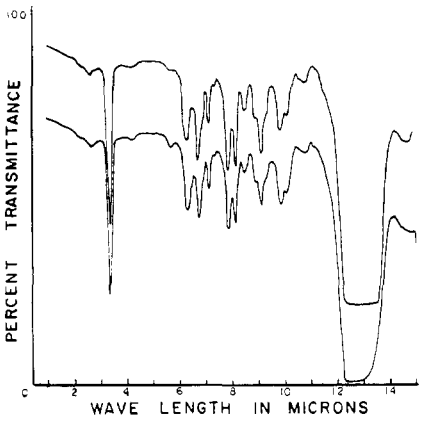

Figure 1. Infrared absorption in chloroform

Upper. Authentic sample of *d*-α-tocopherol
Lower. Antioxidant compound from orange flavedo

that any tocopherol other than the alpha form was present in the sample. This was verified by thin-layer chromatography of samples of the β-, γ-, and δ-tocopherols.

The presence of α-tocopherol in orange oil was suspected by Proctor and Kenyon (*7*), since it appeared to be especially effective as an antioxidant for this oil. Rakieten (*8*), using the bipyridine test of Emmerie and Engel (*3*), reported the α-tocopherol content of orange juice to be not more than 99 mg. per 100 ml. Our results would indicate that the α-tocopherol content of orange flavedo is less than 1 mg. per 100-gram fresh weight. However, the α-tocopherol content of the crude dewaxed oil (24.75 grams) obtained from the *n*-hexane extract of orange peel was 3.9 mg. per gram. This is a high concentration compared to that of many natural oils. While no isolation and identification of α-tocopherol from orange juice were attempted in this study, the authors were unable to detect the presence of any tocopherol-like substance in the juice using the procedure of Emmerie and Engel (*3*). The juice of orange contained little or no antioxidant activity (*9*), and it is doubtful that the α-tocopherol content of the juice would be greater than that of the flavedo.

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PEEL JUICE FLAVOR

Proximate Analyses of Florida Orange Peel Juice Extract for the 1962–63 and 1963–64 Seasons

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Juice was obtained from whole orange peel with a hydraulic press at intervals during the 1962–63 and 1963–64 seasons. In general, maturity and variety did not appear to have much influence on the peel juice bitterness except at the last of the seasons when bitterness was greater. The quantity of benzene extract was considerably higher during the latter season. There appeared to be some agreement between peel juice bitterness and the content of extract neutral fraction. From threshold flavor values, the neutral fraction was estimated to be responsible for 35 to 75% of the total bitterness.

Swift and Veldhuis (*4*) measured soluble solids, reducing sugars, sucrose, total sugars, ascorbic acid, soluble pectic substances, acidity, flavonoids, diacetyl, pH, specific gravity, viscosity, color, and fluorescence in commercial orange juice, peel juice, and segment juice throughout the 1954–55 operating season. Marked differences were noted. Composite peel juice samples for any quarter of the season could be detected by a taste panel when added to good quality segment juice at the 3% level. These experiments did not reveal whether or not bitter substances from peel were present in commercial orange juice. No objective test existed at the time by which such an admixture could be measured.

Subsequently, Swift (*3*) found that the chief volatile constituents present in orange peel juice were linaloöl and α-terpineol and developed a method for their estimation in orange juice. However, as these compounds could not account for the major bitterness of orange peel juice, it was necessary to investigate the nonvolatile substances present. Preliminary work indicated that most of the bitterness was extractable with benzene and that much of the bitterness of the extract resided in its neutral fraction.

The present investigation was undertaken to learn more about the effects of variety and maturity on bitterness and to investigate the relative importance of the neutral fraction in this respect.

Experimental

Orange peel, relatively free of pulp and cell membranes, was obtained from local processing plants over the 1962–63 and 1963–64 seasons. It was frozen at −20° C. and then ground in a Fitzpatrick Model D comminuting mill using a screen with 0.25-inch openings. The ground, frozen peel was weighed, mixed

Freezing weather occurred early in the 1962–63 season, so it was decided to continue the work for an additional year to obtain more nearly normal results. It is intended to use the information gained as a guide in future work on the identity and abundance of individual compounds related to bitterness.

Table I. Proximate Analytical Data on Juice Pressed from Orange Peel during the 1962–63 and 1963–64 Seasons

	1962–63 Season						1963–64 Season					
Collection Date	Oct. 19	Nov. 23	Dec. 13	Jan. 18	Mar. 15	May 24	Dec. 6	Jan. 17	Mar. 13	Apr. 7	Apr. 30	June 3
Yield of peel juice, %	36.5	31.0	37.1	47.6	27.6	21.3	24.5	18.8	21.4	13.5	25.5	21.3
Acid as anhydrous citric, %	3.29	2.07	2.05	1.56	2.29	2.89	1.81	1.82	2.07	2.09	1.70	2.09
Soluble solids uncorrected, %	11.2	12.8	13.2	11.2	11.9	16.4	15.2	14.3	13.0	15.0	12.5	14.0
Taste threshold												
% peel juice	5	5	7	8	5	3	6	5	4	4	5	3
Significance level, %	1.0	1.0	1.0	0.1	5.0	1.0	0.1	1.0	0.1	1.0	1.0	0.1
Benzene extract, g./l.	0.472	0.454	0.396	0.312	0.501	0.397	0.605	0.790	0.629	0.665	0.585	0.956
Fractions												
Acidic, %	46.0	32.8	28.8	24.7	18.8	32.0	28.4	38.7	18.2	25.9	30.4	51.2
Neutral, %	42.6	67.7	63.1	58.0	71.1	56.4	62.0	55.0	67.0	66.3	71.8	44.3
Lactone, %	11.4	0	8.1	17.3	10.1	11.6	9.6	6.3	14.9	7.8	0	4.5
Neutral fraction												
Taste threshold, p.p.m.	28.7	43.8	29.2	30.0	27.0	18.0	29.5	31.0	29.5	29.5	28.0	32.0
Significance level, %	1.0	1.0	1.0	0.1	5.0	0.1	1.0	1.0	0.1	0.1	5.0	0.1

with about 5% diatomaceous filter aid, and stirred in a steam-jacketed kettle until thawing was complete, about 15 pounds being handled at a time. Each batch of thawed peel was folded into a cotton press cloth pack approximately 17 inches square and stacked with other batches in a hydraulic press. A pressure of about 5 tons was maintained for 30 minutes. The expressed juice was weighed, the solids were determined by refractometer (uncorrected for acid), and the acidity was determined by titration. The peel juice volume was reduced by about 25% by vacuum distillation to remove volatile substances. The original volume was then restored with water.

A laboratory staff taste panel of about 12 people was used in the estimation of threshold levels expressed as percentages of peel juice in a typical commercial reference orange juice. Triangular presentation in colored glasses was employed and judgments were made under poor light. Krum's (*2*) table was used in determining significance levels.

One liter of peel juice was extracted with benzene as long as color was removed, emulsions being broken by centrifuging. The combined extract was washed with water and reduced to a 100-ml. volume. An aliquot was evaporated in a tared flask, finished under vacuum at 100° for 30 minutes, and reweighed after cooling to permit calculation of the amount of benzene extract.

The benzene extract, including the aliquot residue, was fractionated by the method of Sethna and Shah (*1*). This involved extraction of phenolic and other acidic substances with 0.5% sodium hydroxide as long as color was removed. The remaining benzene solution was washed and brought to 100-ml. volume, and an aliquot again evaporated and dried as above. From the residue weight of the aliquot, the total remaining weight was calculated and this result subtracted from the total benzene extract gave the amount of acidic substances.

The solvent was evaporated from the remaining benzene extract after adding back the aliquot. The residue was then treated with 40 ml. of aqueous-ethanol (1 + 1) mixture containing 5% potassium hydroxide to decompose lactone substances. After 2.5 hours, the mixture was diluted with 150 ml. of water and the neutral substances were extracted with ether and benzene as long as color was removed. After the ether extract was washed and the solvent evaporated, the washed benzene extract was added and the volume adjusted to 100 ml. The amount of neutral fraction was determined as above from an aliquot residue weight. The amount of lactone fraction was calculated by subtracting the sum of the acidic and neutral fractions from the total benzene extract.

Benzene was eliminated from the neutral fraction by evaporating, adding ethanol, and again evaporating before making to volume with ethanol for taste evaluation. From the known concentration, various levels were made up in the reference orange juice and submitted to the taste panel for threshold determination. In all cases, equivalent amounts of ethanol were added to the control samples. Results are given in Table I with other analytical data.

Results and Discussion

The yield values for peel juices do not represent all of the juice present. However, by standardizing the pressure and the duration of its application, it was thought that relative values could be obtained for the juice content of the peel. It is noteworthy that the yield for the 1962-63 season was consistently higher than for 1963-64, with the exception of the last sample which was the same. The December sample of the first season was already in the bins of the processing plant when the freeze struck. Since it was processed the following morning and the peel sample obtained then, it is not likely that the data for December were affected by the freeze. Differences in acidity and soluble solids were not striking.

In the taste evaluations, the peel juices for the latter season average somewhat more bitter, but the difference is not great. Not enough data are available to draw any firm conclusions as to the effect of freezing temperatures on bitterness or yield. In both seasons under consideration, the most bitter juices were found in the last sample taken. It would appear that relative over-all peel bitterness could be estimated by a consideration of both yield and threshold values.

The amounts of benzene extract were consistently higher during the 1963-64 season. It is possible that factors other than the freezing weather are responsible for this difference, since it shows in the comparison of the December results where maturity and variety were about the same and the freeze damage difference did not exist. In general, the season with the higher average bitterness also had the higher extract content.

Little correlation is apparent between the percentages of the lactone fractions of the benzene extracts and the bitterness of the juices from which these were derived, even if reduced to an absolute content basis by also taking the amount of benzene extract in the peel juice into account. With the phenolic fraction on the same basis, the correlation is a little better but not impressively so. Generally speaking, the average absolute contents are higher for the latter season's more bitter juices. In the case of the neutral fractions, there is better agreement between the average bitterness of the juices and the average absolute content of neutral fraction when the seasons are compared.

The taste threshold determinations of the neutral fractions were made in an attempt to determine their importance in the over-all bitterness of peel juices.

With two exceptions (November 1962 and May 1963) these threshold values varied within the narrow limits of 27.0 to 32.0 p.p.m., which may mean that usually the neutral fraction has approximately the same composition. No trend was discernible. From these thresholds and the other data, it was possible to estimate percentages of the overall peel juice bitterness that was due to the neutral fraction. These percentages, chronologically, are as follows: for 1962-63: 36.8, 35.2, 60.0, 48.3, 41.2, and 37.4; for 1963-64: 76.3, 70.2, 59.5, 59.5, 75.0, and 40.2. These estimations must be viewed with considerable caution, as they are based on the assumption that the tastes are purely additive and that there is no important masking or synergistic effect. However, the neutral fraction is important from a flavor standpoint.

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TASTE MODIFIERS

Taste-Modifying Properties of Miracle Fruit (*Synsepalum dulcificum*)

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Miracle Fruit (*Synsepalum dulcificum*) has a unique taste-modifying property of causing sour materials to taste sweet after the mouth has been exposed to the fruit's mucilaginous pulp. Mild extractive procedures were used in isolation studies of its labile, taste-modifying principles. These principles were concentrated, giving a water-insoluble, tan-colored fraction composed of colloidal materials (mucilages, proteins, lignin, and cellulose). The fruit concentrates by themselves have no detectable sweetness. When activated in the mouth by acidic substances, a sweetness of excellent quality is perceived.

THE botanical plant species of *Synsepalum dulcificum* (Schum.) Daniell, Sapotaceae, is indigenous to tropical West Africa, where it is referred to as Agbayun, miraculous berry, or Miracle Fruit. The shrub has dense foliage and many branches and attains a height of 6 to 15 feet. From December to June it yields ripe red berries, which are ellipsoidal, about 0.75 inch long, and composed of a thin layer of pulp surrounding a single large seed (*2*) (Figure 1). The West African natives often use these fruits to render their stale and acidulated maize bread (kankies) more palatable and to give sweetness to sour palm wine and beer (pitto) (*7*).

Miracle Fruit has the unique property of causing sour materials to taste sweet after the mouth has been exposed to the fruit's mucilaginous material. Sour foods, such as lemons, limes, grapefruit, rhubarb, and strawberries taste pleasantly sweet; dilute organic and mineral acids also taste sweet. Generally, any sour material eaten or drunk for several hours after exposure will taste pleasantly sweet. Salty and bitter taste responses do not appear to be influenced.

The mechanism of the physiological taste response and the chemical character of the active principle are completely unknown. It is known, however, that the active principle of the fresh ripe fruit is a very labile substance.

Experimental

Materials and Procedures. SOURCE OF MIRACLE FRUIT. The major quantities of Miracle Fruit were obtained from Nigeria, Africa. Small quantities came from Togo, Africa, and Florida. The lability of the taste-modifying principles necessitated either rapid transport of one or two days or shipments of frozen fruit.

The fresh fruit was stored in a deep freezer until deseeded by hand. From 100 grams of fruit 60 grams of pulp and 40 grams of seeds were generally obtained. Lyophilization of the frozen pulp gave 9 to 11 grams of dried deseeded Miracle Fruit. The dried pulp was stored in a deep freezer. Stability was good for at least 3 months.

ACTIVE PRINCIPLE ASSAY. The active principle of Miracle Fruit can be detected only by its action on modifying the sense of taste. The current assay procedure is to coat the mouth with the Miracle Fruit mucilaginous material and taste the presence or absence of the sweetness of a lemon slice. A 0.1-gram portion of dried pulp in general was equivalent to one fresh berry and was generally sufficient to give a definite response. The sweetening response is transitory, but repeated tasting of lemon slices will return the sweetness. The effect from one application of the pulp persists for 2 hours or more with diminishing sweetness intensity depending upon the potency of the berry. A potent berry will replace all sourness with sweetness.

The sweetness response is observable with acidic fruits other than lemons, such as limes, grapefruits, and strawberries. The active principle (whole fruit or fractions) was found to have a sweetening effect on citric acid, tartaric acid, acetic acid, hydrochloric acid, lactic acid, phosphoric acid, L-glutamic acid, D-glutamic acid, D-glutamic acid hydrochloride, L-aspartic acid, L-histidine dihydrochloride, and α-pyrrolidone carboxylic acid. No effect was found with ammonium citrate, ammonium