FEATURES

Natural and Synthetic Sweeteners[1]

G. E. Inglett

Cyclamate and saccharin were the only two synthetic sweeteners allowed in U.S. food products until recently. Effective in 1970, cyclamate was banned for general food applications. Saccharin at the present time is still considered safe. The pharmacological evaluations of these synthetic sweeteners has increased the search for a superior sweetener.

The desire for a superior synthetic sweetener has also been encouraged by the recent dietary trend based on a weight - and diet - conscious public and on the appreciable economic advantages of synthetic sweeteners for some applications. The rapid growth of the synthetic sweetener market in the areas of low-calorie soft drinks, presweetened dried drink bases, and dietary canned goods are examples of current dietary foods. Some persons feel that market growth is limited because of saccharin aftertaste. Saccharin is known to have an inherent bitter taste (16), and cyclamate is recorded as possessing some undesirable aftertaste qualities (1).

A large potential market for a pharmacologically acceptable sweetener with superior taste qualities could expand the present sweetener market. The important sweetener candidates were recently discussed by Inglett (5).

Sweet Taste

The mechanism of sweet substances on the taste receptor is not well understood. The sensory response to sweet substances indicates that specific dimensions of a molecule are necessary to activate the taste receptor. Molecular modifications of sweeteners like saccharin, cyclamate, or dihydrochalcones can be made to give new compounds; unfortunately many of these have tasteless or bitter properties. Sodium saccharin, for example, when converted to *N*-methylsaccharin, loses its sweetness. Simple changes in geometric isomerism also can produce drastically different taste responses. For example, interchange of the hydroxyl and methoxyl groups on the B ring of neohesperidin dihydrochalcone gives a tasteless substance.

G. E. Inglett earned his PhD in biochemistry at the State University of Iowa in 1952. He has worked with biological toxins at the University of Illinois, 1952-1954, and in cancer research with the U. S. Public Health Service, 1954-1956. He has been a food chemist with the Corn Products Company, 1956-1960, Griffith Laboratories, 1960-1963, and International Minerals and Chemical Corporation, 1963-1966. Although Dr. Inglett is presently associated with the Cereal Properties Laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture at Peoria, Illinois, this paper is based on experience prior to joining the USDA.

Stereoisomerism can be responsible for differences in taste responses. Many of the D-isomers of amino acids are sweet, whereas the L-isomers generally are tasteless or bitter. Another illustration of minor molecular structure variation producing profound taste differences is the sweet taste obtained from α-D-mannose compared to the bitter taste of its anomer,β-D-mannose (15).

An interesting approach to the mechanism of sweet taste response is the study on isolated protein of bovine taste buds that complexes sugars and saccharin. The binding constants for the sugars determined by refractive index increments follow the order of sweetness of the sugars as measured *in vivo*. Saccharin also gives a high binding constant. The pH dependence of the binding is similar to the *in vivo* sweetness taste response. The sweet-sensitive protein is essentially homogeneous with a molecular weight of 150,000. Its amino acid composition indicates the protein to be rather basic. In a related investigation, epithelial tissue from the back of porcine tongues was isolated (3) as the source of a bitter-sensitive protein. The bitter-sensitive protein gave no response with any sugars tested but gave weak physical absorption of bitter compounds.

An unusual source of sweet taste has been observed from a tropical fruit known as the miracle berry, also known as the miraculous berry of Western Africa. The berries and their taste-modifying effect were first reported in 1852 by Daniell. This berry possesses a taste-modifying substance that causes sour foods to taste sweet. After the mouth has been exposed to the mucilaginous material of the fruit, sour foods such as lemons, limes, grapefruit, rhubarb, and strawberries will taste delightfully sweet. Dilute organic and mineral acids will also taste sweet. Miracle fruit has been known over the years by the botanical names *Bumelia dulcifica*, *Sideroxylon dulcificum*, *Bakeriella dulcifica*, and *Synsepalum dulcificum*. However, it was recently indicated to belong to the genus Richardella and is now called *Richardella dulcifica* (Schum. and Thorn,) Baehni. The shrub is indigenous to tropical West Africa. It has dense foliage and many branches and attains a height of 6-15 ft. The red berries are ellipsoidal, about 0.75 in. long, and are composed of a thin layer of pulp surrounding a single large seed. The West Coast Africans often use these fruits to render their stale and acidulated maize bread, called *Kankies*, more palatable and to give sweetness to sour palm wine and beer.

The type of sweetness induced by the miracle fruit seems highly desirable. Its sweetness lasts in the mouth as long as acidic material is present. More sweetness can be induced by supplying more acid. The process could be repeated continuously for 1-2 hours depending upon the potency of the berry's taste-modifying principle. Isolation studies of the active principle by Inglett et al., (6) established its water insolubility and extreme lability. It was suggested that the active principle could be a glycoprotein based on solubility behavior and lability toward heat, acid, and base. Recently, Brouwer et al., (2) and Kurihara and Beidler (11), working independently succeeded in isolation of the active principle. Solubilization of the active principle, called miraculin was accomplished by using extraction aids such as salmine and a naturally occurring polyamine spermine

---

[1] Received for publication Feb. 5, 1970.

(*N*,*N*'-bis^3-aminopropy^1-1,4-diaminobutane). Miraculin was purified by ammonium sulfate fractionation and gel filtration on Sephadex-50 and Sephadex G-25. The active principle was called taste-modifying protein by Kurihara and Beidler, who solubilized the active principle by extraction with pH 10 buffer for 1 minute. Purification was accomplished by Sephadex chromatography.

The active principle is a glycoprotein as evidenced by the loss of activity on treatment with proteolytic enzymes, the positive test for sugars before and after electrophoresis on polyacrylamide gel, and by the yields of amino acids and sugars by hydrolysis with acids. Miraculin has a molecular weight of 42,000 ± 3,000. It has such a profound action that, as more information is obtained regarding its mechanism of action, it could give useful data regarding the mechanism of taste perception. Beidler has suggested a possible mode of action, but more experimental data are needed to determine more accurately the glycoprotein function in taste physiology.

Table 1. Sweetness of various substances compared to sucrose

Substance	Relative Sweetness, Weight Basis
Sucrose	1
2-Methylcyclohexylsulfamate	15
p-Anisylurea	18
cyclohexylsulfamate (sodium cyclamate)	15-31
Chloroform	40
Methoxy-2-amino-4-nitrobenzene	220
L-Aspartyl-*L* phenylalanine methyl ester	250
p-Phenetylurea (Dulcin)	70-350
6-Chlorosaccharin	100-350
2,3-Dihydro-3-oxobenzisosulfonazole (saccharin)	240-350
Ethoxy-2-amino-4-nitrobenezene	950
Perillaldehyde *anti*oxime (Perillartine)	2,000
1-n-Propoxy-2-amino-4-nitrobenezene (P-4,000)	4,100

Sweet Synthetic Substances

Sweet substances and generalizations concerning the effect of specific groups on sweetness, were collected and discussed by Moncrieff (13). A wide variety of chemical entities were found among the large number of sweet-tasting substances reported. The relative sweetness of some recorded synthetic organic sweeteners relative to sucrose ranges from 15 for 2-methylcyclohexysulfamate to 4,000 for *n*-propoxy-2-amino-4-nitrobenzene (Table 1). One of the better known synthetic sweeteners is saccharin, discovered accidentally in 1879 by Remsen and Fahlberg. Its sweetness is 240 to 350 times as sweet as sucrose. Another common sweetener, cyclamate, was discovered unexpectedly at the University of Illinois by Andrieth and Sveda while studying the antipyretic properties of sulfamic acid derivitives. Andrieth and Sveda have pointed out that both saccharin and cyclamate contain the linkage C-NH-SO_2.

Although there is little similarity between chloroform and cyclamate, both possess a sweet taste approximately 30 times as sweet as sucrose. Urea derivatives that possess sweet taste are illustrated by *p*-anisylurea and *p*-phenetylurea (Dulcin). Reportedly Dulcin is 70 to 350 times as sweet as sucrose and has a more agreeably sweet taste than saccharin; however, because of toxicological reasons, it is not permitted in food products. Likewise, 1-*n*-propoxy-2-amino-4-nitrobenzene (P-4,000) is not allowed in food because of its undesirable toxicological properties. P-4,000 appears to be the sweetest substance known, being 4,100 times as sweet as sucrose (13). It is doubtful that P-4,000 or any of its homologues, 1-ethoxy-2-amino-4-nitrobenzene or 1-methoxy-2-amino-4-nitrobenzene will be allowed in food products although they do not have the after-taste associated with saccharin.

Another interesting product is derived from 1-perillaldehyde found in Shiso oil (beefsteak plant oil), which is commercially available in Japan. The 1-perillaldehyde, that is nearly 50% of Shiso oil, is converted to 1-perillaldehyde *anti*oxime. It is nearly 2,000 times as sweet as sucrose.

A new sugar substitute, L-aspartyl-L-phenylalanine methyl ester, has just been found to be 250 times as sweet as sucrose by Mazur et al., (12). It was synthesized from two nonsweet amino acids, L-aspartic acid and L-phenylalanine. This dipeptide sweetener was reported to have flavor characteristics more similar to sucrose than a cyclamate-saccharin mixture.

A new approach to new sweetening agents has resulted from the discovery of sweet substances produced from naturally occurring flavonoids by Horowitz and Gentili (4) and Krbechek et al., (10). The dihydrochalcones of the naturally occurring flavanones--prunin, naringin, and neohesperidin--are intensely sweet. These three dihydrochalcones are readily prepared from the corresponding flavanones by hydrogenation in an alkaline solution.

Although naringin and neohesperidin dihydrochalcones have a good sweet taste, the aftertaste is completely different from that of any other natural or synthetic sweetener. This aftertaste can be described as menthol-type cooling effect or licorice-like. Although the intense sweetness of neohesperidin dihydrochalcone permits the use of extremely small amounts in a material to be sweetened, the lingering aftertaste presents some problems. Various combinations of neohesperidin dihydrochalcone with saccharin and cyclamate appear to give more acceptable taste qualities (7).

Intensely Sweet Substances Occurring Naturally

In addition to naturally occurring sugars and related carbohydrates of ordinary sweetness, some complex organic substances possessing intense sweetness have been isolated from plant materials (Table 2).

Table 2. Intensely sweet substances occurring naturally.

Botanical Source	Sweetener	Sweetness Relative to Sucrose
Glycyrrhiza glabra L.	Glycyrrhizin	50
Stevia rebaudiana Bertoni	Stevioside	300
Smilax glycyphylla Hassk	---	---
Dioscoreophyllum cumminsii (Stapf Diels)	---	800-1,500
Thaumatococcus Daniellii Benth	---	---

Glycyrrhizin is a saponin of the corresponding sapogenin glycyrrhetinic acid with an attached disaccharide moiety. The sweetner is present in licorice root (*Glycyrrhiza glabra*) as the calcium potassium salt, glycyrrhizic acid, and is isolated from the root as the ammonium salt. The structure of the sugar moiety was confirmed by periodate oxidation of glycyrrhizic acid and by degradation of trimethyl glycyrrhizate pentamethyl ether. Glycyrrhizin in the form of licorice root extracts has been used by pharmacists for years as a flavoring agent to mask unpleasant tasting drugs. Its wider application may be limited because of its inherent physiological activity and some undesirable taste qualities. The triterpenoid glycoside appears to be about 50-100 times sweeter than sucrose.

Another intensely sweet, naturally occurring sweetener is found in the leaves of a small shrub, *Stevia rebaudiana* Bert., that grows wild in Paraguay. The natives use the leaves to sweeten their tea and other foods. Stevioside, the active sweet principle, is obtained in a 6% yield by ethanol extraction of the dried leaves. It is 300 times sweeter than sucrose. Stevioside is a steroid glycoside, the detailed structure of which was established in 1963 by Mosettig et al. (14). It is a diterpenoic acid esterified to one glucose unit and combined in glucosidic linkage with sophorose. The aglycone, steviol, has some interesting biological properties.

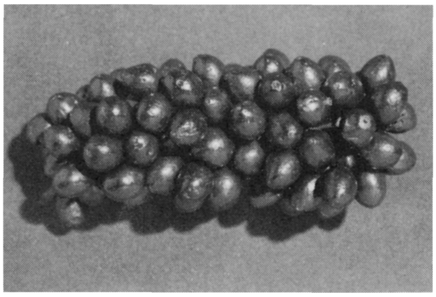

Fig. 1. The Serendipity Berry.

An intensely sweet substance of an unidentified berry from Nigeria was discovered by Inglett and described by Inglett and May (8, 9). Because of the unexpected circumstances surrounding this discovery, it was named "Serendipity." The berry was identified later as *Dioscoreophyllum cumminsii* (Fig. 1). The Serendipity Berry grows in the forests of Nigeria during the rainy season from approximatley July to October. The red berry, approximately ½ inch long, is found growing in grapelike clusters of approximately 50-100 berries. The tough outer skin of the berry encloses a white semisolid mucilaginous material surrounding a friable thorny seed. The fruit is not commonly cultivated or used by the natives in Nigeria. Chromatography of water extracts of the berry on molecular sieves indicated that the sweetener was bound to protein (9). The effect of three proteolytic enzymes-papain, bromelain, and trypsin -- on Serendipity Berry sweetener was studied. Bromelain treatment appeared to separate more completely the sweetener moiety from the proteinaceous material. The sweetener was isolated by chromatography on Sephadex G-50. In another fractionation study, the sweetener recovered from a Sephadex G-200 column was evaluated for sweetness. Results obtained by threshold evaluation gave a mean value of 1,500 times sweeter than sugar. At present this naturally occurring substance is the sweetest ever known.

A large variety of plant materials were examined systematically by Inglett and May (8) for sweetness intensity. Another African fruit containing an intense sweetener was Katemfe. Botanically the plant is *Thaumatococcus Daniellii.* The fruit contains three large black seeds surrounded by a transparent jelly and a light yellow aril at the base of each seed. The mucilaginous material around the seeds is intensely sweet and causes other foods to taste sweet. The seeds were observed in trading canoes in West Africa as early as 1839, and were reported to be used to sweeten bread, fruits, palm wine, and tea. Preliminary studies have indicated a substance similar to the Serendipity Berry sweetener.

There are probably other undiscovered intensely sweet-tasting substances in nature and potentially new sweeteners to be synthesized. The success of any sweetener program will depend on obtaining a superior sweetener that has an advantageous economic cost per sweetness pound, nontoxix properties, and a taste comparable or similar to sucrose.

Literature Cited

1. Bottle, R. T. 1964. Synthetic sweetening agents. *Manufacturing Chemist* 35:60-65.
2. Brouwer, J. N., H. Van DerWel, A. Francke, and G. J. Henning. 1968. Miraculin, the sweetness-inducing protein from miracle fruit. *Nature*220:373-374.
3. Dastoli, F. R., D. V. Lopiekes, and A. R. Doig. 1968. Bitter-sensitive protein from porcine taste buds. *Nature* 218:884-885.
4. Horowitz, R. M. and B. Gentili. 1969. Taste and structure in phenolic glycosides. *J. Agr. Food Chem.* 17:696-700.
5. Inglett, G. E. 1970. *Recent Sweetener Research*. Botanicals, P. O. Box 3034, Peoria, Illinois 61614.
6. Inglett, G. E., B. Dowling, J. J. Albrecht, and F. A. Hoglan. 1965. Taste-modifying properties of miracle fruit (*Synsepalum dulcificum*). *J. Agr. Food Chem.* 13:284-287.
7. Inglett, G. E., L. Krbechek, B. Dowling, and R. Wagner. 1969. Dihydrochalcone sweeteners-sensory and stability evaluation. *Food Res.* 34:101-103.
8. Inglett, G. E. and J. F. May. 1968. Tropical plants with unusual taste properties. *Econ. Bot.* 22:326-331.
9. Inglett, G. E. and J. F. May. 1969. Serendipity berries (*Dioscoreophyllum cumminsii*) -- source of a new intense sweetener. *Food Res.* 34:408-411.
10. Krbechek, L., G. E. Inglett, M. Holik, B. Dowling, R. Wagner, and R. Riter. 1968. Dihydrochalcones. Synthesis of potential sweetening agents. *J. Agr. Food Chem.* 16:108-112.
11. Kurihara, K., and L. M. Beidler. 1968. Taste-modifying protein from miracle fruit. *Science* 161:1241-1243.
12. Mazur, R. H., J. M. Schlatter, and L. Jokay. 1969. New sugar substitute. Inst. of Food Technol. 29th Annual Meet. Abstr., Chicago, Illinois. May 11-14.
13. Moncrieff, R. W. 1967. The Chemical Senses, CRC Press, Cleveland, Ohio.
14. Mosettig, E., Beglinger, U., Dolder, F., Lichti, H., Quitt, P., and Waters, J. A. 1963. The absolute configuration of steriol and isosteriol. *J. Amer. Chem. Soc.* 85:2305-2309.
15. Pangborn, R. M., and R. B. Chrisp. 1966. Gustatory responses to anomeric sugars. *Experientia* 22:612-615.
16. Rader, C. P., S. G. Tihanyi, and F. B. Zienty. 1967. A study of the true taste of saccharin. *Food Res.* 32:357-360.

Index To Advertisers

American Society for
Horticultural Science 134,200