



Extraction Technologies of Steviol Glycosides from *Stevia rebaudiana* and Their Use in Low-Calorie Food Formulations

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Abstract

The global shift towards healthier diets has intensified the demand for natural, low-calorie sweeteners to combat obesity, diabetes, and other metabolic disorders. Steviol glycosides, extracted from the leaves of *Stevia rebaudiana* Bertoni, have emerged as a leading natural, high-potency, zero-calorie sweetening solution. This scientific article provides a comprehensive review and analysis of conventional and innovative extraction technologies—including water and solvent extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and membrane technologies—for recovering steviol glycosides, primarily stevioside and rebaudioside A. It critically evaluates each method's efficiency, yield, purity, scalability, economic viability, and environmental impact. Furthermore, the article details the application of purified stevia extracts across diverse low-calorie food and beverage formulations, such as soft drinks, dairy products, baked goods, and confectionery. It addresses the technical challenges of formulation, including managing the bitter aftertaste of certain glycosides and achieving optimal sweetness profiles and stability. Supported by an analysis of the regulatory status (GRAS, FDA, EFSA) and a discussion on health implications, this work concludes that the strategic integration of advanced, green extraction technologies with innovative food science is essential for meeting global consumer demand for natural, safe, and tasty low-calorie products. Future research directions are suggested to optimize extraction and application processes further.

Keywords: *Stevia rebaudiana*, Steviol Glycosides, Extraction Technologies, Ultrasound-Assisted Extraction, Green Extraction, Low-Calorie Foods, Rebaudioside A, Food Formulation, Natural Sweeteners.

Introduction

The increasing prevalence of obesity, type 2 diabetes, and cardiovascular diseases worldwide has been closely linked to excessive dietary sugar intake, driving both consumers and public health policies towards reducing calories from added sugars. This has created a substantial market demand for safe, tasty, and natural low-calorie sweeteners. Among the alternatives, steviol glycosides from the leaves of *Stevia*

rebaudiana Bertoni, a plant native to South America, have gained significant prominence and regulatory approval globally .

Stevia rebaudiana produces a suite of sweet-tasting diterpene glycosides in its leaves, with stevioside and rebaudioside A being the most abundant and commercially significant. These compounds are 200-400 times sweeter than sucrose (table sugar) yet contribute virtually no calories, making them an ideal sugar substitute . Beyond sweetness, research suggests steviol glycosides may offer additional bioactive benefits, such as anti-hyperglycemic, anti-hypertensive, and anti-inflammatory effects, although these are not the primary basis for their regulatory approval as food additives . The sensory profile varies among glycosides; rebaudioside A is often preferred for its cleaner, sweeter taste with less bitterness and licorice-like aftertaste compared to stevioside .

The journey from stevia leaf to a functional food ingredient hinges on efficient extraction and purification technologies. The core challenge lies in selectively isolating the desired glycosides from the complex plant matrix while achieving high purity, yield, and cost-effectiveness, and preserving the desired sensory properties. Traditional methods often involve hot water or organic solvent extraction, which can be inefficient, time-consuming, and environmentally burdensome due to high energy and solvent use . Consequently, the food industry and scientific community are actively pursuing intensification and "green" extraction techniques.

This article, structured according to the IMRaD (Introduction, Methods, Results, and Discussion) format, aims to provide a systematic review of the extraction technologies for steviol glycosides and their integration into low-calorie food formulations. The **Methods** section will detail the operational principles, parameters, and protocols of both conventional and novel extraction techniques. The **Results** section will present and compare data on the performance of these methods in terms of yield, purity, and economic indicators. Finally, the **Discussion** section will interpret these findings in the context of industrial application, formulation challenges, regulatory frameworks, and future trends, concluding with recommendations for optimizing the value chain of stevia-based low-calorie foods.

1. Methods: Extraction Technologies for Steviol Glycosides

The extraction of steviol glycosides is a critical unit operation that defines the economic feasibility, environmental footprint, and final quality of the sweetener. This section details the methodologies, from leaf pretreatment to advanced isolation techniques.

Raw Material Preparation and Pretreatment

The process begins with the harvesting and drying of *Stevia rebaudiana* leaves. Post-harvest, leaves are typically dried to reduce moisture content, preventing microbial spoilage and stabilizing the glycosides. Sun-drying or controlled hot-air drying are common . The dried leaves are then milled or ground into a coarse powder to increase the surface area for subsequent solvent contact, a step shown to significantly enhance extraction efficiency .

Conventional Extraction Methods

Hot Water Extraction: This is the simplest and most traditional method, akin to brewing tea. Dried leaf powder is steeped in hot water, which solubilizes the glycosides. While solvent-free and simple, it is non-selective, co-extracting chlorophyll, polyphenols, tannins, and other water-soluble compounds, resulting in a crude extract that requires extensive downstream purification. It can also lead to a mushy biomass that is difficult to separate .

Organic Solvent Extraction: Solvents like methanol, ethanol, and isopropyl alcohol are more selective for glycosides. Methanol offers high efficiency but raises food-safety and environmental concerns. Food-grade ethanol is preferred for final product extraction, while isopropyl alcohol is also effective, as demonstrated in studies achieving high rebaudioside A yields . The process involves soaking the leaf material in solvent, often with agitation, followed by filtration and solvent recovery via evaporation.

Advanced and Assisted Extraction Technologies

To overcome limitations of conventional methods, several auxiliary energy-assisted techniques have been developed.

Ultrasound-Assisted Extraction (UAE): UAE uses high-frequency sound waves to create cavitation bubbles in a solvent. The implosion of these bubbles generates extreme local pressure and temperature, disrupting plant cell walls and enhancing mass transfer. A study optimized UAE using 60% isopropyl alcohol, 360W power, and an 18-minute treatment, achieving a rebaudioside A yield of 35.61 g/100g dry leaf . UAE reduces extraction time and solvent consumption but requires optimization of parameters like power, frequency, and temperature.

Microwave-Assisted Extraction (MAE): MAE utilizes microwave energy to heat the solvent and plant matrix internally and rapidly. This heating creates internal pressure that ruptures cells, facilitating the release of glycosides. MAE is known for its rapid extraction kinetics.

Supercritical Fluid Extraction (SFE): SFE, particularly using supercritical CO₂ (scCO₂), is a sophisticated, green technology. CO₂ is pressurized and heated above its critical point, becoming a solvent with gas-like diffusion and liquid-like density. Its solvating power is tunable by adjusting pressure and temperature. While pure scCO₂ is better for non-polar compounds, the addition of a polar modifier like ethanol or water can effectively extract steviol glycosides. SFE is highly selective, leaves no solvent residue, and operates at moderate temperatures, preserving heat-sensitive compounds .

Green Extraction with Water: Emphasizing sustainability, some research focuses on optimizing water as a sole extractant. One study employed a factorial design to optimize parameters (temperature, time, agitation, grinding, leaf-to-water ratio) for a simple, efficient, and cost-effective extraction using only tap water, demonstrating its potential for industrial scale-up .

Purification and Decolorization Techniques

Crude extracts are dark-colored and contain impurities requiring removal to meet purity standards (>95% total glycosides) and improve taste.

Precipitation and Crystallization: A common purification step involves adding calcium oxide (lime) or other agents to precipitate proteins and pigments.

Subsequent selective crystallization from solvents like methanol can isolate specific glycosides. A patented method describes refluxing a dried extract in anhydrous methanol to precipitate stevioside first, then concentrating the filtrate to crystallize rebaudioside A, which can be further recrystallized for higher purity .

Adsorption and Chromatography: Adsorbent resins are widely used for purification and decolorization. Macroporous resins (e.g., Amberlite XAD series, ADS-7) adsorb glycosides from aqueous solutions, allowing impurities like polysaccharides to pass through. The glycosides are then eluted with an organic solvent like methanol. Studies show polymers like Separan AP30 combined with calcium oxide can remove over 65% of color impurities . Ion-exchange resins can remove ionic impurities.

Membrane Filtration: Technologies like ultrafiltration and nanofiltration are emerging for clarifying crude extracts and concentrating glycosides based on molecular size, offering a continuous, low-temperature operation.

Analytical Methods for Quantification

The quantification of steviol glycosides is essential for process optimization and quality control. High-Performance Liquid Chromatography (HPLC) coupled with a Diode Array Detector (DAD) or Mass Spectrometer (MS) is the gold standard. It separates and quantifies individual glycosides (stevioside, rebaudioside A, etc.) against certified reference standards . Other methods include spectrophotometric analysis for total glycosides and refractometry for total soluble solids (°Brix).

Results: Performance Analysis of Extraction and Application

This section presents the outcomes of different extraction strategies and the successful incorporation of stevia extracts into various food matrices.

Comparative Efficiency of Extraction Technologies

The performance of extraction methods varies significantly based on target compounds and process goals. The table below summarizes key findings from the literature.

Table 1: Comparison of Steviol Glycoside Extraction Technologies

Extraction Method	Key Parameters	Reported Yield / Efficiency	Key Advantages	Main Limitations
Hot Water Extraction	High temperature, Long time	Low to moderate purity (≈70% glycosides)	Simple, safe, low-cost solvent.	Low selectivity, high impurity, energy-intensive, bitter taste.
Solvent (IPA) UAE	60% IPA, 360W, 30°C, 18 min	Rebaudioside A: 35.61 g/100g dry leaf	High yield, reduced time & solvent, improved cell disruption.	Capital cost, need for parameter optimization.
Green Water Extraction	Optimized T, time,	Efficient recovery	Sustainable, low-cost, safe,	May require robust

Extraction Method	Key Parameters	Reported Yield / Efficiency	Key Advantages	Main Limitations
	agitation, ratio	(specific data via HPLC)	directly scalable for food use.	downstream purification.
Supercritical CO₂	High pressure, Modifier (EtOH/H ₂ O)	Highly selective, tunable	Solvent-free residue, green, low-temp operation, high purity.	Very high capital and operational cost.
Resin Purification	Resins (Separan AP30, ADS-7, XAD)	>65% color removal, >95% glycoside purity	Effective decolorization and purification, scalable.	Requires prior extraction, solvent for elution.

Formulation in Low-Calorie Food and Beverage Products

Steviol glycosides are successfully integrated into a wide array of products, each presenting unique challenges.

Beverages: This is the largest application sector. Stevia extracts are used in carbonated soft drinks (e.g., Coca-Cola Life), still and flavored waters, juices, and ready-to-drink teas. Their high solubility and stability across a wide pH range make them ideal. Major brands like Truvia and PureVia are prominent in this market. Formulators often use reb A for its cleaner taste or blend glycosides to mask bitterness.

Dairy Products: Stevia sweetens yogurts, flavored milks, and plant-based dairy alternatives. Its stability in these matrices allows for "sugar-free" or "no-sugar-added" claims without compromising shelf-life.

Baking and Confectionery: Application here is more technically challenging because sugar provides bulk, texture, browning (Maillard reaction), and moisture retention. Stevia, being a high-potency sweetener, contributes none of these. Successful formulation requires bulking agents (e.g., erythritol, maltodextrin), and recipe adjustments to leavening, eggs, and fats. It is used in sugar-free candies, chocolates, cookies, and cakes.

Tabletop Sweeteners: Consumer packets, liquids, and dissolvable tablets (brands like Stevia in the Raw, SweetLeaf) are common retail products. These often contain stevia extract blended with carriers like dextrose or erythritol to provide volume for easy measuring.

Table 2: Applications of Stevia Extracts in Low-Calorie Food Formulations

Food Category	Specific Products	Formulation Considerations & Benefits	Common Glycoside Used/Blend
Beverages	Diet sodas, Iced tea, Flavored water	High solubility, pH stability, zero-calorie label claim.	Rebaudioside A (for clean taste), Glycoside blends.
Dairy & Alternatives	Yogurt, Flavored milk, Soy milk	Stability in emulsion, complements tart flavors, no aftertaste masking needed.	Rebaudioside A, Stevioside.
Baked Goods & Confectionery	Cookies, Cakes, Sugar-free chocolate	Requires bulking agents, recipe adjustment for texture/browning.	Often blended with sugar alcohols (erythritol).
Tabletop Sweeteners	Powder packets, Liquid drops	Blended with carriers (e.g., erythritol, dextrose) for volume.	High-purity Stevia Extract (various blends).

Regulatory and Safety Profile

Extensive toxicological studies have led to broad regulatory acceptance. Highly purified steviol glycosides ($\geq 95\%$) are approved as **Generally Recognized As Safe (GRAS)** in the United States by the FDA and have positive safety opinions from the European Food Safety Authority (EFSA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Acceptable Daily Intake (ADI) is set at 4 mg per kg of body weight per day (expressed as steviol). It is crucial to note that regulatory approvals apply to the *purified extract*, not crude stevia leaves or whole-leaf powders.

Discussion

The transition of stevia from a niche natural sweetener to a mainstream food ingredient is a testament to advancements in both extraction technology and food science. This discussion interprets the results within broader industrial, economic, and health contexts.

Interpreting Extraction Performance: Trade-offs and Selection Criteria

The choice of extraction technology is not a straightforward decision but a balance of multiple factors. While UAE and MAE offer excellent intensification—dramatically improving yield and reducing time—they are "enabling technologies" that still require a solvent and downstream purification. Their value is highest in reducing the cost and environmental impact of the primary extraction stage.

The **green water extraction** method presents a compelling case for sustainability and cost, especially if the application can tolerate a less refined extract or if coupled with efficient membrane purification. **SFE** represents the pinnacle of green, selective extraction but its high capital expenditure makes it viable primarily for large-scale producers of high-value botanicals. For most manufacturers, a hybrid approach is likely optimal: an intensified primary extraction (UAE/MAE with food-grade ethanol) followed by sophisticated purification (resin adsorption/crystallization) to achieve $>95\%$ purity as required by regulators.

Overcoming Formulation Challenges in Complex Food Systems

The successful use of stevia extends beyond pure extraction. A major hurdle is the **bitter and licorice-like aftertaste** associated with stevioside and some impurities. This is addressed through:

Glycoside Selection and Blending: Using high-purity **rebaudioside A** (or newer, rare glycosides like reb M and D) which have superior sensory profiles .

Blending with Other Sweeteners: Combining stevia with sugar alcohols (erythritol, xylitol) or bulk sweeteners (allulose, maltitol) mitigates aftertaste, provides necessary volume/texture in baked goods, and can have a synergistic sweetening effect .

Flavor Masking Technology: The use of natural flavors or modifier ingredients can effectively cloak residual bitter notes. Furthermore, the lack of **bulking and functional properties** in high-potency sweeteners is a critical technical barrier in products like bread and ice cream. Here, stevia acts not as a 1:1 sugar replacement but as a sweetening component within a redesigned ingredient system that includes hydrocolloids, fibers, and other texturizing agents .

Health Implications: Beyond Calorie Reduction

The primary health benefit of stevia is its role in reducing dietary sugar and calorie intake, aiding in weight management and glycemic control, which is particularly beneficial for individuals with diabetes or prediabetes . Some studies suggest additional bioactive effects, such as potential anti-hypertensive and antioxidant properties . However, it is vital to distinguish between *food additive use* and *therapeutic use*. Regulatory approvals are for its safety as a sweetener, not as a medicine. Concerns about impacts on gut microbiota or endocrine disruption are occasionally raised, but current evidence from human studies, particularly at typical consumption levels, indicates that highly purified stevia extracts are safe for the general population . Consumers should be advised to use approved products and consult healthcare providers for specific medical conditions.

Economic and Market Trends

The stevia market is growing within the broader natural sweetener sector, driven by consumer demand for "clean-label" ingredients. The development of cost-effective and sustainable extraction processes is key to making stevia price-competitive with artificial sweeteners (e.g., aspartame, sucralose) and other natural options like monk fruit . Continuous innovation in extraction to improve the yield of the most desirable glycosides (like reb A and reb M) will enhance taste and drive further market adoption.

Limitations of the Review and Future Perspectives

This review synthesizes current technologies, but the field is dynamic. Future research should focus on:

Integrated and Continuous Processes: Developing seamless, continuous processing lines from leaf to pure extract to improve efficiency and lower costs.

Biosynthesis and Fermentation: Exploring microbial production of specific steviol glycosides for greater purity and supply chain control.

Advanced Application Science: Deepening research into stevia's interactions with other food components to unlock its full potential in challenging applications like yeast-leavened breads and fermented dairy.

Long-term Human Health Studies: Continuing robust epidemiological studies to confirm long-term health impacts of regular consumption.

Conclusion

The integration of steviol glycosides into the global food supply as a natural, zero-calorie sweetener is a significant achievement in food technology and public health nutrition. The efficacy of this integration is fundamentally dependent on the extraction technology employed. While conventional methods provide a baseline, advanced techniques like Ultrasound-Assisted Extraction, Supercritical Fluid Extraction, and optimized Green Extraction offer pathways to higher yields, superior purity, better sensory profiles, and improved environmental sustainability. The subsequent application of these purified extracts in low-calorie food formulations requires sophisticated food science to overcome challenges related to taste, texture, and stability. With a strong foundation of regulatory safety approval and growing consumer acceptance, the future of stevia is promising. Continued innovation in both extraction and formulation, guided by principles of efficiency and sustainability, will solidify stevia's role as a cornerstone in the creation of healthier, reduced-sugar food products worldwide.

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