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.

Acknowledgment

The author thanks M. K. Veldhuis, and T. J. Kew of this laboratory, for their advice on experimental design and taste panel technique.

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Received for review September 15, 1964. Accepted December 23, 1964. References to specific products of commercial manufacture are for illustration only and do not constitute endorsement by the U. S. Department of Agriculture. The U. S. Fruit and Vegetable Products Laboratory is one of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

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

Figure 1. Miracle fruit (*Synsepalum dulcificum*)

Table I. Treatment of Miracle Fruit Fraction

(Reagents, enzymes, and solvents)

Treatment[a]	Miracle Fruit Active Principle
Water	Insoluble
Water and Tween 81	Insoluble
Lemon juice	Insoluble
Urea solution (5%)	Insoluble
Sodium bicarbonate (5%)	Insoluble
Dimethyl sulfoxide	Insoluble
Dimethyl formamide	Insoluble
Saliva (38° C., 30 minutes)	Insoluble
Hemicellulase (38° C., 30 minutes)	Insoluble
Pectinase (38° C., 30 minutes)	Insoluble
Potassium carbonate solution (5%)	Inactivation
Potassium carbonate solution (1%)	Inactivation
Hydrochloric acid (6*N*)	Inactivation
Sodium metaperiodate solution (5%)	Inactivation

[a] At 23° C. and 20 minutes unless specified differently.

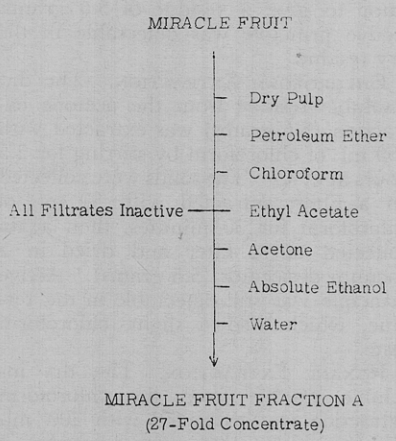

Figure 2. Phase A (nonpolar → polar solvents)

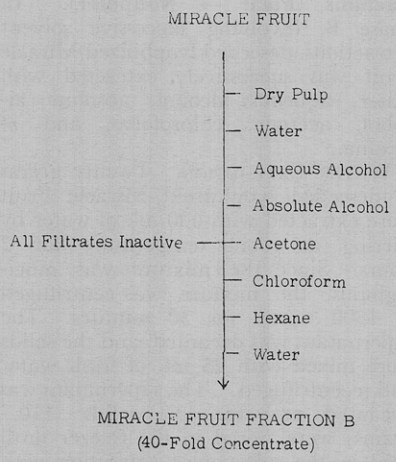

Figure 3. Phase B (polar → nonpolar solvents)

phosphate, or ammonium chloride solutions above pH 4. A slight transient effect was noted with aluminum potassium sulfate solution, pH 3.4.

Dialysis of Ground Miracle Fruit Pulp. The seeds were removed from 5 lyophilized berries. The pulp was ground in a Waring Blendor with 40 ml. of water for 1 minute. The slurry was placed in a cellophane bag (24-A. average pore radius of regenerated cellulose) and dialyzed in 600 ml. of water at 4° C. for 24 hours. The water-soluble materials in the dialyzate were obtained by lyophilization; 0.29 gram of solids was recovered. A 0.06-gram portion of this material was not active. The insoluble material remaining in the cellophane bag contained the active principle.

Water Insolubility of Miracle Fruit Active Principle. The seeds from 14 lyophilized berries were removed and the deseeded material was ground in an Elvehjem-Potter homogenizer with 10 ml. of water. The mucilaginous material was separated by centrifugation at 5° and 5000 r.p.m. for 20 minutes, washed twice with 25-ml. portions of water, and centrifuged. The insoluble slurry was found to contain the active principle. Lyophilization of the slurry gave 0.31 gram of tan-colored solid containing the active principle.

The cloudy decanted solution from the first centrifugation was placed in a cellophane bag and dialyzed at 4° in 1 liter of distilled water with agitation for 24 hours. Lyophilization of the bag contents gave 0.01 gram of light tan-colored material, which was only very slightly active. Lyophilization of the dialyzate (1 liter) gave 0.25 gram of reddish colored solid which contained no detectable activity.

Successive Solvent Extractions. The solvent extraction work is divided into two phases (A and B, Figures 2 and 3).

Phase A involves successive solvent extractions of deseeded lyophilized Miracle Fruit with petroleum ether, chloroform, ethyl acetate, acetone, absolute ethanol, and water (nonpolar solvent to polar solvents).

Petroleum Ether. Ground deseeded lyophilized *Miracle Fruit (40.0 grams)* was extracted with petroleum ether (60° to 110°) three times at room temperature. The material was stirred with 200 ml. of solvent for 2 hours, filtered, and re-extracted. The insoluble residue was air-dried, giving 36.6 grams (91%). This fraction contained the active principle. The yellow extract was evaporated to dryness in vacuo, giving 3.58 grams (9%) of a yellow semisolid which did not contain any active principle. On the addition of acetone, 1.0 gram of white-colored sterols was separated.

Chloroform. The dried residue from the petroleum ether extraction (36.6 grams) was extracted three times with 200-ml. portions of chloroform at *room temperature for 1-hour periods.* The air-dried residue (34.5 grams) contained the active principle. The chloroform extracts on evaporation of the solvent gave 0.71 gram of a greenish rubber-like solid.

Ethyl Acetate. The chloroform-extracted insolubles (34.5 grams) were

Table II. Stability of Miracle Fruit Fraction

Treatment[a]	Miracle Fruit Active Principle
Hot water	Inactivated
Potassium carbonate solution (5%)	Inactivated
Tartaric acid solution (5%)	Insoluble
Sodium chloride solution (5%)	Insoluble
Sodium chloride solution (1%)	Insoluble
Salivary digestion (25° C., 1 hour)	Insoluble
Cold storage (1 month)	Inactivated

[a] At 23° C. and 20 minutes unless specified differently.

extracted twice with ethyl acetate at room temperature with 200-ml. portions for 1-hour periods of time. The air-dried insolubles (34.1 grams) contained the active principle. Evaporation of the ethyl acetate extract gave 0.10 gram of brown material.

ACETONE. The ethyl acetate–insolubles were extracted three times at room temperature with 200-ml. portions of acetone for 0.5-hour periods. Air-dried acetone residue gave 32.5 grams of powder containing the active principle. The acetone extract gave 0.83 gram of brown material devoid of any activity.

ABSOLUTE ETHANOL. The acetone-insolubles (32.5 grams) were extracted three times with 200-ml. portions of absolute ethanol at room temperature for 1-hour periods. The ethanol-insolubles were air-dried, giving 20.0 grams (62%). The solvent was removed from the ethanol extract, giving 14.1 grams (38%) of red sirup, which did not contain the active principle but seemed to reduce the sense of taste. The alcohol-insoluble fraction was treated with reagents, enzymes, and solvents as shown in Table I.

WATER. The ethanol-insoluble fraction was mixed with 200 ml. of water for 2 hours at room temperature. The mixture was very thick, similar to a gum or mucilage. It was centrifuged at 5000 r.p.m. and 5° C. for 20 minutes. The wet solid was lyophilized to give 14.8 grams of light tan-colored powder. A 0.1-gram sample contained the activity. The analysis of this insoluble fraction A is shown in Table IV. The decanted pink solution was not clear; it was passed through cotton and lyophilized, giving 4.63 grams of pink powder, which appeared to have very slight activity at 0.1-gram levels. A 1.0-gram sample was dissolved in water and filtered on Whatman No. 1 paper, and the filtrate was tested. The water-soluble materials contained no activity.

TESTS ON WATER-INSOLUBLE FRACTION A. The stability of Miracle Fruit water-insoluble fraction is shown in Table II.

Table III. Amino Acid in Miracle Fruit Insoluble Fractions[a]

	G./16 G. Nitrogen	
	Fraction A hydrolyzate	Fraction B hydrolyzate
Alanine	5.83	6.64
Arginine	5.05	6.31
Aspartic acid	12.77	12.52
Cystine	Nil	Nil
Glutamic acid	11.89	12.82
Glycine	5.72	6.40
Histidine	3.77	4.28
Isoleucine	5.47	6.31
Leucine	8.95	9.83
Lysine	8.35	9.83
Methionine	0.07	0.08
Phenylalanine	5.11	6.39
Proline	7.46	7.22
Serine	5.75	6.34
Threonine	5.15	5.88
Tryptophan	Nil	Nil
Tyrosine	3.09	3.41
Valine	7.56	8.95

[a] Cysteic acid and methionine sulfone abundant.

Phase B of Successive Solvent Extractions (Polar → Nonpolar). In phase B, involving successive solvent extractions, deseeded lyophilized Miracle Fruit was successively extracted with water, aqueous alcohol, absolute alcohol, acetone, chloroform, and *n*-hexane.

WATER EXTRACTION. Twenty grams of deseeded lyophilized Miracle Fruit were extracted with 600 ml. of water by stirring at room temperature for 2 hours. Since the mixture was mucilaginous, the mixture was centrifuged at 1800 r.p.m. for 30 minutes. The supernatant was decanted, and the solids were mixed with 25 ml. of fresh water and recentrifuged. The supernatant was decanted and the wet solids (110.7 grams) were stored in the freezer until used in the subsequent extraction with alcohol. This residue contained the active principle. The two supernatants were combined and centrifuged at 1950 r.p.m. for 1 hour. Only a small amount of solids were obtained. The slightly cloudy supernatant (579 grams) had only a very small, if any, sweetening effect. Paper chromatography of the water extract revealed the presence of 11 ninhydrin-reactive substances, four acidic and one basic substances, and two reducing carbohydrates.

AQUEOUS ETHANOL EXTRACTION. The wet residue from the water extraction was extracted with 250 ml. of absolute ethanol by stirring for 1 hour at room temperature. The mixture was filtered and the residue dried in a vacuum desiccator to give 6.6 grams of residue, which contained the active principle. The filtrate was evaporated in a vacuum rotary evaporator to give an orange-brown sirupy residue (2.7 grams). No active principle was detectable in the sirup.

ABSOLUTE ETHANOL EXTRACTION. The 6.6 grams of insoluble residue from the aqueous alcohol extraction were extracted with 250 ml. of absolute ethanol by stirring for 3 hours at 5° to 6° C. The solids were filtered off, extracted with 50 ml. of absolute ethanol for 30 minutes, and collected on a filter. The residue was dried in a vacuum desiccator to give 5.7 grams of solid. Active principle was detected in the residue.

Table IV. Analysis of Miracle Fruit Insoluble Fractions A and B[a]

	Fraction A	Fraction B
Protein (N × 6.25), %	19.5	24.3
Fiber, %	19.1	21.7
Methoxyl content, %	2.62	2.73

[a] Dry basis.

ACETONE EXTRACTION. The dry insoluble residue (5.7 grams) from the alcohol extraction was extracted with 200 ml. of acetone by stirring for 3 hours at 5° to 6° C. The solids were collected on a filter, again extracted with 50 ml. of acetone, then collected on a filter and dried in a vacuum desiccator to give a residue of 5.6 grams. Active principle was detectable in the dry residue.

CHLOROFORM EXTRACTION. The dry insoluble residue from the acetone extraction (5.6 grams) was extracted with 200 ml. of chloroform by stirring for 2.5 hours at 6° C. The solids were collected on a filter, extracted with 50 ml. of chloroform for 30 minutes, then again collected on a filter and dried in a vacuum desiccator (5.5 grams). Active principle was still detectable in the residue, which had a slight chloroform taste.

HEXANE EXTRACTION. The dry insoluble residue from the chloroform extraction was extracted with 200 ml. of *n*-hexane by stirring for 2.5 hours at 5° to 6° C. The solids were filtered out, extracted with 50 ml. of *n*-hexane, collected on a filter, and dried in a vacuum desiccator. Weight of final residue, after a small amount was taken for taste test, was 5.0 grams. The active principle was detectable in the residue. An analysis of the insoluble fraction B is shown in Table IV. In another fractionation study, lyophilized Miracle Fruit pulp was extracted successively with water, acetone, petroleum ether, and water.

Hydrolysis of Extracted Insoluble Fractions A and B. One hundred milligram quantities of insoluble fractions A and B were placed in separate pressure bottles with 75 ml. of 6*N* HCl in each and placed in an oil bath at 120° C. for 32 hours. The humin from each sample was collected on a No. 42 Whatman paper and washed with water to a neutral pH. Humin was 35 mg. from preparation B and 15 mg. from preparation A. The filtrates from A and B were reduced to near dryness in vacuo. The volume of B was adjusted to 6 ml. and A to 6.6 ml.

Amino acid analyses by the Moore-Stein Procedure are given in Table III.

Results and Discussion

Mild extractive procedures were used in isolation studies of the Miracle Fruit labile unique taste-modifying principles of the dried pulp. The solvent extraction work was divided into two phases, A and B. From each of these successive solvent approaches an insoluble fraction was obtained that contained the active principle. None of the active principle could be detected in any of the extracts obtained using either phase.

The taste-modifying principles were concentrated 27-fold by phase A, and 40-fold by polar-nonpolar extraction system, phase B. However, these fractions were not nearly as effective on a weight basis as the active principle in the fresh fruit.

The analysis of the insoluble fraction obtained by the two successive extraction procedures is shown in Table IV.

According to the analysis and the solubility characteristics shown in Table I, the Miracle Fruit concentrates are composed of insoluble colloidal materials such as mucilages, proteins, lignins, and cellulose. Eighty per cent alcohol is used to separate the colloidal and noncolloidal carbohydrate and nitrogen compounds in the analysis of plant materials (*3*). The alcohol-insoluble solids, comparable with our Miracle Fruit solids, include the cell wall or structural components, such as cellulose, lignin, proteins, pectic substances, and hemicelluloses.

Examination of the experimental data obtained indicates that the active principle could be a glycoprotein. This possibility is based on the solubility behavior and lability toward heat, acid, and base. Furthermore, the labilities of the products obtained from the two successive solvent approaches suggest a glycoprotein. Phase A, the nonpolar → polar successive solvent fractionation procedure, appears to give a more stable product than the phase B polar → nonpolar successive solvent approach. This denaturation effect is characteristic of some glycoproteins.

Acid hydrolysis of the insoluble colloidal fractions gave amino acid as well as sugar degradation products. The amino acid values of these fractions were obtained by the Moore-Stein procedure. The amino acid content of these hydrolyzates is shown in Table III; the levels of the basic amino acids, arginine, histidine, and lysine, are reasonably high. The sulfur-containing amino acids were oxidized during the isolation procedures to cysteic acid and methionine sulfone. This could explain some of the decrease in activity of the Miracle Fruit active principle during isolation procedures. However, other explanations could also be conceived.

The Miracle Fruit concentrates do not have, by themselves, any detectable sweetness. When they are activated in the mouth by acidic substances, a noticeable sucrose-type sweetness of excellent quality is perceived. The perception appears to be too rapid for the sweetness to be accounted for by acid or enzyme hydrolysis of polymeric carbohydrate substances. This is an intriguing new concept of sweetness and suggests new research approaches for studying mechanisms of taste perception.

Acknowledgment

Appreciation is expressed for the service contributed by William Whitman, Dr. and Mrs. Otto Churney, Julia Morton, Robert Snow, Dennis Dettloff, Mary Chekewicz, and Philip H. Derse, Wisconsin Alumni Research Foundation.

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Received for review August 25, 1964. Accepted December 28, 1964.