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ALTERATION OF TASTE QUALITIES THROUGH NATURAL PRODUCTS

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Feeding our military forces costs some 2 billion dollars a year; the economic importance of research devoted to maintaining the sensory qualities, the highest nutritional standards, and wholesomeness of this immense quantity of food should be clear to all. Research on the psychological, physiological, and chemical aspects of food is justified for many other reasons. One fundamental reason stands out; unpalatable food is rejected and any wholesale rejection by our military forces is costly.

The investigation to be reported concerns the sensory basis of palatability; specifically, the effects on the taste of foods of some rather remarkable substances derived from two plants, _Gymnema sylvestre_ and _Synsepalum dulcificum_ (more commonly called miracle fruit). These substances are of interest not only because of their potential use in enhancing flavor but also because they can help to elucidate the complex relationship between chemical structure and taste quality.

The purpose of this paper is to present historical, chemical, and sensory information on these two plants and to suggest applications.

History

Gymnema sylvestre

Gymnema sylvestre is a woody, climbing plant which runs over the tops of high trees in a large part of southern India as well as in Ceylon and tropical Africa. Many medicinal properties have been attributed to the leaves of the plant. Some of these ascribed powers are apparently without foundation (for example, ingestion of a decoction of the leaves for snake bite); however, others appear to have originated with observation of the effects of the material on taste.

The first published account of the taste properties originated over a century ago with Edgeworth (1). He reported after chewing the leaves that he could not taste the sweetness of the sugar in his tea. The effect lasted, according to his report, for 24 hours. Hooper (20) confirmed the suppression of the sweetness of sugar and also observed a suppression of the bitterness of quinine. Shore (33) and Kiesow (24) confirmed both of these suppression effects and also reported a slight suppression of the saltiness of NaCl. Warren and Pfaffmann (37) and Kurihara (27) demonstrated, in addition, that the suppressing effects extended to the sweetness of saccharine, and the sweet taste of the salts, beryllium chloride and lead acetate.

Miracle fruit

Synsepalum dulcificum berries, known as miracle fruit, thanks to the European travelers who sampled it in its native tropical west Africa, grow on a densely foliated bush which can reach a height of 8 feet (fig. 1). The berries are oval shaped and turn a deep red when ripe. The length of the berry reaches about 3/4 inch but the flesh is rather thin, most of the size of the berry resulting from one large seed (fig. 2).

Miracle fruit was probably first described in the notes of a Frenchman in 1725. Apparently, most travelers who encountered it were amazed by the taste of fruits eaten after eating miracle fruit. Although the flesh of the miracle fruit itself is only mildly sweet, normally sour foods eaten after the miracle fruit, taste extremely sweet. Even lemons can be eaten like oranges. The natives in Africa use miracle fruit to sweeten acidulated maize bread (kankies), palm wine, and pito, a sour beer made from fermented grain. The sweetening of palm wine by miracle fruit described by Daniell in 1852 is particularly interesting. It was necessitated by the scarcity of palm trees in some areas (13). The wine was made near the trees and then transported to areas which lacked them. During the transportation the wine began to sour and the miracle fruit was used to make the wine palatable again.

An American explorer working for the department of Agriculture deserves great credit for interesting Americans in this plant. David Fairchild and several companions sampled miracle fruit on a trip to the Cameroon in the late 1920's. He did not realize at first that the "miraculous" powers resided in the effects on other tastes rather than on the taste of the berries themselves. He dismissed them as "not good enough to become excited over though not at all bad." However, sometime later he was offered beer by a chemist he was visiting and he found that it was sweet. Realizing the interest that such an effect would arouse he gathered seeds for introduction into the United States (18).

Miracle fruit is not easy to grow, however, and the initial attempts in the U.S. failed. Fortunately in about 1957 Dr. and Mrs. Otto Churney and Mr. R. G. Newcomb of Florida obtained two seeds from the Summit Garden in Panama. The plants successfully grown from these seeds are the parent plants for most of the miracle fruit now grown in the U.S.

The earliest description of the effects of miracle fruit suggested that it acts on sour and bitter tastes. Dalziel (12) and Irvine (22) reported that it mitigates the bitterness of quinine but that the effects on acidity are greater. In the opinion of Fairchild (18) "the effect is to paralyze some of the papillae of the tongue so that many things, even acid ones, taste sweet for some time."

Miracle fruit has aroused considerable interest in the popular press, for example, stories in Popular Mechanics, Science News Letter and Food Engineering (2,3,4). Inglett et al.(21) of the research and development division of International Minerals and Chemical Corp. increased interest in miracle fruit by publishing the first effort to identify the active principle. Commercial interest in miracle fruit continues through the Unilever Co. in the Netherlands and through Meditron, Inc. in the U.S. Meditron in particular has extensively investigated growing conditions for miracle fruit plants and has been able to accelerate growth and to increase berry yields.

The great interest in miracle fruit encouraged the U.S. Army Natick Laboratories to undertake a careful examination of the exact effects of the material on taste, but obtaining samples of the berries originally proved very difficult. The first sample of berries tested was generously provided by Dr. and Mrs. Churney. Later, 55 plants were transported from Mr. Newcomb's nursery to greenhouses in Massachusetts. Although a few berries were obtained from the plants, they did not grow well and eventually all but 16 died. Dr. O.B. Dokosi of Ghana attempted to ship berries to the Natick Laboratories but the difficult plane schedules caused all of these shipments to spoil before reaching Massachusetts. Meditron's success in growing miracle fruit finally provided a stable source of material which is now utilized.

Chemical Composition of Active Principles

Work on the chemistry of Gymnema sylvestre leaf samples began with Hooper (20) who in 1887 reported that the active component, which he called gymnemic acid, is a glycoside. In 1959 Warren and Pfaffmann (37) produced a microcrystalline gymnemic acid which also appeared to be a glycoside. Yackzan (38) suggested in addition that gymnemic acid could be a saponin. Stöcklin (36) separated gymnemic acid into four components, A_1, A_2, A_3, and A_4. He concluded that they were probably B-D-glucuronides of different acylated gymnemagenins where gymnemagenin is a hexahydroxy pentacyclic triterpene. The major active constituent found in most leaf samples, A_1, has been shown by Dateo (14) to consist of at least two components.

The first work on the chemistry of miracle fruit by Inglett et al.(21) suggested that the active principle could be a glycoprotein. They were unable, however, to extract it from the fruit. Kurihara and Beidler (26) and Brouwer et al.(11) confirmed the identification and successfully extracted the protein. Kurihara and Beidler estimated the molecular weight of their "taste-modifying protein" at 44,000 and reported that it was 6.7% arabinose and xylose. Brouwer et al. estimated the weight of their "miraculin" at 48,000 and suggested that glucose, ribose, arabinose, galactose, and rhamnose were present.

Experimental Work: Sensory Analyses

Gymnema sylvestre

The first experiment was designed to measure the effects of Gymnema sylvestre on the four "basic" taste qualities. The Gymnema sylvestre leaves were purified by a procedure developed by Dateo (14). The major component of the purified material was the fraction A_1. The study was carried out using the psychophysical method of direct magnitude estimation (35). According to this method, a subject is given a standard solution and asked to assign an arbitrary number to its intensity, for example, "100." Then he is given other solutions and asked to rate their intensities relative to the standard. In the present experiment subjects were asked to describe the quality as well as estimate the intensity of taste solutions. The taste solutions used were sucrose for sweet, sodium chloride (NaCl) for salty, hydrochloric acid (HCl) and citric acid for sour, and quinine hydrochloride (QHCl) and quinine sulfate for bitter.

In order to increase the precision of the results, all solutions were kept at body temperature and were delivered to the tongue through a gravity flow system. Each subject sat in front of the flow system with his tongue extended slightly and his lips resting on his tongue. This prevented taste solutions from entering his mouth and also kept his tongue free of saliva. In addition, his tongue was rinsed with distilled water for 40 sec. before each taste solution was presented.

The Gymnema sylvestre leaves were obtained from the Himalaya Drug Co. in Bombay India. The active components of the leaves were purified by a procedure developed by Dr. George Dateo of the Organic Chemistry Labs at Natick. His procedure produced the active material in a water soluble salt form essentially free of carbohydrate, fats, inorganic salts, proteinaceous material and a large portion of the inactive water soluble constituents and avoided thermal degradation. The major fraction of the resulting material was determined by chromatographic comparison of the Natick sample with ons provided by Dr. T. Reichstein and Dr. W. Stocklin. This fraction is designated A_1 after Stöcklin (36).

Each of the taste solutions used was tested in a separate session, with the first half of the session used to obtain responses without applying the Gymnema fraction. In the second half the subject held 8 ml. of the Gymnema fraction in his mouth for 30 sec. before each taste stimulus was presented.

The subjects were volunteers from the Behavioral Sciences Division at Natick.

The results are shown in fig. 3. The sweetness of sucrose was substantially suppressed by the Gymnema fraction but none of the other taste substances were significantly influenced. This was particularly astonishing since the bitter suppression reported by Hooper, Shore and Kiesow was completely absent. The failure of the fraction to suppress bitterness in this experiment is of especial importance because no other materials are known which suppress only one taste quality without affecting the others. The existence of such a suppressor plays a major role in taste theories and is a powerful tool in the analysis of complex tastes. It offers as well many possible applications in the area of food intake. The importance of this unexpected experimental result demanded that the discrepancy with the earlier results be explained. With hindsight this is not difficult. The early work with Gymnema sylvestre was carried out by applying crude decoctions of the dried leaves to the tongue or by chewing the leaves directly. The leaves have a very intense bitter taste in themselves. The early investigators apparently did not realize that the taste of the leaves would interfere with their tests and they did not carefully rinse the tongue before testing. The exposure to the bitter taste of the leaves adapted the taste receptors to bitter. When the bitter quinine was tested it quite naturally produced very little bitterness from the adapted receptors. In the present experiment the bitterness of the leaves was decreased by the purification and any remaining bitterness was removed with the distilled water rinse so the bitterness of quinine was not suppressed. The suppression of sweetness remains after the rinse showing that it is not a consequence of any cross-adaptation. The failure of the investigators of the late 19th century to interpret properly the apparent bitter suppression of Gymnema sylvestre is readily understandable. During the years when Gymnema sylvestre first came to the attention of taste investigators (1887-1894) the effects of adaptation were only beginning to receive mention in the taste literature. A convincing demonstration of adaptation was not available until Kiesow's work on single taste papillae was published in 1898 (23). This only established the fact that continued stimulation of a single papilla resulted in loss of sensation to that stimulus. That many taste substances of similar quality could cross-adapt with one another was not established until the early 20th century (5). The next experiment was designed to test the effects on several unusual sources of sweetness chosen to be chemically different from sugar. These included Ca cyclamate as well as several naturally occurring sweeteners that have not all, as yet, been completely characterized chemically. All of the plants investigated have been used as sources of sweeteners in the areas where they grow. The leaves of Stevia Rebaudiana are used by natives in Paraguay and the berries of Dioscoreophyllum Cumminsii and Synsepalum dulcificum as well as the sweet mucilage surrounding the seeds of Thaumatococcus danielli are used by natives in tropical west Africa. Gymnema sylvestre suppressed all of these sweeteners. Sweetness can also be produced under special conditions by substances that normally have other tastes or no taste at all. Salts taste sweet when they are relatively weak and distilled water tastes sweet if the tongue has previously been adapted to bitter or sour substances (7,10,17). These sweet tastes are also suppressed by Gymnema sylvestre.

Miracle Fruit

Two theories have been formulated to explain the taste effects of miracle fruit. Dzendolet (16) suggested that the anions of some acids, for example, the citrate ion of citric acid, are sweet but are normally inhibited by the sour taste. Miracle fruit, by blocking the sour receptor sites, would allow the sweet taste of the anion to be perceived. Kurihara and Beidler (25) suggest, on the other hand, that the glycoprotein, miraculin, binds to the receptor membrane near the sweet receptor site. Acids then change the conformation of the sweet receptor site so that it will "fit" the sugar groups attached to the glycoprotein, producing a sweet taste. These two theories were tested with a series of psychophysical investigations.

The first experiment was designed to examine the effects of miracle fruit on the four "basic" taste qualities. The procedure was very similar to that used with Gymnema sylvestre. Subjects first judged stimuli under "normal" conditions, i.e., a distilled water rinse preceding the stimulus but no miracle fruit. Then the subject's tongue was exposed to either a miracle fruit berry or a quantity of freeze-dried miracle fruit and the tests run as in the first part of the experiment. The stimuli tested were sucrose, QHCl, NaCl, HCl and citric acid. The judgments of sucrose, QHCl, and NaCl were not significantly affected by miracle fruit but the effects on HCl and citric acid were dramatic. In fig. 4 the filled circles show the functions obtained before miracle fruit and the x's show those obtained after miracle fruit. The total intensity of the taste of HCl and citric acid is not significantly changed after miracle fruit but the quality of the tastes change dramatically. The hatched area shows the part of the total taste that is sweet. The smaller unhatched area shows the part of the total taste that remains sour. The sourness of a lemon would be located near the highest concentration of cirtic acid in fig. 4. Since the citric acid in the lemon would taste much more sweet than sour as shown by the magnitude estimates, it is not surprising that observers find the effect quite startling.

Dzendolet's theory accounts for the suppression of sourness observed after miracle fruit but it cannot explain the HCl data in fig. 4. The chloride ion should not taste sweet according to his theory (15,16) but HCl is clearly sweetened by miracle fruit.

The next experiment was designed to test the effects of removing the miracle fruit induced sweetness with Gymnema sylvestre. The procedure was similar to the previous experiment. Subjects judged stimuli under normal conditions, after miracle fruit, and then again after Gymnema sylvestre. The results are shown in fig. 5. When the sweetness was removed with Gymnema sylvestre the sourness returned to the normal value. Since Gymnema sylvestre has no direct effect on the sourness of citric acid, the return of sourness to the normal level would appear to be due to the removal of sweetness.

The next experiment was designed to investigate the mechanism of the sourness suppression (9). If miracle fruit suppresses sourness only because it adds sweetness then adding sweetness another way should suppress sourness too. Subjects were given mixtures of citric acid and either xylose or arabinose (two sugars found in miraculin). The amount of citric acid in the mixture was always constant but the amount of sugar varied. After judging the sweetness and sourness of the mixtures, the subjects were given miracle fruit and asked to judge another solution. This final solution was the acid alone. The filled circles show that as the concentration of either xylose or arabinose is increased the sweetness naturally increases; however, the sourness decreases even though the amount of acid is always constant. The x's show the judgments after miracle fruit of the acid alone. The sourness of the acid was suppressed the same amount after miracle fruit that it was by sugar of equivalent sweetness.

The data in figs. 5 and 6 are consistent with the theory of Kurihara and Beidler but this evidence is also consistent with other theories. For example, the miracle fruit protein itself rather than the taste receptor site might be altered by acids to produce a sweet taste. This is a plausible position but Kurihara and Beidler do not favor it. They scaled the sourness of several acids and the sweetness of those same acids after miracle fruit. The resulting curves were similar so they concluded that "...the mechanism of sweetness induction by acid is closely associated to the mechanism of sourness." However, their scaling procedure required the subjects to make relatively difficult judgments. The data in fig. 4 were collected with a procedure slightly easier for the subjects and contradict Kurihara and Beidler. Miracle fruit appears to have a more pronounced sweetening effect on citric acid than it does on HCl.

The last experiment was designed to test directly the statement of Kurihara and Beidler that equally sour acids are equally sweet after miracle fruit. Subjects were asked to choose one solution from a series of concentrations of an acid that was equal in sourness to .01 M HCl. This was done for seven different acids. Later the subjects were given miracle fruit and asked to choose the sweeter of several pairs of stimuli and state how many times as sweet it seemed. For each subject the pairs contained .01 M HCl and the concentration that he chose to be equally sour. The results clearly show that equally sour acids are not equally sweet. However, Dzendolet's argument could provide an explanation of the differential sweetening that would still be consistent with sourness of an acid directly predicting its sweetness after miracle fruit. Perhaps the acids that sweeten more than HCl are producing sweetness from an additional source. Perhaps the anion provides some of this sweetness. If this were true then removing the sourness of such acids should make them taste sweet. The sourness was removed by adapting the tongue with HCl and then testing with the other acids. Since adapting to HCl makes distilled water alone taste sweet (7) the other acids must taste sweeter than water in order to prove that the anion is producing the sweet taste. The acids were not sweetened more than water. Sourness alone can not be the property of acids that cause sweetness after miracle fruit. The results of these experiments suggest that neither theory available adequately explains the effects of miracle fruit.

For purposes of species comparison an experiment was designed to test the effects of miracle fruit on the chorda tympani taste nerve responses of the hamster. Robert Harvey collected the data as part of his dissertation research in the Natick Laboratories under the direction of the author. The results suggested that miracle fruit does not have sweetening effects in hamsters. This is particularly interesting because the hamster taste system is very similar to man's in many respects. In particular, Gymnema sylvestre suppresses responses to sugars in the hamster much like it does in man (6) even though it fails to do so in some other species (30,34). The discovery of a species other than man in which miracle fruit is an effective sweetener would be extremely interesting for taste physiology.

Applications

Both Gymnema sylvestre and miracle fruit are of definite usefulness in taste research. First they are useful because they are powerful tools with which to study taste physiology. For example, the failure of Gymnema sylvestre to suppress the taste of sugar in all mammals suggests that the sweet receptor sites are not common across all species contradicting theories of sweet reception based on only one mechanism. In addition, Gymnema sylvestre suppresses diverse sources of sweetness in man suggesting that even if more than one kind of sweet receptor is present in man all these receptors are similar enough to be inactivated temporarily by the same material. An additional research benefit results from the simplification of complex tastes by the removal of sweet through Gymnema sylvestre. For example, a food product like canned fruit provides complex flavor sensations. By removing sweet the other tastes can more easily be evaluated.

Both Gymnema sylvestre and miracle fruit also have many direct applications in the area of palatability enhancement. There are occasions when sugar has desirable properties in the processing of a food but then leaves the final product too sweet. Gymnema sylvestre can be diluted to suppress sweetness by any desired amount. In addition, it can curb the intake of sweets. A snack containing the proper amount of Gymnema sylvestre would make sweets very unpalatable.

The most obvious application of miracle fruit is as a non-caloric sweetener. Since miracle fruit works on the tongue and not the food it avoids the dangers of additives like cyclamates. The recent reports on potential harm from ingesting cyclamates have focused public attention on food additives as a very dangerous source of environmental pollution. Loss of the use of cyclamates poses great problems for the diets of diabetics and weight watchers and also eliminates other benefits that are of special interest where food transportation costs are important as with military uses of food. Artificial sweeteners are usually very light and eliminate the necessity of transporting and storing large amounts of sugar. A new, safe non-caloric sweetener with excellent taste properties obviously has a very large appeal. Miracle fruit appears to be entirely safe even when eaten in quantities appropriate for a fruit (there are no reports of side effects from its consumption as a fruit in Africa and preliminary tests on mice and hamsters were unable to show any toxicity at all). It sweetens without a bitter aftertaste and makes normally sour fruits like lemons, rhubarb, and grapefruit, very palatable. Fruit based products like jams, pies, and ice creams are also sweetened very well with miracle fruit. Foods not normally sour are not affected by miracle fruit and so, meats, soups, and similar foods are not sweetened. Observers report that the flavor of vegetables is improved with miracle fruit even though vegetables are not normally sour, but this may simply be the result of the addition of a very weak sweet.

The final potential application concerns the flavor problems encountered with some kinds of processed foods and with unconventional foods. The success of processing techniques like dehydration depends to a great extent on the ability to retain flavor when the food is reconstituted. The success of new food sources depends on the ability to give these new foods palatable flavors. Some of the sources for new foods under development - fish flour, algae, vegetable analogues of meat products, food produced by microorganisms like yeast, and protein extractions from green leaves (19,28, 29,31,32) offer enormous nutritional and economic advantages; however, they also pose serious palatability problems. Taste altering substances from plants like Gymnema sylvestre and miracle fruit suggest a new approach to the problems of increasing the acceptance of these nutritionally and economically desirable foods.

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Fig. 1 Miracle fruit tree approximately 8 years old.

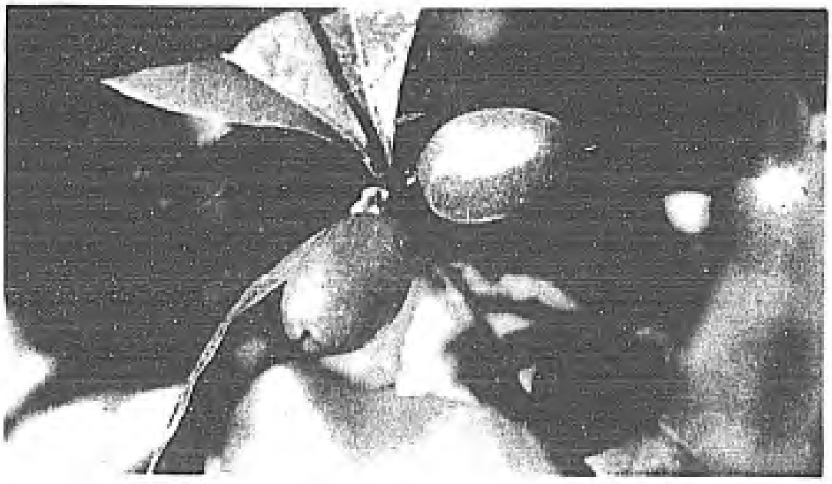

Fig. 2 Miracle fruit. Berries are approximately 3/4 inch long.

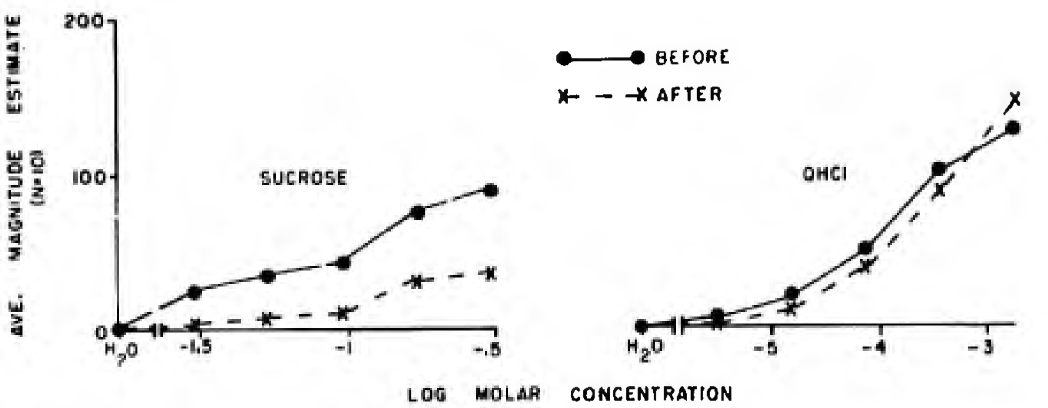

FIG. 3 MAGNITUDE ESTIMATES BEFORE AND AFTER GYMNEMA SYLVESTRE

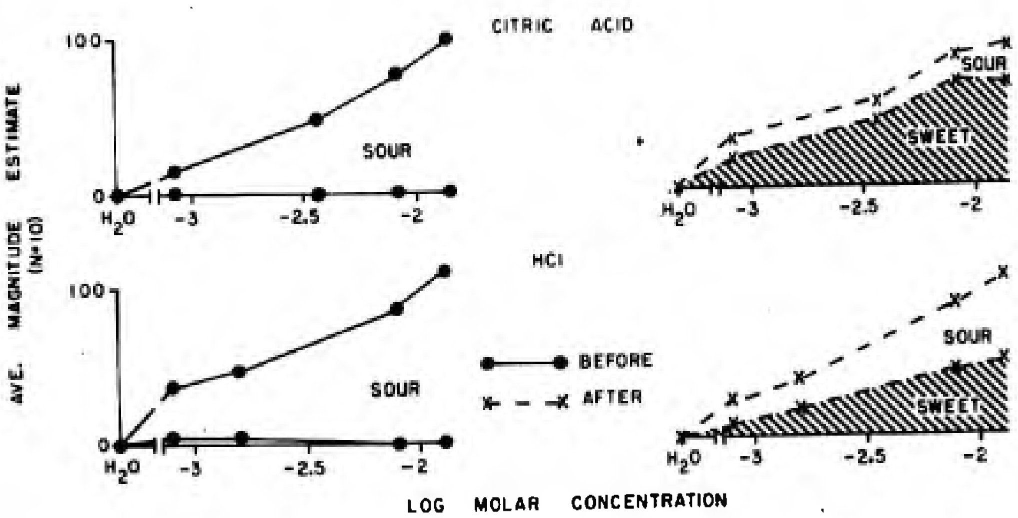

FIG. 4 MAGNITUDE ESTIMATES BEFORE AND AFTER MIRACLE FRUIT

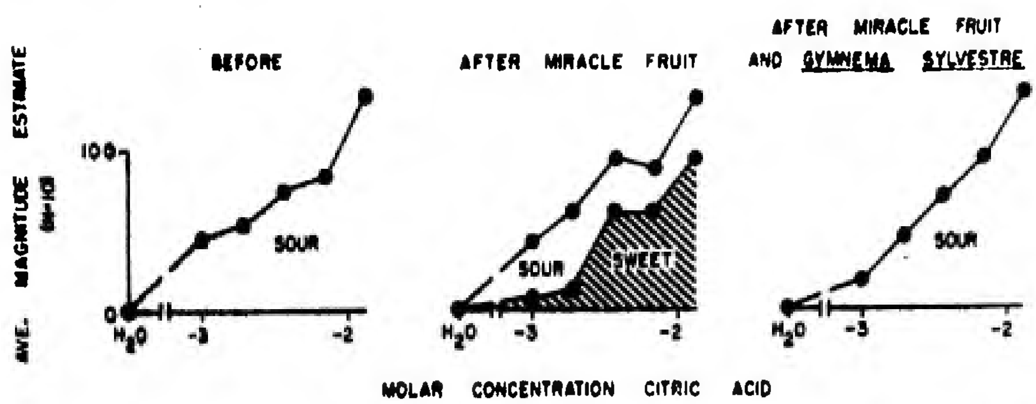

FIG. 5 MAGNITUDE ESTIMATES OF CITRIC ACID BEFORE, AFTER APPLYING MIRACLE FRUIT AND AFTER REMOVING THE SWEETNESS PRODUCED BY THE MIRACLE FRUIT WITH GYMNEMA SYLVESTRE

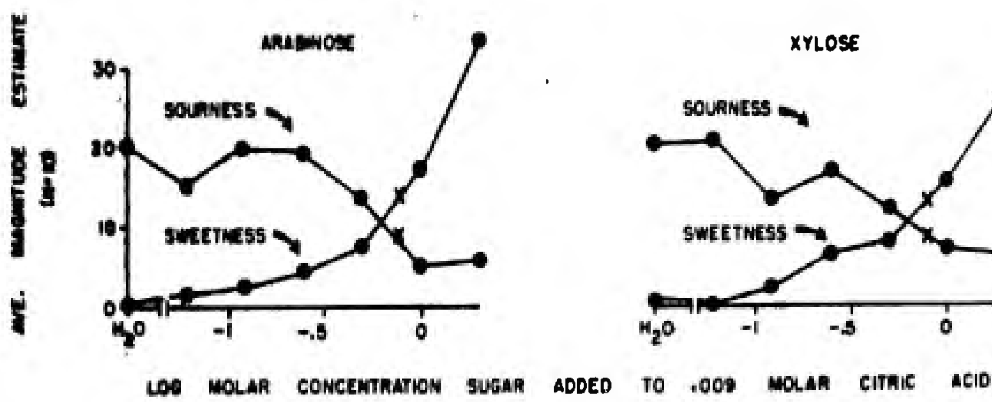

FIG. 6 MAGNITUDE ESTIMATES OF THE SWEETNESS AND SOURNESS OF CITRIC ACID SWEETENED WITH SUGAR COMPARED TO CITRIC ACID SWEETENED WITH MIRACLE FRUIT (X'S SHOW MIRACLE FRUIT VALUES)

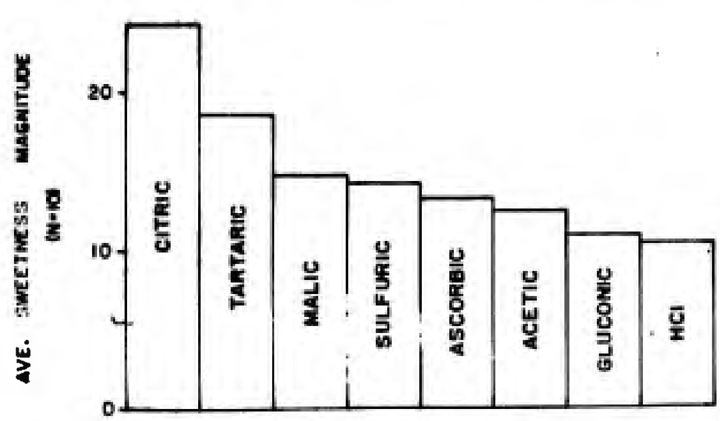

FIG. 7 SWEETNESS OF EQUALLY SOUR ACIDS AFTER MIRACLE FRUIT