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Review Article

An Overview on Stevia: A Natural Calorie Free Sweetener

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ABSTRACT

Various sweetening agents are available in the market. Among all stevia rebaudiana is one which can be utilized by diabetics. Stevia not only imparts the sweet taste but also maintain the normal sugar level and also suitable for high blood pressure patients. Along with that the least quantity can produce sufficient sweetness because it is about 320 times sweeter than table sugar. There are over 80 species of stevia however sweetening properties have been found in Stevia rebaudiana. Due to its sweetness property it has wide range in home-made recipes, in soft drinks, in ayurvedic formulations and allied Industries. It's found calorie free, non toxic in acute toxicity studies with rats, rabbits, guinea pig.

Keywords: Stevia rebaudiana, calorie free, sweetness, non toxic.

INTRODUCTION

Sugar is one of the main ingredients in food habits of human beings. This can be satisfied by sugar cane, sugar beet and others. But these are not recommended for diabetics and calories conscious person who think twice for its consumption.

Stevia is wonderful alternative to sources & artificial sweetener for those who are diabetic. One more reason to recommend Stevia for diabetics is its advantage of safe, non calorie herbal sweetener and also nourishment to the pancreas. It does not lower the blood glucose level in normal subjects.

The leaves of the Stevia are sweeter than cane sugar having slight liquorice sensation and a good alternative for the synthetic sweetener.

Stevia was described by Dr. Bertoni, a South American Natural Scientist who compared it to saccharine, starting studies of Dr. Rebaudi on its healthfulness & ability to sweeten without providing calories¹.

It is a perennial shrub belonging to the Chrysanthemum family, which is indigenous to the northern region of South America².

SPECIES

It is estimated that there are over 80 species of Stevia which grows wild in North America & possibly as many as 200 additional species native to South America. However sweetening properties have been found in *Stevia rebaudiana* & in some species.

Leaves are opposite or upper alternate, often 3 nerved, toothed entire or sometime 3-sects. Flower-heads are white or purplish in panicles corymbs: involucre cylindrical, bract 5 or 6, receptacle flat naked, florets 5, regular, 5-cleft, tubular Achene's narrow, pappus, paleae or bristles, 2 or many. The species mentioned below are perennial herbs unless otherwise stated, succeeding in the open border in summer but needing the protection of a frame in winter.

They are best planted rather deeply in sandy soil & in some regions through the winter outdoors if the crowns are protected.

Few species of Stevia have been discussed here.

1) ***Stevia ivifolia***

About 2 ft. height, erect, Stem shaggy-hairy, corymbosely branched at top, leaves

- rhomboid-lanceolate, deeply sharply toothed, upper sessile. Flower heads white, in fastigiated corymbs; involucre & florets glandular & downy.
- 2) ***Stevia ovata***
About 2 ft. height, Stem erect, Leaves ovate, toothed, wedge shaped at base; upper oblong, sub entire, flower-heads white, in rather compact fastigiated corymbs.
 - 3) ***Stevia purpurea***
About 18 inch height, erect stem velvety hairy much branched. Leaves lanceolate, alternate, lower obovate, channeled, narrowed to stalk, toothed at apex. Flower-heads purple in some what fastigiated corymbs, involucre pale greenish.
 - 4) ***Stevia rebaudiana***
Annual herb, 1- 1½ feet height stem puberulous leaves opposite, oblanceolate, crenulate flower heads very small, whitish in a corymb Leaves have a sugary flavour. Synonym: *Eupatorium rebaudianum*.
 - 5) ***Stevia salicifolia***
Glabrous shrub, 18 in height leaves opposite, narrow, lanceolate, nearly or quite entire, very shortly stalked, almost connate. Flower-heads white in spreading corymbs.
 - 6) ***Stevia serrata***
About 18 inch height stem erect, branched, hairy leaves alternate, somewhat clustered, linear lanceolate, almost glabrous, toothed, entire at base, contracted to stalk. Flower heads white or pink, in fastigiated corymbs². The *Stevia rebaudiana* leaves measure from 2-3 inches long & upto 1 inch wide. When the plant reaches maturity, it is about 2-3 feet tall.

Structure and Sweetening Potential

The herb is 300 times sweetener than table sugar. But 100% calorie free. The fresh leaf of Stevia is itself 3-5 times sweetener than table sugar & dried leaf powder is about 30 times sweetener¹.

The chemical structure of the stevioside is depicted in fig.1 which has glucoside groups attached to a three carbon ring central structure⁴.

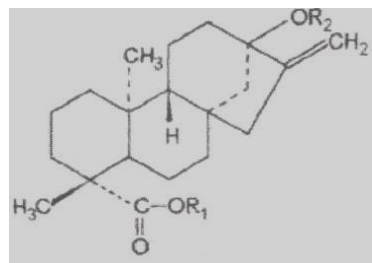


Fig : 1

R1= beta-glucose

R2 =beta -glucose-beta-glucose

Stevioside (5-10%) & Rebaudioside- A (2-4%) are the sweetest compounds besides the related compounds like Rebaudioside – (1-2%) & Dulcoside A & C as well as minor glucosides, including flavonoid glycosides, coumarins, cinnamic acids & some essential oils⁵. The sweet compound represents about 14% constituents of dried leaves & their sweeteners were similar in the structure in that a steviol aglycone connected at C-4 & C-13 to mono, di- or trisaccharides consisting of glucose & or rhamnose residues. The sweetening potencies of the main glycosides present in the leaves of stevia are furnished in table 1⁶.

Table 1: Sweetening potencies of diterpene glycosides present in the Stevia leaves

Glycosides	Percent on dry wt. basis	Percent of Total Glycosides	Sweetening Potency (sucrose=1)	Remark
1) Stevioside	5-10	60-70	250-300	After taste
2) Rebaudioside-A	2-4	30-40	350-400	No after taste
3) Rebaudioside-C	1-2	15-20	50-120	No after taste
4) Dulcoside	0.3	-----	50-120	No after taste

Stevia & its products have bitter after taste which has restricted its usage in the beverages & Other forms. Sources of bitter after taste of stevia are reported to be as under.

Presence of essential oil, tannins & flavonoids.

Presence of sesquiterpene lactones.⁷

Presence of caryophyllene & spathulend.⁸

Distribution of Glycosides

On the whole plant level, the glycoside tends to accumulate as the age advances hence the older leaves have more sweetener content than that of younger leaves. Since chloroplast are important precursors in synthesis, those tissues devoid of chlorophyll, like roots & lower stems contain no or traces of glycosides.

Once the flowering is initiated glycoside concentrations in the leaves begin to decline⁹.

One teaspoonful of dried leaves are claimed to have a sweetening value equal to one cup of sugar¹⁰. Besides being sweet, the herb also scores high on the nutrition chart. It contains beta carotene, aluminium, ascorbic acid, ash, calcium, chromium, iron and magnesium and numerous other phytochemicals¹.

Medicinal Uses of Stevia

1) Hypoglycemic action

Paraguayans say that Stevia is helpful for hypoglycemic and diabetes because it nourishes the pancreas & thereby helps to restore normal pancreatic function¹¹. In semi controlled clinical reports one also encounters this action reported a 35.2% fall in normal blood sugar levels 6-8 hours following the ingestion of Stevia leaf extract¹². Similar trends have been reported in humans and experimental animals by other works^{13, 14}.

It is important to note that Stevia does not lower blood glucose levels in normal subjects. In one study rats were fed crude extracts of Stevia leaves for 56 days at a rate at 0.5 to 1.0 gm extract per day. There procedures were replicated by another team of scientists neither groups observed a hypoglycemic action similar negative results have been obtained by observers^{15, 16, 17}.

2) Cardiovascular Action

Its use as a cardio tonic to normalize blood pressure levels, regulate heart beat, and for other cardiopulmonary indication were 1st reported in the rat studies in 1978. ^{18, 19} neither group observed a hypoglycemic action similar negative result have been obtained by other observers.

3) Antimicrobial Action

Research clearly shows that Streptococcus mutans, Pseudomonas aeruginosa, Proteus vulgaris & other microbes do not thrive in the presence of the non nutritive Stevia constituents.²⁰ This fact, combined with the naturally sweet flavour of the herb, make it a suitable ingredient for mouth washes & for tooth pastes²¹.

4) Digestive Tonic Action

In China, Stevia tea, made from either hot or cold water, is used as a low calorie sweet tasting tea, as an appetite stimulant, as a digestive aid as an aid to weight management and even for staying young. ²²

5) Effects on skin

One of the properties of liquid extract of Stevia that has not yet been investigated experimentally is it apparent ability to help clear up skin problems. The Guarani & other people who have become familiar with Stevia reported that it is effective when applied to acne, seborrhea, dermatitis, eczema, etc. Placed directly in cuts & wounds, more rapid healing, without scarring, is observed. Smoother skin, softer to touch is claimed to result from the frequent application of Stevia poultices & extracts.

Other Medicinal Uses

- As a natural sweetener
- For cavity prevention
- As a weight loss aid
- Diuretics

Uses of Stevia

- As a replacement for sugar & artificial sweeteners
- As a flavor enhancer
- As a herbal tea
- As medicinal plant
- In pharmaceutical products
- In food beverages
- In products such as chewing gum , tooth paste , mouth wash
- Blending with other sweeteners²³
- Blending Stevia ensures value addition to food products
- Breads with stevia proved to show improvement in texture, softness, & shelf life of bread²⁴.

Safety of Stevia

A test of stevioside effect on the hamsters shown no abnormalities in growth / fertility of both sexes, even when high dose of 2500 mg/kg body weight there was no abnormal effect on the growth, weight & fertility of the hamsters.

Group	Daily Dose of Stevioside	Effect on		
		Growth	Fertility	Weight
I – group	500 mg/kg	X	X	X
II – group	1000 mg/kg	X	X	X
III – group	2500 mg/kg	X	X	X
IV – group	Zero	-	-	-
		NS	NS	NS

Substitute for Sugar¹

Shredded leaves can be used as substitute for sugar in cooking. Stevia may replace sugar completely or partly depending on the sugar properties still desired in the final product. A few pharmaceutical companies are said to have evinced interest in the herb to have naturally sweet coated pills & tonics with out any sugar are syrup, which is calorific.

Experiments have shown that Stevia extracts are non-fermenting and do not contribute to the browning reacting of cooked & baked foods. It can be used in any sweet preparation viz- calorie free sweet supari, diabetic ice creams & pastries, low sugar maida & chickpea preparations, sugar free chewing gums, sugar free cola & soft drinks, to sweeten coffee & tea, to sweeten herbal & ayurvedic preparations instead of honey.

Methods of Diterpenoid Glycosides Analysis

A wide range of analytical techniques have been employed to assess the distribution & level of sweet diterpenoid glycosides in *Stevia rebaudiana*. These includes thin layer chromatography^{26, 27, 28} over pressured layer chromatography²⁹ droplet counter- current chromatography³⁰ & capillary electrophoresis.^{31, 32}

Stevioside levels have also been determined enzymatically³³ and by near infrared reflectance spectroscopy³⁴ in plant strains producing mainly stevioside. The most common analytical method however has been high performance liquid chromatography. Although separations have been also achieved using silica gel³⁵ and hydroxyapatite³⁶, hydrophilic³⁷ & size exclusion columns³⁸, amino bonded column have also been used to measure stevioside & related glycosides in food & beverages³⁹. Stevioside & rebaudioside A have also been analyzed by HPLC after conversion to the P- bromophenacyl esters of steviolbioside & rebaudioside B.⁴⁰

Other Studies Carried out on Stevia are

1. Study on the degradation of dioxins by the Stevia extract⁴¹.
2. Stevioside biosynthesis by callus, root, shoot & rooted shoot cultures *in vitro*.⁴²
3. Absorption & metabolism of glycosidic sweeteners of Stevia mixture and there aglycone, steviol in rats and humans⁴³.
4. Effects of chronic administration of *Stevia rebaudiana* on fertility in rats⁴⁴.
5. Trichomes on vegetative & reproductive organs of *Stevia rebaudiana* (Asteraceae) structure & secretory products⁴⁵.
6. Cancer – Chemopreventive effects of natural sweeteners & related compounds⁴⁶.
7. Mutagenicity of steviol & its oxidative derivatives in *Salmonella typhimurium* TM 677⁴⁷.

8. Induction of callusogenesis & organogenesis in *Stevia rebaudiana* leaf & shoot⁴⁸.
9. Chemistry & structure of diterpenes of the Kaurane series: II Reduction of isosteviol⁴⁹.
10. Biological effects of *Stevia rebaudiana* induced by carbon ion implantation⁵⁰.
11. Measurement of relative sweetness of Stevia extract, aspartame, & cyclamate / saccharine blend as compared to sucrose at different concentrations⁵¹.
12. Method of removing off flavour from sweetener extracted from *Stevia rebaudiana*⁵².
13. Examination of Steviol glycosides production by hairy root & shoot cultures of *Stevia rebaudiana*⁵³.
14. Determination of Stevioside in plant material & fruit Teas⁵⁴.

Isolation and Purification

1. Improved isolation and purification of stevioside (Steviosides was isolated from Stevia in 3 main steps, hot water extraction, decolourization by electrolysis and simultaneous decolourization and demineralization by ion exchange)⁵⁵.
2. Extraction of Stevia glycosides with CO₂ + Ethanol, and CO₂ + water + ethanol⁵⁶.
3. Extraction of sweet compounds from *Stevia rebaudiana* Bertoni. (Biochemical method)⁵⁷.
4. Extraction of sweet glycosides from *Stevia rebaudiana* by using ion exchange column⁵⁸.

CONCLUSION

Stevia rebaudiana is a perennial shrub. The glycosides in its leaves, including up to 10% stevioside account for its incredible sweetness, making it unique among the nearly 300 species of Stevia plants, it replaces sugar & artificial sweeteners. Secondly it has been used in various food products, represents empty calories in the diet. Stevia may actually help to prevent cavities. Raw herbal Stevia contains nearly one hundred identified phytonutrients & volatile oil, including trace amounts of rutin, besides this it is safe and can give better development in the industries as well as in home made dishes because it has antimicrobial action too.

REFERENCES

1. McCaleb R., Stevia leaf - Too good to be legal?, Herb research foundation,
2. Handbook of Ayurvedic Medicine & Herbal Formulations, SBP consultants & Engineers Pvt. Ltd., 2002: 179-182
3. Edited by Fred J. Chittenden, OBE; FLS; VMH, Dictionary of Gardening, The Royal Horticultural Society, Oxford Clarendon Press, 1951; 4: 2030.

4. Shock, C.C., Rebaudi's Stevia : Natural non caloric sweeteners, California Agri, 1982; 36 : 94-95.
5. Kinghorn, A.D., Biologically active compounds from plants with reputed medicinal and sweetening properties, Journal of natural products, 1987;50(6), 1009 – 1024 ,
6. Zang, S.Q., Kutowy O and Ashwani Kumar, *Stevia rebaudiana* leaves – A low calorie source of sweetners. Canadian Chemical News, 1999; 5: 22-23.
7. Soejarto DD et al, Potential Sweetening agents of plants origin II. Field search, , Economic Botany, 1983; 37 (1): 71-79.
8. Tury; Tsanova V.P., Sardzhvelade, G..P. and Kharebava, L.G, Studies on the volatile compounds of *Stevia rebaudiana*. Subtropicheskie Kul, 1989; 3: 73-77.
9. Brandle, J.E and Rosa, N., Heritability for yield leaf : stem ratio & stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*, Canadian Journal of plant science, 1992; 72(4): 1263 – 1266,.
10. Midmore, D.J. and Rank, A.H., A new rural industry – Stevia to replace imported chemical sweeteners, RIRDC web publication, project, NO UCQ – 16A, 2002; 2: 16.
11. Soejarto, D. D. et al., Econ Bot, 1983; 37-74,.
12. Oviedo, C. A. et al., “Accion Hipoglicemiant de la *Stevia rebaudiana* Bertoni (Kaa – hee)” Excerpta Medica, 1971; 208: 92-93.
13. Alveres, M. et al., Abstract Pap., Semin. Bras *Stevia rebaudiana* Bertoni 1st, 1981, I: XIII.
14. Suzuki , H. et al., Nippon Nopei Kagaku Kaishi; Influence of oral administration of stevioside on levels of blood glucose & liver glycogen of intact rats, Tokyo; 1977; 51 (3): 171-173.
15. Akashi, H. & Yokoyama, Y., Shokihin Kokyo, Dried leaf extract of stevia, , 1975; 18 (20): 34-43.
16. Hanguk; Sikpum Kwahakhoe, chi.. Lee. C..K. et al., 1979; 11: 224-6.
17. Usami, M. et al.: Horm. Metab. Res, 1980, 12: 705.
18. Boeckh, E. M..A. et al., Avaliacao- clinica do efeito cronico do edulcorante natural *Stevia rebaudiana* bartoni sobre O taste de toleranci a glucose, parametros clinicose electro cardiographicos em individuos nounais V Simposio de plantas medicinais do brasil, sept 1978; 4-6: 208.
19. Humbolt, G et al., Steviosideo : efeitos cardio - circulatorios em ratos V simposio de plantas medicinais do brazil, 1978: 6: 208.
20. Yabu, M. et al., Studies on stevioside, natural sweetener, Horishima daigaku shigaku Tasshi, 1977; 9(1): 12-17.
21. Berry, C.W. & Henry, C..A., J. Dental Res, 1981;690: 430.
22. Kinghorn, D. A, & Soejarto, D. D., Stevioside in economic and medicinal plant research, Academic press, 1991; 7: 157-171,.
23. Shock, C.C., Rebaudis stevia : Natural noncaloric sweeteners, , California agriculture 1982; 36(9): 4-5.
24. Bharathi, N., Sweeter alternative, Times Agri Journal, sept. 2003; 14.
25. Yodyingyuad, V. and Bunyawong, S., Effects of stevioside on growth & reproduction, Human Report, 1991; 6: 158-165.
26. Tanaka, O., Steviol – Glycosides: New Natural Sweetners, Trends Anal Chem 1982, 1: 246 – 248.
27. Metivier, J. Viana ,A.,M., Detemination of microgram quantities of stevioside from leaves of *Stevia rebaudiana* Bert. By two diamentional thin layer chromatography, J Exp Bot , 1979; 30: 805: 810.
28. Kinghorn, A.D, et al., A Phytochemical screening procedure for sweetener – Kaurene glycosides in the genus *Stevia*, J of Natural Product, 1984; 47: 439 – 444.
29. Fullas, F., Kim, J., Compadre, C.M. and Kinghorn, A.D., Seperation of natural product sweetening agents using overpressured layer chromatography, J Chromatogr, 464: 213-219.
30. Kinghorn, A. D., et al., Potential sweetening agents of plant origin I. Purification of *Stevia rebaudiana* sweet constituents by droplet counter current chromatography, J Chromatogr, 1982; 237: 478 – 483.
31. Liu, J, & Li, S.F.Y., Seperation and determination of *Stevia* sweeteners by capillary electrophoresis & high performance liquid chromatography, J Liq chromatogr. 1995;18: 1703 – 1719.
32. Maur, P, Catalano, G., Gardana, C. and Pietta, P., Analysis of *Stevia* glycosides by capillary electrophoresis , Electrophoresis, 1996; 17: 367 – 371
33. Mizukami, H., Shiiba, K. and Ohashi, H., Enzymatic determination of stevioside in *Stevia rebaudiana*, Phytochemistry, 1982; 21: 1927 – 1930
34. Nishiyama, P., Alvares, M. and Vieira, L.G.E. , Quantitative analysis of stevioside in the leaves of *Stevia rebaudiana* by near infrared reflectance

- spectroscopy, J sci food Agric, 1992; 59: 277- 281.
35. Nikolova, Damyanova B., Bankova, V., Popov, S., Separation & quantitation of stevioside & rebaudioside A in plant extract by normal phase high performance liquid chromatography & thin layer chromatography: a comparison, Phytochem Anal, 1994; 81-85.
 36. Kasai, R., Yamaguchi, H. and Tanaka, O., High performance liquid chromatography of glycosides on a new type of hydroxyapatite column, J chromatogr, 1987; 407: 205 – 210.
 37. Hashimoto, Y., Mariyasu, Nakamura S., Ishiguro, S. and Komuro, M., High performance liquid chromatographic determination of Stevia components on a hydrophilic packed column, J Chromatogr, 1978; 161: 403 – 405
 38. Ahmed, M.S. and Dobberstein, R.H., *Stevia rebaudiana* II High performance liquid chromatographic separation and quantitation of stevioside, rebaudioside A and rebaudioside C, J Chromatogr, 1982; 236: 523 – 526.
 39. Change, S.S. and Cook, J.M., Stability studies of stevioside & rebaudioside A in carbonated beverages, J Agric Food chem., 1983; 31: 409 – 412.
 40. Ahmed, M.S., Dobberstein, R.H. and Farnsworth, N.R., *Stevia rebaudiana* I Use of p-Bromophenacyl. Bromide to enhance ultraviolet detection of water soluble organic acids (stevioside and rebaudioside B) in high performance liquid chromatographic analysis, J Chromatography, 1980; 192: 387 – 39.
 41. Kim Byoung, et al, (Environmental and energy research centre, research institute of industrial science and technology, Pohang, 790 – 330, S.Korea) Study on the degradation of dioxins by the stevia extract, Organohalogen Compounds, 54 (Dioxin 2001), 2001: 251 -254.
 42. Swanson, et al, (Coll Pharm, Uni Illinois, Chicago, IL 60612, USA). Stevioside biosynthesis by callus, root, shoot & rooted shoot cultures in vitro, Plant Cell Tissue Organ Cult, 1992; 28 (2): 151 – 157.
 43. Koyama, Eriko., Sakai Norifumi, Ohori – Yuji, Kitazawa Ken, Izawa Osamu, Kakegawa Kunia, Fujino Akiharu, Ui Michio, Absorption & metabolism of glycosidic sweeteners of Stevia mixture and their aglycone, steviol in rats and humans, Food and Chemical Toxicology, June 2003; 41(6): 875 – 883
 44. Melis, M.S., Effects of chronic administration of *Stevia rebaudiana* on fertility in rats, Journal of Ethnopharmacology, Nov 1, 1999; 67 (2): 157 – 161.
 45. Cornara, L., Bonon, M., Tateo, F., Serrato, Valenti G., Mariotti, M.G., Trichomes on vegetative & reproductive organs of *Stevia rebaudiana* (Asteraceae) structure & secretory products, Plant Biosystems, 2001; 135 (1): 25-37.
 46. Konishima Takio, Takasaki Midari (Kyoto Pharmaceutical University, Kyoto 607 – 8414, Japan), Cancer – Chemopreventive effects of natural sweeteners & related compounds, Pure and Applied Chemistry, 2002; 74 (7): 1309 – 1316.
 47. Terai Tadamasu, Ren Huifeng, Mori, G.O., Yamaguchi Yoshihito, Hayashi Tetsuhito (Department of Applied Chemistry, Osaka Institute of Technology, Osaka 535 – 8585, Japan), Mutagenicity of steviol & its oxidative derivatives in Salmonella typhimurium TM 677, Chemical & pharmaceutical Bulletin, 2002; 50(7): 1007 – 1010
 48. Gamkrelidze, K.H., Zarnadzen, Gogoberidze M., Chelidzen (S. Durmishidze institute of biochemistry & bio technology, Georgian academy of science, GA USA), Induction of callusogenesis & organogenesis in *Stevia rebaudiana* leaf & shoot, Bulletin of Georgian academy Sciences, 2001; 164 (1): 147-150.
 49. Al' Fonsov, V.A., Bakaleinik, G.A., Gubaidullin, A.T; Kataev, V.E., Kovylyayeva, G.I., Kononov, A.I., Litvinov, I.A., Strobyskin, I. Yu., Styrobyskin, S.I, Litvinov I.A., Andreeva, D.V., Korochkina M.G (Arbuzov institute of organic and physical chemistry Kazan Research Centre, Russian Academy of Sciences, Kazan, Russia), Chemistry & structure of diterpenes of the Kaurane series II Reduction of isosteviol, Russian Journal of General Chemistry, 2000; 17(6): 953-960.
 50. Shenming Shan, Jiang Xian Zhi, Jin Sen, Chen Liang, Chen Mu Chuan (The Key Lab of Ministry of Education for Cell Biology & Tumour Cell Engineering, Xiamen University, Xiamen 361005, Peop. Rep. China), Biological effects of *Stevia rebaudiana* induced by carbon ion implantation, Zhiwu Xuebao, 2000; 42(9): 892 -897.
 51. Cardello, H.M.A.B, D' Silva M.A.P.A, Damasio M.H., (Department of Food and Nutrition, CP 500-FCF – UNESP,

- Araraquara CEP 14801 – 902, Brazil), Measurement of relative sweetness of Stevia extract, aspartame, & cyclamate / saccharine blend as compared to sucrose at different concentrations, *Plant Foods Hum. Nutr.*, 1999; 54(2): 119-130.
52. Payzant, John Donald, Laidler James Kenneth, Ippolito Robert Maurice (Alberta Research Council, Can.), Method of removing off flavour from sweetener extracted from *Stevia rebaudiana*, Can.Pat. Appl. CA 2185510 AA, 14 Mar 1998: 6.
 53. Yamazaki Takeshi, Flores Hector E, Shimomura Koichiro, Yoshihira Kunitoshi (Dep. Plant Pathol., Pennsylvania State Uni. University Park, PA 16802, USA), Examination of Steviol glycosides production by hairy root & shoot cultures of *Stevia rebaudiana*, *J Nat. Product*, 1991; 54(4): 986-992.
 54. Vanek Tomas, Nepovim Ales, Valicek Pavel (Institute of Organic Chemistry & Biochemistry), Determination of Stevioside in plant material & fruit Teas, *Academy of Science of Food composition and Analysis*, 2001; 14(4): 383 – 388.
 55. Adduci John, Buddhasukh Duang, Ternai Bela (Dep. Chem., La Trobe Univ. Bundoo 3083, Australia), Improved isolation and purification of stevioside, *J. Sci. Soc. Thailand*, 1987; 13(3): 179 –183.
 56. Pasquel, A., Meireles, M.A.A., Marques ,M.O.M., Petenate, A.J., (LASEFI, Departamento de Engenharia de Alimentos (FEA), Extraction of Stevia glycosides with CO₂ + Ethanol , and CO₂ + water + ethanol, Unicap, Campinas 13083 – 970, Brazil) *Braz. J. Chem. Eng.*, 2001; 17(3): 271-282.
 57. Kutowy Oleh, Zhang Shi Qui, Kumar Ashwini (National Research Council of Canada, Can.), Extraction of sweet compounds from *Stevia rebaudiana* Bertoni, US. US 5972120 A, 26 Oct 1999: 7.
 58. Payzant John Donald, Laidler James Kenneth, Ippo Lito Robert Mauric (Alberta Research Council , Can.), Extraction of sweet glycosides from *Stevia rebaudiana* by using ion exchange column, US US5962678A, 5 Oct 1999: 6.