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Agro-production, Processing and Utilization of *Stevia rebaudiana* as Natural Sweetener

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Abstract: ***Leaves of Stevia rebaudiana contains diterpene glycosides which taste sweet but with zero calories in food products. Stevia has been used by native Guarani Indians of South America for centuries as sweetener to counteract the bitter taste of various medicines and beverages. Diterpene glycosides produced by this plant are 100–300 times sweeter than sucrose and are now used as sweeteners to replace sugar in foods and beverages. They remain stable at wide range of temperature and pH conditions in food products during processing and offer no shelf life limitations. These glycosides do not induce glycemic response when ingested, making them suitable for diabetics and obese persons. India has huge demand potentials for this natural sweetener because of increasing diabetic and obese population. In this article effort was made to discuss briefly about plant profile and basic techniques of cultivation, harvesting, drying and extraction of steviol glycosides. Further, this article represents an effort to compile information on safety issues and approval made by regulatory agencies regarding utilization of steviol glycosides in food products around the world.***

Keywords: Stevia; Steviol glycosides; Natural sweeteners; Extraction and Safety issues

1. INTRODUCTION

Stevia rebaudiana (Bert.) Bertoni as a natural sweetener with zero calories has recently found widespread use in the food and pharmaceutical industries. Historically, *Stevia* is an endemic herb grown by people of Rio Monday in the highlands of Paraguay and the Brazilian border for use as a sweetner and herbal remedy [1]. It is the sweetest gift from mother nature called as sweet leaf of Paraguay, sweet herb, honey leaf, honey yerba, candy leaf etc [2]. The leaves of this sweet herb have been used for centuries as sweetener to counteract the bitter taste of various plant based medicines and beverages by indigenous Guarani Indians of the Paraguayan highlands [3].

Leaves of *Stevia* accumulate sweet tasting diterpene glycosides such as stevioside and rebaudiosides which are upto 100-300 times sweeter than sucrose [4-5]. Shade dried leaves of *Stevia* are 10 to 15 times sweeter than sucrose. Glycemic index of sweetening compounds of this plant is zero with no caloric value [6]. The worldwide demand for zero calorie natural sweeteners is increasing because of consumer awarness about harmful effects of artificial sweeteners on human health [7]. Most of the artificial sweeteners are produced from synthetic ingredients by chemical synthesis in the laboratory. In last few decades, consumption of food products enriched with sugars and artificial sweeteners has favored the development of various chronic diseases endangering the human health. Obesity and diabetes are the main characteristic diseases developed due to excessive intake of sugars having high calorific values [8]. Steviodal glycosides from *Stevia* offer a solution for prevention of complex diabetic problems and obesity in modern mankind. Diterpene glycoside does not have mutagenic, teratogenic, and carcinogenic effects [9-10]. Stevioside and rebaudioside of *Stevia* are stable under wide range of temperatures and pH conditions in different food and pharmaceutical products [11-12]. They do not alter the flavour and taste of a food product in which they are used and are also non-fermentative.

Stevia rebaudiana is commercially cultivated in China, Japan, Brazil, Canada, USA, UK, Spain, Belgium, Australia, South Korea, Thailand, Israel and Taiwan [13-14]. China and Japan are the world's major producers and exporters of diterpene glycosides. Japan has approved use of stevioside in many food products including cereals, teas, and soft drinks. In India, *Stevia* has been introduced in the last decade because of high demand potentials particularly considering the huge diabetic population. It has been successfully cultivated in many Indian states like Rajasthan, Maharashtra, Punjab, Kerala and Orissa. High demands for natural sweeteners as compared to artificial ones have driven the farmers in India toward large-scale *Stevia* cultivation [15]. In this article we discussed about some important aspects of Stevia cultivation, production and utilization of zero calorie natural sweeteners.

2. PLANT PROFILE

Stevia rebaudiana is a subtropical perennial herb, belonging to family *Asteraceae*. It has annual, subligneous, more or less pubescent stems with extensive, fibrous and filiform root system [1]. The cultivated *Stevia* plant grows vigorously

giving branched bushy shrub like appearance [16]. It grows up to 60-70 cm in height and bears sessile, oppositely arranged lanceolate to oblancoelate leaves with blunt-tipped lamina having serrate margin from the middle to the tip (Fig. 1A). The upper surface of the leaf lamina is slightly glandular. Plant bears small (10-15 mm) white colour pentamerous flowers (Fig. 1B) in capitulum surrounded by green colour involucral bracts. The capitula are arranged in irregular or sympodial cymes. Seed of *Stevia* is a five-ribbed spindle-shaped achene with feathery pappus (Fig. 1C). Plant is diploid and has 11 chromosome pairs [17].

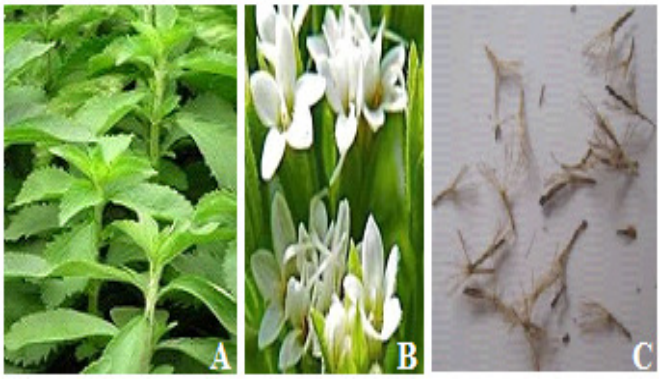

Figure 1: A) *Stevia rebaudiana* growing in field, B) Flowers and C) Seeds

Taxonomical Position	
Kingdom	: Angiospermae
Class	: Dicotyledons
Group	: Monochlamydae
Order	: Asterales
Family	: Asteraceae
Subfamily	: Asteroideae
Tribe	: Eupatorieae
Genus	: *Stevia*
Species	: *rebaudiana*

3. CULTIVATION

In 1964 this crop was cultivated commercially for the first time in Paraguay and later on it has been introduced in a number of countries [18]. *Stevia rebaudiana* is now successfully growing under different cultivation conditions and climatic locations of the world. Seeds produced by *Stevia* plants remain viable for a limited period and have very low germination rate because of their small size. Being highly heterozygous species, there is also variation in plants raised from seeds. They do not produce true to type plant and constant re-selection for type is required in mother-seed plots [19].

Stevia is mainly propagated vegetatively by stem cuttings although it is a labour intensive procedure. Careful selection of plant is required to get more productivity and profitability. Cutting should be obtained from a plant variety which have reduced tendency to flower. Leaves of plant variety selected for propagation should be high in Rebaudioside-A and low in Stevioside associated with bitter after taste. Stem cuttings of selected plant variety should be 3-4 inches long with atleast one or two buds arising from leaf axils. Rooting can be enhanced by using rooting hormones. Treatment of cuttings with synthetic auxins was found beneficial for root formation by *Stevia* growers.

Stevia prefers a well-drained fertile sandy loam or loam soil high in organic matter. It prefers lighter acidic to neutral (pH 6-7) soil for better growth**.** It requires a consistent supply of water but excessive irrigation in water logging soils can cause stem rot disease. *Stevia* requires partial shade during very hot and long summer days. Photoperiod is more critical than intensity of light for *Stevia* cultivation. Long spring and summer days favour leaf growth and short days trigger blossoming in *Stevia* plant. Temperature in the range of 24 to 35 degrees with appropriate soil moisture is required during first two weeks to obtain plantlets from stem cuttings. Initial growth from cuttings is extremely slow and requires good nursery hygiene to prevent disease infection. *Stevia* plants produce two to three shoots depending on the number of buds available on stem segments. These shoots then produce multiple shoots, which is essential for production of good number of leaves for harvest. 20 to 35^{o}C temperature are required for proliferation of shoots from cuttings after field transfer. High temperature and water stress is unfavorable for vegetative growth as it induces flowering during the expected growing season.

Fertilizer requirement for *Stevia* is moderate and varies according to the environment and soil type. *Stevia* plants respond well to fertilizers. Plant prefers low levels of nitrogen but high phosphorus and potassium. Under average climatic conditions and soil type 70 kg Nitrogen, 35 kg Phosphorus and 45 kg potassium per hectare is recommended. The distance between plants should be 20-25 cm. This would give a plant population of around 28 to 30 thousand per acre. Katayama *et al* [20] have tried planting densities ranging from 40000 to 400000 plants per hectare in experiments conducted at Japan. They found that leaf yield increases with increasing planting density up to 83,000 and 111,000 plants per hectare. For *Stevia* cultivation, land should be initially harrowed and then ploughed to get fairly smooth and firm planting surface. Fields should be divided into plots of convenient size for proper drainage and irrigation Formation of raised beds is the most economical way to grow *Stevia* plants as it saves water and avoid the damages caused due to water logging. The raised beds should be of 15 cm in height and 60 cm in width.

4. HARVESTING

Leaves of *Stevia* plants are ready for first harvesting after four months of planting and subsequent harvesting can be done after every 3 to 4 months. On an average one can get three to four commercial harvests in a year depending upon land type, variety and climatic conditions. Young actively growing shoot sections and leaves have high glycoside content and over-matured leaves showing chlorosis have less glycoside content [1]. Shoot tips can also be clipped off along with leaves while harvesting as they contain same amount of diterpene glycosides as do the leaves. The total glycoside content of the leaves start decreasing with onset of flowering so harvesting should be done before the onset of flowering or immediately after flower bud formation.

5. DRYING

Drying is the important activity in post-harvest handling of *Stevia*. Freshly harvested leaves of *Stevia* contain high moisture content and deteriorate if not dried properly. Drying of leaves should be completed immediately after harvesting by placing leaves on a screen or net. Sun drying is the most preferred method as *Stevia* leaves can be quickly dried in moderately warm conditions in about 12 hours. Proper aeration with low density of loading is required for quick drying in full sun. It can also be dried in a simple dryer by passing hot air just above room temperature. Samsudin and Aziz [21] observed that quality of leaves dried at 50°C temperatures in hot air dryer for 6 hours was better in terms of colour, sweetness and nutrient content. Dried leaves with 3-5% moisture content should be packed in air tight container and stored in cool and dry place.

6. EXTRACTION OF STEVIOL GLYCOSIDES

Extraction of steviol glycosides from leaves involve many conventional processes and long purification procedures. It is somewhat similar to the extraction process used in sugar mill. Extraction of glycosides from leaves of *Stevia* involves aqueous or solvent extraction, ion exchange purification, precipitation, filtration, crystallization and drying [22]. Extraction protocol involves dissolving leaves in hot water or alcohols. Some authors have treated leaves with non polar solvents such as chloroform to remove oils, lipids, chlorophyll and other non-polar substances. Afandi *et al* [23] revealed that methanol is the best solvent for the extraction of Rebaudioside-A from *Stevia* leaves in terms of component yield. In their study ethanol and aqueous acetone were also found suitable to extract Rebaudioside-A, but yield was less compared to methanol. Extract was then clarified by precipitation with salt or alkaline solutions, concentrated and redissolved in methanol for crystallization of the glycosides.

The common steps involved in the extraction procedure as described by Rank and Midmore [19] are: soaking the leaves in warm water to dissolve the glycosides, precipitation and filteration of the resultant solution, concentration by evaporation, ion exchange purification, spray drying and crystallization to produce white powder/crystals. Rao *et* al [24] used ultra and nano-filtration membrane to developed simple, inexpensive and eco-friendly process for isolation of steviol glycosides with improved taste profile of the final product. Most of the commercial processing of *Stevia* leaves for the extraction of steviol glycosides is mainly concentrated in China and Japan where factories are located near cultivated areas.

7. SAFETY ASPECTS AND USES

Many biological and toxicological investigations were carried out on steviol compounds of *Stevia* in last 50 years. European Commission's Scientific Committee on Foods (SCF) evaluated safety related issues of this natural sweetener in 1985 and 1999 and raised questions about lack of acceptable purity specifications. In 2004, Joint FAO/WHO Expert Committee on Food Additives (JECFA) established tentative purity specifications which were later made permanent. JECFA have established Acceptable Daily Intake (ADI) of 4 mg/kg bodyweight/day for purified steviol glycosides in 2008 and validated its use as a sweetner in food and beverages.

European Food Safety Authority (EFSA) in 2010 also gave ADI in consistent with that of JECFA and concluded that diterpene glycosides of *Stevia* are safe. Steviol glycosides (E960) have recently been added to the European Union (EU) list of permitted sweeteners. Following approvals by the United States Food and Drug Administration (USFDA) and the European Union (EU), Food Safety and Standards Authority of India (FSSAI) has also recently recommended use of *Stevia* as sweetener in selected food products with ADI limits. Large numbers of studies on these glycosides are available in scientific literature related to safety aspects and most of them supported the validation of regulatory agencies around the world. World Health Organisation has estimated that *Stevia* would replace about 20% of the sugar market. *Stevia* products and extracts are now used in a variety of food products. The steviol glycosides obtained from *Stevia* leaves are used as an alternative to sugars in variety of foods and beverages. *Stevia* sweeteners can also be used in combination with other sweeteners (e.g., sugar, fructose etc) as they act as flavour enhancer in food products. They remain stable in food processes such as extrusion, pasteurisation, baking, canning etc with no shelf life limitations.

Steviol glycoside reduces dental problems as they are free of calories and mild on the teeth. They are especially suitable for diabetics as they do not affect blood sugar levels. FSSAI has recently approved use of steviol glycoside as a non-calorific sweetener in following 11 food items:

- Dairy based flavoured drinks

- Dairy based desserts
- Yoghurt
- Fruit nectars
- Non carbonated water based beverages
- Ice Lollies/Edible Ice
- Jams, jellies and marmalades
- Ready to eat cereals
- Carbonated water
- Soft Drink Concentrate
- Chewing gum

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