tion. Some such change as this is what probably occurs during the alkalizing or conching processes. We have not isolated enough material to permit determining optical rotation. Such a measurement would be desirable.

Since the other two compounds studied have unknown structures, it is not possible to ascribe particular structural changes to them. Their properties generally indicate that they are similar to the catechins and that therefore similar conformational changes probably occur with them during processing. However, the order of change is just the reverse of the order observed with (−)-epicatechin. Apparently the high temperature of roasting has no effect on this compound which is already in the stable cis, aryl-equatorial conformation. On alkalizing or conching the more unstable form appears. By analogy, compounds III and IV may start out in an unstable configuration which changes to a stable configuration during roasting and then back again to the unstable form during conching, but not during alkalizing.

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FRUIT JUICE CONSTITUENTS

Determination of Linaloöl and α-Terpineol in Florida Orange Products

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The principal volatile constituents of orange peel juice that contribute to its bitter flavor have been identified as linaloöl and α-terpineol. A gas-liquid chromatographic method has been developed for their determination in juices and peel oils and examples illustrating possible applications are given. Taste levels of these substances were determined in orange juices.

In recent years, there has been a substantial increase in the yield of juice from a given amount of oranges. Much of it is due to the increased efficiency of mechanical extractors, less good juice being discarded with peel and pulp. There is always some possibility, however, that when extraction pressures are carried to extremes, some juice from the peel may find its way into the final product. This possibility has been noted by Pobjecky (*3*).

Swift and Veldhuis (*5*) reported on a comparison of a number of components and properties in commercial juices, segment juices, and peel juices. Included were soluble solids, acidity, pH, Brix-acid ratio, reducing sugars, sucrose, total sugars, soluble pectic substances, ascorbic acid, flavonoids, and diacetyl as well as viscosity, odor, and fluorescence. This study showed that the general composition of these three types of juice differs considerably, but it was not intended to show which specific compounds might contribute to off-flavors. It was observed that peel juice was bitter and was detectable, when added to the extent of 3 to 5%, to good orange juice.

The present investigation was undertaken for the purpose of identifying certain peel juice constituents capable of contributing bitterness or other off-flavors and of devising means for their determinations. Only relatively soluble, steam-volatile substances are considered in this paper. The principal flavor-influencing constituents found were linaloöl and α-terpineol.

Linaloöl and α-terpineol have both been identified in single-strength orange and grapefruit juices by Kirchner and Miller (*1, 2*). Linaloöl has been found to be a constituent of domestic orange peel oils by Poore (*4*). The present work verifies these findings by different means and describes a method for simultaneous determination of these substances. Some examples of the application of the method are given and certain implications are presented.

Experimental

Identification of Linaloöl and α-Terpineol. Peel juice used in this study was obtained from a commercial plant where peel from juice extractors was being treated for the recovery of peel oil. Juice was squeezed from the peel between fluted rolls and centrifuged to recover oil. This peel juice was taken to the laboratory and clarified with a continuous precoat filter using Hyflo-Supercel filter aid. The filtrate obtained was a bright yellow, apparently homogeneous solution. When a portion of this was distilled, the distillate extracted with ether, and the solvent evaporated from the extract, an oil was obtained in small yield. A portion of this was introduced into an Aerograph gas-liquid chromatographic (GLC) apparatus and it gave two prominent peaks in a region where a normal peel oil showed only very small ones. The areas of the large hydrocarbon peaks of normal peel oils were greatly reduced for the extract. Several runs of the extract were made on the GLC apparatus and the effluent gases corresponding to each of these two large peaks, linaloöl and α-terpineol, respectively, which eluted after the peel oil hydrocarbons were passed through a condensing system and the fractions were accumulated. These were examined as

films on a Perkin-Elmer Model 21 infrared spectrophotometer and found to be linaloöl and α-terpineol by comparison of their spectra with those of authentic compounds.

Development of Method of Analysis. Construction of Reference Curves. The GLC apparatus operating conditions for reference curve construction and for the subsequent analytical procedure were as follows: column, 5-foot stainless steel, 0.25 inch in diameter (O.D.), containing 30% of Carbowax 1540 on 30- to 60-mesh crushed firebrick support; 5-mv. recording potentiometer, chart speed 15 inches per hour, filament current 200 ma., column temperature, 145° C., helium inlet pressure, about 200 mm. of Hg at a flow rate of about 63 ml. per minute. Retention times for linaloöl and α-terpineol were about 25 and 52 minutes, respectively.

A standard solution was prepared containing 10% by weight of both linaloöl and α-terpineol in a distilled hydrocarbon fraction of peel oil originally not showing peaks in either linaloöl or α-terpineol regions. Varying amounts of this mixture were injected into the GLC apparatus, weighing the syringe before and after each injection. From weights of the injected mixture the weights of injected linaloöl and α-terpineol in milligrams were calculated. These were plotted against corresponding peak areas as measured by a planimeter to give the reference curves. The relationship between amounts of the alcohols and peak areas is almost linear.

Analytical Procedure. If the sample is an oil it is only necessary to bring the GLC apparatus to the same operating conditions as used in obtaining the reference curve. Make the injection (20 to 50 μl.) weighing the syringe before and after to obtain the sample weight. The weights of linaloöl and α-terpineol are obtained from their respective peak areas by means of the reference curve.

If the sample is orange juice, bring the pH of about 4 liters to about 8.0 with dry calcium hydroxide to prevent acid conversion of limonene to terpineols during subsequent distillation. Distill 500 ml. into a liter separatory funnel and extract successively with 75-ml. and 25-ml. portions of ethyl ether. Concentrate the combined extracts using a Vigreux or similar fractionating column to prevent loss of oil constituents. When the volume has been reduced to about 5 ml., disconnect the flask, add about 0.5 gram of anhydrous sodium sulfate to the still residue, and let stand for a short time. Transfer the residue quantitatively to a smaller flask (15 to 25 ml.) that has been tared with its glass stopper, using a few milliliters of ether to effect the transfer. Continue distillation through the fractionating column until the volume remaining in the flask is about 0.5 ml. Stopper the flask. With the GLC apparatus under the same operating conditions as used for the standard curve preparation, reweigh the stoppered flask, immediately fill and weigh the syringe, and make the injection. Reweigh the syringe. If these precautions are carried out rapidly, evaporation losses are small. From the weight of the flask contents, the weight of the injected sample, and the weights of the linaloöl and α-terpineol from the reference curves, the amounts of these substances in the original sample can be calculated by using the following equation:

$$\text{Mg. linaloöl and/or } \alpha\text{-terpineol in sample} = \frac{\left(\begin{array}{c}\text{mg. linaloöl and/or } \alpha\text{-}\\ \text{terpineol from curves}\end{array}\right)\left(\begin{array}{c}\text{wt. in mg. of}\\ \text{extract solution}\end{array}\right)}{\text{wt. injected sample in mg.}}$$

Extraction Recoveries of Linaloöl and α-Terpineol from Aqueous Solutions. Four aqueous solutions containing both linaloöl and α-terpineol were prepared in liter amounts. To each of these was added 0.25 ml. of peel oil hydrocarbons to simulate practical conditions as closely as possible. This hydrocarbon fraction, as noted above, was shown by GLC analysis to be free of alcohols. Solutions were mixed thoroughly and extracted once with 150 ml. of ethyl ether, shaking for 1 minute. The ether extracts were processed as outlined in the procedure and the analyses were completed on the GLC apparatus. Concentrations employed and recoveries obtained are given in Table I.

Recoveries of Linaloöl and α-Terpineol from Orange Juice. Sixfold concentrate, containing no cutback juice and hence minimal amounts of essential oil constituents, was diluted to single strength. To 4 liters of neutralized juice was added 20 mg. each of linaloöl and α-terpineol in 4 ml. of ethyl alcohol. Also added was 0.25 ml. of peel oil hydrocarbons as in the previous experiment and for the same reason. The sample was distilled and the distillate was collected in four 100-ml. portions, each of which was extracted with 15- and 5-ml. portions of ether and analyzed separately. The results are presented in Table II.

Applications. Orange Juice Extraction Plant Samples. In order to learn how linaloöl and α-terpineol varied in different extraction plants and at different points within a given plant, several samples were secured from two concerns in the Winter Haven area during February and March 1960. From Plant A were obtained juice from

Table I. Single Extraction Recoveries of Linaloöl and α-Terpineol

Compound	From Aqueous Solutions: Added, mg./liter	Recovered, mg./liter	Recovery, %
Linaloöl	3.00	2.90	96.7
	6.00	6.25	104.1
	10.00	10.51	105.1
	15.00	14.94	99.7
α-Terpineol	3.00	2.97	99.2
	6.00	6.41	106.6
	10.00	10.32	103.2
	15.00	14.31	95.7

Table II. Over-All Recoveries of Linaloöl and α-Terpineol from Orange Juice

Compound	Mg. First[a]	Second[a]	Third[a]	Fourth[a]	Total	Recovery, %
Linaloöl	19.33	0.56	0.096	Trace	19.99	100.0
α-Terpineol	15.84	2.37	0.704	Trace	18.91	94.6

[a] All are 100-ml. fractions.

Table III. Linaloöl and α-Terpineol in Samples from Orange Juice Extraction Plants

Sample Description	Plant A: Linaloöl, Mg./Liter	Plant A: α-Terpineol, Mg./Liter	Plant B: Linaloöl, Mg./Liter	Plant B: α-Terpineol, Mg./Liter
Juice Series				
Juice from blending tank	2.34	1.08	0.74	0.88
Freshly canned juice	0.27	0.80	0.53	0.62
Centrifuged de-oiler condensate (aqueous portion)	..	..	2.08	0.16
Miscellaneous Samples				
Aged canned juice	0.21	5.05	..	..
Concentrate (single strength basis)	0.60	3.52	..	..

Table IV. Linaloöl and α-Terpineol Contents of Citrus Peel

(Per cent)

Type	Peripheral Cell Oil		Oil Distilled from Peel	
	Linaloöl	α-Terpineol	Linaloöl	α-Terpineol
Early season orange	1.29	0.15	1.60	Trace
Valencia orange				
Plant A sample	0.62	0.12	1.56	0.18
Plant C sample	0.62	0.16	1.81	0.21
Grapefruit				
Plant A sample	1.67	0.19	..	0.26
Plant C sample	1.68	0.24	..	0.23

the blending tanks, freshly canned single strength juice, and a sample of concentrate. From this same plant, a sample of canned juice that had been stored 1 year in an unrefrigerated warehouse was also taken. From Plant B were secured samples of fresh juice from the blending tanks, freshly canned single strength juice, and the aqueous phase of a de-oiler condensate. Plant A did not add aqueous de-oiler condensate back to the juice as did Plant B. The analytical data on these samples are presented in Table III. The values given are averages of duplicate determinations.

Peel Oils. Analyses were made on certain extracted and distilled peel oils. The extracted oils were obtained by bending the peels back upon themselves to eject the oil as a spray into a funnel placed in the mouth of a centrifuge tube. After centrifuging, the oil layer was removed with a small pipet. Where this method was not practicable, as with frozen peels, the surfaces of the peels were lightly scarified by pin points thrust through a cork stopper and a folded cloth to act as a sponge. The liquid taken up by the cloth was squeezed into a centrifuge tube, the layers were separated by centrifuging, and the oil layer was removed as before. These methods were used in order to obtain peripheral oil having a composition as nearly as possible to that in fresh fruit. Commercial methods of recovering cold-pressed peel oil involve contact with substantial quantities of water and one would expect loss of the more water-soluble constituents like linaloöl and α-terpineol. To obtain the corresponding distilled oils, 500 grams of cleaned, sliced peel was comminuted with water in a blender, the total volume was brought to 4 liters, and the pH was adjusted at 8 to 10 with calcium hydroxide. In each case, a 500-ml. quantity of distillate was collected, extracted with ether, and the solvent was evaporated from the extract, finishing under brief vacuum, while warming in a water bath to obtain the oil. Early season and Valencia orange as well as grapefruit peel were investigated in this way and the data are given in Table IV.

Peel Juice. This method of analysis was also applied to the original clarified peel juice from which linaloöl and α-terpineol were first isolated. The content of these substances is given elsewhere in this report.

Flavor Evaluation. A laboratory taste panel was used to detect threshold levels of linaloöl and α-terpineol when these substances were added to orange juice. The triangular presentation method was employed in this work. An attempt was also made to characterize the flavors of these compounds by submitting at different levels, both singly and together to panel members. A benzene extract of peel juice was also tasted for flavor after removing the benzene by heating in a vacuum.

Results and Discussion

The results presented in Table I, obtained by a single ether extraction, show satisfactory recovery. In order to ensure a margin of safety for complete recovery in all cases, an additional extraction is recommended in the analytical procedure.

In Table II, recovery of linaloöl and α-terpineol added to orange juice indicates that none of these substances were recovered after the third 100 ml. of distillate. In the analytical procedure it is recommended that 500 ml. be distilled in order to provide an extra margin of safety. Recovery of both linaloöl and α-terpineol from orange juice was considered satisfactory.

In Table III, results of sample analyses are given to illustrate what might be found in commercial samples. In Plant A, the de-oiler condensate was not being freed of oil and returned to the juice, a rather unusual omission in canning plants as it results in a loss in yield. In Plant B, the conventional procedure of centrifuging the condensate free of suspended oil and returning the aqueous portion to the juice was followed. In both cases, the de-oiling procedure reduced the contents of both linaloöl and α-terpineol. In Plant A, linaloöl was highest initially and lowest after de-oiling. The aged orange juice sample which had been in common storage for about 1 year had a terpineol content considerably above normal values. It is considered probable that *d*-limonene was converted to terpineol during storage, but since the initial value is not available, the extent of this change cannot be evaluated. Such a change was observed by Kirchner and Miller (*2*). The α-terpineol value of frozen orange concentrate appears to be high, but the reason is not clear.

The values for extracted oil and distilled oil shown in Table IV were determined to obtain information on the locations of sources of linaloöl and α-terpineol in citrus peel. Presumably, the values for extracted oil are indicative of the amounts present in the peripheral oil cells. In the case of the distilled oil, the entire peel was ground before distilling and the values represent the total amounts present in oil from the whole peel. In this case greater linaloöl or α-terpineol values for distilled oil would imply that these compounds came from other sources as well as from peripheral oil cells. Thus, results seem to show that most of the α-terpineol originated in the peripheral peel oil, while part of the linaloöl came from this oil and part from other portions of the peel. Thus, α-terpineol in freshly extracted orange juice would be expected to follow the peel oil content, but certain extraction procedures might affect the linaloöl value independently. The sampling was limited and is given to illustrate the application of the method as much as to obtain preliminary information. Additional experiments might yield somewhat different results. As indicated previously, the content of both compounds would be reduced by de-oiling, but since the compounds are alcohols and somewhat soluble, part of each would be returned to the juice with the aqueous portion of the de-oiler condensate in conventional practice. In concentrate production, the compounds would be removed with the water vapors and those found in the final product would be expected largely from cutback juice or added oil. The values of linaloöl in grapefruit oil distilled from the peel have been omitted because a new peak that overlapped that for linaloöl appeared on the trace. Thus, at present, the method cannot be recommended for the estimation of this compound in grapefruit oil distilled from comminuted whole peel, although oil from the peripheral cells behaves like that from orange peel.

In taste tests, the laboratory panel could detect added linaloöl and α-terpineol in orange juice at the 7.6- and 2.5-mg. per liter levels, respectively. At higher concentrations, the panel members were asked to indicate the levels at which these substances became objectionable in orange juice. Judgments varied widely, but averaged 23

mg. per liter for linaloöl and 12.6 mg. per liter for α-terpineol, their approximate concentrations in peel juice. Three out of 14 described linaloöl as conferring a bitter flavor, but only one out of 11 thought α-terpineol bitter when each was added singly. However, when 8 mg. of linaloöl and 3 mg. of α-terpineol were added together to 1 liter of juice, the taste was judged objectionable and bitter. These concentrations were about one third those of the peel juice used which contained about 23 mg. of linaloöl and 8.5 mg. of α-terpineol per liter. However, peel juice is much more bitter than can be accounted for by its content of these alcohols, but they do appear to contribute to its total taste effect.

As noted in the experimental section it is necessary to neutralize orange juice before distillation to prevent conversion of *d*-limonene to terpineol. If sodium or potassium hydroxide is used for this purpose, uncontrollable foaming results, probably because of the soluble soaps formed from the lipide matter of the juice. Calcium hydroxide, which forms insoluble soaps, avoids this difficulty.

Conclusion

Linaloöl and α-terpineol have been identified as highly flavored constituents of orange peel juice. A method has been devised for their determination in orange juices and oils. Part of the linaloöl may come from other locations in the peel than the peripheral oil cells. Taste tests were conducted to establish the taste thresholds, levels at which they became objectionable, and flavor character. These substances added individually were not particularly bitter but, when added together, they were. It was not possible, however, to account for a major part of peel juice bitterness on the basis of linaloöl and β-terpineol contents.

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ONION FLAVOR AND ODOR

Enzymatic Development of Pyruvic Acid in Onion as a Measure of Pungency

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Pyruvic acid appears enzymatically in onion tissue disintegrated by comminution. Over 95% of the maximum amount of pyruvic acid is produced within 6 minutes after the start of comminution. The total amount produced appeared to depend on the generally accepted degree of pungency of the onion lot investigated. Weak onions produced 2 to 4 μmoles, those of intermediate strength 8 to 10 μmoles, and strong onions 15 to 20 μmoles of pyruvic acid per gram of onion. The enzymatic basis of the method, as well as its relation to other methods of estimation of pungency, is discussed.

A SURVEY of recent literature on the chemical and enzymological properties of the onion strongly suggests that its pungency arises as a result of the interaction of *S*-substituted L-cysteine sulfoxide derivatives and enzymes of the alliinase type when the integrity of the onion tissue is destroyed by comminution or other means:

$$RS(O)CH_2CHNH_2COOH + H_2O \dashrightarrow [RSOH] + NH_3 + CH_3COCOOH$$

(pyruvic acid)

The presumed unstable initial products, RSOH [sulfenic acids (*22*)], can then react in several ways to form sulfur-containing odoriferous substances which impart the characteristic pungency to homogenates of onion.

The existence of such amino acids in the onion is evidenced by paper chromatographic identification (*19, 23*) and more recently by the actual isolation and positive identification of *S*-methyl-L-cysteine sulfoxide (MCSO) and *S*-propyl-L-cysteine sulfoxide (PCSO) (*25*) from onion, and of cycloalliin (*24*) (3-methyl-1,4 - thiazane - 5 - carboxylic acid - 1-oxide). The existence in onion of the appropriate enzyme and its partial purification have recently been reported (*20*). Evidence exists that a substantial part of the volatile reaction products of the hypothetical sulfenic acid intermediates are present in onion homogenates as methyl and propyl esters of methyl and propyl thiosulfinic acids (RS(O)SR). Thus, they react with thiamine to give rise to allithiamine analogs (*14*); with *N*-ethyl maleimide after appropriate extraction procedures (*4, 20*); and after heating, give rise to families of alkyl di- and trisulfides which have been identified after separation by gas chromatography (*3*). In addition, the following volatile sulfur-containing compounds have been reported to be present in onion volatiles: *n*-propanethiol (*3, 6, 16*); *n*-propylthioaldehyde (*10*); hydrogen sulfide and sulfur dioxide (*4, 16*).

The presence of relatively large