

New approach to the application of ionizing radiation on vegetal products: the stevia case

Nova abordagem da aplicação da radiação ionizante em vegetais: o caso de Stevia

Nuevo enfoque para la aplicación de radiaciones ionizantes en productos vegetales: el caso de la estevia

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ABSTRACT

The application of irradiation in food treatment aims to prevent foodborne illnesses, inactivate spoilage-causing organisms, and control or destroy insect pests. Food products can also be sterilized through exposure to substantially higher radiation levels (<https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know>). Irradiation thus serves primarily as a safety measure for both the food and pharmaceutical industries. At the same time, growing evidence highlights the role of irradiation in enhancing plant bioactive components. This effect, once considered a secondary outcome, may become commercially significant, complementing pathogen control. While high doses of ionizing radiation are harmful to plant growth, lower or moderate doses can act as physical elicitors, triggering stress responses that boost protective compounds such as antioxidants and secondary metabolites. This mechanism is applied in mutation breeding to develop new varieties with improved traits, including increased nutritional value. Irradiation can raise levels of certain plant biocomponents, such as phenolic compounds and essential oils, with effects depending mainly on dose and plant species. Among several examples, stevia—a natural zero-calorie sweetener derived from the *Stevia rebaudiana* Bertoni plant—stands out for its rich profile of bioactive compounds, particularly steviol glycosides. This article discusses the current and potential perspectives of stevia irradiation.

Keywords: ionizing radiation, stevia, *Stevia rebaudiana* Bertoni, steviol glycosides.

RESUMO

A aplicação da irradiação no tratamento de alimentos visa prevenir doenças transmitidas por alimentos, inativar organismos que causam deterioração e controlar ou destruir pragas de insetos. Os produtos alimentícios também podem ser esterilizados através da exposição a níveis de radiação substancialmente mais altos (<https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know>). A irradiação, portanto, serve

principalmente como uma medida de segurança para as indústrias alimentícia e farmacêutica. Ao mesmo tempo, evidências crescentes destacam o papel da irradiação no aumento de componentes bioativos de plantas. Este efeito, antes considerado um resultado secundário, pode se tornar comercialmente significativo, complementando o controle de patógenos. Embora altas doses de radiação ionizante sejam prejudiciais ao crescimento das plantas, doses mais baixas ou moderadas podem atuar como elicitores físicos, desencadeando respostas de estresse que impulsionam compostos protetores, como antioxidantes e metabólitos secundários. Este mecanismo é aplicado no melhoramento por mutação para desenvolver novas variedades com características aprimoradas, incluindo maior valor nutricional. A irradiação pode aumentar os níveis de certos biocomponentes vegetais, como compostos fenólicos e óleos essenciais, com efeitos dependendo principalmente da dose e da espécie vegetal. Entre vários exemplos, a estévia — um adoçante natural de zero caloria derivado da planta *Stevia rebaudiana* Bertoni — destaca-se por seu rico perfil de compostos bioativos, particularmente glicosídeos de esteviol. Este artigo discute as perspectivas atuais e potenciais da irradiação da estévia.

Palavras-chave: radiación ionizante, stevia, *Stevia rebaudiana* Bertoni, steviol glycosides.

RESUMEN

La aplicación de la irradiación en el tratamiento de alimentos tiene como objetivo prevenir las enfermedades transmitidas por los alimentos, inactivar los organismos que causan el deterioro y controlar o destruir las plagas de insectos. Los productos alimenticios también pueden esterilizarse mediante la exposición a niveles de radiación sustancialmente más altos (<https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know>). Por lo tanto, la irradiación sirve principalmente como una medida de seguridad tanto para la industria alimentaria como para la farmacéutica. Al mismo tiempo, cada vez hay más pruebas que destacan el papel de la irradiación en la mejora de los componentes bioactivos de las plantas. Este efecto, que antes se consideraba un resultado secundario, puede llegar a ser comercialmente significativo, complementando el control de patógenos. Si bien las altas dosis de radiación ionizante son perjudiciales para el crecimiento de las plantas, las dosis bajas o moderadas pueden actuar como elicitores físicos, desencadenando respuestas de estrés que potencian los compuestos protectores, como los antioxidantes y los metabolitos secundarios. Este mecanismo se aplica en el fitomejoramiento por mutación para desarrollar nuevas variedades con rasgos mejorados, incluido un mayor valor nutricional. La irradiación puede elevar los niveles de ciertos biocomponentes vegetales, como los compuestos fenólicos y los aceites esenciales, con efectos que dependen principalmente de la dosis y de la especie vegetal. Entre varios ejemplos, la estevia —un edulcorante natural sin calorías derivado de la planta *Stevia rebaudiana* Bertoni— destaca por su rico perfil de compuestos bioactivos, en particular los glucósidos de esteviol. Este artículo analiza las perspectivas actuales y potenciales de la irradiación de la estevia.

Palabras clave: radiación ionizante, estevia, *Stevia rebaudiana* Bertoni, glucósidos de esteviol.

1 INTRODUCTION

Ionizing irradiation is a non-thermal processing technology that has been widely used to improve microbial safety and reduce food loss [1] (Fan and Niemira, 2020). Physiological responses to ionizing radiation have been described across a wide range of plant species (Wi *et al.*, 2007). Ionizing radiation can damage DNA directly or indirectly by generating reactive oxygen species (ROS), which induce oxidative stress (Sies and Jones, 2020). Phyto-genetic improvement through induced mutation accelerates the natural mutation process, enabling the development of new plant varieties with enhanced quality, higher yield, and greater resilience to climate change. Irradiation can influence cell differentiation and growth *in vitro*, thereby facilitating mutation breeding; low doses of gamma irradiation are among the most widely used physical methods for this purpose (Kovacs and Keresztes, 2002). Several authors [(Moghaddam *et al.*, 2011; Hasbullah *et al.*, 2012; Ognyanov *et al.*, 2022) provide evidence that radiation doses applied to seeds (up to 300 Gy) can be used to mitigate abiotic stress, enhancing tolerance to salinity or drought stress through mechanisms related to the ROS-scavenging system (Katiyar *et al.*, 2022; Hadipanah, 2025).

The food and pharmaceutical industries decontaminate raw materials using high doses of ionizing irradiation, as radiation can eliminate microbial contamination (Pereira, *et al.*, 2015; Balakrishnan *et al.*, 2022; Iqbal *et al.*, 2016). High-dose radiation can disrupt the structure of lignocellulose and enhance the effectiveness of enzymatic hydrolysis, accelerating the dissolution of water-soluble carbohydrates. Observed plant responses to irradiation include both stimulation and inhibition of germination and seedling growth, as well as other physiological changes. For example, irradiation at 4 kGy has been associated with increased total phenolic content in almond skin (Harrison *et al.*, 2007; Pereira *et al.*, 2015). In plant tissues, some authors attribute radiation-induced increases in phenolics to the radiolytic degradation of tannins (Variyar *et al.*, 1998). In this article, we focus on the effects of irradiation on stevia (*Stevia rebaudiana* Bertoni) with implications for improved yields of key bioactive constituents.

2 STEVIA: STRUCTURE, PROPERTIES, METABOLISM, EXTRACTION OF BIOCOMPONENTS, GROWTH, AND IMPORTANCE FOR THE FOOD INDUSTRY

Stevia leaves have been used for more than 1,500 years by the Guarani people. Although endemic to South American regions with humid, wet environments—mainly Paraguay—stevia is now grown in many countries, including China, Japan, Taiwan, the USA, Canada, Paraguay, Brazil, Argentina, Peru, Colombia, and Mexico (Jarma-Orozco *et al.*, 2022; Arcos-López *et al.*, 2023). An image of stevia leaves is presented in Figure 1.

Figure 1. Stevia leaves and extract



Source: royalty-free image from the web.

There are reports that stevia was already known in Spain in the 16th century. However, Europeans learned about the plant only in the late 19th century, after stevia was introduced and promoted by the botanist Moisés Santiago Bertoni, who learned of stevia from indigenous guides while exploring Paraguay's eastern forests.

In 1905, stevia's sweetening qualities were first described in a botanical journal by Bertoni: "A fragment of the leaf only a few square millimeters in size suffices to keep the mouth sweet for an hour; a few small leaves are sufficient to sweeten a strong cup of coffee or tea." Bertoni renamed it from *Eupatorium rebaudianum* to *Stevia rebaudiana* (Carakostas *et al.*, 2008). In 1931, French chemists Bridel and Lavieille isolated the glycosides that give stevia its sweet taste. These compounds were named stevioside and rebaudioside A (Barriocanal *et al.*, 2008). During World War II, stevia began to be actively consumed in the United Kingdom due to sugar shortages and rationing (Peteliuk

et al., 2021). The name "stevia" is used to refer both to the stevia plant (*Stevia rebaudiana* Bertoni) and to the sweet compounds extracted from its leaves. The commercially available sweetener is often referred to as "stevia" or, more specifically, as high-purity stevia leaf extract, which is highly attractive to consumers due to its naturalness (Saraiva *et al.*, 2020).

Stevia is a perennial flowering plant that belongs to the Asteraceae family (also known as the daisy or sunflower family) and thrives in tropical and subtropical climates but is usually grown as an annual in cooler climates (Evans *et al.*, 2015). It is known for its sweet-tasting leaves. The classification places it among a large group of herbs and shrubs native to tropical and subtropical regions of the Americas. When extracts of its leaves are processed into a powder, stevia is used as a sugar substitute in most developed countries. Stevia is commercially valuable for its steviol glycoside content, which has been discovered in only a few plant species (Ceunen and Geuns, 2013). Natural constituents of the plant include ent-kaurene diterpene glycosides—stevioside, rebaudiosides A, B, C, D, and E, dulcoside A, and steviolbioside. The foremost sweet-tasting, non-toxic tetracyclic diterpene steviol glycosides of particular interest are stevioside and rebaudioside A.

The backbone of all steviol glycosides is steviol, to which various glycoside or glucose groups attach to form the variety of sweet compounds in stevia. The structure of steviol glycosides consists of a naturally produced diterpenoid: a diterpene ent-kaurene skeleton linked to a number of glucose units. Steviol and isosteviol, tetracyclic diterpenoids obtained by hydrolysis of stevioside, also exhibit extensive pharmacological activities (Yang *et al.*, 2024). Out of approximately 230 species in the North and South American genus *Stevia*, *S. rebaudiana* has been found to produce these sweet-tasting steviol glycosides at high concentration levels; stevioside (triglucosylated steviol) is the most predominant ent-kaurene-type diterpene glycoside, constituting approximately 3–8% of dried leaves. It was first isolated in the first decade of the twentieth century in impure form (Gupta *et al.*, 2016). Although stevioside and rebaudioside A are the most abundant and best analyzed, more than 30 additional steviol glycosides have been described in the scientific literature (Wölwer-Rieck, 2012). The stevia plant is a good source of vitamins and is rich in a number of minerals, including K, Ca, Na, Mg, Cu, Mn, Fe, and Zn, as well as essential amino acids, fatty acids, and other health-beneficial bioactive compounds including non-glycosidic labdane diterpenes, flavonoids, phenolic compounds, crude fiber, phytosterols, chlorogenic acids, triterpenes, and hydrocarbons.

Stevia is used mainly as food or food additive but is also employed as a solubilizing or foaming agent, among other uses (Ahmad *et al.*, 2020; Myint *et al.*, 2020).

The extraction of steviol glycosides from stevia leaves involves many process steps, often including extraction by organic solvents. However, other alternatives have also been reported, such as ultrasound-assisted extraction (Covarrubias-Cárdenas *et al.*, 2018). Pressurized Hot Water Extraction (PHWE) was first optimized with Response Surface Methodology in terms of maximizing rebaudioside-A yield and minimizing color components. PHWE was then combined with pressing in a wine press, resulting in higher efficiency for extracting steviol glycosides compared to reported methods in the literature [37](Nemeth and Janosi, 2019). Enzyme-assisted extraction of bioactive compounds from plant sources, particularly for food and nutraceutical purposes—such as enzyme-assisted extraction of stevioside from *Stevia rebaudiana*—has been considered an example of a process of potential value to the food industry (Puri *et al.*, 2012).

No uptake of stevia or any component or by-product has been found in the gastrointestinal tract, and steviol glycosides pass through the upper gastrointestinal tract fully intact. Gut bacteria in the colon hydrolyze steviol glycosides into steviol (the only metabolite found in feces) by cleaving off their glucose units. Steviol is absorbed via the portal vein and primarily metabolized by the liver, forming steviol glucuronide—steviol glucuronide (SV glu) was found at maximum concentrations of 33 $\mu\text{g/mL}$ (21.3 μg steviol equivalents/mL)—which is primarily excreted in the urine. Energy from fermentation of glucose units (usually assessed as 2 kcal/g) is so low that it is minimal; thus, effectively, stevia can be said to provide zero kilojoules. High-purity stevia leaf extract is not metabolized, so it provides zero kilojoules (Geuns *et al.*, 2007).

Besides sweet-tasting chemicals known as steviol glycosides, stevia contains several bioactive compounds showing strong antioxidant potential, anti-inflammatory, antiproliferative, antimicrobial, and hepatoprotective activities owing to the presence of various compounds with medicinal significance, such as phenolic compounds, flavonoids, diterpene glycosides, condensed tannins, anthocyanins, and phenolic acids. Since inflammation and oxidative stress play critical roles in the pathogenesis of many diseases, stevia emerges as a promising natural product that could support human health (Ameer *et al.*, 2020; Orellana-Paucar, 2023).

As stevia originates from a subtropical climate, it is sometimes difficult to acclimatize it in different places. Factors that govern and control vegetal developmental processes are both intrinsic and extrinsic to the plant [43] (Witzany, 2025). Lighting

conditions are an important controller of plant growth and development, and they affect secondary metabolite synthesis. High solar radiation and air temperature are the main influencing factors for plant growth and development in tropical climates. Therefore, it is necessary to use adaptive strategies for growing *Stevia rebaudiana* in different environments.

Stevia cultivated in medium-altitude lands shows good adaptability with a high photosynthesis rate, percentage of open stomata of 93.15%, leaf thickness of 382.56 μm , and xylem diameter of 30.97 μm , which is no different from the highlands. Thus, medium-altitude lands have the potential as new areas for *stevia* development in tropical climates, considering the increasingly competitive use of agricultural land in the highlands. Semenova *et al.* (2024) explored the effect of additional UV irradiation on flavonoid and steviol glycoside accumulation in *Stevia rebaudianaplants*. The total flavonoid content was significantly higher (by 74%) under UV-B irradiation. The highest concentration of steviol glycoside was observed during budding and flowering under UV-B and UV-C irradiation (by 13.2% and 11.3%, respectively).

Maximal content of steviol glycosides in *stevia* is achieved at or just before flowering, and delayed flowering with long days provides longer duration for steviol glycoside accumulation.

Daily light influences steviol glycoside biosynthesis and the relative abundance of specific glycosides in *stevia*, as already mentioned. Germination from seed is difficult, and most plants are grown from cuttings. In field conditions, the growth, flowering, and quality of sweet glycosides of the *stevia* crop are affected by solar radiation, day length, temperature, soil moisture, and wind. In vitro and under greenhouse conditions, *stevia* is also affected by nitrogen source, mannitol, and glutamine concentration. As it thrives in warm, humid climates, the plant requires rich, well-drained soil, with solar radiation and air temperature being the main influencing factors for plant growth and development in tropical climates. Gomes *et al.* (2018) studied the effects of plant densities and harvesting times on productive and physiological aspects of *Stevia rebaudiana*Bertoni grown in southern Brazil and concluded that harvesting at the beginning of flowering promoted higher dry leaf biomass yield and, when associated with the lowest planting density, promoted higher levels of rebaudioside A and stevioside. The lowest planting density resulted in greater leaf biomass accumulation, whereas the highest density promoted higher yields per area.

Differences in altitude generate differences in temperature and humidity. In research conducted in Malang, East Java, stevia was exposed to temperatures above 20°C during growth in the highlands for 36.46% of the time, while in medium to low lands it was exposed for 92.85%–93.96% of the time. Changes in temperature and humidity affected anatomical and physiological characteristics. *Stevia rebaudiana* adapts to high temperature and low humidity environments by increasing stomatal density, trichomes, and photosynthesis rate. In addition, it reduces stomata width, stomata aperture, open percentage of stomata, leaf thickness, palisade length, xylem diameter, conductance, and transpiration rate. The response of stevia in the highlands and medium-altitude lands environment indicates good adaptability (Khuluq *et al.*, 2022). Increased stevia leaf yields proved highly influenced by genotypes, environments, and their interactions, where environmental effects contributed 92.38% of the total variation (Amien *et al.*, 2022).

There are regional experiments on the macro- and microelements in the soil and their relationship with the content of steviol glucosides in *Stevia rebaudiana* Bert. (Combatt Caballero *et al.*, 2021). Therefore, to increase the content of steviol glycosides in the stevia plant, adaptive strategies to environmental change based on anatomy and physiology characteristics are necessary, along with a combination of optimizing environmental factors, using specific agronomic practices, and employing biotechnological techniques such as micropropagation, *in vitro* mutagenesis, or organo-mineral fertilization (Díaz-Gutiérrez *et al.*, 2020).

1. Cultivars of *Stevia rebaudiana* (Bert.) were evaluated for their tolerance to abiotic stresses, including salinity and drought, and many examples of positive results were found (Pal *et al.*, 2023; Nikraftar *et al.*, 2024). *Stevia* is commercially available as dried leaves in different countries; some articles have reported the efficiency of different drying methods (Lakshmi *et al.*, 2019; Huang *et al.*, 2021). The legal status of stevia extracts as food additives and supplements varies from country to country. Steviol glycosides (SGs) have been approved by the foremost regulatory authorities worldwide for use in foods and beverages due to the absence of harmful effects on human health. *Stevia*, as a natural alternative to sugar, has been approved for use in South Africa with the promulgation (10 September 2012) of new sweetener regulations (Regulations Relating to the Use of Sweeteners in Foodstuffs). Regulation R733, Regulations Relating to the Use of Sweeteners in Foodstuffs, allows the use of extracts of *Stevia rebaudiana*, in composition and quantities in line with Codex standards, in food and beverages.

The European Union approved stevia sweeteners in 2011. Steviol glycosides (E 960) are an authorized food additive in the European Union for use in several food categories, and specifications have been adopted for them. Presently, those specifications stipulate steviol glycosides (E 960) as a final product containing not less than 95% of 11 identified steviol glycosides—stevioside, rebaudiosides A, B, C, D, E, F, and M, steviolbioside, rubusoside, and dulcoside in any combination and ratio (EFSA Panel on Food Additives and Flavourings (FAF), European Food Safety Authority Journal, v. 18, issue 4, 2020).

Steviol glycosides extracted from the stevia plant were declared GRAS (generally recognized as safe) in the United States of America in December 2008 by the United States Food and Drug Administration (FDA). It is believed to hold close to 10% of the USA sugars and sweeteners market and is sold under several trademarks such as Truvia®, PureVia®, and Enliten®. Data based on FDA sources across the web about the sweetness intensity of allowed sweeteners compared to table sugar are presented in Table 1.

Table 1. Sweetness intensity of FDA-allowed sweeteners compared to table sugar

FDA Allowed Sweeteners	Times Sweeter
Advantame	20,000
Neotame	7,000 to 13,000
Thaumatococin	2,000 to 3,000
Saccharin	200 to 700
Sucralose	600
Steviol Glycosides (SGs)	200 to 400
Luo Han Guo	100 to 250
Aspartame	200
Acesulfame Potassium (Ace-K)	200

from sources across the web

As stevia is used as a sugar substitute in foods, packaged non-alcoholic beverages, and as a functional food ingredient in beverages and as a solubilizing agent, commercial products have been formulated from stevia derivatives (Tireki, 2021; Muñoz-Labrador *et al.*, 2023). Scientific evidence encourages the use of stevioside and rebaudioside A as sweetener alternatives to sucrose and supports their use based on their absence of harmful effects on human health and beneficial effects against various diseases; they can be further exploited as nutraceuticals (Uçar *et al.*, 2017; Ahmad *et al.*, 2020).

Stevia is heat-stable, pH-stable, and not fermentable. It is safe for people with diabetes, high blood pressure, and obesity, and it does not increase the risk of dental caries.

Based on the JECFA (Joint Expert Committee on Food Additives) declaration, safe consumption of steviol glycosides for humans is determined to be 4 mg/kg body weight per day. Stevioside (triglucosylated steviol) is the most predominant ent-kaurene-type diterpene glycoside, constituting approximately 3–8% of dried leaves.

Stevia leaves are used in infusions or as part of food preparation. In 2020, the production of diet cereal bars using stevia leaves directly as a sweetener was reported (Formigoni *et al.*, 2020). In that case, stevia shrubs were previously oven-dried with forced air circulation at 60°C until moisture content was below 10%. The leaves were manually separated from the stalks and branches, pretreated with ethanol, and then kept in identified sterile plastic bags.

Stevia products, including the core aglycone steviol, steviol glycosides, chemically synthesized or modified derivatives of the above, as well as various extracts, manifest potent cancer cell growth inhibitory effects, may help kill breast cancer cells, and prevent cancer growth. Stevia glycosides might also be toxic to particular stomach, lung, and leukemia cancer cells. Several groups report cytotoxic effects against cancer cells comparable to those exhibited by conventional anticancer drugs, such as 5-FU, and one study has even reported less toxicity on non-cancerous cells, making stevia products attractive candidates with prophylactic and restorative function in the disease (Iatridis *et al.*, 2022; Martinez-Rojo *et al.*, 2020; Zhang *et al.*, 2025; Khatun *et al.*, 2021).

Several works from the literature showed that the antioxidant activity of leaf extracts from *Stevia rebaudiana* Bertoni can act protectively against various diseases or present antimicrobial action against, for instance, *Listeria monocytogenes* (Rivas *et al.*, 2016).

Studies have found that stevia leaf extract protected rat livers from radiation-induced toxicity. The treatments reversed tissue damage, improved liver function, and restored depleted antioxidants after oral pretreatment before radiation-induced toxicity in rats. The butanol fraction, which has a higher concentration of stevioside and other compounds such as flavonoids and caffeoylquinic acid derivatives, showed the best protective effect (Abdallah *et al.*, 2022; Papaefthimiou *et al.*, 2023).

The stevia market size was valued at USD 796.04 million in 2022 (data from the web). Once limited to the health-food market as an unapproved herb, this plant-derived sweetener is now widely available and rapidly replacing artificial sweeteners in consumer products. Global stevia sales are predicted to reach about \$1.4 billion by 2028.

3 STEVIA AND RADIATION

In recent decades, research has explored the effects of gamma radiation on stevia seeds, in vitro tissues, or stevia plants, particularly to enhance growth and increase the content of steviol glycosides (Subrahmanyeswari *et al.*, 2024; Kumar *et al.*, 2024). Gamma radiation was used to assess the existence of considerable amounts of genetic diversity and stevioside and rebaudioside contents among different varieties of stevia plants (Ali *et al.*, 2019).

The radiation doses used for induced mutation vary widely depending on the organism, tissue type, and desired outcome, typically ranging from tens to hundreds of gray (Gy). The optimal dose is usually determined by the median lethal dose (LD₅₀) or the 50% growth reduction dose (GR₅₀) for the specific vegetal material. Many reports describe gamma radiation-induced mutations in stevia plants and stevia shoots, leading to improved growth traits and higher concentrations of steviol glycosides. Higher accumulation of SGs occurs in the vegetative phase followed by a decrement during the reproductive phase. Irradiated stevia plants have shown increased biomass production and higher levels of rebaudioside A and stevioside compared to non-irradiated plants. In some experiments, authors found that 325 Gy is the suitable dose for the improvement of stevia by acute gamma irradiation [77](Khalida *et al.*, 2022). There are published works describing the effects of gamma rays on seed germination, survival rate, prolonged vegetative phase, and morphology of *Stevia rebaudiana* [78–80](Singh *et al.*, 2020; Pande *et al.*, 2011). Mutagenesis could be one of the ways to produce stevia with delayed flowering. Irradiated seeds exposed to doses >500 Gy failed to germinate; the plant height and number of leaves in irradiated plants were highest in treatment with 100 Gy (Abdullah *et al.*, 2021). Studies have shown the radio-stimulation of stevia seed germination using gamma rays, where a dose of 10 Gy showed the better effect (González and Nakayama, 2015).

Chiew and coworkers reported that acute gamma irradiation of *Stevia rebaudiana* Bertoni enhanced particular types of steviol glycosides. In that case, mutation induction with gamma irradiation was effective in generating genetic variations and developing new plant varieties with desired traits. The LD₅₀ was found to be at 23 Gy, and irradiation of 900 shoot tip explants at 23 Gy produced 468 surviving shoot tips, which were all capable of developing and successfully sub-cultured until the fourth generation, M4. These M4 in vitro mutant plantlets exhibited a significant increase in the

number of leaves and average leaf size. High-performance liquid chromatography (HPLC) analysis performed in parallel further revealed the mutant plants contained higher concentrations of stevioside (387.04 ppm), rebaudioside A (670.18 ppm), and rebaudioside D (106.26 ppm) compared to the non-irradiated plantlets, which exhibited 96.87, 194.42, and 28.25 ppm, respectively (Chiew *et al.*, 2019; Ahmad *et al.*, 2015).

Therefore, through mutation breeding, it is possible to create new varieties of stevia with enhanced characteristics. Gamma radiation applied to stevia seeds or in vitro systems induces enhancement in growth characteristics and production of bioactive compounds in *Stevia rebaudiana* Bert.

4 NEW APPROACH TO THE APPLICATION OF IONIZING RADIATION ON STEVIA

Stevia has exceptional potential for improvement through induced mutation, especially by using gamma rays on plants, cuttings, seeds, seedlings, or in vitro systems, as has already been reported in different works mentioned above. One of the targets of mutation induction on stevia is to obtain new varieties able to produce increased steviol glycosides. In that case, the percentages of steviol glycosides were raised as a result of radiation treatment.

Mutations can be induced by chemical or physical agents. Mutation induction by gamma irradiation has been shown to be useful for generating genetic variations as well as developing new plant varieties from which desired mutants were successfully selected. The ionizing radiation doses used to induce mutations typically range from tens to hundreds of gray (Gy), where 1 Gy is a unit of absorbed radiation dose equal to one joule of energy per kilogram of matter. On the other hand, radiation doses used for decontamination are in the range of kGy, depending on the application and the type/level of contamination.

Other ways to produce enhancement of steviol glycosides in stevia, besides induced mutation, have been investigated. The most promising approach is described in some works that reported the effect of radiation that occurs when irradiation is performed on dried leaves (Ahmad *et al.*, 2021; Eliwa *et al.*, 2025). Stevia dried leaves for herbal infusion preparation are easily available commercially. The samples were packed, sealed in polythene bags, and gamma irradiated doses of a few kGy, as was reported in the two last-mentioned works.

Dried aromatic and medicinal herbs are frequently decontaminated using ionizing irradiation, as radiation can eliminate microbial contamination while preserving flavor, taste, and aroma and ensuring shelf-life extension. It seems that in the case of irradiated stevia dried leaves, the flavor, taste, and aroma are not only preserved but also present enhancement of their important steviol glycosides. Therefore, as a complement to pathogen control, irradiation would promote an increase in desirable important biocomponents.

5 CONCLUSIONS AND PERSPECTIVES

Gamma irradiation has been used to improve the plant's tolerance to abiotic stresses, such as salinity, drought, and temperature extremes, making stevia more resilient and potentially more productive in challenging environments. There is strong demand for natural zero-calorie sweeteners, and stevia, rich in steviol glycosides (SGs), may be the most promising candidate, based on its health benefits, industrial applications as food and food ingredient, use as a sucrose replacer, and absence of harmful effects on human health. Higher yields and improvements in the levels of the best-tasting SGs are currently objectives for stevia breeding. All ways to increase SGs are presently the object of investigation. Ionizing radiation can induce mutations in stevia plants, leading to higher concentrations of SGs. Increases in SGs have also been reported through irradiation of stevia dried leaves. From a practical point of view, this approach appears to offer plenty of possibilities, as that effect is obtained at the same range of irradiation doses where radiation is employed to prevent foodborne illnesses.

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