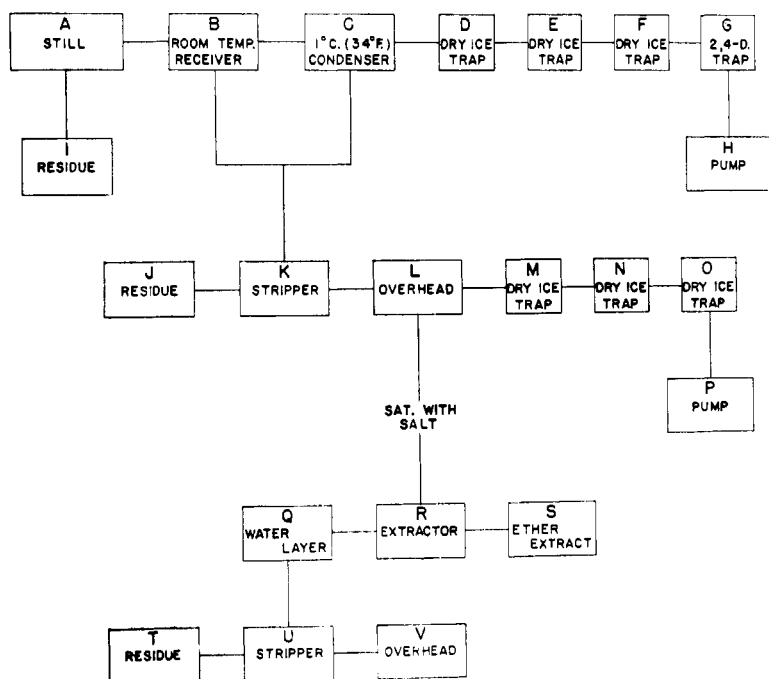[1] Present address, Tenco, Inc., Linden, N. J.

Figure 1. Flowsheet for separation of volatile constituents from orange juice

of stored canned juice held at room temperature for 3 years.

A flowsheet describing separation of the volatile material from the juice appears in Figure 1. The frozen, fresh Valencia orange juice (3000 gallons) was thawed by running through a Seprosieve into a warm water-jacketed kettle equipped with a stirrer, and then fed directly into the evaporator, A, while still cold. Distillation was carried out at 40 mm. of mercury absolute, with the evaporator contents maintained below 45° C. (113° F.) at all times, until 75% of the juice had distilled. The evaporator was heated by steam at 8-pound gage pressure (112° C., 233.6° F.). [It was found by test runs that orange juice lost its volatile oil less readily than grapefruit juice (16), so it was necessary to distill off 75% of the juice before essentially all of the oil was removed.] The still was a long vertical-tube, natural-circulation, vacuum evaporator with an external vertical-liquid separator. The vapors passed from the top of the evaporator through two water-cooled stainless steel condensers into flask, B, at room temperature. This flask was connected to the vacuum pump, H, through another condenser, C, refrigerated with a 1° C. (34° F) brine system, and a series of traps, D,E,F, maintained at $-78°$ C. ($-108°$ F.) with dry ice and alcohol. A trap, G, containing 2,4-dinitrophenylhydrazine in sulfuric acid was inserted just ahead of the pump to trap acetaldehyde. The condensate from the room temperature, B, and refrigerated receivers, C, was pumped to a stainless steel stripping column, K, in order to concentrate the volatile water-soluble materials. The stripping col-

umn was also operated under vacuum with a 1° C. (34° F.) condenser and refrigerated receiver, L, connected through a series of dry ice traps, M,N,O, to the vacuum pump, P, in order to collect the volatiles. By this means the evaporator distillate was reduced to 150 gallons. The distillate was saturated with salt and extracted with diethyl ether in a continuous extractor, R, under a pressure of 360.7 mm. of mercury absolute. Ether used in this work was anhydrous analytical grade containing not more than 0.01% alcohol. Prior to use it was checked to make sure no peroxides were present. Approximately 5 gallons of ether were used to each 1000 gallons of juice. The water layers in the various cold traps were separated from the oils, saturated with salt, and extracted with ether. All of the ether-extracted water fractions were then combined, freed of excess salt and ether, and concentrated in the stripping column to 5 gallons in

order to recover the volatile water-soluble material, V. This 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 Hyper-Cal column. Low-boiling alcohols present in this fraction were fractionally distilled on this column. The combined oil layers from the condensers, B,C, the cold traps, D,E,F,M,N,O, and the oil remaining after removal of the low-boiling alcohols from the ether extract, were analyzed.

The freshly canned juice and the juice that had been stored for 3 years were distilled in a similar manner.

As the residue, M, from stripping the evaporator distillate from stored juice was acidic, a portion was worked up to determine which acids were present. Thirty gallons of the residue were neutralized with sodium hydroxide and evaporated to dryness, and 4.76 grams of sodium salts were recovered. From a similar treatment of 58 gallons of residues from fresh juice, and 14 gallons from freshly canned juice there were obtained 1.28 and 0.7 grams of sodium salts. This material was converted to the p-phenylphenacyl esters (7) for chromatographic separation and identification of the acids (17).

Table I gives the approximate amounts of the compounds found in the volatile water-soluble fractions of the three juices.

Results and Discussion

Carbonyls. A crystalline 2,4-dinitrophenylhydrazone (melting point 167.5°C.) was identified as that of acetaldehyde by a mixed melting point with a known sample. It was also identified by the dimedone derivative (melting point 143° C.), and by the preparation of ethylidene di-2-naphthyloxide (melting point 173° C.).

Table I. Volatile Water-Soluble Constituents of Fresh, Freshly Canned, and Stored Canned Valencia Orange Juices

	Mg. per Kg. of Juice		
	Fresh	Freshly canned	Stored canned
Acetaldehyde	3.0	3.0	0.8
Furfural	Trace	Trace	5.1
Acetone	Trace	Trace	Trace
Diacetyl	...	...	Trace
Ethyl alcohol	380	550	484
Methanol	0.8	Present	62
Hydrogen sulfide	Trace	Trace	None
Acetic acid	2.8	5.8	18.6
Propionic acid	...	...	0.1
Butyric acid	...	...	Trace
Isovaleric acid	...	...	0.4
C_5H_7COOH	0.1	0.1	0.7
Other acids	0.1	...	...

Fresh juice contained 3.0 mg. of acetaldehyde per kg. based upon analysis with 2,4-dinitrophenylhydrazine. Values for the freshly canned and stored canned juices were 3.0 and 0.8 mg., respectively.

Furfural was detected in trace amounts in both the fresh and the freshly canned juices by means of the p-toluidine acetate test. In the stored canned juice this aldehyde was found to the extent of 5.1 mg. per kg. It was positively identified by means of a semicarbazone derivative melting at 190–192° C., which showed no melting point depression when mixed with an authentic derivative. The 2,4-dinitrophenylhydrazone melted at 225–227° C. This furfural probably arises from the carbohydrate fraction of the juice.

Traces of acetone were found in all three juices. The 2,4-dinitrophenylhydrazones were mixtures which could not be resolved by recrystallization. They were resolved chromatographically (28) and were a mixture of acetone and acetaldehyde 2,4-dinitrophenylhydrazones. The presence of acetone had been demonstrated by Hall and Wilson (10) by means of a potassium nitroprusside test for methyl ketones and an iodoform test.

A trace of diacetyl was found in the stored canned juice. It was identified by preparing the semicarbazone which melted at 279° C.

Alcohols. Ethyl alcohol was determined semiquantitatively by specific gravity and refractive index measurements. It was positively identified by means of its 3,5-dinitrobenzoate derivative melting at 92–93° C., and was present in the fresh juice to the extent of 406 mg. per kg. (0.04%). Hall and Wilson (10) reported a value of 0.018%, but did not indicate the method of determination. The freshly canned juice contained 554 mg. per kg., and the stored canned juice 484 mg. per kg.

A small quantity of methanol (0.8 mg. per kg.) was found in the fresh orange juice as determined by its oxidation to formaldehyde and analysis for formaldehyde by the chromotropic acid test (3). The results were checked against the actual quantity of methanol isolated by fractionation. Qualitatively it was identified by the isolation of the 3,5-dinitrobenzoate (melting point 108–109° C.). It gave no melting point depression with an authentic sample of methyl 3,5-dinitrobenzoate. Unfortunately, the freshly canned juice fraction containing the methanol was lost before a quantitative determination could be made. It was, however, shown to be present qualitatively. The stored canned juice was found to contain 62 mg. per kg. of juice. The absolute amounts in the various alcohol fractions were determined by the refractometer method (33). The increase in methanol

in the stored juice probably results from the breakdown of pectins.

Acids. The p-phenylphenacyl esters obtained from the acids in the stripper residues were separated by chromatographic adsorption on silicic acid (17). The major portion of the derivatives in all three juices was found to be the derivative of acetic acid and represented 2.8, 5.8, and 18.7 mg. per kg., respectively, in the fresh, freshly canned, and stored canned juices. The melting point of the ester was 109–111° C., and a mixed melting point with p-phenylphenacyl acetate gave no depression.

Other acids were found in the stored canned juice. Propionic acid was present to the extent of 0.1 mg. per kg. of juice. The p-phenylphenacyl ester of this acid melted at 102–103° C. and showed no depression with p-phenylphenacyl propionate. Isovaleric acid (0.4 mg. per kg. of juice) was identified as the p-phenylphenacyl ester melting at 75–77° C., and a trace of butyric acid was found with p-phenylphenacyl ester melting at 82° C. Traces of other acids were found in the fresh juice but in amounts too small to be identified.

The p-phenylphenacyl ester of one of the unsaturated acids found in grapefruit juice (16) was also found in all three orange juices. The p-phenylphenacyl ester melts at 120° to 126° C. and analyzes for the derivative of an acid with the formula $C_6H_8O_2$. The amounts of this acid in the fresh, freshly canned, and stored canned juice are respectively, 0.1, 0.1, and 0.7 mg. per kg. of juice.

Miscellaneous. During distillation of the juice small quantities of hydrogen sulfide were detected (7, 18) in both the fresh juice and the freshly canned juice, but not in the stored canned juice. Passage of nitrogen through unheated freshly reamed juice showed hydrogen sulfide to be present as such and not as an artifact produced by heating.

Oil-Soluble Constituents

Experimental

The oil layers obtained from the low-temperature distillation of the three lots of juice were separated into zones of similar R_f values by chromatographic processes. The individual components were obtained by further chromatographic separations, by distillation, or by chemical means.

The chromatographic methods used, establishment of the purity of isolated compounds, and determination of the physical properties of pure compounds have been described (13–15, 20, 21). In the identification of the compounds, mixed chromatograms with known compounds were run when these were available for comparison. The adsorbent used for preparing chromatostrips and

adsorbent columns was reagent grade silicic acid.

An example of the separation of one of the oil layers serves to illustrate the procedures used. From the low-temperature distillation of the fresh lot of 3000 gallons of orange juice, the combined oil layers collected in the cold traps consisted of 1010 grams of a nearly colorless oil of n_D^{25} 1.468, $\alpha_D^{20} + 100°$ after drying over anhydrous sodium sulfate. (The oil obtained by ether extraction of the water layers was treated separately.) This oil was adsorbed on a column packed with 200 grams of silicic acid as a slurry of the adsorbent with 1200 ml. of benzene-free hexane. The column was washed with hexane until tests on chromatostrips showed all hydrocarbons had been removed. Development was continued using successively stronger solutions of ethyl acetate in hexane until chromatostrip tests showed all of the material had been washed into the eluate, which was collected in 10-ml. portions.

The hydrocarbon portion was kept as one fraction and separated by vacuum distillation on a Podbielniak Hyper-Cal column as a material having a lower boiling point than limonene, as limonene, and as a material having a higher boiling point than limonene. The low-boiling material was adsorbed on silicic acid with hexane as developer and separated into α-thujene (?) and β-myrcene. The high-boiling material was adsorbed on silicic acid with hexane as the developer and separated into two fractions, sesquiterpene I and sesquiterpene II.

The nonhydrocarbon eluate fractions from the original column were systematically tested on chromatostrips with 15% ethyl acetate in hexane as the developer, with fluorescein-bromine (15) and 2,4-dinitrophenylhydrazine in 2N hydrochloric acid used as indicators. Fractions of similar R_f value were combined and the solvent evaporated at reduced pressures. In this way the nonhydrocarbon fractions were divided into eight fractions. After examination on chromatostrips for the number of compounds present and the best solvent for separations, each fraction was adsorbed on columns of silicic acid and separated into components. For less strongly adsorbed compounds chloroform or benzene was most often used as a developer. For compounds of intermediate or strong adsorption 5% ethyl acetate in benzene, 15% diisopropyl ether in hexane, 5% ethyl acetate in chloroform, 5% ethyl acetate in benzene, or 5% ethyl acetate in hexane was used. For maximum separation of components of close R_f values, 1 to 1.5 grams of the oil mixture was used with 100 grams of silicic acid of 200 mesh and approximately 5% moisture content. It was often necessary to perform more than one separation on chromatographic columns to

effect separation of sufficient quantities of material for identification purposes.

Methods of testing the purity of compounds with one- and two-dimensional chromatograms and crude distillations of 50- to 100-mg. samples were the same as reported in the work on grapefruit volatile oil constituents (14). Spots on the chromatograms were detected by spraying with fluorescein-bromine, 2,4-dinitrophenylhydrazine, o-dianisidine, or concentrated sulfuric-nitric acid.

In contrast to finding a majority of the components of the volatile oil from grapefruit juice separable by chromatography, the orange oil constituents were most often separated into chromatographically homogeneous fractions which consisted of two or more compounds. It was often necessary to resort to distillation or chemical methods to separate these mixtures. These methods are described for the individual compounds in the summary of identification work for that compound.

Results and Discussion

The following compounds were isolated from the three juices.

Hydrocarbons

d-LIMONENE. $\alpha_D^{24} + 103.8°$, n_D^{25} 1.4708.
β-MYRCENE. Analysis. Calculated for $C_{10}H_{16}$, C 88.16; H 11.84. Found, C 87.69; H 11.94, α_D 0, n_D^{25} 1.4648, boiling point at 18 mm. 68.5° C., at 36 mm. 82.2° C.

Derivatives. Maleic anhydride adduct melting point 33–35° C. (from methanol) (lit. 34–35° C.).

α-THUJENE (?). Analysis. Calculated for $C_{10}H_{16}$, C 88.16; H 11.84. Found, C 87.95, H 12.21, α_D^{25} + 31.14, n_D^{25} 1.4552, boiling point at 30 mm. 71° C.

Derivatives. An authentic sample of α-thujene could not be found for comparison with this unknown. This compound can be separated chromatographically from both limonene and β-myrcene, but is indistinguishable chromatographically from α-pinene. The only derivative which could distinguish between α-pinene and α-thujene was the hydrochloride. Repeated attempts to form the hydrochloride of α-pinene resulted in a solid compound melting at 98° to 118° C. after four recrystallizations from methanol (lit. 132° C.). It was not possible to isolate a crystalline hydrochloride from the unknown. On the basis of physical properties this compound is tentatively identified as α-thujene.

$C_{15}H_{24}$ (I). Analysis. Calculated for $C_{15}H_{24}$, C 88.16; H 11.84. Found, C 87.41; H 11.55, α_D^{25} + 86.18, n_D^{25} 1.5012, d_4^{25} 0.9053, boiling point at 16 mm. 140.5° C.

Derivatives. Bromide, melting point 140–142.5° C. (from ether-alcohol). Analysis. Calculated for $C_{15}H_{24}Br_4$, C 34.36; H 4.70. Found, C 34.38;

H 4.62. This compound absorbs 2 moles of hydrogen per mole and, therefore, contains two double bonds. Repeated attempts to form a crystalline hydrochloride by saturating an ether or an acetic acid solution of the hydrocarbon with dry hydrogen chloride, or by adding the equivalent amount of hydrogen chloride in ether to the hydrocarbon in ether, were unsuccessful.

The properties of this compound do not agree with those of any of the well-characterized sesquiterpenes found in the literature.

$C_{15}H_{24}$ (II). Analysis. Calculated for $C_{15}H_{24}$, C 88.16; H 11.84. Found, C 86.10, H 11.52. α_D^{25} −6.56, n_D^{25} 1.5008, boiling point at 10 mm. 115° C., at 21 mm. 132.5° C.

Nonhydrocarbons

Esters. ETHYL ISOVALERATE. The ester was hydrolyzed with potassium hydroxide in diethylene glycol. The 3,5-dinitrobenzoate of the alcohol portion melted at 92–93° C. A mixed melting point with ethyl 3,5-dinitrobenzoate was not depressed. The p-phenylphenacyl ester of the acid portion melted at 77–79° C. A mixed melting point with p-phenylphenacyl isovalerate was not depressed.

This ester, isolated chromatographically, was found mixed with hexanal, octanal, and ethyl $C_6H_8O_2$. It was separated from the mixture by distillation at 30 mm. in a microdistillation column.

ETHYL $C_6H_8O_2$. The ester was hydrolyzed with potassium hydroxide in diethylene glycol. The 3,5-dinitrobenzoate of the alcohol melted at 93° C. The p-phenylphenacyl ester of the acid melted at 124–126° C. By comparison on chromatostrips, and by the melting point of the acid derivative, the acid portion of this ester was determined to be the same as the $C_6H_8O_2$ acid isolated from the distillation water of the juice.

This ester, isolated by adsorption from silicic acid, was not separable by chromatography from hexanal, octanal, and ethyl isovalerate. It was separated from these compounds by distillation at 30 mm. in a microdistillation column.

METHYL α-ETHYL n-CAPROATE. Analysis. Calculated for $C_9H_{18}O_2$, C 68.31; H 11.46. Found, C 67.57; H 11.35. Boiling point at 30 mm. 80° C., n_D^{25} 1.4101.

Derivatives. The compound was hydrolyzed with potassium hydroxide in diethylene glycol. The p-phenylphenacyl derivative of the acid melted at 54.5–55.5° C. (from 95% alcohol) (lit. 53–54° C.). Analysis. Calculated for $C_{22}H_{26}O_3$, C 78.07; H 7.74. Found, C 78.06; H 7.80.

The chromatographically homogeneous fraction in which this compound was found contained citronellyl acetate and citronellal. The aldehyde was removed by reaction with semicarbazide hydro-

chloride and separated from the esters by adsorption on silicic acid and development with ethyl acetate. The two esters were then separated by distillation in a microdistillation column.

CITRONELLYL ACETATE. Boiling point at 8 mm. 110.5° C., n_D^{25} 1.4422.

Derivatives. The ester was saponified with potassium hydroxide in diethylene glycol. The alcohol portion compared with citronellol on chromatostrips showed no differences. The p-phenylphenacyl ester of the acid melted at 107–108° C. (from 95% alcohol) (lit. 111° C.).

TERPINYL ACETATE. Analysis. Calculated for $C_{12}H_{20}O_2$, C 73.42; H 10.27. Molecular weight 196. Found C 71.70; H 10.34. Molecular weight 194.

Derivatives. The ester was saponified with potassium hydroxide in diethylene glycol. The alcohol portion compared with α-terpineol on chromatostrips showed no differences.

This compound was found as a mixture with $C_{15}H_{24}O$ (II) and was separated from it by reacting the aldehyde with semicarbazide hydrochloride and separating the two on a silicic acid column with ethyl acetate as developer.

Carbonyls. HEXANAL. Boiling point 113° C., n_D^{25} 1.4175.
Derivatives. 2,4-Dinitrophenylhydrazone melting point 105–106° C. (from 95% alcohol) (lit. 106–107° C.). Analysis. Calculated for $C_{12}H_{16}N_4O_4$, C 51.42; H 5.75; N 19.99. Found, C 51.79; H 5.93; N 19.87. Semicarbazone melting point 106–108° C. (from benzene) (lit. 106° C.).
OCTANAL. Derivatives. 2,4-Dinitrophenylhydrazone melting point 109° C. (from 95% alcohol). Analysis. Calculated for $C_{14}H_{20}N_4O_4$, C 54.33; H 6.54; N 18.17. Found, C 55.16; H 7.01; N 18.19. Mixed melting point with octanal 2,4-dinitrophenylhydrazone showed no depression.
DECANAL. Derivatives. 2,4-Dinitrophenylhydrazone melting point 104–106° C. (lit. 104° C.). The derivative melting point was not depressed when mixed with the 2,4-dinitrophenylhydrazone of decanal.
2-DODECENAL (?). Boiling point at 10 mm. 117° C., n_D^{25} 1.4585.

Derivatives. The 2,4-dinitrophenylhydrazone was formed and separated from reagent by adsorption on silicic acid and development with benzene. The resulting hydrazone melted at 74–77° C. (from 95% alcohol), but the product was amorphous. The semicarbazone was formed and separated from reagent and unreacted compound by adsorption on silicic acid and development with ethyl acetate. (Semicarbazones could be detected on developed chromatostrips by spraying with a solution of 50 mg. of bromophenol blue and 200 mg. of citric acid in 100 ml. of water. The semicarbazone was a blue spot on a yellow background.) The semicarbazone was waxy, even after precipitation from methanol and water, hexane, or ethyl acetate. A characteristic melting point could not be obtained.

CITRONELLAL. This compound was removed from a mixture with citronellyl acetate and methyl α-ethyl n-caproate by reacting with semicarbazide hydrochloride. The mixture was resolved by adsorption on silicic acid and developing with ethyl acetate. Semicarbazone melted at 85–86° C. from 50% methanol (lit. 86° C.). The 2,4-dinitrophenylhydrazone prepared by adding reagent to the mixture with the esters and recrystallizing from 95% alcohol melted at 76–77° C. (lit. 76.5–78° C.).

$C_{15}H_{24}O$ (I). Analysis. Calculated for $C_{15}H_{24}O$, C 81.76; H 10.98. Molecular weight 220. Found, C 81.93; H 11.01. Molecular weight 237. Boiling point at 12 mm. 125.5° C., n_D^{25} 1.5019.

Derivatives. The 2,4-dinitrophenylhydrazone was amorphous and melted at 183–186° C. (from 95% alcohol). The semicarbazone melted at 215–217° C. from methanol. The compound reacts with o-dianisidine and is probably an aldehyde.

$C_{15}H_{24}O$ (II). Analysis. Calculated for $C_{15}H_{24}O$, C 81.76; H 10.98. Found, C 81.85; H 10.39. Boiling point at 6 mm. 141° C., n_D^{25} 1.5038.

Derivatives. Semicarbazone melting point 126–129° C. from 50% methanol. Analysis. Calculated for $C_{16}H_{17}N_3O$, C 69.27; H 9.81; N 15.15. Found, C 69.32; H 9.36; N 14.95. 2,4-Dinitrophenylhydrazone melts at 60–62° C. from 95% alcohol (amorphous). When chromatographed on chromatostrips this compound appeared as an ultraviolet absorbing spot which reacted with 2,4-dinitrophenylhydrazine and with o-dianisidine. It was concluded to be a sesquiterpene aldehyde.

$C_{15}H_{22}O$. Boiling point at 3 mm. 146.5° C., n_D^{25} 1.5210.

Derivatives. Semicarbazone, melting point 192–194° C. (from 50% methanol). This compound was judged to be the same as the $C_{15}H_{22}O$ compound found in grapefruit juice by comparison on chromatostrips and by odor.

CARVONE. Derivatives. 2,4 - Dinitrophenylhydrazone melting point 185–188° C. The melting point of the derivative was not depressed when mixed with an authentic sample of carvone 2,4-dinitrophenylhydrazone. The sample has a strong odor of caraway.

Alcohols. $C_{15}H_{26}O$ (I). Analysis. Calculated for $C_{15}H_{26}O$, C 81.02; H 11.79. Molecular weight 222. Found, C 80.24; H 12.08. Molecular weight 232. Melting point 94–96° C. (from 80% methanol).

Derivatives. The allophanate was formed by adding the compound in benzene to cyanic acid, extracting the reaction mixture with hot 90% ethyl acetate in hexane, evaporating the solvent, and recrystallizing the product from 90% methanol. After 24-hour standing at 5° C. well-formed crystals were present (melting point 153–153.5° C.).

The compound was mixed with benzene, pyridine, and 3,5-dinitrobenzoyl chloride, but did not form the benzoate derivative, as shown by chromatography of the reaction mixture, and by recovery of the unchanged compound. The compound failed to give the alkyl xanthate test for primary or secondary alcohols (8). It was concluded to be a tertiary sesquiterpene alcohol. Its properties did not agree with those of any of the known sesquiterpene tertiary alcohols.

$C_{15}H_{26}O$ (II). Analysis. Calculated for $C_{15}H_{26}O$, C 81.02, H 11.79. Molecular weight 222. Found, C 80.61; H 12.03. Molecular weight 242. Boiling point at 4 mm. 151° C., n_D^{25} 1.5013.

Derivatives. The compound reacted with cyanic acid and the formation of an allophanate was shown by chromatographing the reaction mixture on chromatostrips. A low R_f compound which reacted with fluorescein-bromine spray was formed. A crystalline derivative could not be obtained from hexane, dilute methanol or hexane–ethyl acetate mixtures. The formation of an allophanate indicates this compound is an alcohol.

LINALOÖL. Analysis. Calculated for $C_{10}H_{18}O$, C 78.39; H 11.19. Molecular weight 154. Found, C 77.89; H 11.13. Molecular weight 183. Boiling point at 32 mm. 115° C., $α_D^{24}$ + 16.47, n_D^{25} 1.4645.

Derivatives. Phenylurethane melting point 61–64° C. (from hexane) (lit. 65–66° C.). The compound was oxidized with chromic anhydride in glacial acetic acid, diluted with water, and extracted from the mixture with hexane. The product had the odor of citral, and when chromatographed on chromatostrips with several solvents, it was indistinguishable from citral, and gave colored spots when sprayed with o-dianisidine and 2,4-dinitrophenylhydrazine.

CARVEOL. Boiling point at 6 mm. 100° C., $α_D^{26}$ + 9.30, n_D^{25} 1.4932.

Derivatives. 3,5 - Dinitrobenzoate melting point 119° C. (from alcohol-ethyl acetate, 3 to 1) (lit. dl-trans-carveol, 119° C.). The compound was oxidized with chromic anhydride in glacial acetic acid, extracted with hexane from the aqueous reaction mixture, compared to carvone chromatographically, and reacted with 2,4-dinitrophenylhydrazine. The 2,4-dinitrophenylhydrazone melted at 191° C. (from 10% ethyl acetate in alcohol) (lit. 189° C.). It was not depressed when mixed with carvone 2,4-dinitrophenylhydrazone.

1-DECANOL. This alcohol was found mixed with carveol. Fifty milligrams of the mixture were oxidized with 1 ml. of a saturated solution of chromic anhydride in glacial acetic acid. After dilution with water and extraction with hexane, the mixture was chromatographed on chromatostrips. The reaction products

of decanal and carvone were identified by comparison to known samples. The 2,4 - dinitrophenylhydrazones formed from the mixture were adsorbed on silicic acid and developed with benzene. After removal of the solvent, the single band formed was dissolved in 95% alcohol and carvone 2,4-dinitrophenylhydrazone filtered from the cooled solution. After removal of as much red carvone derivative as possible the supernatant liquors were evaporated and yellow crystals obtained by crystallization from 95% alcohol. After several recrystallizations the melting point was 92–94° C. Mixed with octanal 2,4-dinitrophenylhydrazone of melting point 109° C., the melting point of the mixture was depressed to 86–95° C. Mixed with decanal 2,4-dinitrophenylhydrazone of melting point 104° C., the melting point of the mixture was 96–100° C. The amount of 1-decanol present in the mixtures was calculated from the refractive index of the original fractions.

1-OCTANOL. This alcohol was always found in the α-terpineol fractions. The mixture was oxidized with chromic anhydride, compared to octanal on chromatostrips, the 2,4-dinitrophenylhydrazone formed, and separated by adsorption on silicic acid with benzene as the developer. Melting point 105–107° C. (from 95% alcohol) (lit. 106°C.). The melting point was depressed with the 2,4-dinitrophenylhydrazones of decanal, hexanal, and dodecanal, but not with octanal. The amount of 1-octanol present in the mixture with α-terpineol was calculated from the refractive index of the original fractions.

α-TERPINEOL. Melting point 37–38° C. (from hexane) (lit. 38–40° C.), $α_D^{24}$ + 86, n_D^{25} 1.4815.

Derivatives. Nitrosochloride melting point 105–108° C. from ethyl acetate (lit., 107–108° C.).

1-HEXANOL. Analysis. Calculated for $C_6H_{14}O$, C 70.53; H 13.81. Found, C 70.72; H 13.82. Boiling point at 58 mm. 90.5° C., n_D^{25} 1.4182.

Derivatives. 3,5-Dinitrobenzoate melting point 59–59.5° C. from 95% alcohol (lit. 60–61° C.). Allophanate melting point 163–164° C. from 10 to 1 hexane–ethyl acetate (lit. 165° C.). A portion oxidized with chromic anhydride in glacial acetic acid and compared on chromatostrips to hexanal showed no differences. The 2,4-dinitrophenylhydrazone of the oxidized sample melted at 99–101° C. from 95% alcohol, and its melting point was not depressed when mixed with a known sample of hexanal 2,4-dinitrophenylhydrazone.

3-HEXEN-1-OL. This compound was difficult to separate chromatographically from 1-hexanol. It could not be separated by distillation. The best fraction obtained was shown to contain some 1-hexanol.

Analysis. Calculated for $C_6H_{12}O$, C 71.87; H 12.07. Molecular weight 100. Found C 72.51; H 13.79. Molecular weight 126. Boiling point at 50 mm. 90° C., n_D^{25} 1.4275.

Derivatives. 3,5-Dinitrobenzoate melting point 47° C. from 95% alcohol (lit. 49° C.). The allophanate prepared by reaction of the best fraction with cyanic acid was separated by recrystallization from ethyl acetate into a derivative melting at 164° C., and shown by mixed melting point to be identical with the allophanate of 1-hexanol. Another derivative was obtained from this preparation by separating from an ethyl acetate solution all crystals melting above 153° C., and recrystallizing the supernatant liquors after evaporation of the solvent from 90% ethyl acetate in hexane to a constant melting point of 146–148° C. (lit. 146° C.). The original fraction was oxidized with chromic anhydride solution and the 2,4-dinitrophenylhydrazones formed from the reaction products were separated by adsorption on silicic acid and development with benzene into two hydrazones. The higher R_f derivative melted at 99–100° C., and its melting point was not depressed when mixed with known hexanal 2,4-dinitrophenylhydrazone. The other derivative melted at 150–151° C. from 95% alcohol (lit. 144° C.).

$C_7H_{16}O_2$. Boiling point at 38 mm. 95.5° C., n_D^{25} 1.4272. Derivatives. 3,5-Dinitrobenzoate melting point 63–64° C. (from 95% alcohol). Analysis. Calculated for $C_{14}H_{18}N_2O_7$, C 51.53; H 5.56; N 8.59. Found, C 51.16; H 5.16; N 8.53.

Polyoxygenated Compounds. Material which appeared in the volatile fractions of very low R_f values was isolated from the adsorption columns. These fractions were mostly insoluble in hexane, soluble in ethyl acetate, and of a viscous nature. The fractions gave an acid reaction with pH paper. By analogy to similar fractions obtained from grapefruit juice, these fractions were concluded to be a mixture of compounds with more than one oxygen in the molecule.

Other Compounds. Three other chromatographically homogeneous fractions were isolated from the canned stored orange juice. These fractions were set aside at 38° F. for several months before final identification was undertaken. During this period two of the fractions had badly decomposed as shown by chromatostrip tests and identification was impossible. By comparison on chromatostrips before the sample decomposed it was possible to determine that these compounds were not present in either the fresh or freshly canned juices. The fractions reacted with fluorescein-bromine spray and were of high R_f value, indicating an unsaturated ester, carbonyl, oxide, or high-molecular-weight type compound. One of

the three fractions had not decomposed as much as the other two. It was adsorbed on a column and about one fourth of the original fraction was obtained in an unchanged form. When a distillation test showed it to be a mixture, an amount too small for identification remained. The sample was of high R_f value and gave a fluorescein-bromine test for unsaturation. Its boiling point was about 100° C. at 20 mm., and n_D^{25} 1.472. When compared to 1,8-cineol it had the same R_f values but would not give the resorcinol addition product. It appeared to react slowly with 2,4-dinitrophenylhydrazine. From the evidence it did not appear to contain either 1,8-cineol or 1,4-cineol.

Table II contains a summary of the compounds found with an estimation of the amounts obtained in the three orange juices. The amounts were estimated by weighing the oil before separation procedures, weighing the separated fractions obtained, and correcting the weight of the separated fractions for the over-all yield. As pointed out in the earlier work on grapefruit juices (14), the losses experienced were almost entirely

confined to the process of removing developing solvent from the fractions. Corrections could not be made for differences in volatility of the components of a mixture because of its complexity. Where fractions were left as mixtures the components were estimated from refractive index data and observations of chromatographed portions on chromato-strips. Therefore, the quantitative figure in Table II must be regarded as estimations.

Other compounds have been reported present in orange peel or juice (Table III) which were not found in the orange juice samples examined in this study. For example, citral was isolated by chromatography from commercial orange peel oil, but was not found in the oil fractions of any of the juices tested. Isopulegol has been reported as a constituent of citrus oils (25), which also contain citronellal. Isopulegol was prepared by refluxing citronellal with acetic anhydride and hydrolyzing the mixture with potassium hydroxide (31). The purified compound was obtained by adsorbing the steam-distilled mixture on silicic acid with 50% isopropyl ether

Table II. Volatile Oil Constituents of Orange Juices

| | Mg. per Kg. of Juice | | |
	Fresh	Freshly canned	Canned stored
Oil present by Clevenger analysis	105.00	88.06	62.36
Total oil obtained[a]	91.58	76.36	54.44
Hydrocarbons	88.39	70.96	45.96
Nonhydrocarbons	3.17	5.40	8.48
Hydrocarbons			
Limonene	80.10	63.60	43.0
β-Myrcene	1.98	1.14	0.69
α-Thujene (?)	0.30	0.30	0.03
$C_{15}H_{24}$(I)	5.80	5.78	2.04
$C_{15}H_{24}$(II)	0.20	0.18	0.21
Nonhydrocarbons			
Esters			
Ethyl isovalerate	0.01	0.01	0
Ethyl $C_6H_8O_2$	0.03	0.03	0
Methyl α-ethyl n-caproate	0.06	0.10	0.02
Citronellyl acetate	0.10	0.04	0.02
Terpinyl acetate	0.08	0.01	0
Carbonyls			
Hexanal	0.04	0.03	0
Octanal	0.06	0.06	0
Decanal	0.05	0.04	0.02
2-Dodecenal (?)	0.06	0.06	0
Citronellal	0.04	0.04	0.02
$C_{15}H_{24}O$(I)	0.14	0.10	0.15
$C_{15}H_{24}O$(II)	0.07	0.12	0
$C_{15}H_{22}O$	0.09	0.09	0.09
Carvone	0	0	0.08
Alcohols			
$C_{15}H_{26}O$ (I)	0.07	0.24	0.23
$C_{15}H_{26}O$ (II)	0.14	0.16	0
Linaloöl	0.93	1.10	0.12
Carveol	0.06	0.17	0.86
1-Decanol	0.10	0.09	0.07
1-Octanol	0.21	0.23	0.19
α-Terpineol	0.32	1.72	4.08
1-Hexanol	0.10	0.14	0.08
3-Hexen-1-ol	0.10	0.18	0.06
$C_7H_{16}O_2$	0.07	0.08	0.09
Polyoxygenated compounds	0.12	0.15	0.75
Other compounds			0.59

[a] Total oil obtained from 3000 gallons of fresh juice, 2500 gallons of freshly canned juice, and 1520 gallons of canned stored juice.
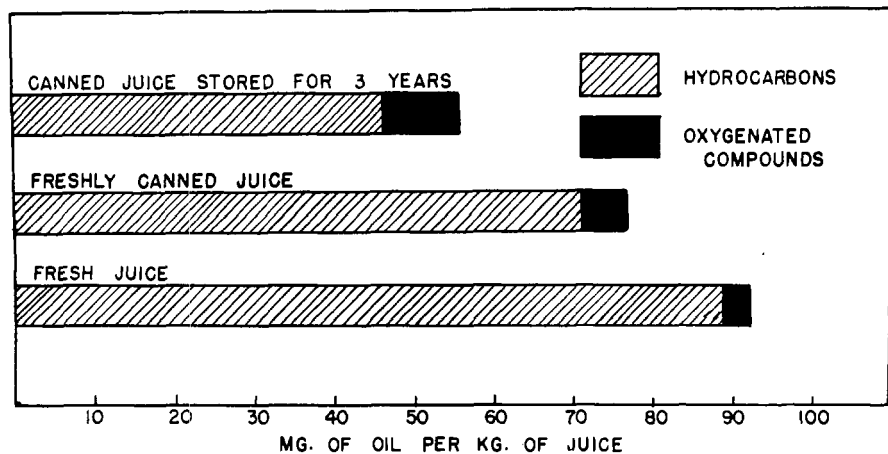
Figure 2. Volatile oils from orange juice

in hexane as the developer. The product, $[n_D^{25}$ 1.4696, allophanate melting point 219° C. (lit. 219° C.)], was chromatographed on chromatostrips in various solvents and compared to fractions of similar R_f values from the juices. Isopulegol was not present in the isolated oil fractions. It was felt that citronellol should be a constituent of the volatile oils from these juices, particularly since citronellyl acetate and citronellal were shown to be present. None of the fractions contained citronellol by comparison on chromatostrips. Citronellal 2,4-dinitrophenylhydrazone was prepared from citronellol after oxidation with chromic anhydride. It was separable by chromatography from the 2,4-dinitrophenylhydrazones of octanal, decanal, and carvone which are the oxidation products of the compounds with R_f values similar to citronellol. By oxidation of a synthetic mixture of geraniol, 1-octanol, α-terpineol, 1-hexanol, and conversion of the

oxidized products to their 2,4-dinitrophenylhydrazones it was possible to isolate the 2,4-dinitrophenylhydrazone of the citral (oxidation product of geraniol) in pure form by chromatography on silicic acid with benzene as developer. Since these alcohols have R_f values close to geraniol, and no such derivative was found by similar treatment of orange juice fractions, it was further concluded that geraniol was not present in the oil fractions. Nerol would react in the same way as geraniol in these tests.

The data presented in Figures 2, 3, and 4 show that the volatile oil portion of orange juice is greatly changed during pasteurization and subsequent storage. The total volatile oil decreases markedly, hydrocarbons are lost, and the nonhydrocarbons increase. There is a general decrease in the volatile esters, aldehydes, linaloöl, and the $C_{15}H_{26}O$ (II) compound (which may be farnesol). Other aliphatic alcohols remain essen-

tially unchanged, and there is an increase in carveol, α-terpineol, $C_{15}H_{26}O$ (I), and polyoxygenated compounds. These findings agree in general, but with important differences, with the work of Blair and coworkers (2) and Henry and Clifcorn (11). Blair and coworkers concluded from infrared studies of oil isolated from heated and stored orange juices, that changes in flavor were caused by hydrolysis of esters and the production of 1,4-cineol, α-terpineol, and terpinolene from the hydrocarbons. Their experiments showed a decrease in recoverable oil and the production of oil-soluble volatile acids during storage. The work of Henry and Clifcorn presents evidence that the products responsible for some of the off-flavor of canned orange juice are volatile unsaturated alcohols and hydrocarbons. Results of the analysis of the volatile oils from the various orange juices are in contrast to the findings from a similar analysis of grapefruit juice (14). In grapefruit juice the recoverable oil does not decrease on canning and storage and the hydrocarbons are apparently changed to volatile alcohols without as great a conversion to α-terpineol. The nature of the changes in the volatile oil fractions of grapefruit juice reflect the greater stability of this juice over orange juice.

An attempt was made to evaluate the significant changes in the composition of the volatile oils from the three juices in relation to their effect on the flavor of orange juice. A mixture of 5 mg. of α-terpineol, 1 mg. of carveol, 0.1 mg. of carvone, 1 mg. of polyoxygenated compounds, 5 mg. of furfural, 62 mg. of methanol, and 16 mg. of acetic acid was added to 1 liter of freshly squeezed orange juice and compared to an aliquot
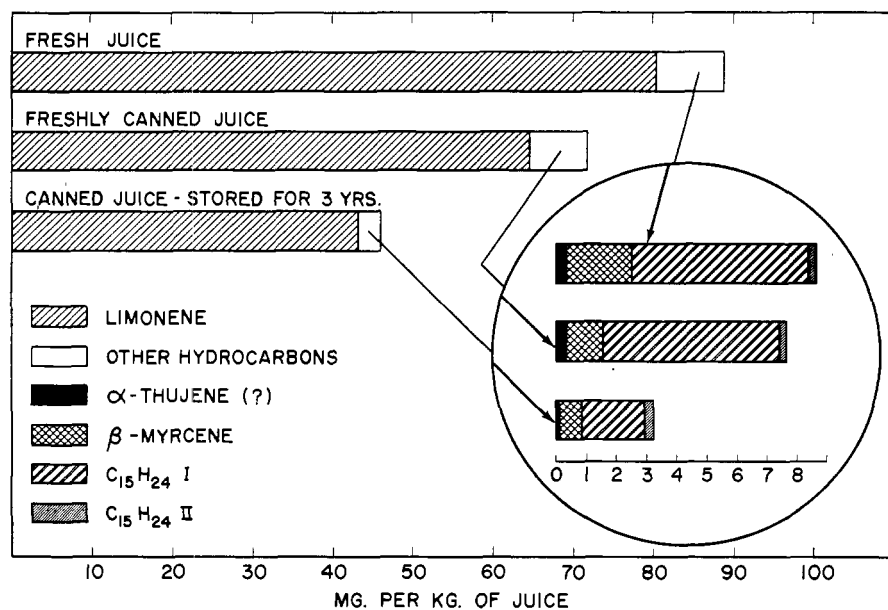

Figure 3. Composition of hydrocarbons from orange juice

Table III. Volatile Oil Constituents Previously Reported in Peel and Juice of Sweet Orange

Hydrocarbons	Aldehydes
Limonene	Citral
Myrcene	Decanal
Ocimine(?)	Nonanal
Terpinolene(?)	Octanal
α-Terpinene	2-Decenal
Terpinene	2-Dodecenal
Carene	Citronellal
Cadinene	

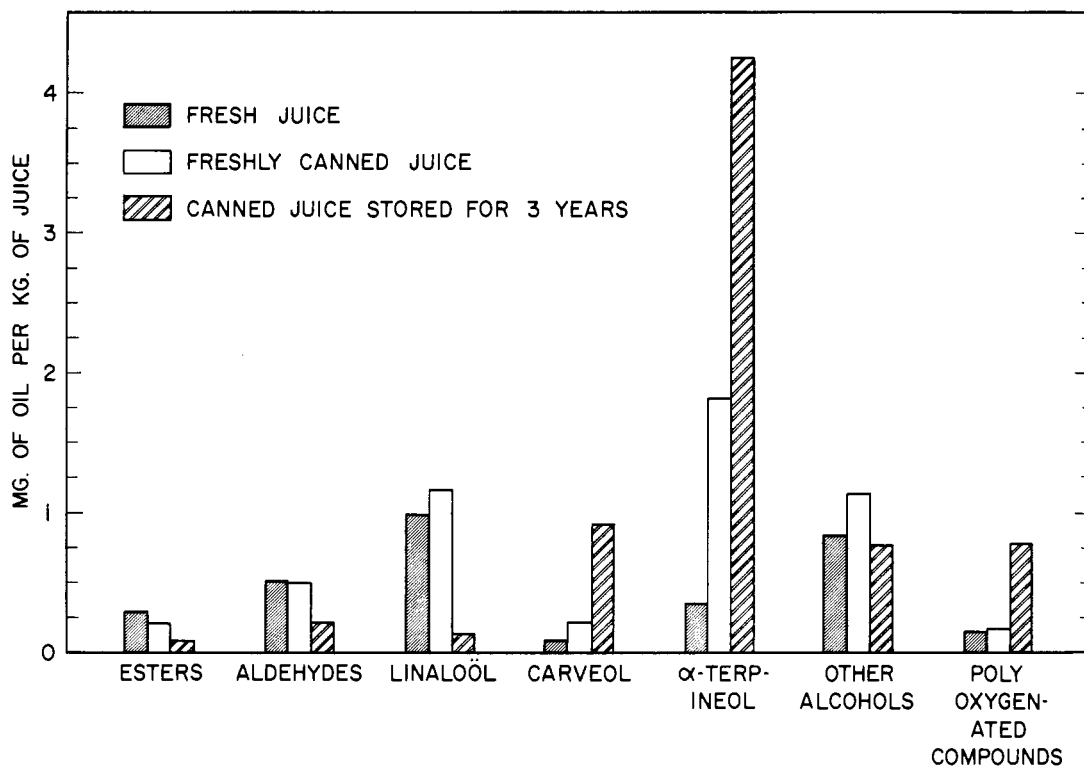| Alcohols	Esters
Linaloöl	Methyl anthranilate
1-Octanol (as ester)	Methyl N-methyl anthranilate
1-Nonanol	Nonyl caprylate
d-Terpineol	$C_{10}H_{17}OOC_8H_{15}$
l-Terpineol	
Nerol	
1-Decanol(?)	Esters (acid portion)
Geraniol(?)	
Farnesol (or nerolidol)	Formic acid
	Acetic acid
Ceryl alcohol	Capric acid
$C_{13}H_{17}OH$	Caprylic acid
2-Phenylethanol	Butyric acid

Figure 4. Changes in oxygenated constituents of orange juice

of the same juice without added oils. The flavor of the treated juice was easily distinguishable from that of the untreated juice. The flavor was described as stable and musty, but it did not resemble the flavor and odor of canned stored orange juice. As one of the findings of the work on the volatile oils in orange juice appears to be that the flavorful components are destroyed by canning and storage, a concentrate was prepared by low-temperature evaporation which was substantially free of any volatile oil (less than 0.001%). The reconstituted

juice had little orange flavor but it did not have a canned or pasteurized taste. Addition to this juice of α-terpineol, carveol, carvone, polyoxygenated compounds, furfural, methanol, and acetic acid in the amounts found in stored canned orange juice produced a decided stale flavor which could be identified as characteristic of aged canned orange juice but which was not identical with canned juice ordinarily encountered. The $C_6H_8O_2$ acid found in the water-soluble volatile fractions was isolated only as its p-phenylphenacyl ester and its

flavor contribution could not be studied.

Experiments were conducted to determine the effect of volatile oil decomposition on the flavor of orange juice and to determine, if possible, the extent of the contribution of the decomposition of the volatile oils to the flavor of canned orange juice. To accomplish this, freshly reamed juice was evaporated at low temperature to remove oil. After diluting to the original Brix, part of the lot was canned with added peel oil (same lot of oil as described below) and part without oil. All samples were pasteurized by agitating

Table IV. Triangular Taste Tests on Canned Orange Juice

Sample No. and Lot	Oil Level during Storage, %	Storage Temp., °F.	Oil Added for Flavor Evaluation[a]	Storage Temp. of Oil Added, °F.	Compared to Sample No.	Sample Preferred	Correct Judgments Out of 18, %	Statistical Significance[b]
1	0.002	40	Terpeneless	70	2	1	69	++
2	0.002	40	Whole oil	70				
3	0.002	40	Whole oil	0	4	3	83	+++
4	0.002	40	Whole oil	70				
5	0.002	40	Terpeneless	0	6	5	83	+++
6	0.024	40	None	..				
7	0.002	40	Terpeneless	0	8	..	23	−
8	0.002	40	Terpeneless	70				
9	0.002	70	Terpeneless	70	10	9	55	+
10	0.024	70	None	..				
11	0.002	70	Whole oil	70	10	11	60	+
12	0.024	40	None	..	10	12	73	+++
1A	0.001	40	Terpeneless	0	2A	2A	44	−
2A	0.001	40	Whole oil	0				
3A	0.016	40	None	..	2A	2A	67	++
4A	0.016	70	None	..	5A	..	55	+
5A	0.001	70	Whole oil	70				
6A	0.016	70	None	..	3A	3A	89	+++

[a] Oil added just before tasting to contain equivalent of nonhydrocarbon constituents as found in 0.024% whole oil in first lot and 0.016% in lot A.

[b] — no significance, + significant at 5% level, ++ significant at 1% level, +++ significant at 0.1% level.

the cans in a 200° F. water bath for 2.25 minutes and stored for 4 months at 40° and 70° F. A second lot of juice was treated in a similar fashion. A single lot of commercial orange oil of good fresh odor was deterpened by a chromatographic process (*13*). Part of this oil was stored at 0° F. and part at 70° F. for 4 months. A portion of the same lot of whole peel oil was stored at similar temperatures. Juices stored without oil were reconstituted with these oil samples to the same oil level (calculated on the basis of nonhydrocarbon content) as juices stored with oil before taste tests were made. The triangular taste test method was used to rate the samples. The results of these experiments are shown in Table IV.

The flavor of both lots of juice without any volatile oil initially present was distinctly bad. This is a strong indication that part of the unacceptable flavor of pasteurized orange juice does not arise from the volatile flavor fraction. The findings of Guyer and Boyd (*9*) substantiate this observation. The results of the taste tests shown in Table IV can be summarized as follows:

The storage temperature of either the juice or the flavoring oil had a great effect on the taste of the juice.

Terpeneless oil as added flavor was preferred over whole oil if the storage temperature was high.

Terpeneless oil as added flavor was not affected by storage.

Juice stored without oil and reconstituted with oil prior to tasting was preferred to juice stored with oil.

At high storage temperatures the off-flavor contributed by the nonvolatile fraction of the juice was the dominant flavor.

These conclusions and the results of the analysis of the volatile fractions show that contributions to the off-flavors in orange juice are produced by alterations of the hydrocarbon portion of the oil present in the juice, but that the off-flavor produced by the volatile oil decomposition is only part of the off-flavor of canned and stored canned juices.

The flavor of orange juice (*10, 23, 27*) and of heated and stored orange juice (*2, 4, 5, 9, 11, 12, 19, 29, 30*) has been the subject of numerous investigations. Hall and Wilson (*10*) investigated the volatile material from a large volume of fresh orange juice but largely ignored the contribution of the volatile oils to the flavor and aroma. Boyd and Peterson (*4*) recognized that the presence of volatile oils in fresh juice gave it the pleasant aroma and flavor and showed certain effects of storage, heating, and oxygen removal on the flavor quality of the juice. Henry and Clifcorn (*11*) distinguished between cooked flavor and off-flavor of canned orange juice. The off-flavor was claimed to be volatile, whereas the cooked flavor was nonvolatile and

water-soluble. Theories of flavor change in orange juice have been reviewed by Swift (*30*). In addition, Swift (*30*) and Huskins, Swift, and Veldhuis (*12*) investigated the changes in the lipides on heating and storage and concluded that these compounds were not responsible for flavor change. Recently Guyer and Boyd (*9*) have shown that orange juice packed with low volatile oil deteriorated as fast as juice packed with a normal oil content.

Results of the analysis of the volatile fractions from fresh, freshly canned, and stored canned orange juice and the taste texts of variously treated juices allow tentative conclusions to be made about the nature of the flavor of orange juice. Most of the characteristic flavor of fresh orange juice is due to volatile oils. Low-temperature removal of volatile oils from fresh juice leaves a liquid which can still be distinguished as being of orange juice origin but which is insipid and flat. The flavorful components of fresh juice are largely destroyed by heating and storage. The volatile hydrocarbons appear to be degraded by heating and storage to alcohols, polyoxygenated compounds, and probably nonvolatile components. Furfural, acetic acid, and methanol are produced by heating and storage. These compounds probably arise from the nonvolatile portion of the juice. An unidentified acid ($C_6H_8O_2$) was found present in fairly large amounts in canned and stored juices, but its flavor effect was not evaluated. The addition to fresh orange juice of the major volatile products found in heated orange juice produces a definite off-flavor. The off-flavor produced by this addition still lacks some of the characteristics of canned and stored canned orange juices. These juices are dominated by an off-flavor derived from the nonvolatile portion of the juice. This off-flavor is produced by heating and its nonvolatile origin can be demonstrated by heating juices with the volatile flavors removed.

Acknowledgment

The authors wish to express appreciation to G. J. Keller and R. G. Rice for assistance in handling the fresh and freshly canned juices; and in the water-soluble work, to G. J. Keller for preparation of taste test packs, to R. G. Rice for supervision of the taste tests. Thanks are due to A. J. Haagen-Smit and Glen Swinehart for performance of the elementary analyses.

Literature Cited

(1) Bartholomew, E. T., Sinclair, W. B., Janes, B. E., *Hilgardia* **12**, 473 (1939).
(2) Blair, J. S., Godar, E. M., Masters, J. E., Reister, D. W., *Food Research* **17**, 235 (1952).
(3) Boos, R. N., *Anal. Chem.* **20**, 964 (1948).
(4) Boyd, J. M., Peterson, G. T., *Ind. Eng. Chem.* **37**, 370 (1945).
(5) Curl, A. L., Veldhuis, M. K., *Fruit Products J.* **26**, 329 (1947).
(6) Davis, W. B., *Am. J. Botany* **19**, 101 (1932).
(7) Drake, N. L., Bronitsky, J., *J. Am. Chem. Soc.* **52**, 3715 (1930).
(8) Feigl, F., "Qualitative Analysis by Spot Tests," tr. by J. W. Mathews, p. 271, New York, Nordemann, 1939.
(9) Guyer, R. B., Boyd, J. M., *Food Technol.* **8**, 295 (1954).
(10) Hall, J. A., Wilson, C. P., *J. Am. Chem. Soc.* **47**, 2575 (1925).
(11) Henry, R. E., Clifcorn, L. E., *Canning Trade* **70** (31), 7 (Feb. 23, 1948).
(12) Huskins, C. W., Swift, L. J., Veldhuis, M. K., *Food Research* **17**, 109 (1952).
(13) Kirchner, J. G., Miller, J. M., *Ind. Eng. Chem.* **44**, 318 (1952).
(14) Kirchner, J. G., Miller, J. M., J. AGR. FOOD CHEM. **1**, 512 (1953).
(15) Kirchner, J. G., Miller, J. M., Keller, G. J., *Anal. Chem.* **23**, 420 (1951).
(16) Kirchner, J. G., Miller, J. M., Rice, R. G., Keller, G. J., Fox, M. M., J. AGR. FOOD CHEM. **1**, 510 (1953).
(17) Kirchner, J. G., Prater, A. N., Haagen-Smit, A. J., *Ind. Eng. Chem., Anal. Ed.* **18**, 31 (1946).
(18) Kirchner, J. G., Rice, R. G., Miller, J. M., Keller, G. J., *Arch. Biochem.* **25**, 231 (1950).
(19) Loeffler, H. J., *Ind. Eng. Chem.* **33**, 1308 (1941).
(20) Miller, J. M., Kirchner, J. G., *Anal. Chem.* **24**, 1480 (1952).
(21) *Ibid.*, **25**, 1107 (1953).
(22) Moore, E. L., Atkins, C. D., Wiederhold, E., MacDowell, L. G., Heid, J. L., *Proc. Inst. Food Technol., 6th Conf.* **1945**, 160.
(23) Morgan, D. A., Veldhuis, M. K., Eskew, R. K., Phillips, G. W. M., *Food Technol.* **7**, 332 (1953).
(24) Natarajan, C. P., Mackinney, G., *Ibid.*, **3**, 373 (1949).
(25) Naves, Y. R., *Parfums France* **9**, 60 (1931).
(26) Pennzig, Otto, Erdi Botta, Rome, Italy, Ministerio di Agricoltura e Comercio. Annali di Agricoltura, No. 116 (1887).
(27) Rice, R. G., Keller, G. J., Beavens, E. A., *Food Technol.* **6**, 35 (1952).
(28) Rice, R. G., Keller, G. J., Kirchner, J. G., *Anal. Chem.* **23**, 194 (1951).
(29) Riester, D. W., Braun, O. G., Pearce, W. E., *Food Inds.* **17**, 742 (1945).
(30) Swift, L. J., *Proc. Florida State Hort. Soc.* **64**, 181 (1951).
(31) Tiemann, F., Schmidt, R., *Ber.* **30**, 27 (1897).
(32) Trout, S. A., Tindale, G. B., Huelin, F. E., Australia, Council Sci. and Ind. Research. Pamphlet **80**, 22 (1938).
(33) Woodman, A. G., "Food Analysis," 4th ed., p. 511. McGraw-Hill, New York, 1941.