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Review Article

Botanical and Protein Sweeteners

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Abstract: Plant species with unusual taste properties such as bitterness, sourness or sweetness and others with a taste- modifying components; have long been known to man, although their exploitation has been limited. Exponential growth in the number of patients suffering from diseases caused by the consumption of sugar has become a threat to mankind's health. Artificial low-calorie sweeteners available in the market may have severe side effects. It takes time to figure out the long-term side effects and by the time these are established, they are replaced by a new low-calorie sweetener. Saccharine has been used for centuries to sweeten foods and beverages without calories or carbohydrate. It was also used on a large scale during the sugar shortage of the two world wars but was abandoned as soon as it was linked with the development of bladder cancer. Naturally occurring sweet and taste modifying proteins (Thaumatin, Curculin, Miraculin, Brazzein, Pentadin, Monellin, Mabinlin) present in plants such as *Thaumatococcus daniellii* (Marantaceae), *Curculigo latifolia* (Hypoxidaceae), *Synsepalum dulcificum* (Sapotaceae), *Pentadiplandra brazzeana* (Pentadiplandraceae), *Dioscoreophyllum cumminsii* (Menispermaceae), *Capparis masaikai* (Capparaceae) are being seen as potential replacements for the currently available artificial low calorie sweeteners. Most protein sweetener plants such as *S. dulcificum, P. brazzeana, C. masaikai,* are shrubs; *C. latifolia, T. daniellii,* are perennial herbs while *D. Cumminsii* is an annual liana.

Keywords: Nutritive and Non-Nutritive Sweeteners, Protein sweetener, Natural Sugar, Artificial Sugar.

1. Introduction

The role sweeteners play in the diet is constantly debated. Sweeteners of one kind or another have been found in human diets since prehistoric times. Terms such as sugar-free, sugar alcohols, sucrose, corn sweeteners, etc. can be confusing. Each of the sweeteners available to consumers has specific applications and certain limitations. A variety of sweeteners exist to help consumers satisfy their desire for sweetness. Sweeteners are used in foods for several reasons, besides adding sweetness. Sugar is used as a preservative in jams and jellies; it provides body and texture in ice cream and baked goods; it aids in fermentation in bread and pickles (Birch G. Gerard, 2000).

Sweeteners that supply energy (calories) are referred to as nutritive sweeteners, even though they lack other nutrients essential for growth and health maintenance. Nutritive sweeteners provide four calories per gram. For most sugars, this is about 17 calories per teaspoon. Sweeteners that do not supply calories are referred to as non-nutritive sweeteners. Synthetic (artificial) sweeteners may be nutritive or non-nutritive. Synthetic sweeteners must pass the approval of the Food and Drug Administration (FDA) before they can be marketed in the United States. The three synthetic sweeteners currently approved by the FDA are aspartame, saccharin and acesulfame-K. Aspartame is a nutritive sweetener. Saccharin and acesulfame-K are non-nutritive sweeteners (Halliday, 2008).

1.1 Nutritive Sweeteners

Sugars: All natural sugars are composed of simple carbohydrates. Carbohydrates are classified into three categories: monosaccharides (one unit of sugar), disaccharides (two units of sugar) and polysaccharides (many units of sugars).

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The simpler forms of carbohydrates are called sugars, and the more complex forms are either starches or dietary fibers. The simple carbohydrates follow:

Classification of Most Common Carbohydrates

Monosaccharides	Disaccharides	Polysaccharides
(Single Sugars)	(Double Sugars)	(Complex Sugars)
Glucose	Sucrose	Starch
Fructose	Lactose	Glycogen
Galactose	Maltose	Cellulose (fiber)

Fructose: Fructose is the sweetest tasting of all the natural sugars. It is found in fruits and honey. Fructose is known as the fruit sugar. It also has been referred to as levulose. Fructose is one and one-half times as sweet as sucrose.

Glucose: Glucose is in nearly all plant foods. Glucose is known as the "blood sugar" because it is the main sugar circulating in blood and is the main form into which the body converts other sugars and carbohydrates.

Another name for glucose is dextrose. Glucose is rapidly absorbed into the bloodstream from the intestines. Glucose is unusual in that it can be absorbed into the bloodstream to some degree through the lining of the mouth.

Sucrose is the most abundant sugar in plants. Sucrose is a disaccharide composed of two simple sugars and when sucrose is digested it separates the two chemically attached simple sugars, glucose and fructose, for the body to absorb and use for energy. Sucrose is the most common sugar for household and industrial use. It is produced by concentrating the sugar from sugar cane or sugar beet juice.

Traditionally, the word sugar has been used to imply sucrose, but this can cause confusion because there are so many other sugars currently on the market. Sucrose is purified and granulated to various stages to provide raw, white, brown and powdered sugars. During processing, a dark coloured liquid, molasses, is produced.

Lactose: Lactose is the sugar found in milk. Lactose is primarily used in products to provide bulk. It is seldom used as a sweetener because it is the least sweet of the sugars. Lactose has two monosaccharide parts, glucose and galactose, which are normally broken apart by the enzyme lactase. Some individuals may lose the ability to digest lactose and become lactose intolerant.

Cheese, although a product of milk, has less lactose because the lactose changes during the fermentation process.

Maltose: Maltose is produced during bread making and beer brewing. When you eat or drink a food source of maltose, the maltose is split into two glucose units so they can be absorbed.

Honey: Honey is a natural syrup made from plants by honeybees. The same sugars in honey are found in table sugar, fructose and glucose. Honey is more concentrated than crystalline table sugar, so it contains more calories per equal measure than table sugar.

Honey is often proclaimed as more nutritious than sugar. It does provide trace minerals and B vitamins in very minute amounts, but it is misleading to actually say honey is more nutritious. The contribution of these nutrients to the overall diet is insignificant.

Maple sugar: Maple sugar is produced by boiling off the liquid from the syrup derived from the spring flow of the sap of mature sugar maples. Maple sugar is mainly sucrose.

In human nutrition, the primary role of carbohydrates is to supply energy. A gram of carbohydrate contains 4 calories (energy). The body prefers this source of energy over all other available sources of energy nutrients.

Carbohydrates are known as the "energy sparing nutrient". The energy available from carbohydrates spares protein from being used for energy so that protein may build and repair body tissue. When carbohydrates are absent from the diet, protein is used for energy.

Carbohydrates are found in almost all plant foods and one animal source -- milk. There are nutrient differences between these sugar sources.

Fruit furnishes the same monosaccharides and the same calories per monosaccharide gram of weight as refined table sugar and honey. The difference is that in fruit the sugars are diluted in a large quantity of water that contains vitamins, minerals and fiber.

The significant difference between sugar sources is not between "natural" and "refined," but between concentrated sugars (honey, table sugar, concentrated fruit juices, corn sweeteners) and the diluted, naturally occurring sugars in foods such as oranges, corn, milk, and potatoes.

There is strong evidence that sugar plays a part in dental cavities. Dental cavities are caused by the acid by-product of bacterial growth in the mouth, and bacteria thrive on carbohydrates. Therefore, sugars are implicated as the cause of cavities. Any carbohydrate-containing food, including bread, bananas, milk and concentrated sugars, can support bacterial growth.

It takes about 24 hours for a large enough build-up of bacteria to accumulate on a tooth to produce cavity-causing acid. Brushing after eating carbohydrates and once-a-day, flossing may effectively prevent cavity formation, regardless of the carbohydrate content of the diet. Some individuals may never get cavities because they have inherited resistance to them.

Corn Syrup: This glucose sweetener was developed in the 1920s by treating cornstarch with acid, heat and/or enzymes. Corn syrup is not as sweet as sucrose but is often used with or in place of sucrose to provide "body" and "texture" in food.

High-Fructose Corn Syrup: Made from corn syrup by converting glucose into fructose, this unique enzymatic process, developed in 1970, provides a much sweeter product, allowing a reduction in quantity used. Currently, the soft drink industry uses high-fructose corn syrup as its main nutritive sweetener. It may be listed as corn sweetener or high fructose corn syrup on a label.

Sugar Alcohols: These sweeteners are commercially produced from glucose, or derived from fruits and vegetables. The most common sugar alcohols are sorbitol, mannitol, maltitol, and Xylitol. Prunes have the largest amount of the naturally occurring sorbitol of any fruit normally eaten in the United States. Apples and pears also are high in sorbitol. Sugar alcohols are found in dietetic candies, chewing gums, and as a coating on the tablets and gums.

Products containing sugar alcohols may carry the label "sugarless," "sugar-free" or "no sugar," but they are still carbohydrates and supply calories. Sugar alcohols do not promote tooth decay. They are absorbed more slowly than sugars, which may be the reason they can have a laxative effect.

Aspartame: This artificial sweetener was discovered in 1965. In 1981, the FDA approved aspartame as a table-top sweetener and as an ingredient in dry foods, such as dry bases for beverages and cold cereals. In 1983, approval was extended to carbonated beverages. Aspartame is marketed in the U.S. under the trade names of NutraSweet and Equal. Aspartame provides the same energy as any protein, four calories per gram because aspartame is the methyl ester of two amino acids (proteins) -- aspartic acid and phenylalanine.

Aspartame is 180-200 times sweeter than sucrose; it does not contribute a significant amount of calories due to the small amount needed to sweeten products.

Due to a possible excess of phenylalanine for phenylketonuric (PKU) children, aspartame must carry a warning label stating: "Phenylketonuric: contains phenylalanine". Phenylketonuria is a genetic disease in which the body cannot produce the enzyme necessary for the body to use phenylalanine.

The Acceptable Daily Intake set by the FDA is 50 milligrams of aspartame per kilogram of body weight. In terms of aspartame-sweetened soft drink usage, this equates to four to five 12 ounce cans for a 40 pound child or about 17 cans for a 150 pound adult. Adequate data is not available to establish the safety of aspartame for children less than two years of age. There are few, if any, reasons to use a sugar substitute for children less than two years of age. Children need energy to grow.

Over 200 commercial products have aspartame as one of their ingredients. The use of aspartame is limited at high or prolonged temperatures because it breaks down and loses its sweetness. Although there have been over 6,000 unsolicited complaints to the FDA concerning aspartame, the FDA supports the safety of aspartame for the general population. The centres for Disease Control state that some individuals may have an unusual sensitivity to aspartame. Statistically, some sensitivity to aspartame could be expected with over 100 million U.S. individuals consuming products containing aspartame.

1.2 Non-Nutritive Sweeteners

Non-nutritive sweeteners do not provide any calories when consumed. There are two non-nutritive sweeteners currently approved by FDA; Saccharin and Acesulfame-K.

Saccharin was discovered in 1879 and has been marketed for over 80 years. Due to the length of its safe use, saccharin was given GRAS (generally regarded as safe) status.

In 1977, FDA proposed removing saccharin from public use because of a Canadian study of saccharin and bladder cancer in rats. Public opposition led Congress to pass a moratorium on the FDA's action to take saccharin off the market. This moratorium is still in effect. Products containing saccharin must carry a warning label that states: *Use of this product may be hazardous to your health. This product contains saccharin which has been determined to cause cancer in laboratory animals.*

Saccharin has not been shown to cause cancer in human beings. Saccharin is approximately 300 times as sweet as sucrose and is stable within a wide range of temperatures.

Acesulfame-K was approved by FDA in July 1988 as a free-flowing table-top sweetener and for use in dry base beverage mixes, puddings and desserts, chewing gum and dairy product analogues (including toppings). Acesulfame-K is marketed under the names of Sunette and Sweet One. Acesulfame-K is about 200 times sweeter than sucrose. Acesulfame-K is heat stable and can be used in baking. Petitions for its use in soft drinks and baked goods have been filed.

Cyclamates were removed from the open market in 1969 because of research that linked cyclamates as a possible cancer-causing agent in rats. Petitions have been filed to bring back cyclamates as an alternative sweetener.

Traditional methods to sweeten foodstuffs, feed, and other consumer products have relied on the use of low-molecular-weight sweetening agents, especially sucrose. In recent years, however, there has been an increasing demand for low-calorie sweeteners. Together with this trend, there is also an increase in the demand for healthy and natural eating products. Therefore, In order to address this need, there is an intense and ongoing search for alternative sweeteners. The alternative sweeteners that have been developed show a very intense sweetening profile. They are several orders of magnitude sweeter than sucrose on both a molar and weight basis. Therefore, in order to supply the same sweetening effect as sucrose, these compounds can be used in minute amounts. This results in almost

negligible addition to the calorie count, as well as a product that does not contribute to tooth decay. Moreover, the alternative and intense sweetening additives also can be used in diabetic foods, since they do not trigger a demand for insulin in these patients. Finally, some intense sweeteners also have been found to be strong flavor enhancers, thus expanding their range of applications.

The prevalence of obesity and diabetes has increased dramatically in recent years in the United States, with similar patterns seen in several other countries, including India as well (Levine *et al.*, 2003). Diabetes mellitus is a chronic disease caused by an inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced (Pinget and Boullu-Sanchis, 2002). Artificial sweeteners like Saccharin, Aspartame, Cyclamate and Acesulfame-K are used worldwide as low-calorie sweeteners by patients affected by diseases linked to the consumption of sugar, e.g. diabetes, hyperlipemia, caries, obesity etc. but they have side effects such as psychological problems, mental disorders, bladder cancer, heart failure and brain tumors. Sweet proteins have the potential to replace these artificial sweeteners, by acting as natural, good, low-calorie sweeteners, as we know that proteins do not trigger a demand for insulin in these patients whereas sucrose does.

In humans, the sweet taste is mainly due to the recently discovered T1R2-T1R3 receptor, two of the three members of the T1R class of taste-specific proteins hypothesized to function in combination as a heterodimer. The human T1R2-T1R3 receptor recognizes natural and synthetic sweetness and T1R1-T1R3 recognizes the umami taste (Nelson, 2002). So far there are seven known sweet and taste-modifying proteins, namely Brazzein, Thaumatin, Monellin, Curculin, Mabinlin, Miraculin and Pentadin (Faus, 2000). The key residues on the protein surface responsible for biological activity have not yet been identified with certainty for any of these proteins. Monellin was found to be 100000 times sweeter than sucrose on a molar basis (Hung *et al.*, 1999) followed by Brazzein and Thaumatin which are 500 times (Ming *et al.*, 1994) and 3000 times sweeter than sucrose (van der Wel and Loeve, 1972) respectively (the latter two on a weight basis). All of these proteins have been isolated from plants that grow in tropical rainforests. Although most of them share no sequence homology or structural similarity, Thaumatin shares extensive homology with certain non-sweet proteins found in other plants. The potential industrial applications of these proteins are the low-calorie sweetener industry and the cola, snacks, food and chocolate industries.

CHAPTER TWO

2. Protein Sweeteners

2.1 Miracle Fruit

- **Scientific names:** *Synsepalum dulcificum*, *Synsepalum subcordatum*
- **Family:** Sapotaceae
- **Common name:** Miracle Fruit
- **Origin:** Ghana (Tropical West Africa)

It is one of the strangest tropical fruits. The most unusual thing about it is the effect it has on one's taste after this miraculous berry has been consumed. The "miracle" is that if lemon or other sour food is eaten after the miracle fruit, the sour tastes sweet as if sugar has been added. That kind of magical experience is unforgettable! The interest in this plant is so high that anyone who has a plant always finds eager volunteers to test its sweetening properties. A natural chemical in the fruit masks the tongue's sour taste buds so that lemons taste like lemonade or lemon pie, or lemon candy. What causes the miracle? The fruit has a unique taste changing glycoprotein that inhibits the taste buds' perception of sour taste. The sweet sensation lasts for half an hour to a few hours (Slater and Joanna, 2007).

2.1.1 History

The plant was discovered in West Africa, where the native diet revolved around a few basic foods, mostly of sour taste. Just imagine the delight of the people when someone ate a few red berries and later ate a meal of sour foods, to find everything suddenly sweet! The West African natives use the fruit to sweeten sour palm wine (beer) Pito, and fermented maize bread - Kenkey.

This West African wonder was not botanically identified and named until the middle of the 19th century as *Synsepalum dulcificum*, a member of the Sapotaceae family, relative of the Sapodilla (*Manilkara zapota*) (Theerasilp *et al.*, 1989).

Bill Whitman was the first to grow the plant successfully in the US. He had a seedling all of 7" tall and a cutting of 4" bearing fruit upon the young twigs.

2.1.2 The Plant

This small, evergreen shrub grows very slowly to a height of 4-6 ft in container, and 10-15 ft in natural habitat. Eventual size depends on where the plant is grown; a 10 year old plant might be easily only 4-5 feet tall. It forms an oval to pyramidal shaped bush or small tree. Inconspicuous brown-and-white 1/2 inch flowers are followed by bright scarlet, 1 inch football-shaped fruit, sweet and pleasant tasting. Most of the fruit is taken up by a single large seed, but the pulp around it can be nibbled off and then for the next hour or so, anything one eats that is sour has a sweet flavor. The plant starts fruiting when only 1 ft tall. It produces fruit practically year around. In native habitat, two large crops are available yearly, each after a rainy season. The mature bushes usually have a few fruits hanging around all year. Seed to fruit in 2 to 3 years and Flower to fruit in 30 to 45 days.

One of the many virtues of miracle fruit is its ease of cultivation, although, like with any plant, it would be nice if you learn its likes and dislikes before inviting it into your home or garden. If planted in alkaline limestone-based soils, the plant may die. These plants seem to live for acid, thriving in it, and then converting it! They must have rich, well-drained soils that are acid in pH, with lots of peat moss, and require constant supply of micronutrients. On alkaline soils, they often are grown in large containers with generous amounts of peat moss for sustained success in fruiting. It makes an excellent container tree, which gives it the added benefit of mobility. Since it is so easily containerized, almost anyone can grow this plant whether they have an outside planting area or not. Miracle fruit can be a rewarding indoor plant. It is not a fast-growing plant, which is another benefit for those who would like to grow it in their house or greenhouse. It thrives under warm temperatures and high humidity. A 10 inch plant is happy in a one-gallon pot. One that size will flower and fruit at least twice a year, probably more frequently.

Miracle Fruit thrives best in partial shade. When plants are small they are subject to frost damage, so they should be container-grown and kept indoors or moved to protected locations when frost or freeze threatens. Older plants may sustain some leaf and minor twig damage but may sustain cooler temperatures without being killed. Although not thought to be frost tolerant, the *Synsepalum* plants have been observed growing in Florida in protected from wind locations as far north as Tampa.

When propagating miracle fruit, the seeds are sown in a rich, well-drained medium, just barely covered, and water lightly every other day. Seeds generally come up in about eight to ten weeks, but grow slowly the first year, often being only two to three inches tall at the end of almost one year of growth. It really takes three to four years before the plants reach a height of more than fifteen to twenty inches, and then they start to grow more rapidly. However, when growing this plant from seed, it takes few years before the first fruit is been enjoyed. If possible, it is always recommended to get a mature full specimen, at least 1 ft tall that is ready to bloom**.**

There are 2 known species of *Synsepalum* that carry miracle fruits. *Synsepalum dulcificum* is a smaller-leaf version (leaves are narrow) and is somewhat slower growing plant. *Synsepalum subcordatum* (Giant Miracle Fruit) is a larger leaf

variety and grows into a small tree. The fruit is slightly bigger than those of *S. dulcificum*, and are produced more profusely, especially in the first years. With age, the fruit crop amounts of these two species become about the same (Theerasilp *et al.*, 1989).

2.1.3 Miraculin

Miraculin is a natural sugar substitute, a glycoprotein extracted from the fruit of *Synsepalum dulcificum* (Theerasilp and Kurihara, 1988). The berry, which contains active polyphenols, was first documented by explorer Chevalier des Marchais, who searched for many different fruits during a 1725 excursion to its native West Africa. The protein is a single polypeptide with 191 amino acid residues (Nirasawa *et al.*, 2001).

Miraculin itself is not sweet. However, after the taste buds are exposed to miraculin, ordinarily sour foods, such as citrus, are perceived as sweet. This effect lasts up to an hour. Thus, miraculin has the unusual property of modifying sour taste into a sweet taste (Temussi, 2002).

Miraculin works by binding to the sweet receptors on the tongue. The Miraculins effect lasts as long as the protein is bound to the tongue, which can be up to an hour. It makes most acidic foods taste sweet, but does not improve the taste of bitter things (Aaron Rowe, 2006).

The active substance, isolated by Prof. Kenzo Kurihara a Japanese scientist, was named miraculin after the miracle fruit when Kurihara published his work in *Science* in 1968.

2.1.4 Glycoprotein Structure

Miraculin was first sequenced in 1989 and was found to be a glycoprotein consisting of 191 amino acids and some carbohydrate chains (Theerasilp *et al.*, 1989).

Miraculin occurs as a tetramer (98.4 kDa), a combination of 4 monomers group by dimer. Within each dimer 2 miraculin glycoproteins are linked by a disulfide bridge (Kurihara, 1992).

The molecular weight of the glycoprotein is 24.6 kDa, including 3.4 kDa (13.9% of the weight) of sugar constituted (on molar ratio) of glucosamine (31%), mannose (30%), fucose (22%), xylose (10%) and galactose (7%).

The taste-modifying protein, miraculin, has seven cysteine residues in a molecule composed of 191 amino acid residues. Both tetramer miraculin and native dimer miraculin in its crude state have the taste-modifying activity of turning sour tastes into sweet tastes (Igeta *et al.*, 2006).

2.1.5 Sweetness Properties

Miraculin, unlike curculin (another taste-modifying agent), is not sweet by itself, but it can change a sour beverage into a sweet beverage, even for a long period after consumption. The anti-sweet compound, Gymnemic acid suppresses the sweet taste of miraculin, as it does for sucrose. The duration and intensity of the taste-modifying phenomena depend on various factors: miraculin concentration, duration of contact of the miraculin with the tongue, and acid concentration. Maximum sweet-induced response has been shown to be equivalent to the sweetness of 17% sucrose solution (Matsuyama *et al.*, 2009).

Glycoprotein is sensitive to heat: when heated over 100°C, miraculin loses its taste-modifying a property. Miraculin activity is inactivated at pH below 3 and pH above 12 at room temperature. The sweet modifying effect stays at pH 4 (in acetate buffer), for 6 months at 5°C.

The detailed mechanism of the taste-inducing behaviour is still unknown. It has been suggested that the miraculin protein can change the structure of taste cells on the tongue. As a result, the sweet receptors are activated by acids, which are sour in general. This effect remains until the taste buds return to normal. The two histidine residues (i.e. His29 and His59) appear to be mainly responsible for the taste-modifying behavior. One site maintains the attachment of the protein to the membranes while the other (with attached xylose or arabinose) activates the sweet receptor membrane in acid solutions. Further research is being conducted at the University of Tokyo using a system of cultured cells that allowed the testing of human taste receptors at various pH values to uncover the mechanism. As already known miraculin binds strongly to the sweet taste receptors on our tongues; however, it does not activate receptors at neutral pH. Once an acid is introduced, the miraculin protein changes shape in such a way that it turns on the sweet receptors, it is bound to, causing an ultra-sweet sensation without affecting other flavors tasted. Once the acidic food is swallowed, miraculin returns to its inactive shape until the next acidic food comes along. This can continue for about an hour while the miraculin protein is still bound to the taste receptor (Matsuyama *et al.*, 2009).

Amino acids sequence of glycoprotein miraculin unit adapted from Swiss-Prot biological database of protein sequence.

SIGNAL (29)	MKELTMLSLS FFFVSALLAA AANPLLSAA
1-50	DSAPNPVLDI DGEKLRTGTN YYIVPVLRDH GGGLTVSATT PNGTFVCPPR
51-100	VVQTRKEVDH DRPLAFFPEN PKEDVVRVST DLNINFSAFM PNPGPETISS
101-150	WCRWTSSTVW RLDKYDESTG QYFVTIGGVK FKIEEFCGSG FYKLVFCPTV
151-191	CGSCKVKCGD VGIYIDQKGR GRRLALSDKP FAFEFNKTVY F

2.1.6 As a Sweetener

As miraculin is a readily soluble protein and relatively heat stable, it is a potential sweetener in acidic food (e.g. Soft drinks). Japanese researcher's more or less successful attempts to mass produce it is focused on recombinant technology. While attempts to express it in yeast and tobacco plants have failed, researchers have succeeded in preparing genetically modified *E. coli* bacteria, lettuce and tomatoes that express miraculin. The scientists' crops resulted in 40 micrograms of miraculin per gram of lettuce leaves, which was considered a large amount. Two grams of lettuce leaves produced roughly the same amount of miraculin as in one miracle fruit berry (Slater and Joanna, 2007).

Miraculin was never approved for use as a sweetener by the United States Food and Drug Administration (FDA). In the 1970s the Miralin Company planned on bringing miraculin to market and was founded with investments by Reynolds Metals, Barclays, and Prudential. Legal advice and contact with the FDA indicated that miraculin would be approved as generally recognized as safe as the berries had been eaten for centuries in Africa with no reports of adverse reactions (substances used in food prior to January 1, 1958, through either scientific procedures or through experience based on common use in food can be designated GRAS). However, on the eve of the product's launch in 1974 the FDA determined that miraculin would be considered a food additive and thus require years more testing. At this point, the company's investment capital could not sustain them and Miralin folded. Afterward, Miralin requested the FDA documents under the freedom of information act. The documents were nearly completely blacked out, and the rationale for the sudden change in regulation was not revealed (Theerasilp and Kurihara, 1988).

Limitations: Miraculin is a non-heat-stable protein, subject to denaturation from heating, and thus miracle berries are not taste-bud active when cooked.

While miraculin changes the perception of taste, it does not change the food's chemistry, leaving the mouth and stomach vulnerable to the high acidity of some foods, such as lemon juice, that may cause irritation if eaten in large quantities.

Plate A: Showing the Shape of the Fruit.

Plate B: Showing the mesocarp of the fruit.

Plate C: Showing the potted shrub.

Plate D: Showing the prolificity of the shrub.

2.2 Lemba

- **Scientific names:** *C. latifolia, C. capitulata, C. racemosa, C. orchioides*
- **Family:** Hypoxidaceae
- **Common name:** Lemba
- **Origin:** Malaysia

The genus Curculigo belongs to the family Hypoxidacea, which consists of approximately 20 species of exclusively tropical origin (Kocyan, 2007). In Malaysia, it is locally known as Lemba and the common species are *Curculigo latifolia* Dryand which is a perennial herb with specialized underground stems. The four other species found are namely *C. latifolia, C. capitulata, C. racemosa* and *C. orchioides.* The plants are not cultivated but found abundantly distributed in both Peninsular Malaysia and Borneo. The plants grow about two meters tall and the petiole up to 1 meter long that consist of blade elliptical leaf, 30-100cm x 5-l0cm,

subglabrous. The leaf stalks are one-third the length of the leaves and they overlap one another at their bases to form a thick stem. Inflorescence ovoid to flowers cylindrical, compact, 2-6cm x 2-6cm, bracts, 1-6cm long; green, flower color; yellow; fruit ovoid, 10-25mm, tiny seeds and sweet.

The leaves are tough, thin and broad, who makes leaves them very suitable for wrappings (Buchanan, 1999). It grows well in a hilly area and it is a shade-loving plant, thriving partly in shaded or sunless conditions, with abundant water supply. It prefers fertile, well-drained soils, rich in organic matter. Leaves are elliptic with numerous distinct parallel primary veins and the plants can easily be mistaken for ground orchids or young palms. Flowers are yellow with the exception of *C. orchioides*, which have pink flowers. In Borneo, traditionally the leaves are used for wrapping foods or pounded and wrapped around the knees to ease joint pains. The remarkable feature, however, lies in the fruits. Interest in Lemba has increased owing to their roles as alternative sweeteners and the possible beneficial implications in the prevention of diabetes.

The fruit contained sweet proteins called curculin, which exhibit taste-modifying activity (Barre *et al.*, 1997). Curculin modifies taste bud sensations by changing acidic or tasteless food into sweetness. Curculin extract was first developed into natural sweeteners by Yamashita *et al*., (1990) but commercialized products are hindered by fruit productions as these plants are yet to be cultivated. Preliminary studies on germination rate of Lemba seeds showed that seeds were unable to germinate on germinating trays, jiffy bags or blotter papers. It was suspected that seeds may be recalcitrant or may require special germinating media or pre-treatments. In addition to this, success of seed germination may be affected by fruit ripeness and maturity, where fruit developmental studies and ripening of this plant was never reported. The difficulty in using seed as propagules has enticed the needs for other means of propagation. As these plants are monocots, the most suitable vegetative propagules are corms and rhizomes.

2.2.1 Curculin

Curculin is a sweet protein that was discovered and isolated in 1990 from the fruit of *Curculigo latifolia* (Hypoxidaceae) (Yamashita *et al.*, 1990) a plant from Malaysia. Like miraculin, curculin exhibits taste-modifying activity; however, unlike miraculin, it also exhibits a sweet taste by itself. After consumption of curculin, water and sour solutions taste sweet. The taste-modifying activity of the protein (discussed below) remains unchanged when it is incubated at 50°C for 1 hr between pH 3 and 11. The plant is referred to locally as 'Lumbah'.

2.2.2 Protein Structure

The active form of curculin is a heterodimer consisting of two monomeric units connected through two disulfide bridges. The mature monomers each consists of a sequence of 114 amino acids, weighing 12.5 kDa (curculin 1) and 12.7 kDa (curculin 2), respectively. While each of the two isoforms is capable of forming a homodimer, these do not possess the sweet taste nor the taste-modifying activity of the heterodimeric form. To avoid confusion, the heterodimeric form is sometimes referred to as "neoculin" (Suzuki *et al.*, 2004). These sweet proteins are postulated to evoke activities by interacting with the T1R2-T1R3 heterodimeric sweet taste receptor similar to low molecular mass sweet compounds, such as sugars and aspartame.

2.2.3 Sweetness Properties

Curculin is considered to be a high-intensity sweetener, with a reported relative sweetness of 430-2070 times sweeter than sucrose on a weight basis (Yamashita *et al.*, 1990).

A sweet taste, equivalent to a 6.8% or 12% sucrose solution, was observed after holding curculin in the mouth in combination with clear water or acidified water (citric acid), respectively. The sweet taste lasts for 5 minutes with water and 10 minutes with an acidic solution. Sweetness was also observed with other acids such as ascorbic acid (vitamin C) and acetic acid (Yamashita *et al.*, 1990).

The taste-modifying activity of curculin is reduced in the presence of ions with two positive charges (such as Ca^{2+} and Mg^{2+}) in neutral pH solutions, although these ions have no effect in acidic solutions. In the same way, monovalent ions (such as Na^{+} and Cl^{-}) have no effect in solutions with either neutral or acidic pH.

Although the "sweet-inducing" mechanism is unknown, it is believed that one active site of curculin strongly binds to the taste receptor membranes while a second active site fits into the sweet receptor site. The latter site is thought to be responsible for the induction of sweetness. Presence of Ca^{2+} and/or Mg^{2+}, water and acids tune the binding of the active site of curculin to the receptor site and therefore modify perceived sweetness (Yamashita *et al.*, 1990).

Amino acid sequence of sweet protein curculin adapted from Swiss-Prot biological database of protein sequences.

SIGNAL (22)	MAAKFLLTIL VTFAAVASLG MA
1-50	DNVLLSGQTL HADHSLQAGA YTLTIQNKCN LVKYQNGRQI WASNTDRRGS
51-100	GCRLTLLSDG NLVIYDHNNN DVWGSACWGD NGKYALVLQK DGRFVIYGPV
101-114	LWSLGPNGCR RVNG
PROPEP (22)	GITVAKDSTE PQHEDIKMVI NN

2.2.4 As a Sweetener

Like most proteins, curculin is susceptible to heat. At a temperature of 50°C (122°F) the protein starts to degrade and lose its "sweet-tasting" and "taste-modifying" properties, so it is not a good candidate for use in hot or processed foods. However, below this temperature both properties of curculin are unaffected in basic and acidic solutions, so it has potential for use in fresh foods and as a table-top sweetener.

Because curculin is not widely found in nature, efforts are underway to produce a recombinant form of the protein. In 1997, curculin was expressed in *E. coli* and yeast, but the recombinant protein did not exhibit "sweet-tasting" or "taste-modifying" activity (Kurihara *et al.*, 1997). However, a 2004 study obtained a recombinant curculin, expressed in *E. coli*, exhibiting "taste-modifying" and "sweet-tasting" properties (Suzuki *et al.*, 2004).

Plate E: Showing the perennial monocotyledonous plant.

Plate F: Showing the arrangement of the flowers.

Plate G: Showing the arrangement of the flower in acropetal succession.

Plate H: Showing the fruit of the plant containing Curculin.

In addition to the challenges related to commercial production of the protein, there are many regulatory and legal issues remaining to be resolved before it can be marketed as a sweetener. Curculin currently has no legal status in the European Union and the United States. However, it is approved in Japan as a harmless additive, according to the List of Existing Food Additives established by the Ministry of Health and Welfare (English publication by JETRO).

2.3 Oubli

- **Scientific name:** *Pentadiplandra brazzeana*
- **Family:** Pentadiplandraceae
- **Common name:** Oubli
- **Origin:** Gabon and Cameroon

The plant grows in Gabon and Cameroon, where the fruit has been consumed by the apes and local people for a long time. The berries of the plant are incredibly sweet. African locals call them "Oubli" (French for "forgot") in their vernacular language because their taste helps nursing infants forget their mother's milk, as once they eat them they forget to come back to the village to see their mother (Stein, 2002)

2.3.1 Brazzein

Brazzein is smaller, more heat-stable and pH-stable member of the set of proteins known to have intrinsic sweetness. The protein, consisting of 54 amino acid residues, is reported to be between 500 and 2000 times sweeter than sucrose and represents an excellent alternative to available low-calorie sweetener. It was originally isolated from the fruit of an African plant *Pentadiplandra brazzeana* Baillon (Jin *et al.*, 2003). Heat and pH stability of the protein makes it an ideal system for investigating the chemical and structural requirements of a sweet-tasting protein. It was first isolated as an enzyme by the University of Wisconsin–Madison in 1994 (Ming and Hellekant, 1994).

Brazzein is found in the extracellular region. The tissue it's found in is the pulp surrounding the seeds.

With pentadin, discovered in 1989, brazzein is the second sweet-tasting protein discovered in this African fruit (Van der Wel, 1989).

Like the other natural sweet-proteins such as monellin and thaumatin, it is highly sweet.

2.3.2 Protein Structure

The monomer protein, consisting of 54 amino acid residues, is the smallest of the sweet proteins with a molecular weight of 6.5 kDa (Hellekant and Danilova, 2005). The amino acid sequence of brazzein, adapted from the Swiss-Prot biological database of protein, is as follows: QDKCKKVYEN YPVSKCQLAN QCNYDCKLDK HARSGECFYD EKRNLQCICD YCEY.

The structure of brazzein was determined by proton nuclear magnetic resonance (NMR) at a pH 5.2 and 22 degrees C. Brazzein has four evenly spaced disulfide bonds and no sulfhydryl groups.

3D analysis of brazzein showed one alpha-helix and three strands of anti-parallel beta sheet. It is not similar to either of the other two sweet-tasting proteins, monellin and thaumatin (Izawa *et al.*, 2000).

However, a recent 3D study shows that these three proteins possess similar "sweet fingers" believed to elicit the sweet taste

Residues 29–33 and 39–43, plus residue 36, as well as the C-terminus, were found to be involved in the sweet taste of the protein. The charge of the protein plays also an important role in its interaction with the sweet taste receptor.

Based on this knowledge a synthesized improved brazzein, called pGlu-1-brazzein, was reported to be twice sweet as the natural counterpart (Jin *et al.*, 2003).

2.3.3 Sweetness Properties

On a weight basis, brazzein is 500 to 2000 times sweeter than sugar, compared to 10% sugar and 2% sugar solution respectively (Izawa *et al.*, 2000). Its sweet perception is more similar to sucrose than that of thaumatin with a clean, sweet taste with lingering aftertaste and with a slight delay longer than aspartame in an equi-sweet solution (Pfeiffer *et al.*, 2000).

Brazzein is stable over a broad pH range from 2.5 to 8 and heat stable at 98°C for 2 hours (Hellekant *et al.*, 2005).

2.3.4 As a Sweetener

Brazzein represents an alternative to available low-calorie sweeteners. As a protein, it is safe for diabetics and very soluble in water (> 50mg/mL) (Birch G. Gerard, 2000).

When blended with other sweeteners, sweeteners such as aspartame and stevia, brazzein reduces side aftertaste and complements their flavor (Halliday, 2008).

Unlike other natural sweeteners, apart from thaumatin, its sweet profile is closer to sucrose. Unlike other sweet-tasting proteins, it can withstand heat, which makes it suitable for any industrial food manufacture (Hellekant *et al.*, 2005).

Increasing interest of brazzein makes it difficult to source naturally from Gabon, but it can also be synthesized by a solid-phase method. Recombinant proteins were successfully produced via *E. coli*.

The Texas companies Prodigene and Nectar Worldwide were among the licensees to use Wisconsin Alumni Research Foundation patents on brazzein, and genetically engineer the enzyme into maize. Brazzein then can be commercially extracted from maize through ordinary milling. Approximately one ton of maize yields 1-2 kilograms of Brazzein. It can also be engineered into plants like wheat to make pre-sweetened grains, e.g. for cereals (Halliday, 2008).

2.3.5 Application

Sweet carbohydrates have several problems associated with their use, such as high caloric content, tooth decay and diabetes mellitus. This leads to demand for non-sugar alternatives. However, non-sugar alternatives show also deficiencies such as being unsuitable in most cooking or baking applications. Their organoleptic qualities are also inferior and concern from a toxicological point of view can be raised for sweeteners, such as synthetic sweeteners, which lack a history of human consumption.

Brazzein combines a long history of human consumption, small size with high sweet potency, solubility and exceptional thermostability. It tastes purely sweet with no sourness, saltiness or bitterness. These qualities make it a very good alternative. However, as is a characteristic of many high-intensity sweeteners, the sweetness of brazzein grows slightly slower than that of sucrose. The sweetness of brazzein is readily washed from the tongue and neither mouth cooling nor extensively lingering occurs. It often improves the mouthfeel of beverages when blended with other sweeteners and works well in both citric acid and phosphate beverage systems.

Brazzein combines well with most high-intensity sweeteners such as acesulfame-K and aspartame, providing both quantitative and qualitative synergy. Also, it improves stability, flavor and mouthfeel when blended with acesulfame-K and aspartame, either alone or blended. It typically reduces the side taste of other sweeteners; for example, a blend of stevioside and brazzein is superior in taste quality to stevioside alone. Brazzein has been expressed in yeast (Guan *et al.*, 1995), fruits and vegetables to increase their sweetness and in grains to be economically extracted and used as sweetened flour (Faus, 2000).

2.3.6 Brazzein Controversy

Despite the fact that the sweet taste of the berries was well known in West Africa, the University claims that the sweet compound (brazzein) is its own invention and admit to no connection with Gabon.

This fact, which involved the appropriation of legal rights by means of patents over indigenous

biomedical knowledge without compensation to the indigenous groups, is considered an act of Biopiracy by GRAIN and Green Peace (The European Patent Directive, 2008).

Plate I: Showing the oval shape of the fruit.

Plate J: showing the arrangement of the fruit.

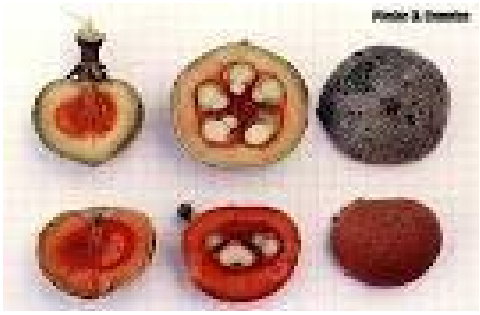


Plate K: showing the fruit mesocarp containing the sweet protein.

2.4 Sweet Prayer Plant

- **Scientific name:** *Thaumatococcus daniellii* Benth.
- **Family:** Marantaceae
- **Common name:** Sweet prayer plant, Katemfe
- **Origin:** West Africa

The sweet prayer plant or katemfe (*Thaumatococcus daniellii* Benth.) grows throughout the hot, humid tropical rainforest and coastal zone of West Africa. Its natural habitat is the undergrowth of forest trees. *T. daniellii* is particularly found in southern parts of Ghana, Cote d'Ivoire and Nigeria. It is also known to exist in the Princes Islands, Angola, the Central African Republic, Uganda and Indonesia.

Katemfe is a rhizomatous, perennial and monocotyledonous herb, propagating itself by rhizomes (Onwueme *et al.*, 1979). About 2 or 2.5m long petioles arise from the rhizomes depending on the age and the environment of the plant. At the end of these long petioles are large, broad and oval papery, tough, versatile leaves that are about 45cm long and 30cm broad. The leaves are ovate-elliptic rounded, truncate at the base, and shortly acuminate at the apex.

The inflorescence of *T. daniellii* usually arises from the lowest node and may be simple or forked with spikes about 8 to 10cm in length and bracts, usually umbricate, about 3 to 4cm in length (Tomlinson, 1961). The flowers that may be as long as the bracts form in short spikes close to the ground at the base of a swollen petiole. Sepals are broadly linear and about 1cm in length. Corolla tubes are short and lobes are oblong and about 2.5 to 3cm long. As many as 10 to 12 purplish-pink flowers may form on each inflorescence, but usually only 2, 3 or 4, rarely more than 4, of these form matured fruits. The plant flowers most of the year, but is most prolific from July until late October, followed by fruit formation, maturing and ripening from January until mid-April (Onwueme *et al.*, 1979).

The fruit grows on short stalks close to the ground and may be covered with plant debris as it clusters on the soil surface within the reach of insects and rodents. It is pyramidal or trigonal in shape, maturing from a dark-green through brown to crimson or bright-red colour when fully ripe and may weigh between 6 and 30g depending on whether it has one, two or three seeds. Within the fruit are the black hard seeds that are covered by a thin layer of sticky, transparent gel. The seeds look more like stones when dried, obviously showing its hardness and impervious nature. It also has a soft, fleshy and juicy cap called an aril, which contains sweeter substances (Onwueme *et al.*, 1979).

Local uses are multipurpose, ranging from cultivation as a fetish plant in Gabon to collecting leaves for wrapping and boiling food in Ghana (Facciola, 1998) (Fig. 2). Large quantities of the fruit are consumed by the local people to sweeten over fermented palm wine and sour foods. From the aril of *T. daniellii*, an intensely sweet, non-toxic and heat stable protein – thaumatin - is extracted, used as a sweetener or a taste modifier in beverages, desserts, chewing gum and pet foods.

2.4.1 Thaumatin

Thaumatin is a low-calorie sweetener and flavour modifier. The substance, a natural protein, is often used primarily for its flavour-modifying properties and not exclusively as a sweetener (Green, 2001).

The thaumatins were first found as a mixture of proteins isolated from the katemfe fruit

(*Thaumatococcus daniellii* Bennett) of West Africa. Some of the proteins in the thaumatin family are natural sweeteners roughly 2000 times more potent than sugar. Although very sweet, thaumatins taste is markedly different from sugars. The sweetness of thaumatin builds very slowly. Perception lasts a long time, leaving a liquorice-like aftertaste at high usage levels. It consists of 207 amino acid residues with eight intramolecular disulfide bonds and contains no free cysteine residues. It aggregates upon heating at pH 7.0 above 70^{o}C, whereupon its sweetness disappears (Kaneko and Kitabatake, 1999). Thaumatin is highly water soluble, stable to heating, and stable under acidic conditions.

2.4.2 Biological Role

Thaumatin production is induced in katemfe in response to an attack upon the plant by viroid pathogens. Several members of the thaumatin protein family display significant *in vitro* inhibition of hyphal growth and sporulation by various fungi. The thaumatin protein is considered a prototype for a pathogen-response protein domain. This thaumatin domain has been found in species as diverse as *Oryza sativa* and *Caenorhabditis elegans*. Thaumatins are pathogenesis-related (PR) proteins, which are induced by various agents ranging from ethylene to pathogens, and are structurally diverse and ubiquitous in plants (Palomares *et al.,* 2008). They include thaumatin, osmotin, tobacco major and minor PR proteins, alpha-amylase/trypsin inhibitor, and P21 and PWIR2 soybean and wheat leaf proteins. The proteins are involved in systematically acquired resistance and stress response in plants, although their precise role is unknown. Thaumatin is an intensely sweet-tasting protein (on a molar basis about 100,000 times as sweet as sucrose) found in the West African shrub *Thaumatococcus daniellii*: it is induced by an attack by viroids, which are single-stranded unencapsulated RNA molecules that do not code for protein. The thaumatin protein I consist of a single polypeptide chain of 207 residues.

Like other PR proteins, thaumatin is predicted to have a mainly beta structure, with a high content of beta-turns and little helix. Tobacco cells exposed to gradually increase salt concentrations develop a greatly increased tolerance to salt, due to the expression of osmotin, a member of the PR protein family (Singh *et al.,* 1999). Wheat plants attacked by barley powdery mildew express a PR protein (PWIR2), which results in resistance against that infection. The similarity between this PR protein and other PR proteins to the maize alpha-amylase/trypsin inhibitor has suggested that PR proteins may act as some form of the inhibitor (Mauch *et al.,* 2004).

Thaumatin-like protein has been found in olive fruit, and it can cause allergic reactions. A worker in an olive-oil mill in Jaen, Spain had respiratory symptoms at work. His blood serum was used to extract a 23-kilodalton protein from olive pulp, which was purified and confirmed to cause an allergic reaction in a skin prick test. The protein had amino acid sequence ATFXIVNQXTYTVXAAASP, which is similar (homologous) to that of thaumatin-like proteins (Palomares *et al.*, 2008).

2.4.3 Production

Within West Africa, the katemfe fruit has been locally cultivated and used to flavor foods and beverages for some time. The fruit's seeds are encased in a membranous sac, or aril, that is the source of thaumatin. In the 1970s, Tate and Lyle began extracting thaumatin from the fruit. In 1990, researchers at Unilever reported the isolation and sequencing of the two principal proteins found in thaumatin, which they dubbed thaumatin I and thaumatin II. These researchers were also able to express thaumatin in genetically engineered bacteria.

Thaumatin has been approved as a sweetener in the European Union (E957), Israel, and Japan. In the United States, it is a Generally Recognized as Safe flavoring agent (FEMA GRAS 3732).

Thaumatin is a pertinacious substance with a very high sweetening capacity than sugar (3000 times). The gene to produce Thaumatin has been genetically engineered into *Escherichia coli* and other micro-organization so that it can be produced on a large scale.

2.4.4 Uses of the Fruit

The fruit of *T. daniellii* has diverse uses because of the presence of the protein, thaumatin which is utilized for various uses. Industrially, thaumatin which is present in the mesocarp of the fruit has a high pharmaceutical potential in drugs, confectioneries and mineral drink manufacturing as a sweetener.

They indicated it to be 100,000 times sweeter than sugar; hence, it is used as a sweetener and flavor enhancer. Thaumatin contains many amino acids, some of which give it a lingering aftertaste that limits its human use to products such as medicines. It is also used to enhance flavor of pet foods and to flavor beverages (Higginbotham, 1986).

The thaumatin gene can be engineered directly into selected fruits and vegetable crops to improve their flavor and sweetness (Zemanek and Wasserman, 1995). Generally, natural sweeteners may be both nutritive and flavourable and thus popular both as food and flavouring. However, because common sugar and other nutritive sweeteners such as honey and corn syrup are associated with health problems (such as obesity and tooth decay) or even a principal cause of this life threatening sickness (diabetes), efforts have been made from 19th century to produce non-nutritive sweetness which may be natural or synthetic includes saccharin, aspartame, cyclamates and thaumatin. It combines well with monosodium glutamate and is used in typical

Japanese seasonings as well as in chewing gum (www.Britannica.com).

2.4.5 Uses of Stalk and Roots

The stalk *Thaumatococcus daniellii* is used for weaving mats, fish trap and ornamental bags and is also used as a sponge for pulping roll.

Plate L: Showing the aril of the fruit.

Plate M: Showing the trigonal shape of the fruit.

Plate N: Showing the long petiole of the leaf.

Plate O: Showing the leaf shape.

The outer bark of the stalk is used mainly for ornamental bags. The inner bark is used as a sponge and pulp while the stalk is used to wave fish trap. The roots are used for medicinal purpose to cure ailments such as stomach ache and typhoid fever (Van Der Wel and Loeve, 1972).

2.5 Serendipity Berry

- **Scientific name:** *Dioscoreophyllum cumminsii*
- **Family:** Menispermaceae
- **Common name:** Serendipity berry
- **Origin:** West Africa

The search for natural sweeteners has prompted intensive research on plants with sweetening properties. *Dioscoreophyllum cumminsii* (Stapf) Diels is a dioecious, semelparous annual Liana found as a late successional, understorey species in West African semi-deciduous forests. The fruits and subterranean tubers are intensely sweet and are both edible. The active principle in *Dioscoreophyllum cumminsii* Diels fruits (commonly known as serendipity berries) as a low molecular weight protein named monellin (after the Monell Chemical Senses Centre in Pennsylvania, U.S.A). The sweetening substance in *D. cumminsii* is a protein (monellin), which is 3000 times as sweet as sugar. As a non-carbohydrate sweetener, monellin has potentially been used in combating tooth decay, in low-calorie diets for diabetics and dieters and as a sweetener in the food industry. Unfortunately, it is one of the threatened plant species in the country because of massive habitat loss and fragmentation. Conservation is now needed as a salvage program. Population size is a major determinant of extinction risk. This has prompted the application of growth and population dynamics models to viability analysis of wild species. The aim of population viability analysis (PVA) is to determine the minimum viable population size (MVP) or area (MVA) of a particular species. In this paper, the MVP was estimated for *D. cumminsii* uses genetic models. The models simulated minimum effective population size of 6040 individuals' ha^{-1} in the ratio of 5032 males to 1008 females. This suggests that to retain evolutionary potential, *D. cumminsii* requires an effective population size of more than the 500–5000 range considered adequate for many other species.

2.5.1 Monellin

Monellin, a sweet protein, consists of two non covalently associated polypeptide chains, an A chain of 44 amino acid residues and a B chain of 50 amino acid residues. The protein can be purified from the fruit of *Dioscoreophyllum cumminsii* grown in West Africa and is approximately 100,000 times sweeter than sugar on a molar basis and several thousand times sweeter on a weight basis (Kohmura, 1998). Single-chain monellin (SCM), which is an engineered 94-residue polypeptide,

has proven to be as sweet as native two-chain monellin and is more stable than the native monellin at high temperature and in acidic environments. Native monellin is relatively sensitive to heat or acid treatment, which may cause separation of the sub-units and denaturation of the protein. Despite misgivings about the stability of the protein to heat and acid, downstream processes have been established. Its D-enantiomer has been crystallized and analyzed by X-ray crystallography at 1.8 Å resolutions. Two crystal forms (I and II) were found under crystallization conditions similar, but not identical, to the crystallization conditions of natural L-monellin (Lee *et al*., 1999). One NMR study of a non-sweet analog in which the Asp^{B7} of protein was replaced by Abu^{B7} (L-2-Aminobutylic acid), showed similar 3-dimensional structures of these two proteins, indicating that the lack of the beta-carboxyl group in the Abu^{B7} analog is responsible for the loss of sweetness. Recent research on identifying binding sites on the receptor by means of structure-taste relationships, found that four monellin analogues, [AsnA16]-, [AsnA22]-, [GlnA25]-, and [AsnA26]-monellin were 7500, 750, 2500, and 5500 times as sweet as sucrose on a weight basis, respectively. Thus, among them, [AsnA22]-monellin and [GlnA25]-monellin were less sweet than the native monellin (Tancredi *et al*., 2004).

Plate P: Showing the mode of stem (liana).

Plate Q: Showing the type of fruit.

2.6 Mabinlang

- **Scientific name:** *Capparis masaikai*
- **Family:** Capparaceae
- **Common name:** Mabinlang
- **Origin:** China

Capparis masaikai, known as Mabinlang, grows in the subtropical region of the Yunnan province of China and bear fruits of tennis-ball size. The mature seeds are used in traditional Chinese medicine. *Capparis* is a flowering plant genus in the family Capparaceae. These plants are shrubs or lianas and are collectively known as caper shrubs or caper bushes. *Capparis* species occur over a wide range of habitat in the subtropical and tropical zones.

Shrubs or climbers, to 7.5m tall. New branches reddish, slightly flat, with vertical ridges and stipular stripes, densely shortly rust-colored tomentose. Stipular spines to 5mm but often absent on flowering twigs, stout, recurved, sulcate, hollow, base inflated, apex sharp. Petiole 1.2-2.1cm, ca. 2mm in diam., trichomes like those on branches; leaf blade elliptic, oblong, or sometimes elliptic-obovate, 7-20 × 3.5-9cm, nearly leathery, often dark reddish brown when dry, abaxially densely rust-colored shortly tomentose but glabrescent, adaxially almost glabrous, midvein slightly broad, abaxially lavender and raised, and adaxially impressed, secondary veins 6-10 on each side of midvein and abaxially lavender and slightly raised, reticulate veins not obvious, base rounded to broadly cuneate, apex rounded to obtuse or sometimes acute to acuminate. Inflorescences axillary subumbels or axillary and terminal together forming a 10-20cm panicle, 3-8-flowered, densely rust-colored shortly tomentose, often with abortive leaflets; peduncle 1-5cm. Pedicel 3-4cm. Sepals 8-12 × 5-8mm, outside densely rust-colored shortly tomentose, inside glabrous; sepals of outer whorl inwardly concave to hemispheric, leathery; sepals of inner whorl slightly inwardly concave, thin.

They are also used as sweets; when chewed the seeds elicit a sweet taste (Liu, 1993). The origin of the sweet taste was identified as sweet-tasting proteins named Mabinlins. They are highly sweet, 100-400 times sweeter than sucrose on a weight basis (Nirasawa, 2001). Caper bushes are mainly used by humans for their fruit, which are rich in micronutrients. The fruit of other species, such as *Karir* (*C. decidua*), are also used for cooking; *C. mitchellii* and the Wild passionfruit (the local subspecies of *C. spinosa*) are well-known bush tucker in Australia. Mabinlang seeds (*C. masaikai*) are eaten as sweets.

Mabinlang is also used in Traditional Chinese Medicine. *Aspalathos*, the root of a shrub contained for example in the sacred Ancient Egyptian incense (kyphi), is sometimes considered to be *C. spinosa*. Other species have also recorded uses in herbalism and folk medicine; dedicated research is largely lacking, however. Mabinlins are sweet-tasting proteins found in Mabinlang seed (and possibly in other *Capparis* species); at least one of them is highly resistant to heat. The market for mabinlins is presently not large, but this is mainly due to insufficient supply rather than to lack of demand.

Plate R: Showing the type of flower.

Plate S: Showing seeds which contain the sweet protein.

Plate T(a): Showing the berry.

Caper bushes from arid regions - chiefly *C. decidua* - are highly useful in landscape gardening, afforestation and reforestation. They can stop soil erosion and preserve agricultural land. Any large-flowered species can be used to attract butterflies. The Crimson Rose (*Atrophaneura hector*), a spectacular swallowtail butterfly of South Asia, likes to visit flowers of *C. spinosa* in the winter.

2.6.1 Mabinlin

Mabinlin is a sweet protein with the highest known thermostability. It is derived from *Capparis masaikai* and its sweetness was estimated to be around 400 times that of sucrose on weight basis. It consists of an A chain with 33 amino acid residues and a B chain composed of 72 residues. The B chain contains two intramolecular disulfide bonds and is connected to the A chain through two intermolecular disulfide bridges (Guan *et al.*, 2000). Its heat stability is due to the presence of these four disulfide bridges. The sweetness of Mabinlin-2 is unchanged after 48 hr incubation at boiling point and of Mabinlin-3 and -4 is unchanged after 1 hr at 80°C (Kohmura and Ariyoshi, 1992).

Plate T(b): Showing the berry.

Table 1. Showing the comparison between the sweet proteins.

Protein	Miraculin	Curculin	Brazzein and Pentadin	Thaumatin	Monellin	Mabinlin
Family of Plant	Sapotaceae	Hypoxidaceae	Pentadiplandraceae	Marantaceae	Menispermaceae	Capparaceae
Scientific Name	*Synsepalum dulcificum*	*Curculigo latifolia*	*Pentadiplandra brazzeana*	*Thaumatococcus daniellii*	*Dioscoreophyllum cumminsii*	*Capparis masaikai*
Common Name	Miracle fruit	Lemba	Oubli	Sweet prayer plant	Serendipity berry	Mabinlang
Origin	West Africa	Malaysia	Gabon and Cameroon	West Africa	West Africa	China
Mode of Stem	Shrub	Perennial herb	Shrub	Perennial herb	Annual liana	Shrub
Degree of Sweetness in Relation to Sucrose	2000	430-2070	500-2000	3000	100000	400
Thermostability	100^{o}C for 1hr	50^{o}C for 1hr	98^{o}C for 2hrs	70^{o}C for 1 hr	50^{o}C for 1hr	100^{o}C for 48hrs
Ph Range	3-12	3-11	2.5-8	1-6	2-9	4-7.5
Number of Amino Acid (Residue)	191	228	54	207	94	105

CHAPTER THREE

3. Interaction of Sweet Proteins and their Receptor

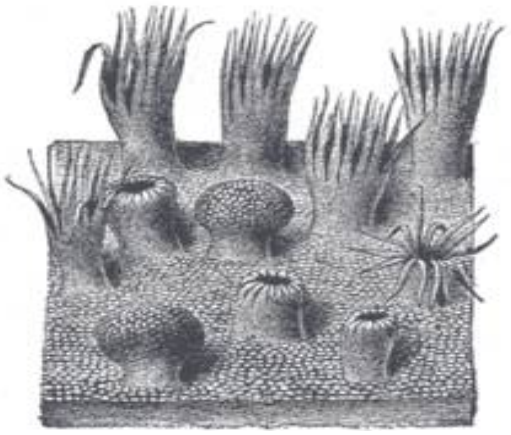
Humans detect taste with taste receptor cells. These are clustered in the taste buds. Each taste bud has a pore that opens out to the surface of the tongue enabling molecules and ions taken into the mouth to reach the receptor cells inside. There are five primary taste sensations salty, sour, sweet, bitter and umami. Sweet and umami (the taste of monosodium glutamate) are the main pleasant tastes in humans. T1Rs are mammalian taste receptors that assemble two heteromeric G-protein-coupled receptor complexes T1R1+T1R3, an umami sensor, and T1R2+T1R3, a sweet receptor (Zhao *et al.*, 2003).

Sweet and taste-modifying proteins interact with the T1R2-T1R3 receptor with a different mechanism compared to small molecular weight compounds (Tancredi *et al.*, 2004). Recently, it has been shown that the T1R2-T1R3 receptor has many characteristics similar to the mGluR, apart from some minor differences in the active site region.

The major work by Kunishima *et al*., solving the crystal structure of the N-terminal active site region of the subtype 1 of mGluR both free and complexed with glutamate has helped a lot in understanding the mechanism of interaction between ligand and T1R2-T1R3 receptor. Their structural work on mGluR and its N-terminal domain showing considerable conformational change induced by the glutamate complexation (Kunishima *et al.*, 2000). The 'Active' and 'resting' conformations of m1-LBR, an extracellular ligand binding region of mGluR, is modulated by the dimer interface. The protomer can form 'open' or 'closed' confirmations and is made up of two domains namely LB1 and LB2. The population of active conformers depends on ligand binding, i.e. the so called 'closed-open_A'. The ligand-free receptor exists as two different structures, free form I (open-open_R), the 'resting' conformation with two open protomers and free form II (closed-open A), nearly identical to the complexed form. The mechanism suggested by these structures is that the receptor is in dynamic equilibrium, and that ligand binding stabilizes the 'active' dimer. There are thus two ways, in principle, to activate the receptor: first, to complexate form I with the proper ligand (glutamate for the mGluR, aspartame or any other small molecular weight sweetener for the T1R2-T1R3 receptor) and second, by shift the equilibrium between free form I and free form II in favor of free form II.

The exact mechanism of interaction of sweet proteins with the T1R2-T1R3 sweet taste receptor has not yet been elucidated. Low molecular mass sweeteners and sweet proteins interact with the same receptor, the human T1R2-T1R3 receptor. Studies have shown that the T1R3 receptor protein is encoded by the Tas1r3 gene involved in transduction of sweet taste (Inoue *et al.*, 2004).

Recently, it has been found that T1R3-independent sweet- and umami-responsive receptors and/or pathways also exist in taste cells (Damak *et al.*, 2003).



CHAPTER FOUR

4. List of Sugar Substitutes and their Equivalent Sweetness to Sugar

The three primary compounds used as sugar substitutes in the United States are saccharin, aspartame, and sucralose. Maltitol and sorbitol are often used, frequently in toothpaste, mouthwash, and in foods such as "no sugar added" ice cream. Erythritol is gaining momentum as a replacement for these other sugar alcohols in foods as it is much less likely to produce gastrointestinal distress when consumed in large amounts. In many other countries, xylitol, cyclamate and the herbal sweetener stevia are used extensively.

4.1 Natural Sugar Substitutes

Table 2. Showing the sweetness and energy densities of natural sugar substitutes in comparison to those of sucrose.

Name	Sweetness by weight	Sweetness by food energy	Energy density	Notes
Brazzein	800			Protein
Curculin	550			Protein
Erythritol	0.7	14	0.05	
Glycyrrhizin	50			
Glycerol	0.6	0.55	1.075	E422
Hydrogenated starch hydrolysates	0.4–0.9	0.5×–1.2	0.75	
Inulin				
Isomalt	0.45–0.65	0.9–1.3	0.5	E953
Lactitol	0.4	0.8	0.5	E966
Luo Han Guo	300			
Mabinlin	100			Protein
Maltitol	0.9	1.7	0.525	E965
Malto-oligosaccharide				
Mannitol	0.5	1.2	0.4	E421
Miraculin				A protein that does not taste sweet by itself, but modifies taste receptors to make sour things taste sweet temporarily
Monatin				Naturally-occurring sweetener isolated from the plant *Sclerochiton ilicifolius*
Monellin	3,000			Protein; the sweetening ingredient in serendipity berries
Osladin				
Pentadin	500			Protein
Sorbitol	0.6	0.9	0.65	E420
Stevia	250			Extracts known as rebiana, Truvia, PureVia; mainly containing rebaudioside A, a steviol glycoside
Tagatose	0.92	2.4	0.38	
Thaumatin	2,000			Protein; E957
Xylitol	1.0	1.7	0.6	E967

4.2 Artificial Sugar Substitutes

Table 3. Showing the sweetness and energy densities of artificial sugar substitutes in comparison to those of sucrose.

Name	Sweetness (by weight)	Trade name	FDA approval	Notes
Acesulfame potassium	200	Nutrinova	1988	E950
Alitame	2,000		(Withdrawn)	Pfizer
Aspartame	160–200	NutraSweet, Equal	1981	E951
Salt of aspartame-acesulfame	350	Twin sweet		E962
Cyclamate	30		(Banned, 1969)	E952, Abbott
Dulcin	250		(Banned, 1950)	
Glucin	300			
Neohesperidin dihydrochalcone	1,500			E959
Neotame	8,000	NutraSweet	2002	E961
P-4000	4,000		(Banned, 1950)	
Saccharin	300	Sweet'N Low	1958	E954
Sucralose	600	Kaltame, Splenda	1998	E955, Tate & Lyle

CHAPTER FIVE

5. Conclusion

As it has been found that sweet proteins are thousands of times sweeter than sucrose and are of low caloric value, these proteins can be used as natural, low-calorie sweeteners by people suffering from diseases linked to the consumption of sugar e.g. obesity, diabetes and hyperlipemia.

There are no artificial sweeteners proven absolutely safe. Sugar and other "natural" sweeteners are hard to be considered as food with no ill effects. Protein sweeteners are natural, healthy and harmless sweetener! Due to their safety, it may have a great potential and practical use in everyday life and become a gift to humanity, a way to improve our diets and our health.

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