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Chemical and functional properties of *Stevia rebaudiana* Bertoni stems as an agro-industrial byproduct for potential use in functional foods

Propiedades químicas y funcionales de tallos de *Stevia rebaudiana* Bertoni como subproducto agro-industrial para uso potencial en alimentos funcionales

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Abstract Stevia leaves (*Stevia rebaudiana* Bertoni) are considered natural, harmless, non-caloric sweeteners. The stems of the stevia plant hadn't been as researched and are considered waste or agro-industrial byproducts, yet a good source of potentially techno-functional fiber, which could be considered as a food ingredient. The aim of this work was to evaluate the chemical and techno-functional properties of stevia stems as a potential ingredient for functional foods. The stems were obtained from stevia plantations in the state of Yucatan – Mexico, and their proximal chemical composition and techno-functional profile were determined considering water and oil retention, water absorption and adsorption, and the organic molecules absorption capacity. The analytical determinations were performed in triplicate and a one-way analysis of variance was used for the statistical analysis of the data obtained. They presented a high content of carbohydrates (54.84 %), crude fiber (32.33 %), ash (6.99 %), and protein (4.46 %). The stems retained five times their weight in water and oil, with 5.57 and 5.00 g/g, respectively. Their organic molecules absorption capacity was 2.09 g/g, with 4.49 g/g for water absorption and 0.29 g/g for water adsorption. As an agricultural byproduct of stevia leaves harvesting, the stems are a functional alternative to use in food preparations with potential physiological effects.

Keywords: dietary fiber, natural sweetener, physiological effect, physicochemical properties.

Resumen Las hojas de stevia (*Stevia rebaudiana* Bertoni) son consideradas edulcorantes naturales, bajos en calorías e inocuos. Por su parte, los tallos de stevia han sido menos estudiados y se consideran subproductos agroindustriales, aunque representan una buena fuente de fibra con propiedades tecnofuncionales que podría utilizarse como ingrediente alimentario. El objetivo del presente trabajo fue evaluar las propiedades químicas y tecnofuncionales de los tallos de stevia como potencial ingrediente de alimentos funcionales. Los tallos fueron obtenidos de plantaciones de stevia en Yucatán – México, y se les determinó la composición química proximal y su perfil tecnofuncional considerando la retención de agua y de aceite, la absorción y adsorción de agua y la absorción de moléculas orgánicas. Las determinaciones analíticas fueron realizadas por triplicado y se utilizó un análisis de varianza de una vía para el análisis estadístico de los datos obtenidos. Estos presentaron un alto contenido de carbohidratos (54,84 %), fibra cruda (32,33 %), cenizas (6,99 %) y proteína (4,46 %). Los tallos retuvieron cinco veces su peso en agua y en aceite (5,57 y 5,00 g/g), respectivamente. La capacidad de absorción de moléculas orgánicas fue de 2,09 g/g, la absorción de agua iguala a 4,49 g/g y la adsorción de agua de 0,29 g/g. Los tallos como subproducto agrícola de la cosecha de hojas de stevia son una alternativa funcional para usar en la elaboración de alimentos con potenciales efectos fisiológicos.

Palabras clave: efecto fisiológico, endulzante natural, fibra dietética, propiedades fisicoquímicas.

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is an herbaceous plant native to Paraguay and frequently cultivated in several regions of different countries such as: China, Japan, the United States of America, Indonesia, Brazil, and Canada (Gayosso-Rodríguez *et al.*, 2023). The botanist Moisés Santiago Bertoni first described the plant, and its significant commercial exploitation began in the decade of the 70s, when it was promoted through research on the extraction and refinement of its sweetening components, such as the steviol glycosides present in its leaves (Peteliuk *et al.*, 2021).

Functional foods can be defined as those that have been innovatively developed to contain substances or live microorganisms that provide benefits to improve the health of consumers or help prevent disease. They must be used in a safe concentration, sufficient to achieve the desired benefit (Temple, 2022). Kurek and Krejpcio (2019), have reported the potential therapeutic benefits of *Stevia rebaudiana* extracts and its pure compounds in promoting health in various *in vitro* and *in vivo* models.

Stevia leaves produce diterpene glycosides with high-potency sweeteners that can replace sucrose and artificial sweeteners like aspartame, acesulfame potassium (Ace-K), neotame, saccharin, sucralose, and advantame (Basson *et al.*, 2021). Glycosides are non-nutritive and non-toxic. To date, more than thirty such compounds have been identified, with Stevioside and Rebaudioside A being the most investigated. Stevioside is the main component in the native variety of stevia; it represents 4 to 13 % of all glycosides, its taste is bitter or astringent and its sweetening power after the molecule has been purified is 300 times sweeter than sucrose at a concentration of 0.4 % (Fasiha *et al.*, 2020).

It is precisely because of this non-caloric sweetening capacity that steviol glycosides and their extracts have been widely used in the technological development of food products. The scientific literature has reported their use in the production of beverages, bread, cookies, dairy products, and confectionery, among other products (Jaimes *et al.*, 2018; Gupta *et al.*, 2017). The versatility of steviol glycosides for use in the food industry also lies in their physicochemical properties, such as their thermal stability (~200 °C), their stability against different acidity and alkalinity conditions (pH 2 - 10), their non-fermentability, and their high organoleptic acceptability (Gasmalla *et al.*, 2014).

Another important application of stevia is in the field of functional foods, defined as foods that have a potentially positive effect on health beyond basic nutrition, due to the physiological effects as antioxidant, antihyperglycemic and antilipid, attributed to it. Stevia has been shown to have therapeutic potential due to its ability to regulate blood glucose, improve renal and hepatic functions, and reduce arterial hypertension. It is also important to mention the safety of its consumption as toxicological studies have shown that, in general, the plant's metabolites do not have teratogenic, mutagenic, carcinogenic, or allergenic effects (Mathur *et al.*, 2017).

Regarding their nutritional characteristics, stevia leaves, in addition to being rich in carbohydrates, contain a high proportion of minerals, proteins, and dietary fiber. In this regard, the functional potential of stevia leaves has been confirmed, mainly due to their contribution of dietary fiber, phenols, flavonoids, vitamins, and minerals (Jahangir-Chughtai *et al.*, 2020). The understanding of these peculiarities of the stevia leaves has served as a basis for the various technological and therapeutic applications of the different parts of the plant.

Stevia production has increased to an estimated 4.000 tons annually, which has led to the generation of a large amount of waste and agro-industrial byproducts, mainly its stems, that could have a wide range of applications. The potential to exploit stems as a source of antioxidants has been demonstrated with hot water Stevia leaf extracts that presented greater antioxidant activity compared to fish oil oxidation (Yu *et al.*, 2017). Nogueira *et al.* (2024) carried out *in vitro* studies to evaluate the antioxidant, antidiabetic, and anti-obesity capacity of methanolic and hydroalcoholic extracts of Stevia stems. The results indicated the presence of bioactive compounds such as polyphenols and flavonoids that have great importance in health. The extracts presented 17 % phenolic compounds, an antioxidant activity of 65 to 89 %, lipase enzyme inhibition activity of 98 % for the methanolic extracts, and inhibition of α-amylase and α-glucosidase of the hydroalcoholic extract, with 30 and 90 %, respectively.

Bondarev *et al.* (2003) analyzed by high-performance liquid chromatography the content of the three main steviol glycosides: stevioside and rebaudioside A and C in vegetative and generative organs during the ontogeny of *S. rebaudiana*. The highest glycoside content was found in the upper sections of actively growing young shoots, while the lower sections of senescent shoots exhibited the lowest amount. During ontogeny, a gradual increase in glycoside content was observed in both mature leaves and stems of stevia, and this process continued until the budding phase and the onset of flowering, making the stems a potential source of these sweetening compounds. However, although the stems have not yet been recognized as a source of sweeteners, they also possess highly significant nutritional and functional characteristics in addition to their sweetening compounds. For example, the high amount of fiber (Atteh *et al.*, 2011) and antioxidant compounds (Yu *et al.*, 2017) found in the stems, allows expanding the study of the plant towards the utilization of all its parts, since the interest has been mainly focused on the leaves and the stems have been categorized as by-products or waste.

Barbosa-Martín *et al.* (2022) indicated that *S. rebaudiana* stems, could be an alternative for the use and valorization of waste or the development of functional products based on the circular economy, due to their dietary fiber content. The stems could provide a benefit that, in some way, contributes to reduce overweight and obesity, with a hypoglycemic effect in patients with type 2 diabetes mellitus. Therefore, the goal of the present work was to evaluate the chemical components and techno-functional properties of stevia stems as agro-industrial byproducts. The results are expected to contribute to the recognition of stems as relevant ingredients for the development of functional foods.

Materials and methods

Stem collection and processing

S. rebaudiana, Morita II variety stem samples were obtained from plantations of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) at the Campo Experimental de Mococha, Yucatan, Mexico. Plants of the selected variety had thick, branched stems with elliptic leaves arranged in opposite directions. Samples were obtained from the first harvest of leaves at three months of age from the experimental plots and dried for three days in sun-shade periods. The stems were then collected, dried in a Fisher Scientific convection oven at 50 °C for 24 h, processed in a Thomas Wiley industrial mill to reduce the particle size, and finally sieved on a 2 mm mesh. The powders obtained were stored in amber glass bottles until use.

Chemical characterization

Proximal chemical evaluation was performed according to the methods established by the Association of Official Analytical Chemists (AOAC). All analyses were performed in triplicate and the results were expressed on a dry basis (% d.b.).

- The moisture (M) content was measured in terms of the weight loss of the sample after drying it in a convection oven at 110 °C for 2 h, following method 925.10 (AOAC, 2005a).

- The fat (F) content was obtained from an extraction with hexane for 1 h in accordance with the Soxhlet principle, using a Soxtec™ 8000 system (Tecator, Höganäs, Skåne län, Sweden), following method 2003.06 (AOAC, 2012).

- The amount of ashes (A) or mineral residues was calculated based on the weight of the sample after burning it at 550 ºC for 2 h, following method 923.02 (AOAC, 2005b).

- The nitrogen (N_2) content was quantified according to the Kjeldahl method using a Kjeltec™ 8200 digestion system (Tecator, Höganäs, Skåne län, Sweden) using cupric sulfate and potassium sulfate catalysts, according to 992.23 method (AOAC, 2000).

- Protein (P) content was calculated as nitrogen × 6.25 since there is no specific conversion factor for fibrous stevia residues.

- For the crude fiber (CF), the Weende method 962.09 was used with a Fibertec™ 8000 system (Tecator, Höganäs, Skåne län, Sweden), according to the AOAC (1990). The sample was digested with 1.25 % sulfuric acid and then with 1.25 % sodium hydroxide. The residue that consisted of fiber was determined by drying to constant weight, calcining, and reweighing.

- Nitrogen-free extract (NFE) was calculated and expressed as total carbohydrate. This value was estimated as the difference between the other components, as indicated in **Equation 1**:

Eq 1.

$$\% NFE = 100 - P - F - A - CF$$

Where:

(P) = represents protein,

(F) = indicates fat,

(A) = represent ash,

(CF) = indicates crude fiber.

Functional properties

The functional properties of the samples were determined in triplicate using the following methods:

Water Retention Capacity (WRC)

It was performed by adapting the technique reported by Chau *et al.* (1997), as modified by Barbosa-Martin *et al.* (2016). Using 20 mL of distilled water, 1 g of sample was hydrated and centrifuged at 2550 *x g* for 30 min at 25 °C. Subsequently, the supernatant was measured, and the WRC was expressed as the grams of water absorbed divided by the grams of sample.

Oil Retention Capacity (ORC)

It was developed based on the technique proposed by Chau *et al.* (1997) and adapted by Barbosa-Martín *et al.* (2016). To 1 g of sample (S), 10 mL of corn oil was added and centrifuged at 2550 *x g* for 30 min at 25 °C and the oil retained (OR) was weighed. The density of the oil used was 0.91 g/cm^3. Finally, the volume of supernatant (VS) was measured, and the ORC was calculated as indicated in **Equation 2**.

Eq 2.

$$ORC = \frac{OR\ (VS\ x\ 0.91)}{S}$$

Organic Molecule Absorption Capacity (OMAC)

According to the technique reported by Segura-Campos *et al.* (2014), 3 g of sample were saturated with corn oil, left to stand for 24 h at 25 °C, and centrifuged at 2000 *x g* for 15 min at 25 °C. The OMAC was estimated as the grams of oil absorbed between the grams of sample.

Water Absorption Capacity (WAC)

Following the 88-04 technique by the American Association of Cereal Chemists (AACC, 1984) reported by Barbosa-Martin *et al.* (2016), 5 grams of sample were saturated with distilled water (SV) and centrifuged at 2000 *x g* for 10 min. The precipitate was weighed, and the approximate water absorption capacity (ApWAC) was estimated by the difference in weights between the grams of sample. Subsequently, the amount of sample to be weighed for the determination was calculated according to **Equation 3**.

Eq 3.

$$Sample\ weight = \frac{SV}{(ApWAC + SV)}$$

Next, the samples were tested in 4 systems considering the volumes of distilled water described below. The amount of water necessary to complete the sample weight to 18 g was determined (18 - Sample weight), and, to this calculated volume, +1.5, +0.5, -1.5, and -0.5 mL of water was added. The solutions were shaken, then centrifuged at 2000 *x g* for 10 min, and the volume of water absorbed was calculated. Finally, the WAC was expressed as the grams of water absorbed between the grams of the sample.

Water Adsorption Capacity (WAdC)

It was performed through the methodology proposed by Chen et al. (1984) and adapted by Barbosa-Martín et al. (2016). The dried and milled stevia stems were placed in an equilibrium microenvironment with a relative humidity of 98 %, which was generated by placing 20 mL of saturated potassium sulfate saline solution in hermetically sealed glass flasks and placing them in desiccators at 25 °C. The sample was left in the microenvironment until it reached constant weight (48 h). Water adsorption capacity was measured as the grams of water adsorbed between grams of sample.

Statistical analysis

The results obtained were analyzed with descriptive statistics and one-way analysis of variance (95 % confidence level) using the statistical software package Statgraphics Centurion version 18 (Statgraphics Technologies, Inc., The Plains, VA, USA). Duncan's test was applied to determine differences between the means of the average values of the proximal composition and functional properties of the stems at a 95 % confidence level.

Results and Discussion

Chemical characterization

The results revealed that the stevia stems sample presented a high content of carbohydrates expressed as nitrogen-free extract (54.84 %), fiber (32.33 %), ash (6.99 %), and protein (4.46 %), as well as moisture content of 7.14 %. (**Table 1**). This was expected since the stems of herbaceous plants, such as stevia, are usually rich in assimilable carbohydrates (Jahangir-Chughtai *et al.*, 2020). These results are in agreement with those reported by Atteh *et al.* (2011), in a study where the stems of the plant were recognized as a good source of fiber, minerals, and protein. These authors also reveal that, if consumed, the stems provide a very low metabolizable energy content, which is associated with their high fiber content. This is relevant as the use of the stems as food ingredients could generate products with a very low energy density that could be included in calorie-restricted dietary plans. Other fiber properties that stevia stems could contribute to the diet include increased faecal mass, improved intestinal transit, antioxidant effects, early satiety generation and low energy density, among others (Mugdil & Barak, 2013).

Table 1

Chemical-proximal composition (% dry basis) of Stevia rebaudiana stems compared with reference data

Gluten seco	Gluten seco Current study	Gluten seco Atteh *et al*. (2011).
Fat	1.38 ± 0.03 a	1.1 ± 0.01
Protein	4.46 ± 0.09 b	6.7 ± 0.1
Ash	6.99 ± 0.19 c	5.6 ± 0.6
Fiber	32.33 ± 0.54 e	45.1 ± 0.8
Carbohydrates as (nitrogen free extract)	54.84 ± 0.83 f	41.5 ± 0.6

Note. Data are expressed as mean ± standard deviation. [a-f] Different letters indicate a significant difference ($p < 0.05$) between the chemical components.

Likewise, the stems could be used as a source of affordable protein as they have a better protein assimilation rate than the leaves when consumed by monogastric animals. In terms of minerals expressed as inorganic matter, according to reports by Atteh *et al.* (2011), the stems would provide sodium and potassium, mainly. In addition, stevia stems can act as non-caloric sweeteners due to their stevioside (0.69 %) and rebaudioside A (0.3 %) contents (Atteh *et al.*, 2011), as prebiotics in a similar manner to inulin and fructooligosaccharides (Sanches-Lopes *et al.*, 2017), and as antioxidants due to the presence of phenolic compounds (Yu *et al.*, 2017).

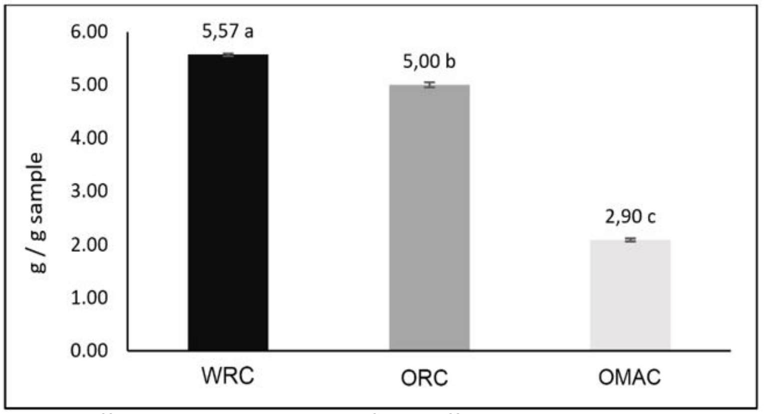
Functional properties

The functional potential of stevia stems is mainly due to their contribution to fiber content. The main reason is based on the interactions that fiber has with other nutrients, usually expressed as water retention capacity (WRC), oil retention capacity (ORC), and organic molecule absorption capacity (OMAC). The results of the functional evaluation of the stems showed that they were able to retain five times their weight in water and oil (5.57 ± 0.12 g/g and 5.00 ± 0.15 g/g, respectively) and twice their weight in organic molecules (2.09 ± 0.09 g/g) (**Figure 1**). It is worth mentioning that water retention capacity (WRC) is in turn composed of both physical (water adsorption capacity, (WAdC) and chemical (water absorption capacity, WAC) interactions. For the stems, the distribution of water retention was 4.49 ± 0.02 g/g absorption and 0.29 ± 0.03 g/g adsorption, respectively (**Figure 2**).

The observed WRC can be explained mainly by the fiber and protein contents present in the stems (**Table 1**), since both elements are prone to form interactions with water molecules. In this sense, water retention may have benefited from the soluble portion of fiber, such as the mucilage and gums, and the interaction of the hydrogen bonds generated with the proteins (Aminlari *et al.*, 2009). In the stevia stems, a higher amount of WAC than WAdC was observed, most likely due to the fiber's ability to form gels and bind water by absorption phenomena through its exposed hydrophilic groups (Segura-Campos *et al.*, 2014).

Figure 1

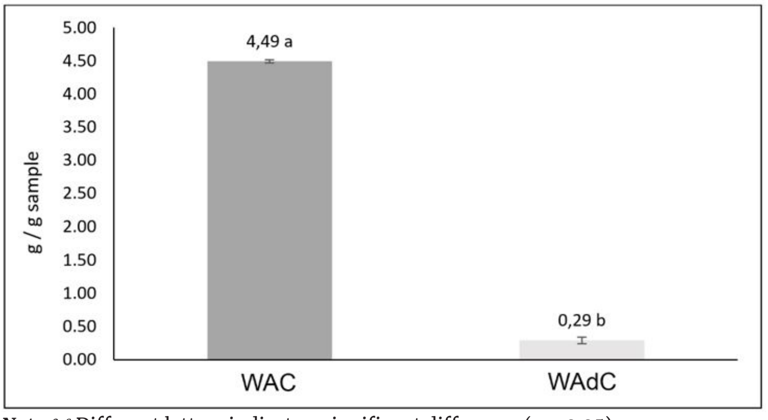
Water retention capacity (WRC), oil retention capacity (ORC), and organic molecule absorption capacity (OMAC) of stevia (Stevia rebaudiana Bertoni) stems



Note. a–c Different letters indicate a significant difference ($p < 0.05$).

Figure 2

Water absorption capacity (WAC) and water adsorption capacity (WAdC) of stevia (Stevia rebaudiana Bertoni) stems



Note. a–c Different letters indicate a significant difference ($p < 0.05$).

The influence of water retention can be seen in the physiological field since when fiber is associated with water, it produces biological effects such as an increased fecal mass, improved intestinal transit, early generation of satiety, low energy density, and reduction of glucose absorption, among others (Mugdil & Barak, 2013).

Furthermore, from a technological point of view, water retention leads to the formation of gels that could be used in foods such as sauces, beverages, jams, etc.

Additionally, the oil retention capacity (ORC) is also related to the fiber content present in the stems, specifically to its insoluble fraction, which consists mainly of cellulose, lignin, and insoluble hemicelluloses. This characteristic is related to the capacity to absorb fat under the action of a mechanical force. When this retention is low, it provides a non-greasy sensation in fried products, but when it is high, it imparts juiciness and better texture to meat products. Moreover, physiologically speaking, this property is relevant since it involves the uptake and elimination of lipid elements such as triglycerides and cholesterol from dietary foods.

According to Vázquez-Ovando *et al.* (2009), the organic molecule absorption capacity (OMAC) is closely related to the insoluble fiber content and could indicate the ability of the stems to intestinally bind to proteins, carbohydrates, bile salts, cholesterol, drugs, toxic compounds, and carcinogens.

Finally, to highlight the functional potential of stevia stems, their properties were compared with the same plant leaves and by-products from other plant sources (**Table 2**). Compared to stevia leaves, the stems retained a higher proportion of water and organic molecules. They also retain more water than other sub-products, such as pomegranate peel, coffee husk, and avocado seeds. Regarding oil retention, the stems have a lower capacity compared to stevia leaves and other byproducts like avocado seeds.

Table 2

Comparison of the functional potential of stevia (Stevia rebaudiana Bertoni) stems

Vegetal source	WRC (g/g sample)	ORC (g/g sample)	OMAC (g/g sample)	Reference
Stevia Stems	5.57 ± 0.12	5.00 ± 0.15	2.09 ± 0.09	Own study
Stevia leaves	4.07	6.79	1.81	Segura-Campos *et al*., (2014)
Pomegranate peel	3.53	4.05	nr	Hasnaoui *et al*., (2014)
Coffee husk	5.11	4.72	nr	Ballesteros *et al*., (2014)
Avocado seed	4.13	6.37	nr	Barbosa-Martín *et al*. (2016)

Note. nr: not reported.

Conclusions

Stevia stems are a potential source of fiber with attractive functional properties for use as functional food ingredients, since they retain five times their weight in water and oil and have twice their absorption capacity for organic molecules.

Due to their fiber content and functional properties, the stems of *S. rebaudiana* can be an alternative to use and valorize agro-industrial wastes with a circular economy approach for the development of functional foods with an added value due to their potential beneficial physiological effects for people with diabetes, obesity, and associated ailments.

These findings indicate that the stems are a potential ingredient in diet and health food products, such as powders, nutrition bars, breads, and cookies. However, more scientific and technological studies related to the contribution of dietary fiber and its fractions, sweetening properties, and studies of bioactive compounds and their effects on the health of consumers are required. This co-product must be further studied so that it can be incorporated into the stevia production chain, adding value and contributing to sustainable development since it is currently considered a waste product. This increases the potential value of stem waste in terms of its viability for commercial applications such as food additives, feed additives, health supplements, and nutraceuticals.

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