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RECENT TRENDS OF SOME NATURAL SWEET SUBSTANCES FROM PLANTS

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ABSTRACT

Two types of sweeteners are available: natural sweeteners of plant origin and artificial or synthetic sweeteners. Sweetening agents either evoke sweet taste or enhance the perception of sweet taste. Natural sweetening agents are preferred over synthetic sweetening agents since they do not have any adverse impact on health. Non-saccharide natural sweetening agents are low calorific, nontoxic and super sweet (100 to 10,000times sweeter than sugar) in nature and can overcome the problems of sucrose and synthetic sweeteners. Natural sweeteners are useful sugar substitutes for diabetic patients. Common and scientific names of natural sweeteners along with their properties, chemical structure of sweet principles, have been presented in this paper.

KEYWORDS: Diterpene glycosides, Sweet proteins, Natural sweetening agents.

INTRODUCTION

"Sugar is deep, deep ancient cravings," said Daniel Leiberman, an evolutionary biologist at Harvard University. Our relationship with sugar starts at birth as we are born with a sweet tooth. The instant 'lift' we get from sugar is one of the reasons we turn to it at times of celebration or when we crave comfort and reward.[1]

Man's addiction to sweet foods is presenting ever increasing health problems such as obesity is becoming a global epidemic. Today, the average sugar intake in the U.S. is 22 teaspoons per person per day, which is four times the amount that the World Health Organization suggests is healthy. Eating too much sugar is linked to a laundry list of negative effects, including diabetes, obesity and high blood pressure. There are several commercial natural and synthetic sweeteners available but all of them do not satisfy the ideal sweetener.[2]

SWEETENING AGENTS SHOULD HAVE THE FOLLOWING IDEAL PROPERTIES

1. They are required to be effective when used in small concentration.
2. They must be stable at a wide range of temperature to which the formulations are likely to be exposed.
3. Prolonged use of these agents containing preparations should not produce any carcinogenic effects.
4. They should have very low or non-calorific value.
5. They should be compatible with other ingredients in formulations.
6. They should be readily available and inexpensive.
7. Many synthetic sweeteners, which are widely used are proved to be carcinogenic and are non-nutritive. Hence, demand greatly increased for natural sweetening agents, especially for non-sacchariferous sweetening agents, because they are highly potent, useful, safe and low-calorie sugar alternatives. Two classes of such sweeteners are certain sweet glycosides and sweet proteins.[3]

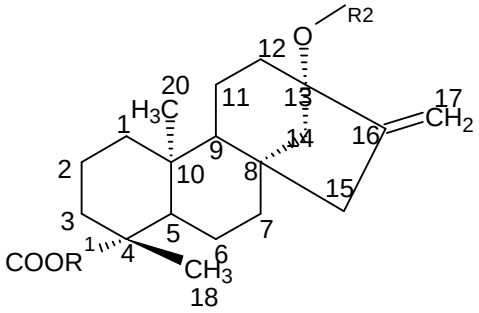
DITERPENE GLYCOSIDES

Ent-kaurene Diterpene Glycosides from the leaves of *Stevia rebaudiana*

Stevia, a natural sweetener plant having medicinal and commercial importance is being used all over the world. *Stevia rebaudiana* Bertoni is the botanical name of stevia. It is a perennial shrub belongs to the (Asteraceae) Compositae family. Stevia is native to Paraguay and Brazil and it is often referred to as ‘‘the sweet herb of Paraguay’’. It is also known as “honey yerba” and “honey leaf” and by some other variations of these names.[4] The plant *Stevia rebaudiana* Bertoni has been studied in depth because it was discovered that this plant is the source of six sweet-tasting diterpene glycosides.[5,6,7] They are steviosides, rebaudiosides A, C, D, E and dulcoside A. (Table 1). These sweet diterpene glycosides, as well as a complex mixture of organic compounds of which more than a hundred compounds have been identified, are found mainly in the leaves of the plant.

Stevioside is the most abundant sweet-tasting compound in the leaves. Bridel and Lavielle isolated the crystalline glycoside, stevioside from an alcoholic extract of S. *rebaudiana* and found it to be 300 times sweeter than sucrose.[8]

Table 1. Diterpene glycosides isolated from Stevia leaves



Diterpene glycoside	R1a	R2a	Sweetening potency (sucrose=1)
Steviobioside	H	glc(2→1)glc	100-125
Rubusoside	Glc	glc	100-120
Stevioside	glc	glc(2→1)glc	150-300
Rebaudioside A	glc	glc(2→1)glc, glc(3→1)glc	250-450
Rebaudioside B	H	glc(2→1)glc, glc(3→1)glc	300-350
Rebaudioside C (dulcoside B)	glc	glc(2→1)rham, glc(3→1)glc	50-120
Rebaudioside D	glc(2→1)glc	glc(2→1)glc, glc(3→1)glc	250-450
Rebaudioside E	glc(2→1)glc	glc(2→1)glc	150-300
Dulcoside A	glc	glc(2→1)rham	50-120

a glc,-D-glucopyranosyl:rham,α-L-rhamnopyranosyl.

SWEET PROTEINS

Sweet proteins have the potential to replace the commercial artificial sweeteners, by acting as natural low calorie sweeteners because it is known that proteins do not trigger a demand for insulin whereas sugar such as sucrose and fructose do. There are seven known sweet and taste modifying proteins, namely (1) Monellin (2) Thaumatin (3) Mabinlinand (4) Curculin (5) Pentadin (6) Brazzeinand (7) Miraculin. The properties and characteristic of these proteins are illustrated in Table 2. There are several recent reviews relating to the sweet proteins. Apart from Curculin and Mabinlin, the other known sweet proteins were discovered in West Africa (Table2).

The key group on the protein surface responsible for biological activity has not yet been identified with certainty for any of the known sweet proteins. The sweet taste in man is mainly due to recently discovered T1R2-T1R3 receptor. The human T1R2-T1R3 receptor recognizes natural and synthetic sweeteners and T1R1-T1R3 recognizes umami taste.[9]

BRAZZEIN

Brazzein is a sweet-tasting protein extracted from the West African fruit of the climbing plant Oubli (*Pentadiplandrabrazzeana* Baillon). It was first isolated by the University of Wisconsin–Madison in 1994.[10]

Brazzein is the smallest, most heat-stable and pH-stable member of the set of proteins known to have intrinsic sweetness. The protein is about 6473 Dalton in size and consists of 52 amino acid residues. It is reported to be between 2000 times sweeter than sucrose in a 2% solution than a 2% sucrose solution [11, 12] and represents an excellent alternative to commercially available low calorie sweeteners.

Table 2. Compression of Thaumatin, Monellin, Mabinlin, Pentadin, Brazzein, Curculin and Miraculin

	Thaumatin	**Monellin**	**Mabinlin**	**Pentadin**	**Brazzein**	**Curculin**	**Miraculin**
Source	ThaumatococcusdanielliBenth	Dioscoreophyllum Cumminsii Diels	Capparismasakai Level	Pentadiplandra brazzeana Baillon	Pentadiplandra Brazzeana Baillon	Curculingolatifolia	Richadeladulcifica
Geographic distribution	West Africa	West Africa	China	West Africa	West Africa	Malaysia	West Africa
Varients	I, Π, a, b, c[a]	-	I-a, Π,	-	-	-	-
Sweetness factor (weight basis)	3000	3000	100	500	2000	550	-
Molecular mass (active form, kDa)	22.2	10.7	12.4	12.0[b]	6.5	24.9	98.4
Aminoacid	207	45(A chain) 50(B chain)	33(A chain) 72(B chain)	?	54	114	191
Active form	Monomer	Dimer (A+B)	Dimer (A+B)	?	Monomer	Dimer (A+A)	Tetramer (A+A+A+A+A)

Unlike most other sugar substitutes, brazzein is a protein, not a carbohydrate; In addition, brazzein has fewer calories than sugar. Also, unlike sugar, it can be eaten by diabetes without any problem. .

The amino acid sequence of brazzein was determined by peptide sequencing (Fig. 1) [13] and the three-dimensional structure of brazzein was solved by homonuclear H NMR spectroscopy.[14] The protein has a highly compact structure consisting of one short α-helix and three anti-parallel β-strands held together by four disulfide bridges.

Asp Lys Cys Lys Lys Val Tyr GluAsn Tyr Pro Val Ser Lys CysGln
1 5 10 15
Leu Ala AsnGlnCysAsn Tyr Asp Cys Lys Leu Asp Lys His Ala Arg
20 25 30
Ser Gly GluCysPhe Tyr Asp Glu Lys ArgAsn Leu GlnCys Ile Gly
35 40 45
Asp Tyr Cys Gly
50

Fig.1. Brazzein comprising 52 amino acids.

THAUMATIN

Thaumatin is a low-calorie sweetener and flavour modifier. The protein is often used primarily for its flavour-modifying properties and not exclusively as a sweetener. The Thaumatin were first found as a mixture of proteins isolated from the katemfe fruit (*Thaumato coccus daniellii* Bennett) of west Africa.[15]

Naturally occurring Thaumatin consists of six closely related proteins (I, II, III, a, b, c) all with a molecular mass of 22 kDa (207 amino acids).The protein crystallizes in a hexagonal lattice after a temperature shift from 293 to 277 K. The structure has been solved at 1.6 A° resolution. It's fold was found to be identical to that found in three other crystal forms grown in the presence of crystallizing agents of differing chemical natures. It consists of 207 amino acid residues with 8 intermolecular disulfide bonds and contains no free cysteine residues (figs. 2a, 2b).

It aggregates upon heating at pH 7.0 above 77°C, whereupon its sweetness disappears.

1 MATEFIVNRC SYTVWAAASK GDAALDAGGR QLNSGESWTI NVEPGTNGGK IWARTDCYFD

61 DSGSGICKTG DCGGLLRCKR FGRPPTTLAE FSLNQYGKDY IDISNIKGFN VPMNFSTTR

121 GCR GVRCAAD IVGQCPAKLK APGGGCNDACTVFQTSEYCCTTGKCGPTEY SRFFKRLCPD

181 AFSYVLDKPT TVTCPGSSNY RVTFCPTA

Fig. 2a. Amino acid sequence of Thaumatin I

1 MAATTCFFFL; FPFLLLLTLS RAATFEIVNR CSYTVWAAAS KGDAALDAGG RQLNGESWT

61 INVEPGTKGG KIWARTDCYF DDSGRGICRT GDCGGLLQCK RFGRPPTTLA EFSLNQYGKD

121 YIDISNIKGFNVPMDFSPTT RGCRGVRCAA DIVGQCPAKL KAPGGGCNDA CTVFQTSEYC

181 CTTGKCGPTE YSRFFKRLCP DAFSYVLDKP TTVTCPGSSN YRVTFCPTAL ELEDE

Fig. 2b. Amino acid sequence thaumatin Π

Table 3. Table of the 20 Amino acid names, Three- and one-letter Standard Abberviations, and Linear Structures

Name	Abbreviation	Linear structure
Alanine	ala A	CH3-CH(NH2)-COOH
Arginine	arg R	HN=C(NH2)-NH-(CH2)3-CH(NH2)-COOH
Asparagine	asn N	H2N-CO-CH2-CH(NH2)-COOH
Aspartic Acid	asp D	HOOC-CH2-CH(NH2)-COOH
Cysteine	cys C	HS-CH2-CH(NH2)-COOH
Glutamic Acid	glu E	COOH-(CH2)2-CH-(NH2)-COOH
Glutamine	gln Q	H2N-CO-(CH2)2-CH(NH2)-COOH
Glycine	gly G	NH2-CH2-COOH
Histidine	his H	NH-CH=N-CH=C-CH2-CH(NH2)-COOH
Isoleucine	ile I	CH3-CH2-CH(CH3)-CH(NH2)-COOH
Leucine	leu L	(CH3)2-CH-CH2-CH(NH2)-COOH
Lysine	lys K	H2N-(CH2)4-CH(NH2)-COOH
Methionine	met M	CH3-S-(CH2)2-CH(NH2)-COOH
Phenylalanine	phe F	Ph-CH2-CH(NH2)-COOH

Proline	pro P	NH-(CH2)3-CH-COOH
Serine	ser S	HO-CH2-CH(NH2)-COOH
Theronine	thr T	CH3-CH(0H)-CH(NH2)-COOH
Tryptophan	trp W	Ph-NH-CH=C-CH2-CH(NH2)-COOH
Tyrosine	tyr Y	HO-Ph-CH2-CH(NH2)-COOH
Vaniline	val V	(CH3)-CH-CH(NH2)-COOH

MONELLIN

Monellin, a sweet protein, was discovered in 1969 in the fruit of the West African shrubknown as serendipity berry (*Dioscoreophyllumcumminsii*).It was first reported ascarbohydrate.The protein was named in 1972 after the Monell Chemical SensesCenter in Philadelphia, U.S.A., where it was isolated and characterized. Monellin, a sweet protein, consists of two noncovalently associated polypeptide chains, an A chain of 44 amino acid residues and a B chain of 50 amino acid residues (figs. 3a, 3b).[16]

FREIKGYEYQ LYVYASDKLF RADISEDYKT RGRKLLRFNG PVPPP

Fig. 3a Chain A 44 amino acid residue.

GEWEIIDIGP FTQNLGKAV DEENICIGQYG RLTFNKVIRP CMKKTIYEEN

Fig .3b.Chain B 50 amino residues.

CURCULIN

Curculin which is extracted with 0.5 M sodium chloride from the fruits of *Curculigolatifolia* and purified by ammonium sulphate fractionation, CM- sepharose ion-exchange chromatography and gel filtration. The protein acts as a low calorie sweetener and has a maximum sweetness equal to 0.35M of sucrose. In addition to its sweetness, Curculin also has taste modifying abilities since water and sour substances elicit a sweet taste after consumption of Curculin. Currently, there is no other protein that has both sweet taste and taste modifying abilities.

The taste modifying activity of the protein remains unchanged when it is incubated at 50°C for 1 hour between ph 3 and 11. Curculin elicits a sweet taste. It is 20, 000 times sweeter than sucrose on a molar basis and 550 times sweeter then sucrose on a weight basis.[17]

1 KFLLTILVTF AAVASLGMAD NVLLSGQTLHADHSLQAGAYTLTIQNKCNL VKYQNGRQIW

61 ASNTDRRGSG CRLTLLSDGN LVIYDHNNND VWGSACWGDN GKYALVLQKD GRFVIYGPVL

121 WSLGPNGCRR VNGGITVAKD STEPQHEDIKMVINN

Fig.4. Amino Acid sequence of Curculin

MABINLIN

Mabinlin the sweet tasting polypeptide exists in the fruits of Chinese plant *C masaikaiaikai.* This protein is comprised of two polypeptide chains, of 33and 72 amino acids respectively, which are tightly associated through non-covalent interactions. It is about 100 times sweeter than sucrose on a weight basis. Mabinlins, as proteins, are readily soluble in water and found to be highly sweet, however Mabinlin-2 with its high heat stability has the best chance to be used as a sweetener.[18]

PENTADIN

Pentadiplandra brazzeana Ballion, a climbing shrub found in some countries of tropical Africa (such as Gabon), contain 12-kDa sweet-tasting protein, first isolated by van der Wel et al (1989). Electrophoretic studies in the presence and absence of 2-mercaptoethanol suggested that the mature protein consists of subunits coupled by disulfide bonds. The sweetness intensity was estimated to be around 500 times that of sucrose on a weight further work has been reported towards characterization of this sweet-tasting protein.[19]

MIRACULIN

The taste- modifying protein, miraculin has the unusual property of beingableto modify a sour taste into a sweet taste. *Richardella dulcifica (S*apotaceae), a shrub native to tropical West Africa, produces red berries that have an active ingredient, glycoprotein molecule with some trailing carbohydrate chains called, miraculin, a taste modifying protein that cause citric acid, ascorbic acid, and acetic acid, which are normally sour, to perceived as sweet after the berry has been held in the mouth. The maximum sweetness after exposure to 0.4 µm miraculin induced by 0.02M citric acid was estimated to be around 400000 times that of sucrose on a molar basis. The taste modifying effect lasts for usually 1-2 hr. Miracle fruit is available as freeze dried granules or in tablets –this form has a longer shelf life than fresh fruit. Tablets are made from compressed freeze dried fruit which causes the texture to be clearly visible even in tablet form.[20]

CONCLUSION

There may be a bright future for the high molecular weight sweet proteins for commercial application as food additives. They are much sweeter than sugar, and in general, are more stable to heat and lighter than the small molecular weight natural sweeteners such as stevioside and glycyrrhizin. There is still controversy about the safety of the natural sweeteners such as stevioside and rebudioside A. The FDA has allowed the sweet proteins to be available for the consumers market. They would be extremely useful as natural low calorie sweeteners for people suffering from diseases linked to consumption of sugar such as obesity, diabetes and hyperlipemia.

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