

Comparison of Chemical and Functional Properties of *Stevia rebaudiana* (Bertoni) Varieties Cultivated in Mexican Southeast

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ABSTRACT

The leaf powders from two varieties of *Stevia rebaudiana* (Bertoni) cultivated in Yucatan, Mexico were analyzed for their proximate composition, dietary fiber composition and functional properties. The leaf powders were a good source of carbohydrates (64.06% - 67.98%), protein (12.11% - 15.05%), and crude fiber (5.92% - 9.52%). Total dietary fiber content in the *S. rebaudiana* leaf powders were 28.61 (Morita II) and 29.12 (Criolla) g/100g sample, with most of this content represented by insoluble dietary fiber 87.79% (Morita II) and 70.02% (Criolla). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were higher in Criolla (19.29%, 17.77%, 8.98%) than Morita II variety (18.11%, 14.16%, 2.28%). Hemicellulose and cellulose were higher in Morita II (3.96%, 11.78%) than criolla variety (1.51%, 8.79%). Functional properties of leaf powder from Morita II and Criolla were, water-holding capacity (2.87 - 4.07 g/g sample), oil-holding capacity (6.49 - 6.79 g/g sample), water-absorption capacity (3.41 - 3.44 g/g sample), water-adsorption capacity (0.25 - 0.28 g/g sample), and organic molecule absorption capacity (1.13 - 1.81 g/g sample). These suggest that *S. rebaudiana* (Bertoni) leaf powders may be used as dietary supplement or as food additive.

KEYWORDS

Stevia rebaudiana (Bertoni); Physico-Chemical Properties; Dietary Fiber; Functional Properties

1. Introduction

Stevia is a perennial shrub that extensively grows in places like Brazil, Central America and Israel but is native to Paraguay. The genus *Stevia* belongs to Asteraceae family, tribe Eupatorieae and comprises of 240 species. This plant grows mostly at the altitude of 500 - 3000 m above sea level in semidry mountainous terrain. Different species of *Stevia* contain several potential sweetening compounds; *Stevia rebaudiana* (Bertoni) is the sweetest of all [1]. For centuries this herbal sweetener has been used in native cultures to counteract the bitter taste of

various plant based medicines and beverages. Nowadays the extraction of sweeteners from stevia leaves is a growing industrial and commercial worldwide sector; more than 750 tons of stevia leaves per year are used as crude extract for consumption and extraction of glycosides [2]. The sweetening property is associated with their contents of several glycosides, stevioside, steviobioside, rebaudiosides A to F, dulcoside A and steviol. These glycosides and their derivatives are known to account for 4% - 20% of the dry weight of stevia leaves [3].

Stevia and its extract have been studied widely from the sweetener point of view. However, a search through literature show little information on the no-sweetening

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components that make up 80% - 90% dry weight of this plant [4] and no information for varieties adapted for cultivation in Mexico. Green vegetables have long been recognized as the cheapest and most abundant potential sources of proteins, starch, dietetic fiber and other micro-nutrients because of its ability to synthesize these kinds of compounds from a wide range of easily available primary materials such as water, carbon dioxide, and atmospheric nitrogen [5]. According to Aletor and Adebayo [6], leaves and leaf protein concentrates could be used to improve not only the nutritional contribution of food products but also their functional properties. This means that stevia leaf powder or the residue obtained after extraction of sweetening components could be used as additives for the food industry.

Therefore the objectives of this study were to characterize the proximal content, dietary fiber composition and functional properties of the *Stevia rebaudiana* (Bertoni) leaf powder cultivated in Yucatan, Mexico.

2. Materials and Methods

2.1. Raw Material and Chemicals

Stevia rebaudiana (Bertoni) varieties (Morita II and Criolla) were obtained from plots established in Yucatan, México (Figure 1). The plantation had a crop management according to the production technology described by Ramirez *et al.* [7]. Samples were obtained from the first cut of the plot at an age of three months; samples consisted on branches of leaves and stems. All chemicals were reagent grade or better and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Proximate Composition

The proximate composition of *S. rebaudiana* leaf powders were determined using AOAC [8] methods: moisture content (Method 925.09), ash (Method 923.03), crude fat (Method 920.39), crude protein using a 6.25

nitrogen-protein conversion factor (Method 954.01) and crude fiber (Method 962.09). The carbohydrate content was estimated as the nitrogen-free extract (NFE).

2.3. Total Dietary Fiber

This parameter was determined with the gravimetric enzymatic method proposed by Prosky *et al.* [9]. Briefly, 1 g of sample was weighed into each of four flasks and 50 mL of phosphate buffer (50.0 mM, pH 6) were added to each. The flasks were then placed in a water bath at 100°C, 0.1 mL of thermostable α -amylase enzyme (Sigma A-3306) was added to each and then they were agitated at 60 rpm for 15 min. After cooling, pH was adjusted to 7.5. The flasks were returned to the bath at 60°C, 0.1 mL protease (Sigma P-3910) added to each and then they were agitated at 60 rpm for 30 min. After cooling, pH was adjusted to 4.0. The flasks were again placed in the bath at 60°C, 0.3 mL amyloglucosidase (Sigma A-9913) added and then they were agitated for 30 min. Finally, ethanol 95% (v/v), preheated to 60°C, was added at a 1:4 (v/v) ratio. In a vacuum, flask content was filtered into crucibles containing celite (Sigma C-8656). The residue remaining in the flask was washed three times with 20 mL of ethanol 78% (v/v), twice with 10 mL of ethanol 95% (v/v) and twice with 10 mL acetone. Crucible content was dried at 105°C. Protein ($N \times 6.25$) was determined for the residue in two crucibles and the residue in the remaining two was burned at 550°C for 4 h.

$$\text{TDF}(\%) = \frac{[\text{Residue weight (g)}] - \text{protein (g)} - \text{ash (g)} \times 1000}{\text{Sample weight (g)}}$$

2.4. Insoluble Dietary Fiber (IDF)

This was determined following the method of Prosky *et al.*, [9], which is similar to that for TDF, except that addition of ethanol 95% (v/v) at 1:4 (v/v) is omitted.



Figure 1. Morita II and Criolla *Stevia rebaudiana* varieties, respectively.

2.5. Soluble Dietary Fiber (SDF)

Calculated by the difference between TDF and IDF:

$$\text{SDF (\%)} = \text{TDF} - \text{IDF}.$$

2.6. Fiber Components

2.6.1. Acid Detergent Fiber (ADF)

Samples were oven dried at 55°C to dry matter, then ground to pass a 1 mm screen. Dry 50 mL fritted glass crucibles overnight at 100°C and hot weigh (W1), recording weight to nearest 0.1 mg. Thoroughly sample was weighted (W2) (approximately 0.9 to 1.1 g, record weight accurate to 0.1 mg) into Berzelius beaker. A volume of 100 mL acid-detergent solution (20 g of Cetyltrimethylammonium bromide technical grade in 1 L of sulfuric acid 1.0 N) was added at room temperature. Sample was heat to boiling in 5 - 10 min; heat was reduced to avoid foaming as boiling begins. The sample was refluxed 60 min from onset of boil, adjusting boiling to slow, even level. After 30 min, using a minimal amount of acid detergent solution, the beaker was washed. The sample was filtered through tared fritted glass crucible, using minimal vacuum. The Berzelius beaker was washing with boiling water while inverted over the crucible to insure quantitative transfer of all fiber particles into the crucible. The residue was rinse twice with 30 - 40 mL acetone by filling crucible each time with vacuum off, allowing a minimum of 15 to 30 sec (2 min recommended) before vacuuming dry. Finally the residue was dried overnight in forced-air oven at 100°C and weigh hot, recording weight (W3) to nearest 0.1 mg. Percent Acid Detergent Fiber (ADF) was calculated as follows [10]:

$$\% \text{ADF (DM basis)} = (W3 - W1/W2) \times 100$$

where:

W1 = tare weight of crucible in grams,

W2 = initial sample weight in grams,

W3 = dry weight of crucible and dry fiber in grams.

2.6.2. Neutral Detergent Fiber (NDF)

Samples were processed similar than method reported for ADF using a volume of 100 mL neutral-detergent solution. The solution was prepared with distilled water, sodium borate, EDTA, lauryl sulfate, 2-ethoxyethanol and disodium phosphate. The pH was adjusted at 6.9 - 7.1 [10].

2.6.3. Acid Detergent Lignin (ADL)

Samples were processed similar than method reported for ADF using a volume of 100 mL 72% H₂SO₄ solution. The crucible with sample was ignited in a muffle furnace at 500°C for 2 hours. The crucible was weighted to the

nearest 0.1 mg (W3) [11].

2.6.4. Cellulose and Hemicellulose

These parameters were calculated as follows:

%Cellulose = ADF (Cellulose, lignin, cutin) – ADL (Lignin, cutin);

%Hemicellulose = NDF (Hemicellulose, cellulose, lignin, cutin) – ADF (Cellulose, lignin, cutin).

2.7. Water-Holding (WHC) and Oil-Holding Capacity (OHC)

Both capacities were determined following Chau *et al.* [12]. Briefly, 1.0 g (d.b.) of sample was weighed and then stirred into 10 mL distilled water or corn oil (density = 0.89 g/mL) for 1 min in a vortex. These suspensions were centrifuged at 2200 × g for 30 min and supernatant volume measured. Water-holding capacity was expressed as g of water held per g of sample, and oil-holding capacity as g of oil held per g of sample.

2.8. Water Absorption Capacity

The procedure of Chau *et al.* [12] was used. 10 mL of water added to 1.0 g (d.b.) of sample, the suspension was then stirred using magnetic stirrer for 5 min, the suspension was transferred into centrifuge tubes and centrifuged at 2000 × g for 30 min. The supernatant obtained was measured using a 10 ml measuring cylinder. The density of the water was assumed to be 1 g/mL. The water absorbed was calculated as the difference between the initial water used and the volume of the supernatant obtain after centrifuging. The result was expressed as g of water per g of sample.

2.9. Water Adsorption Capacity

This property was determined according to Chen *et al.* [13]. Briefly, 1.0 g (d.b.) of sample was placed in an equilibrium micro-environment at 98% relative humidity, generated by placing 20 mL of saturated potassium sulfate saline solution in tightly sealed glass flasks and placing these in desiccators at 25°C. The sample was left in the microenvironment until reaching constant weight (72 h). Water adsorption capacity was expressed as g of water per g of sample.

2.10. Organic Molecule Absorption Capacity

Organic molecule absorption capacity was determined according to Zambrano *et al.* [14]. A 3.0 g (d.b.) sample was placed in an excess quantity of corn oil (approx. 10 mL) for 24 h at 25°C, and then centrifuged at 2000 × g and 25°C for 15 min. Organic molecule absorption capacity was expressed as the absorbed hydrophobic com-

ponent and calculated in terms of sample weight gain (g oil/g sample).

2.11. Statistical Analysis

Three replicates were performed for each study. All results were analyzed using descriptive statistics with a central tendency and dispersion measures. One-way ANOVAs were run to evaluate proximate composition, dietary fiber content and functional properties. All analyses were done according to Montgomery [15] and processed with the Statgraphics Plus version 5.1 software.

3. Results and Discussion

3.1. Proximate Composition

On dry weight basis, ash, protein and carbohydrate content of *S. rebaudiana* leaf powders were found to be higher whereas fat and crude fiber was estimated to be less (Table 1). The differences in the proximal components of both varieties could be explained by different growth conditions, as described by Rahmesh *et al.* [16].

Ash content is associated with the amount of minerals contained in a sample; these minerals are involved in all aspects of growth, health and reproduction, participating also in the formation of cells, tissues and organs [17]. Mishra *et al.* [18] reported an ash content of 11% for leaves of *S. rebaudiana* from India with a significant content of macronutrients like Ca (464 mg/100g), P (11 mg/100g), Na (190 mg/100g), K (1800 mg/100g), and Mg (349 mg/100g); and micronutrients like Fe (55 mg/100g) and Zn (1.5 mg/100g). Varieties of *S. rebaudiana* cultivated in Mexico contain high amounts of ashes (Table 1), so that could be an important source of macro and micronutrients. The proximal component of lower content was fat 3.23% (Morita II) and 3.04 (Criolla). Tadhani and Subhash [19] reported a fat content of 4.34% for leaves of *S. rebaudiana* from India; these authors identified six fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids) in the leaf oil

with a high content of linolenic acid (21.59 g/100g). Varieties of *S. rebaudiana* contain similar amounts of fat, so that could be an important source of linolenic acid. The protein content of both varieties was higher than reported by Abou-Arab *et al.* [20] that reported (11.4%) for leaves of *S. rebaudiana* from Egypt; these authors identified seventeen amino acids, including all of the indispensable amino acids with the exception of tryptophan. With high levels of protein the varieties of *S. rebaudiana* could be a valuable source of indispensable amino acids for health products. Braz de Oliveira *et al.* [21] isolated from root and leaf of *S. rebaudiana* inulin-type fructooligosaccharides, a naturally occurring plant polysaccharide with important functional properties related to prebiotics, dietary fiber, role lipid metabolism and diabetes control. *S. rebaudiana* are a good source of carbohydrates and crude fiber, this indicates a possible application of leaf powder as a dietary supplement

3.2. Total (TDF), Soluble (SDF) and Insoluble (IDF) Dietary Fiber

Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine [22]. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. In addition an analogous carbohydrate is defined as those carbohydrates-based food ingredients that are non-digestible and non-absorbable, and which are similar to plant dietary fiber [23]. The adequate intake of dietary fiber through the high consumption of fruits and vegetables makes possible to achieve a healthy diet, a correct body weight, an acceptable lipoprotein profile, and a normal blood pressure [24]. TDF content in the *S. rebaudiana* leaf powders were 28.61 (Morita II) and 29.12 (Criolla) g/100g sample, with most of this content represented by IDF 87.79% in Morita II leaf powder and 70.02% in Criolla leaf powder. The remainder was constituted by SDF (Figure 2).

Dietary fiber has been widely studied for its health benefits. It is considered a preventive factor for cancer, serves as a substrate for colonic bacteria, promotes intestinal food transit, and decreases bile acid reabsorption thereby altering micelle formation and contributing to lowering blood cholesterol levels [25]. The *S. rebaudiana* leaf powders differed in their IDF/SDF proportion 8.47 for Morita II and 2.33 for Criolla. The relatively high IDF percentages in *S. rebaudiana* Morita II leaf powder suggest possible applications in dietetic physiological products. Intake of this kind of fiber is linked to a sensation of satiety, since as the fiber absorbs water it takes up space in the stomach and diminishes the need to consume more food. It also increases the volume and

Table 1. Proximate composition of *Stevia rebaudiana* (Bertoni) varieties (% d.b.).

Component	Morita II	Criolla
Moisture	(7.45) ^b	(7.80) ^a
Ash	7.82 ^b	11.93 ^a
Fat	3.23 ^a	3.04 ^a
Protein	12.11 ^b	15.05 ^a
Crude fiber	9.52 ^a	5.92 ^b
Carbohydrates	67.32 ^a	64.06 ^b

^{a,b}Different superscript letters in the same row indicate statistical difference ($p < 0.05$). Data are the mean of three determinations.

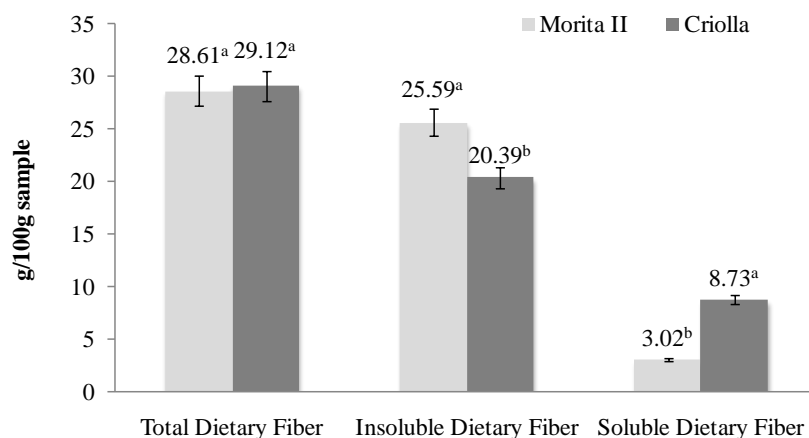


Figure 2. Dietary fiber fractions of *Stevia rebaudiana* (Bertoni) varieties (g/100g sample). ^{a,b}Different superscript letters in the same property indicate statistical difference ($p < 0.05$). Data are the mean \pm standard deviation.

weight of the fecal bolus, promoting improved functioning of the digestive system and preventing disorders such as constipation and colon cancer.

3.3. Fiber Components

Neutral detergent fiber and acid detergent fiber are widely used analytical methods for the estimation of the nutritive qualities of feed. They were originally proposed by Van Soest [26], but have undergone significant changes over the years [10]. Van Soest's fractionation makes it possible to measure the total cell wall content (NDF), lignocellulose content (ADF) and lignin content (ADL). In particular, ADF determination allows the evaluation of practically all the cellulose and lignin. Because of the long history of these methods, there exist large amounts of NDF and ADF data for many different potential celulosic ethanol feed stocks. Briefly, both techniques reflux a sample of biomass in a boiling aqueous surfactant solution. The NDF solution is kept at neutral pH, while the ADF solution contains 1.00 N sulfuric acid. After refluxing for 1 h, the solids are filtered, rinsed multiple times with water and acetone, and then dried. The remaining biomass is weighed, and reported as either a fractional value remaining (%), or in g/kg (parts-per-thousand, ppt). A related procedure, acid detergent lignin, is performed on samples after the ADF procedure, and uses concentrated (72% w/v) sulfuric acid to dissolve any remaining structural carbohydrates, leaving insoluble lignin. **Figure 3** shows NDF, ADF and ADL content of *S. rebaudiana* varieties. NDF includes mainly insoluble hemicellulose, cellulose, lignin and cutin. The NDF content was slightly higher in creole variety. On the other hand, ADF that include principally cellulose, lignin and cutin, and ADL that include lignin and cutin were higher in creole variety. **Figure 4** shows the hemicellulose and cellulose contents of *S. rebaudiana* varieties. Both com-

ponents were higher in Morita II variety. Hemicellulose content was estimated by difference between NDF and ADF. Cellulose content was estimated by difference between ADF and ADL. The properties of hemicellulose may be important in human physiology. This fraction is largely degraded in the colon and has been reported to increase fecal output. Hemicellulose has other important properties such as ion-binding capacity and water-holding capacity. The effects of fiber components has been demonstrated in different studies as realized by Hillman *et al.* [27] who demonstrated that dietary supplementation with maximally tolerated amounts of pectin, cellulose or lignin is associated with significant changes in serum cholesterol levels in healthy normolipidemic subjects in the short term. In general, dietary fiber has been recognized as a healthy food component. Health benefits associated with adequate intake of substances as cellulose, hemicellulose and lignin include: lower blood cholesterol and sugar levels, reduced risk of constipation, obesity, diabetics, heart complications, colon and rectal cancer, gallstone, piles and hernia. These health benefits reflect the nutritional significance of dietary fiber, and have attracted the consumer to fiber rich foods. Public health organization also recommended an increase in the daily consumption of dietary fiber [28]. For these reasons, the determination of the dietary fiber content of food as *Stevia* has been receiving much attention for the last few years.

3.4. Functional Properties

Figure 5 showed the functional properties of *S. rebaudiana* leaf powders from Morita II and Criolla varieties. Morita II showed higher values of water holding and organic molecule absorption capacity than Criolla variety. The water holding capacity of the *Stevia* leaf powders may be due to the high protein content (**Table 1**).

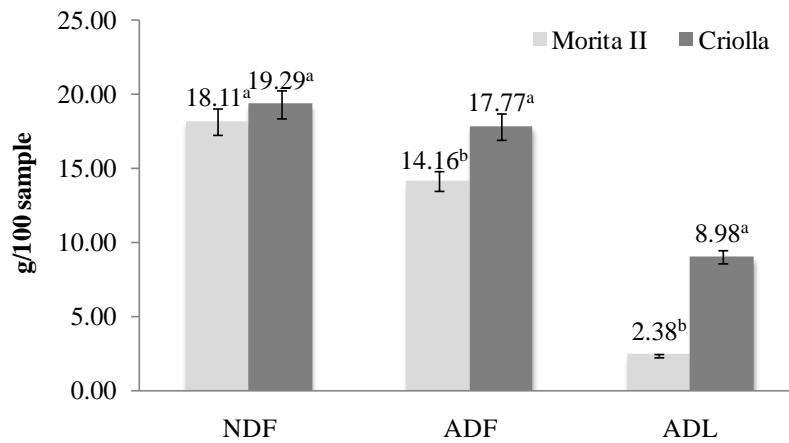


Figure 3. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) content of *Stevia rebaudiana* (Bertoni) varieties. ^{a,b}Different superscript letters in the same property indicate statistical difference ($p < 0.05$). Data are the mean \pm standard deviation.

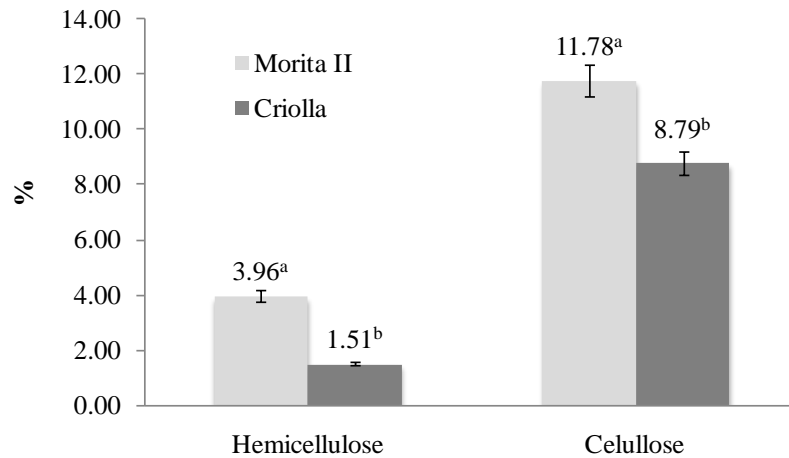


Figure 4. Hemicellulose and cellulose content of *Stevia rebaudiana* (Bertoni) varieties. ^{a,b}Different superscript letters in the same property indicate statistical difference ($p < 0.05$). Data are the mean \pm standard deviation.

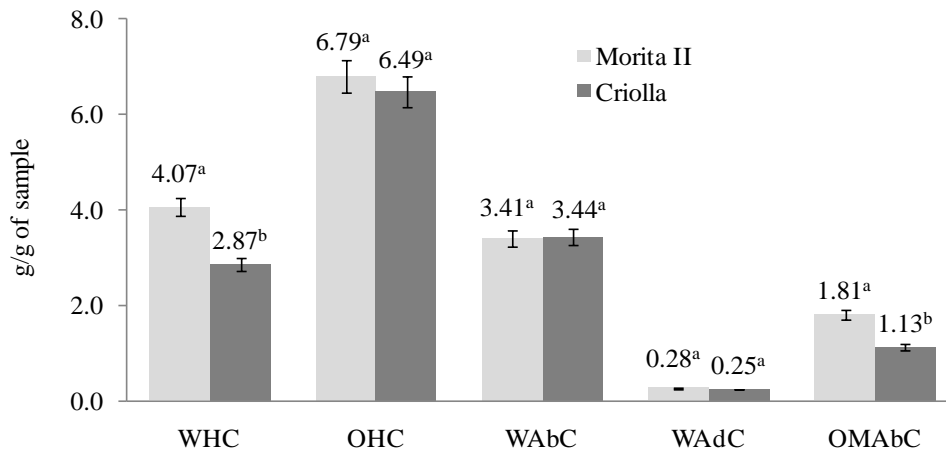


Figure 5. Functional properties of *Stevia rebaudiana* (Bertoni) leaf powder. Water Holding Capacity (WHC), Oil Holding Capacity (OHC), Water Absorption Capacity (WAbC), Water Adsorption Capacity (WAdC), and Organic Molecule Absorption Capacity (OMAbC). ^{a,b}Different superscript letters in the same property indicate statistical difference ($p < 0.05$). Data are the mean \pm standard deviation.

Proteins would increase water holding capacity, thus enhancing the swelling ability [29], an important function of protein in preparation of viscous foods such as soups, gravies, dough and baked products.

Both varieties showed similar oil holding capacity (Figure 5), this functional property has been attributed to the physical entrapment of oil. *Stevia* leaf powders seems to possess an adequate fat absorption capacity, allowing it to play an important role in food processing, since fat acts on flavor retainers and increases mouth feel of foods [30]. The *S. rebaudiana* leaf powders had similar values of water absorption capacity (WAbC) (3.4 g/g sample) and water adsorption capacity (WAdC). Water absorption capacity is indicative of a structure's aptitude to spontaneously absorb water when placed in contact with a constantly moist surface or when immersed in water and water adsorption capacity is the ability of a structure to spontaneously adsorb water when exposed to an atmosphere of constant relative humidity. It is initially a surface phenomenon but at higher hydration levels absorption can occur inside the structure, leading to swelling and eventual solubilization [14]. In this case, the proteins would have a large number of exposed hydrophilic sites that could be interacting with water and increasing WAbC [31].

The *S. rebaudiana* leaf powders showed values of water adsorption capacity of 0.28 and 0.25 g water/g sample, which are substantially lower than values for carrot (0.82 g water/g sample) and beet (1.58 g water/g sample) [14]. The insoluble dietary fiber is responsible for most WAdC since it adsorbs water like a sponge; however, no direct relation between IDF and WAdC was observed here. The *S. rebaudiana* organic molecule absorption capacities (OMAbC) were 1.81 and 1.13 g/g sample. Given this OMAbC values, the *S. rebaudiana* leaf powders could function efficiently interacting with fats, biliar acids, cholesterol, drugs and even toxic or carcinogenic compounds at the intestinal level and then be eliminated in the feces. Lignin, an IDF component, is probably the main compound responsible for OMAbC [32], in the *S. rebaudiana* powders since it is among the fiber components with the highest capacity to link to organic molecules.

4. Conclusion

Stevia rebaudiana (Bertoni) leaf powders from Morita II and Criolla varieties cultivates in Mexican Southeast are good sources of carbohydrates, protein and dietary fiber which are vital for human nutrition and maintain a good health. It is suggested that they may be used in enhancing the nutritive value of low nutrient density foods. Beside their functional properties indicate that could be applied as binders or extenders in food systems.

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Abbreviations

ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; ANOVA: Analysis of Variance; AOAC: Association of Official Analytical Chemists; IDF: Insoluble Dietary Fiber; NDF: Neutral Detergent Fiber; NFE: Nitrogen-Free

Extract; OHC: Oil-Holding Capacity; OMAbC: Organic Molecule Absorption Capacity; TDF: Total Dietary Fiber; SDF: Soluble Dietary Fiber; WAbC: Water Absorption Capacity; WAdC: Water Adsorption Capacity; WHC: Water-Holding Capacity.