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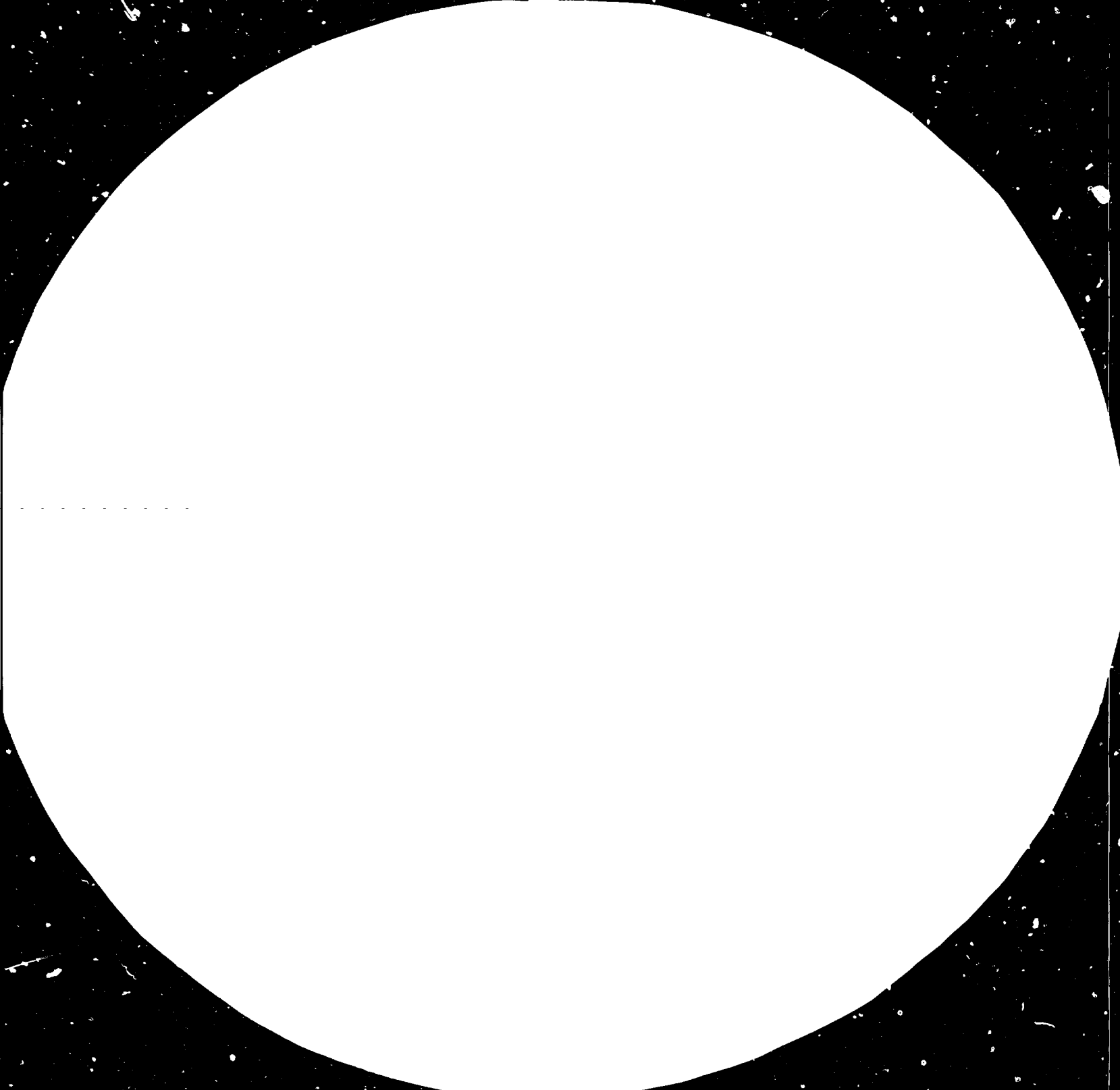
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POTENTIAL FOR THE DEVELOPMENT OF A PROTEIN-
SWEETNER INDUSTRY IN AFRICA*

by

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I. INTRODUCTION

Since the ban on cyclamates in 1970 and the question of possible long-term safety of saccharin, there has been an intensive search for alternative low-cost, low-calorie sweeteners for foods, soft drinks and other consumer products such as gum, candies, toothpastes, mouthwashes, medicinals, etc. At the present time, Aspartame (Searle and Co., U.S.A.) and saccharin are the major non-caloric sweeteners used in the United States. Saccharin is banned in Canada so Aspartame is the only non-caloric sweetener currently used there. Aspartame is considerably more expensive than saccharin but the demand for sweeteners for low-calorie soft drinks and also sweeteners for coffee, tea, etc. is so great that the consumers are willing to pay the price. Alternative low-calorie, natural sweeteners are needed.

Although there are a number of possible natural sweeteners such as the leaves of the shrub, Stevia rebaudiana used by the natives of Paraguay to sweeten tea and other foods, and "glycyrrhizin" isolated from the root of the licorice plant Glycyrrhiza glabra, the most promising alternatives would appear to be the protein sweeteners in Africa: miraculin from Richardella dulcifica (Synsepalum dulcificum), monellin from Dioscoreophyllum cumminsii, and thaumatin from Thaumatococcus daniellii, (Inglett, 1971). All three have been considered seriously for commercialization. An American based company, the Miralin Co., attempted to process and distribute miraculin as a tablet as part of a reducing diet. Although they reportedly spent about \$5 million on studies to demonstrate its safety, the U.S. Food and Drug Administration required still more tests and the company went out of business (Higginbotham, 1979).

Monellin was carefully considered and finally rejected for commercialization by Tate and Lyle Ltd. (Higginbotham et. al., 1981). Thaumatin, called "Talin" under a trademark owned by Tate and Lyle, Reading, England is well on the way to commercial production and worldwide distribution. It is already being marketed in Japan as a sweetener and flavour enhancer. It has been accepted as a sweetener for medicinals in the U.K. It is expected that Talin will be given official acceptance as a food additive in the U.K. in the near future, and in West Germany and the U.S.A. at a later date.

It seems clear that there will be a worthwhile potential market for the fruits of the Thaumatococcus daniellii plants for use in production of thaumatin/Talin sweeteners. This could be a valuable agricultural product for Africa. It also is likely that there will be a market in the future for miraculin and the fruits of Richardella dulcifica (Synsepalum dulcificum) could become an important agricultural crop. More research is needed on

miraculin to demonstrate its safety as a food additive. Discussions should be held with the U.S. Food and Drug Administration to determine what additional information is needed to demonstrate the safety of miraculin as a food additive.

Monellin is considered to be the least likely of the protein sweeteners to be commercialized (Higginbotham, 1979); however, monellin is still worthy of further production and investigation, particularly for research purposes.

In addition, further research is desirable on the protein sweeteners, their methods of extraction, and potential uses in the food industry. The primary nutritional problem in the West is overconsumption of calories leading to over-weight and resulting diseases such as high blood pressure and cardiac disease. Thus there is a huge potential market for low-calorie, safe sweeteners that can be added to foods and beverages. It is likely that the protein sweeteners of Africa will play an important role in this regard in the future.

II. AFRICAN PLANTS YIELDING PROTEIN SWEETENERS - [Inglett, 1971; Van der Wel, 1974; Higginbotham and Hough, 1977; Higginbotham, 1979; Higginbotham et.al., 1981]

There are at least three African plants that yield protein sweeteners. The first is Richardella dulcifica (Synsepalum dulcificum) a bush or small tree indigenous to tropical West Africa from Ghana to the Congo, that produces the miraculous berry or fruit. The fruits contain a single large seed surrounded by a thin pulp which contains the protein called "miraculin". Miraculin itself is not sweet, the berries have no particular flavour but the "sweet-modifying" pulp produces a remarkable i.e., miraculous sweetness in any acid or sour food subsequently eaten. For example, lemon juice becomes sweet. This is a persistent effect lasting several hours. A drawback is that it causes acid foods eaten later in the meal to also become sweet when perhaps the consumer wishes to enjoy an acid flavour.

Miraculin is a glycoprotein, molecular weight 42000 ± 3000 , containing 373 amino acids with an isoelectric point about 9.0. It is the largest known molecule able to elicit a sweet taste. One kilogram of berries yields about 50 mg of miraculin. Maximum yield is 200 mg/kg berries. Miraculin is thermolabile and is inactivated below pH 3.0 and above pH 12.0. Sweetness is evoked by 100 ug of miraculin making it many times sweeter than sucrose (Higginbotham and Hough, 1977; Higginbotham et. al., 1981). Miraculin has been suggested as a sweetening agent for yogurt, mouthwashes, vitamin C and aspirin. It has been marketed as an aid for dieting.

The second African plant producing protein sweetener is Dioscorephyllum cumminsii whose fruit, "serendipity berries" yield "monellin". The fruits are eaten by natives in the Congo. D. cumminsii is a dioecious (male and female plants) herbaceous perennial indigenous to tropical West Africa, the Sudan, and equatorial Africa growing in Zimbabwe Mozambique and Kenya. D. cumminsii berries grow in grape-like clusters. Each berry contains a single black seed surrounded by a white mucilage which is extremely sweet. Monellin is a protein with a molecular weight of about 11,500 \pm 1000) and an isoelectric point of 9.03. It contains 94 amino acids. Yield of monellin is 3 to 5 g/kg fruit. Monellin has a sweetness of 1500 to 2000 (to perhaps even 300) times that of sucrose at a 7% sucrose level. It is unstable in acid soft-drinks and when heated above 65°C. It is tasteless below pH 2.0 and above pH 9.0 depending upon the temperature and ionic environment. Denaturation or proteolytic hydrolysis results in loss of sweetness but some sweetness may be regained under certain circumstances. Monellin is perceived as sweet by man and old world monkeys. It is not sweet to new world monkeys, rats, guinea pigs, dogs, hamsters, pigs or rabbits. It is considered to be the least likely of the three African protein sweeteners to be commercialized (Higginbotham, 1979).

The third African plant producing the protein sweetener called "thaumatin" or "Talin", the registered trademark of Tate and Lyle Ltd., is Thaumatococcus daniellii which grows in dense thickets throughout the West African rain forest from Gambia to Zaire. The plants grow in Ivory Coast, Nigeria, Ghana, Central African Republic, Angola and Togo. Tate and Lyle Ltd. and the Crops Research Institute, Bunso, Ghana are cooperating on experimental growing of T. daniellii. Yields of 2 to 8 tons of T. daniellii fruit have been obtained per hectare in Ghana. It is estimated that 30 tons could be produced under proper conditions. Up to 6 grams of thaumatin can be extracted from each kg of fruit. Aluminum salts increase the extractability of thaumatin. T. daniellii fruits contain 1 to 3 black seeds, each capped with a soft membranous sac called an aril containing the sweet material. This is surrounded by a thin layer of transparent gel. Thaumatin (Talin when combined with aluminum salts and extracted by the patented Tate and Lyle process) is 5000 to 5500 times as sweet as sucrose on a weight basis (at the sweetness threshold 0.6% sucrose) and 2500 to 2700 times as sweet as sucrose on a weight basis at an 5% sucrose level (Higginbotham, 1979). Talin is the sweetest natural compound so far discovered.

Thaumatococcus ("Katemfe" in West Africa) is used by natives to sweeten palm wine and fruit drinks. It is a mixture of closely related sweet proteins designated as thaumatin I, thaumatin II, thaumatin To, etc. Molecular weights range from 22,208 for thaumatin I to 22,293 for thaumatin II to 21,500 \pm 100 for thaumatin To. Thaumatin I and II contain 207 amino acids and thaumatin To contains 203 amino acids. The isoelectric points are all very basic ranging from pH 11.5 to 12.5.

Talin is soluble to greater than 10% (100 μ g/ml) in cold water. Normal levels of use are 3 mg/100 ml. 10% solutions can also be obtained in 60% ethanol.

Freeze-dried Talin is stable indefinitely. Soft drinks may be preserved for several years at ambient temperatures without loss of sweetness.

Talin sweetener is characterized by an initial delay in sweetness followed by a rapid rise to maximum sweet intensity and a gradual loss of sweetness followed by a licorice-like aftertaste. Aldohexuronic acids, their salts, amides and lactones reduce the lingering sweet aftertaste and increase the intensity of perceived sweetness (Higginbotham et. al., 1981). Arabinogalactan also reduces the aftertaste. Addition of DL-alanine and food acids as has been done in Japan to produce a mixture called ST-1 sweetener provides a more sucrose-like taste and doubles the sweetness intensity, because such small quantities are necessary to produce sweeteners, there is no "body" to the sweetener.

Stability of Talin is enhanced at lower pHs. It can be heated at 100°C for several hours without loss of sweetness if the pH is below 5.5. Talin is most stable to heat between pH 2.7 and 6.0 with a maximum stability at 2.8 to 3.0. Monovalent and divalent cations decrease sweetness but aluminum salt extraction yields a thaumatin-aluminum combination (Talin) which has double the sweetness of thaumatin alone. Gelatin stabilizes sweetness while carrageenans are incompatible with Talin sweeteners. Dilute salt (NaCl) solutions (2% or less) cause a momentary loss of sweetness that is regained as saliva rinses the salt from the taste buds.

Thaumatococcus is not only a sweetener but it is also a flavour enhancer. Thaumatococcus enhances the flavour of peppermint, spearmint, cinnamon, menthol, ginger, chocolate and coffee (Higginbotham et. al., 1981). Thaumatococcus can be used to provide not more than 50% of the total sweetness in soft drinks. Beyond that level the aftertaste becomes noticeable.

Added to chewing gum at a level of 100 ppm, Talin extends the flavour duration to about 20 minutes compared with about 5 to 10 minutes without Talin.

III. CONCERNS OF PROTEIN SWEETENERS

Both monellin and Talin show a significant delay in sweetening (compared with sucrose) followed by a build-up and lingering sweetness. Since the protein sweeteners are used in small concentrations, they provide practically no body or viscosity to the carrier food. They also have overtones of flavour other than sweetness that not everyone may like.

Miraculin causes acids and acid foods to taste sweet for a considerable period after consumption and, as part of a meal, may cause acid to taste sweet when actually an acid flavour is preferred i.e.- lemon juice on fish, vinegar on salad, etc.

IV. SAFETY

All new sweetening agents must be shown conclusively to be free of any toxicity or carcinogenic properties. They also must be acceptable in flavour to the consumers in the products to which they are added. The overfed Western World is very much concerned with the use of non-caloric sweetening agents. Food additive status requires from 5 to 7 years testing costing as much as 20 million dollars. Cyclamates, saccharin aspartame and xylitol all used for sweetening certain products have had questions raised regarding their safety. Thus, there is a need for new, safe sweetening agents.

Tate and Lyle Ltd., Reading, England are far along in their efforts to commercialize thaumatin (Talin). They have been investigating the safety of Talin as a food additive since 1976. Tate and Lyle Ltd. have demonstrated that Talin is free of acute and short-term toxicity and found no allergenic, immunogenic, teratogenic or mutagenic effects. Long-term carcinogenic testing has not been completed. Talin has been accepted as a safe and natural food in Japan since May 1979, and October 1981, Talin was accepted as a safe excipient for medicines by the U.K. Committee on Safety of Medicines. It is expected that Talin will be given official approval as a food additive in the U.K. in the near future. Efforts to get approval of Talin as a food additive in Germany, Canada and the U.S.A. are underway. Getting such approval combined with toxicity testing cost millions of dollars. This insures that, as soon as Talin has been officially approved as a food

additive, commercialization will proceed very rapidly. At that point, there should be a good market for the fruits of Thaumatococcus daniellii. While Tate and Lyle Ltd. hold the trademark on the name Talin and hold certain patents on methods of extracting the thaumatin, there are still excellent opportunities to develop new processes and to market thaumatin to other food companies throughout the world. Therefore, there should be an excellent market for the fruits, the crude fruit extracts or the purified extracts as a newly developed agricultural product.

An American company (based in Massachusetts), the Miralin Co. tried to commercialize Miraculin. They developed plantations for growing Richardella dulcifica (Synsepalum dulcificum) in the West Indies and Brazil and produced tablets containing miraculin. The tablets were designed to reduce caloric intake when combined with prescribed diets. The company invested an estimated 5 million dollars in toxicological testing, but the U.S. Food and Drug Administration (FDA) concluded that the data provided was still incomplete and inadequate to establish the safe use of the substance as a food additive. Further studies would be needed to establish Miraculin as a safe food additive "sweetener". According to information received from the Food and Drug Administration no petitions so far been submitted to the American FDA on behalf of monellin or thaumatin.

V. PROCESSING OF PROTEIN SWEETENERS

Miraculin

The pulp surrounding the seeds is removed, collected and mixed with water. The mixture is then dialyzed to remove low molecular weight compounds. A cellophane dialysis bag retains the miraculin which can be recovered by centrifugation and lyophilized to yield a tan coloured powder. Miraculin can be purified by ion exchange column chromatography. Miraculin following recovery by centrifugation of the aqueous suspension can be solubilized in carbonate buffer at pH 10.5. Colour can be removed by passage through a diethyl amino ethyl-Sephadex-25 column (Kurihara and Beidler, 1968).

Monellin

Monellin is difficult to extract. Inglett et. al. (1965) were unsuccessful in solubilizing it in water, salt solutions, organic solvents

or treatments with enzymes. It can be extracted in saliva, or aqueous solutions of neuraminic acid derivatives, tannin-binding agents like polyethylene glycol, gelatin or casein at pH 7.0. Better extractions are obtained with aqueous solutions of strongly basic compounds like spermine or other polyamines like spermine. The extracts can then be further purified by ammonium sulfate fractionation and successive gel filtration on Sephadex G-50 and G-25. Wlodawer and Hodgson (1975) produced crystals of monellin by vapor diffusion of 20% ethanol into a buffered solution of monellin.

Thaumatococcus

The seeds are removed from the fruits and the arils are removed from the seeds and homogenized in water. Aqueous extracts of thaumatococcus contain up to 6 residues of Al^{3+} per molecule. Dilute aluminum salts facilitate extraction of thaumatococcus from the arils. The suspension is then centrifuged to remove the insolubles. By the Tate and Lyle patented process (U.S. Patent No. 4,011,206), Talin may be extracted with water or with dilute solutions of aluminum salts (Higginbotham, 1977). The Talin is then purified by a series of ultra-filtrations, gel-filtration and physical processes to remove small molecules from the protein sweetener. The product may be further purified by passage through Sephadex-50 and ion-exchange chromatography. The final clear, brown liquor is freeze or spray dried to yield a light, fluffy buff-coloured product (Higginbotham, 1981). The final product is very soluble in water and has a sweetness approximately 5000 times as sweet as sucrose at the threshold level and 2000 to 3000 times as sweet as sucrose at normal sucrose levels (6 to 10% sucrose).

VI. CONCLUSION

The African protein sweeteners can be expected to play an important role as safe, low-calorie sweeteners for many foods, soft drinks, medicinals, gums, toothpastes, mouthwashes, etc. in the future. Although considerable research has been done on the chemical structures and other characteristics of the protein sweeteners, much more research is needed on high yielding varieties of plants, more efficient methods of extraction, establishing their safety as food additives and on possible uses for these interesting products. Producing the fruits used for extraction of the protein sweeteners could be an important new agricultural industry in Africa. Production of the sweeteners themselves for sale to the international food industry could be a profitable new business for Africa.

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