Journal of Materials and Environmental Sciences
ISSN : 2028-2508

JMES, 2017 Volume 8, Issue 3, Page 1015-1022

http://www.jmaterenvironsci.com /

Nutritional and biochemical properties of natural sweeteners of six cultivars of *Stevia rebaudiana* Bertoni leaves grown in Morocco

A. Khiraoui[1,2], M. Bakha[2,3], F. Amchra[2,4] , S. Ourouadi[1], A. Boulli[1], C. Al-Faiz[2], A. Hasib[1*]

1 Equipe Environnement et Valorisation des Agro-Ressources, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, BP: 523, Mhgila Beni Méllal, Morocco.

2 Institut National de la Recherche Agronomique, UR Plantes Aromatiques et Médicinales et Produits de terroirs, INRA, CRRA-Rabat, PB 6570, 10101 Rabat, Morocco.

3 Laboratoire de Biologie et Santé, Faculté des sciences, Université Adbelmalek Essaadi, BP 2121, 93002 Tétouan, Morocco.

4 Laboratoire de Matériaux, d'Electrochimie et environnement Faculté des sciences, Université Ibn-Tofail, B.P 133 Av. Université, Kénitra 14000, Morocco.

Received 08 Feb2016,
Revised 26 Oct 2016,
Accepted 30 Oct 2016

Keywords

- ✓ *Stevia rebaudiana*,
- ✓ proximal composition,
- ✓ carbohydrates, protein,
- ✓ crude fiber,
- ✓ fat,
- ✓ minerals,
- ✓ stevioside.

azhasib@yahoo.fr
(A. Hasib);
Tel (212) 61 38 69 02;
Fax: (212) 48 52 01

Abstract

In order to know the variability of a breeding on six cultivars from different areas in Morocco, *Stevia rebaudiana* leaf was investigated for its proximate composition, mineral content and biochemical screening. Further, stevia plants are a good source of carbohydrates, protein and crude fiber contents were found to be (51.50 – 56.72%), (11.75 – 16.23%) and (17.43 – 19.13%) on dry weight basis, respectively. In this order, stevia leaves also contained (12.47 to 17.35 %) on dry weight basis for total soluble carbohydrates. Minerals content analysed by spectrometric ICP-AES showed that the high content of K, Ca, Mg, Na of stevia were present in the leaf; whereas, Mn, Fe, Cu, Zn, and Cr were found as trace amount. The responsible compounds for the natural sweetness of stevia leaves include a diversity of diterpenoid glycosides derived from a steviol skeleton. These steviol glycosides also exhibit a low calorific value as well as promising therapeutic applications. The stevioside was extracted by hot water (60 °C) from the dried stevia leaves and analyzed by HPLC. Our results showed that the stevioside content was obtained at (6.26 - 10.10 %) on dry weight basis. So, stevia leaf produces a variety of high potential natural-source and low calorie sweetener. These results strongly suggest that due to its all favourable properties; stevia could be used in either food or cosmetic and pharmaceutical products.

1. Introduction

There is an increasing interest in *S. rebaudiana* Bertoni leaves as they are a good source of non-caloric sweeteners due to the presence of steviol glycosides that has a great potential in food industry as a strategy to reduce sugar consumption [1].

Moreover, *S. rebaudiana* is a perennial shrub of the Asteracease (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is often known as "the sweet herb of Paraguay" [2]. The plant is also cultivated in China and Southeast Asia [3]. Stevia sweeteners, crude extract from leaves, are used to sweeten soft drinks, soy sauce, yogurt, and other foods in Japan, Korea and Brazil [4]. The dry extract from the leaves of stevia contains flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxycynnamic acids (caffeic, chlorogenic, etc.), oligosaccharides, free sugars, amino acids, lipids and trace elements [5].

Stevia, the common name for the extract stevioside from the leaves of *S. rebaudiana Bertoni*, is new promising renewable raw food stuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners [6]. The natural sweeteners of stevia leaves, called steviol glycosides, are diterpenes, isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside [7].

The study of Mohammad *et al* [8] identified nine amino acids in stevia leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine and methionine. Abou-Arab *et al* [9] found still more amino acid in the stevia leaves. Altogether seventeen amino acids were determined and classified as essential and non-essential amino acids.

In *S. rebaudiana* roots and leaves, inulin-type fructooligosaccharides, a naturally occurring plant polysaccharide with important functional properties related to prebiotics, dietary fiber, role lipid metabolism and diabetes control, have been isolated by Braz de Oliveira *et al* [10]. In the leaf oil of stevia, Tadhani and Subhash [11] identified six fatty acids using methyl ester standards. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were identified in the leaf oil.

Kim *et al* [12] studied the amounts of water-soluble vitamins in the stevia leaf and callus extracts, and determined that the contents of folic acid, vitamin C and vitamin B2 in the leaf extracts were significantly higher than those of the callus extracts. In the leaf extract, folic acid was found to be the major compound, followed by vitamin C. In the callus extract, vitamin C was the major compound, followed by vitamin B.

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [13].The phytochemicals present in *S. rebaudiana* are austroinullin, b-carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside and thiamine [14]. The presence of macro and micronutrients in foods is important for the development and maintenance of vital body functions. They are involved in all aspects of growth, health and reproduction, participating also in the formation of cells, tissues and organs [15].

S. rebaudiana extracts have been suggested to exert beneficial effects on human health, including antihypertensive, antimicrobial, antiobesity and antioxidant activities and also thought to influence glucose metabolism and renal functions [16]. *S. rebaudiana* does not lower blood glucose levels in normal subjects [17].

Diabetes mellitus is a major public health problem in the world, the prevalence of diabetes mellitus is increasing with ageing of the population and lifestyle changes associated with rapid urbanization. The World Health Organization estimates that 3% of the world's populations (194 million) have diabetes and is expected to double (6.3%) by the year 2025. In Morocco, the statistics are alarming. According to the latest figures from the Ministry of Health issued on the occasion of World Diabetes Day, Morocco has more than 2 million diabetes in over 20 years.

The inhibition for the corrosion of mild steel in sulphuric acid solution by the extract of Stevia rebaudiana leaves has been studied using electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization techniques. Inhibition was found to increase with increasing concentration of the leaves extract. The effect of temperature on the corrosion behavior of mild steel in 0.5M H2SO4 with addition of extract was also studied [18]. Introduction of green corrosion inhibitor can stop the use of expensive and harmful synthetic inhibitor [19].

In many Moroccan regions, stevia Leaves powdered are just used to sweeten tea and the tisanes. Until now, nobody has investigated the potential nutritive of biochemical constituents of stevia leaves in Morocco. Therefore, the main aim of this study is to evaluate the biochemical characteristics that were investigated in six different areas of Morocco.

2. Material and Methods

2.1 Raw Materials

The stevia leaves (*Stevia. rebaudiana* Bretoni*),* were collected from different areas in Morocco (Table 1, Figure 1) at *Agadir* (30°30' N, 9°33' W), *Berkane* (34°55' N, 2°19' W), *Larache* (35°09' N, 6°08' W), *Marrakech* (31°37' N, 8°30' W), *Rabat* (34°01' N, 6°51' W) and *Sefrou* (33°49' N, 4°51' W). The stevia plants that reached the maximum growth stage (mature stage before flowering) were harvested at August 2014 by cutting the plant at 5 - 10 cm from the ground. The brown and yellow leaves were removed. The plants were also dried under an aired greenhouse at temperature ranged from to 25 - 30°C for 48 - 72 h.

Table1. Geographical characteristics of the regions studied

	Soil characteristics	**Bioclimat**
Agadir	Sandy	Arid
Berkane	Very fine silty	Semi-arid
Larache	Sandy loam	Sub-humid
Marrakech	Sandy clay	Semi-arid
Rabat	Sandy loam	Sub-humid
Sefrou	Silty clay	Sub-humid

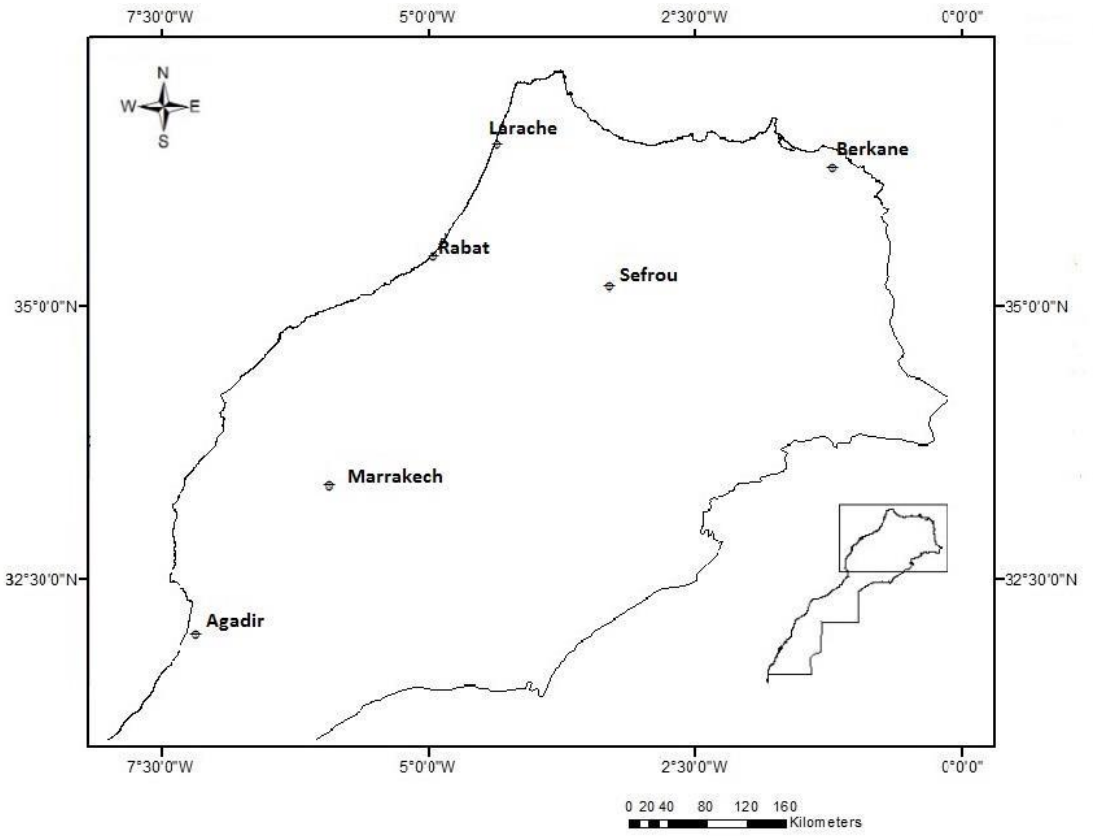

Figure 1: Location map of the different study locations.

2.2 Experimental

Harvested leaves were blended to powder form with a high speed blender (Microtron MB 550), KINEMATICA AG (Switzerland) and were kept in glass vials to be stored at 4 ± 1°C in a refrigerator until used.

Moisture, contents of stevia leaves samples were established according to (AOAC, 1990) [20] method; the ground samples were dried in an oven at 105°C for 24 h. The percentage of loss in weight was expressed as the moisture content. The dry matter of harvested plants was determined by difference [20]. Ash was determined by combustion of the samples in a muffle furnace at 550°C for 12 h [20].

The sugar content was established by using the Bertrand method [20]. This dosage consists of collecting the precipitate of the copper (II) oxide formed by reduction of the cupro-alkaline liqueur in presence of reducing sugars, and to measure it out by manganimetry. Bertrand's tables give the directly correspondence between the volume of KMnO4 (0,1N) used and content of glucose of the trial hold.

The fat content of the samples was extracted with hexane in a Soxhlet apparatus. Proteins content was determined from the nitrogen content by Kjeldahl method using factor 6.25 and calculated as N x 6.25 [21].

Crude fiber is loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 and NaOH under specific conditions [20].

Total energy (Calorific value) was determined according to the method described by Sukker [22] using the Atwater factor. By this determination, 1g of carbohydrate provides 4 kcal; 1g of protein provides 4 kcal and 1 g fat provides 9 kcal.

Conventional extraction method of stevioside was performed with 1g of stevia leaves, by adding 40 ml of distilled water maintaining at 60 °C for 120 min with magnetic. Extracts were diluted if necessary and the amount of stevioside were determined by HPLC. All the extracts were diluted with deionized water and filtered through a 0.45 µm cellulose membrane filter (Xilab, Belgium) before being subjected to HPLC analysis. The HPLC system consisted of a Dionex P680 pump, a Dionex ASI-100 automated sample injector, a Dionex Ultimate 3000 Column Compartment (Thermo Scientific, France), a diode array detector (DAD) (Hewlett Packard, France) and piloted by a Chromelon chromatography data system (Thermo Scientific, France) to obtain chromatographic profiles of the extracts. Separation was performed with the mobile phase consisted of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B) (69:31 v/v) as isocratic at flow rate of 1 ml/min for 30 min. Samples were injected (20 µl) onto an apolar reversed-phase C18 column (250 - 4.6 mm ID, 5µ,

Phenomenex, Le Pecq, France) at 40°C. Chromatograms were recorded at 200nm. The standards used in the experiments were weighed and dissolved in deionized water. Calibration curves were generated with concentrations ranging from 50 to 500 mg/l of stevioside.
For mineral analysis, samples were incinerated in muffle furnace at 550°C for 12h. Ashes were quantified gravimetrically. The ashes were diluted with hydrochloric acid 0,1N and with distilled and demineralised water. The dosage of the studied trace elements (Ca, K, Mg, Na, Mn, Fe, Cu, Zn and Cr) was created by the water method regia and analysis by spectrometric Plasma Atomic Emission Inductively Coupled argon (ICP-AES). The water method regia's policy sample dissolution in a mixture of hydrochloric and nitric acid, Then analyzed by ICP-AES. The used apparatus was an ICP-AES (Varian-Vista), equipped with an ultrasonic nebulizer Cetac. The main analytical parameters of the device are:

- Rf power: 0.7 - 1.5 kilowatt (kW 1.2-1.3 for axial);
- Flow rate of plasma gas (Ar): 10.5- 15 l / min (radial), 15 l / min (axial);
- Auxiliary gas flow (Ar): 15 l / min (axial);
- Viewing size: 5-12 mm;
- Copy and playback time: 1-5s (maximum 60 s);
- Copy Time: 3 s (maximum 100 s).

2.3 Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 20.0 for window. All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3. Results and Discussion

The analysis of variance (ANOVA) showed a variability of biochemical characters measured highly significant ($p < 0.01$) than the threshold 0.05 (Table 2).
The results obtained by the biochemical analysis of dried stevia plants are presents in Table 2. An ANOVA analysis was performed to evaluate if there were significant. Differences in data showed that leaves had moisture ranged from 4.97 to 8.31 % in *Marrakech* and *Agadir* regions respectively. These moisture levels are similar to those reported by Goyal *et al* [23], and Kaushik *et al* [24].
The results of dry matter and the ash's data shows that they vary widely from one area to another and ranges from 91.68 to 95.02 % and 7.37 to 11.28 % respectively. *Marrakech* cultivar records the highest value of dry matter and ash compared to other regions, confirming also the results found by Goyal et *al* [23] and Tadhani & Subhash [11], with 95.35 % for dry matter and 13.1 % for the ash.
Stevia dry mass production from the six regions has yielded ranged from 11.75 and 16.23 % for *Marrakech* and *Sefrou* respectively. *Sefrou* cultivar records the highest value of proteins compared to other regions and is found of the low amount of total proteins in *Marrakech* cultivar (Table. 2). These values were important than those found by Serio [25], Wölwer-Rieck [1] and Tadhani & Subhash [11].
The leaves stevia analysed contain quite high percentage of carbohydrates ranging from 51.50 to 56.72 % of dry matter. *Agadir* cultivar was also found to contain the highest value while a low value was found in *Rabat* and *Berkane* cultivars. These results are in agreement with those reported by Mishra et *al* [26] and Abou-Arab et *al* [9], in this order, stevia leaves also contained 4.06 to 5.87 %, 9.17 to 11.47 % and 12.47 to 17.35 % for reducing sugars, total sugars and total soluble carbohydrates, for *Rabat* and *Marrakech* respectively (Table 2).
The stevia leaves contained higher amounts of dietary fiber in the range of 17.43 to 19.13 % of dry matter in *Rabat* and *Berkane* cultivars respectively, which is in accordance with those reported by [23] and [16]. Thus, stevia leaves could be considered as a potential fiber to be used in food formulations. High ash content indicates that the stevia leaves are also good source of inorganic minerals.

Table 2. Chemical composition of dried stevia leaves (*S. rebaudiana*) (g /100 g dry weight basis) from different geographical areas in Morocco.

	Agadir	***Berkane***	***Larache***	***Marrakech***	***Rabat***	***Sefrou***
Moisture	$8.31^{a} \pm 0.19$	$5.95^{bc} \pm 0.36$	$7.04^{ab} \pm 0.34$	$4.97^{c} \pm 0.24$	$8.24^{a} \pm 0.23$	$5.01^{c} \pm 0.22$
Dry matter	$91.68^{c} \pm 0.19$	$94.04^{ab} \pm 0.36$	$92.95^{bc} \pm 0.34$	$95.02^{a} \pm 0.24$	$91.75^{c} \pm 0.23$	$94.98^{a} \pm 0.22$
Ash	$8.13^{d} \pm 0.04$	$9.83^{b} \pm 0.16$	$7.37^{e} \pm 0.06$	$11.28^{a} \pm 0.08$	$10.34^{b} \pm 0.11$	$8.82^{c} \pm 0.10$
Protein	$12.36^{c} \pm 0.04$	$15.24^{b} \pm 0.15$	$14.89^{b} \pm 0.15$	$11.75^{d} \pm 0.06$	$14.90^{b} \pm 0.07$	$16.23^{a} \pm 0.04$

Fat	$3.91^{cd} \pm 0.05$	$4.27^{c} \pm 0.01$	$4.05^{cd} \pm 0.10$	$4.94^{b} \pm 0.05$	$5.78^{a} \pm 0.05$	$3.86^{d} \pm 0.10$
Crude fiber	$18.85^{ab} \pm 0.21$	$19.13^{a} \pm 0.08$	$19.02^{ab} \pm 0.22$	$18.08^{bc} \pm 0.16$	$17.43^{c} \pm 0.09$	$17.69^{c} \pm 0.21$
Carbohydrates*	$56.72^{a} \pm 0.20$	$51.50^{d} \pm 0.19$	$54.66^{bc} \pm 0.18$	$53.93^{b} \pm 0.16$	$51.52^{d} \pm 0.20$	$53.37^{c} \pm 0.15$
Reducing sugar	$5.17^{b} \pm 0.08$	$4.81^{b} \pm 0.16$	$4.06^{c} \pm 0.09$	$5.87^{a} \pm 0.08$	$3.30^{d} \pm 0.21$	$3.87^{cd} \pm 0.04$
Non-reducing sugars	$10.78^{a} \pm 0.13$	$9.86^{b} \pm 0.13$	$9.71^{b} \pm 0.14$	$11.47^{a} \pm 0.20$	$9.17^{b} \pm 0.09$	$11.31^{a} \pm 0.09$
Total soluble carbohydrates	$15.96^{b} \pm 0.27$	$14.67^{c} \pm 0.05$	$13.77^{d} \pm 0.02$	$17.35^{a} \pm 0.17$	$12.47^{e} \pm 0.02$	$15.19^{bc} \pm 0.16$
Stevioside	$10.10^{a} \pm 0,30$	$6.66^{b} \pm 0,20$	$6.26^{b} \pm 0,56$	$7.90^{b} \pm 0,23$	$7.73^{b} \pm 0,53$	$7.83^{b} \pm 0,13$

* Calculated by difference.
- All values are means of triplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different ($P < 0.05$).

From this study and other investigations, stevia leaves are a good source of carbohydrates, protein, and crude fiber which are the essential factors for maintenance of health. Beside, compared to other stevia leaves fat contain values in different studied regions found by Serio [25] and Mishra et *al* [26], our results are less, ranging between 3.86 to 5.78 % in *Sefrou* and *Rabat* regions respectively.

The extract prepared from the leaves of *S. rebaudiana* contains sweet steviol glycosides, mainly stevioside. After HPLC investigations, our results showed that stevioside varies between 6.26 to 10.10 % in *Larache* and *Agadir* regions respectively. These results of stevioside are similar to those reported by Goyal *et al* [23], and Kinghorn & Soejarto [4].

Stevia, the common name for the extract stevioside from the leaves of *S. rebaudiana* Bertoni, is a new promising renewable raw food stuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners [6]. Stevioside has the chemical formula of a diterpene glycoside ($C_{38}H_{60}O_{18}$) and, as an active component in stevia leaves is responsible for the edulcorant properties. Its use has been approved in Brazil, Argentina and Paraguay as well as in China, Korea and Japan. These molecules are highly stable in aqueous solutions within a broad range of pH and temperature [9]. And also stevioside, an abundant component of *S. rebaudiana* leaf, has become well-known for its intense sweetness (250–300 times sweeter than sucrose) and is used as a non-caloric sweetener in several countries. A number of studies have suggested that, beside sweetness, stevioside and rebaudioside A, may also offer therapeutic benefits, as they have anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory actions [27].

Stevia leaves have recorded a higher value of proteins, crude fiber and carbohydrate comparing to different aromatic and medicinal plants. However, the quantity of fat was so low comparing to these plants (Table 3).

Table 3. Stevia biochemical compounds comparison to different aromatic and medicinal plants (mg/ 100 g) of dried weight

	Stevia	***Tea*** [28]	***Olive leaves*** [29]	***Oregano*** [30]	***Stinging nettle*** [31]
Carbohydrates	51.50 - 56.72	25	27.5	1.16	ND
Proteins	11.75 - 16.23	15	5.4	0.2	6.5
Crude fiber	17.43 - 19.13	ND	7	0.8	5.3
Fat	3.86 - 5.78	2 - 3	6.5	0.18	1.6
Ash	7.37 - 11.28	5	3.6	ND	5.6

ND, not determined.

Data presented in Table 4 indicates the mean concentrations of macro minerals (Potassium, Calcium, Sodium and Magnesium) and micro elements that is heavy metals (Copper, Manganese, Chromium, Iron and Zinc) determined in dried stevia leaves.

The stevia leaves are considered as a good source of minerals. Our results showed that the stevia leaves are rich in potassium, detected at high content: 1421.24 to 2787.11 mg/100 g, followed by calcium: 579.68 to 734.57 mg/100 g and Sodium: 69.87 to 190.14 mg/100g. The relatively lowest level of major minerals was Magnesium, detected at concentration 179.57 to 198.18 mg/100 g.

Regarding to heavy metals, the leaves contained lower amount of Mn, Fe, Cu, Zn, and Cr which recorded 6.48 to 12.73, 5.73 to 35.44, 0.35 to 1.08 and 1.71 to 5.32 and 0.39 to 2.51 mg/100 g on dry weight basis, respectively.

Table 4. Minerals content (mg 100 g^{-1}) of dried stevia leaves from different geographical areas in Morocco

	Agadir	***Berkane***	***Larache***	***Marrakech***	***Rabat***	***Sefrou***
K	$1421.24^{f} \pm 0.17$	$2551.34^{b} \pm 0.08$	$1633.25^{e} \pm 0.10$	$2787.11^{a} \pm 0.03$	$1940.71^{d} \pm 0.06$	$1987.19^{c} \pm 0.76$
Ca	$579.68^{d} \pm 0.43$	$606.71^{b} \pm 0.03$	$733.77^{a} \pm 0.16$	$734.57^{a} \pm 0.14$	$599.74^{c} \pm 0.40$	$598.77^{c} \pm 0.37$
Na	$97.83^{e} \pm 0.07$	$69.87^{f} \pm 0.05$	$185.18^{b} \pm 0.11$	$123.28^{c} \pm 0.13$	$190.14^{a} \pm 0.07$	$108.31^{d} \pm 0.19$
Mg	$182.90^{d} \pm 0.55$	$184.21^{cd} \pm 0.07$	$188.42^{b} \pm 0.26$	$185.36^{c} \pm 0.06$	$198.18^{a} \pm 0.08$	$179.57^{e} \pm 0.14$
Mn	$7.80^{b} \pm 0.04$	$6.71^{c} \pm 0.06$	$12.73^{a} \pm 0.17$	$6.52^{c} \pm 0.08$	$5.40^{d} \pm 0.15$	$6.48^{c} \pm 0.02$
Fe	$19.56^{b} \pm 0.33$	$35.44^{a} \pm 0.04$	$12.60^{d} \pm 0.09$	$19.89^{b} \pm 0.04$	$5.73^{e} \pm 0.11$	$14.33^{c} \pm 0.12$
Cu	$0.35^{b} \pm 0.01$	$1.08^{a} \pm 0.01$	$0.56^{b} \pm 0.12$	$1.08^{a} \pm 0.03$	$0.53^{b} \pm 0.08$	$0.36^{b} \pm 0.04$
Zn	$2.16^{c} \pm 0.01$	$3.28^{b} \pm 0.02$	$2.87^{b} \pm 0.01$	$2.17^{c} \pm 0.02$	$5.32^{a} \pm 0.11$	$1.71^{d} \pm 0.09$
Cr	$0.39^{d} \pm 0.004$	$1.92^{a} \pm 0.06$	$2.38^{b} \pm 0.05$	$1.12^{c} \pm 0.01$	$0.96^{c} \pm 0.04$	$2.51^{b} \pm 0.04$

- All values are means of duplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different ($P < 0.05$).

The mineral pattern depends on the fruit origin and factors of cultivation site. Similar results have been reported in the literatures [1, 11, 23, 26, and 32]. But we found that the mineral Sodium in the region of *Marrakech*, thus the mineral Manganese in the region of *Marrakech*, and also Chromium in *Sefrou* and *Larache*, generally higher than those reported by Erna [32].

Minerals have many important functions in the human body. The main elements are sodium, magnesium, phosphorus, sulphur, chlorine, potassium, and calcium which are classified as macronutrients while the minor elements, considered as micronutrients, are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine [15, 33]. Stevia contains substantial amounts of these important nutrients, which further establishes it as a mineral loaded ingredient needed to protect the body, regulate and maintain the various metabolic processes. Potassium, Calcium, Magnesium, and Sodium, which are nutritionally important, were found in a reasonable amount in stevia leaves [34].

Iron, zinc and manganese are considered as, antioxidant, micro nutrients; and their presence could therefore boost the immune system [35] and in prevention of free radical mediated diseases at each level. Iron is an essential element for synthesis of hemoglobin. The higher amount of iron in stevia leaves is again useful in maintenance of normal hemoglobin level in the body. Moreover, stevia leaves could also be used to prepare various sweet preparations for combating iron deficiency as anemia. Which is the major nutritional disorder of developing countries [9].

Nutrient composition of stevia (Table 5) which was analyzed on dry weight basis indicated that energy value being analyzed were found to be (3.05 - 3.17 Kcal /g) which may be entitled as the status of low calorific sweetener due to its intense sweetness in comparison to other available low calorie sweeteners. These results are in agreement with those reported by [26] and [36]; they reported that total energy were (2.7 and 3.6 Kcal/g) respectively. Intense sweetness includes acesulfame potassium (Calorie free), aspartame (4.0 Kcal/ g), saccharin (1.7 Kcal/g) and sucralose (0.9 Kcal/g) [37]. Calorie contribution to the diet by commonly used sucrose being considered high as it gets utilized by the body more completely and has a potential to escalate towards overweight status. In this concern, the use of stevia as a low calorie sweetener could be of immense help in restricting the calorie intake in the diet of affluent and also where calorie restricted diets are prescribed.

Table 5: Total energy of dried *S. rebaudiana* leaves from different geographical areas in Morocco (Kcal /100 g dry weight basis).

	Agadir	***Berkane***	***Larache***	***Marrakech***	***Rabat***	***Sefrou***
Carbohydrate (Kcal/100g)	226.88 ± 0.80	206.00 ± 0.76	218.64 ± 0.72	215.72 ± 0.64	206.08 ± 0.80	213.48 ± 0.60
Protein (Kcal/100g)	49.44 ± 0.16	60.96 ± 0.60	59.56 ± 0.60	47.00 ± 0.24	59.60 ± 0.28	64.92 ± 0.16
Fat (Kcal/100g)	35.19 ± 0.45	38.43 ± 0.09	36.45 ± 0.90	44.46 ± 0.45	52.02 ± 0.45	34.74 ± 0.90
Total energy (Kcal/100g)	311.51 ± 1.41	305.39 ± 1.45	314.65 ± 2.22	307.18 ± 1.33	317.70 ± 1.53	313.14 ± 1.66

In safety studies, no negative side effects were reported [38]. Stevia was recently approved for use as a sweetener by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives [39], and has also recently received GRAS approval from the Food and Drug Administration. However, no study to date has examined the effect stevia has on food intake and satiety levels [6].

Conclusion

After a scientific study, considering the biochemical composition like protein, total sugar, fat, stevioside and dietary fiber content. *S. rebaudiana* could be used as part of the nutrition for human beings. So, it could be concluded that stevia plants are a good source of carbohydrates, protein, and also could be considered as an excellent source of stevioside, crude fiber and minerals. Finally, the determined concentration of heavy metals in leaves of *S. rebaudiana,* were in normal range level. The implication of the findings may be taken into consideration, while using the herbs for food safety in preparation of herbal products and standardized extracts, should be collected from an unpolluted natural plants.

Acknowledgments-The authors are pleased to acknowledge the National Institute for Agricultural Research, UR Medicinal and Aromatic Plants INRA, CRRA-Rabat, Morocco for providing the facilities for the research. Also the laboratory of Environment and Valorization of Agro-Resources, Faculty of Sciences and Technology, University of Sultan Moulay Slimane, Beni-Mellal, Morocco is gratefully acknowledged for their facilities for the characterization.

Reference

1. Wölwer-Rieck, U. The leaves of *Stevia rebaudiana* Bertoni, their constituents and the analyses thereof: a review. *J Agric Food Chem*. 60 (2012) 886–895.
2. V.S.P. Chaturvedula, J.F. Clos, J. Rhea, D. Milanowski, U. Mocek, G.E. DuBois, I.Prakash, Minor diterpenoid glycosides from the leaves of *Stevia rebaudiana*. *Phytochemistry Lett*. 4 (2011) 209–212.
3. Koyama, E., Sakai, N., Ohori, Y., Kitazawa, K., Izawa, O., Kakegawa, K., *et al*. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. *Food Chem Toxicol*. 41(2003) 875-883.
4. Kinghorn, A. D., & Kim, N. C. Highly sweet compounds of plant origin. *Arch Pharm Res.* 25 (2002) 725-746.
5. Komissarenko, N. F., Derkach, A. I., Kovalyov, I. P., & Bublik, N. P. Diterpene glycosides and phenylpropanoids of *Stevia rebaudiana* bertoni. Rast Research. 1 (1994) 53-64.
6. Anton, S., Martin, C., Han, H., Coulon, S., Cefalu, W., Geiselman, P., *et al*. Effects of stevia, aspartame, and sucrose on food intake, satiety and postprandial glucose and insulin levels. *Appetite*. 55 (2010) 37–43.
7. Geuns, J. Stevioside. *Phytochemistry*. 64 (2003) 913–921.
8. Mohammad, M., Mohammad, U., Sher, M., Habib, A., etIqbal, A. *In vitro* clonal propagation and biochemical analysis of field established *Stevia rebaudiana* Bertoni. *Pakistan J Bot*. 39 (2007) 2467–2474.
9. Abou-Arab, A., Abou-Arab, A., et Abu-Salem, M. F. Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant. *Afr J Food Sci*. 4 (2010) 269–281.
10. Braz de Oliveira, A. J., Correia Gonçalves, R. A., Cantuaria Chierrito, T. P., Müller dos Santos, M., Mera de Souza, L., Gorin, P. A. J., *et al*. Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni. *Food Chem*. 129 (2011) 305–311.
11. Tadhani, M., & Subhash, R. Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. *J Med Sci*. 6 (2006a) 321–326.
12. Kim, I., Yang, M., Lee, O., et Kang, S. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT – *Food Sci and Tech*. 44 (2011) 1328–1332.
13. Edeoga, H., Okwu, D., et Mbaebie, B. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotech*. 4 (2005) 685–688.
14. Jayaraman, S., Manoharan, M., et Illanchezian, S. *In-vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. *Trop J Pharm Res*. 7 (2008) 1143–1149.
15. Szefer, P., & Nriagu, *J. Mineral components in foods*. New York: CRC Press. (2007) 10.
16. Savita, S., Sheela, K., Sunanda, S., Shankar, A., et Ramakrishna, P. *Stevia rebaudiana* – A functional component for food industry. *J Human Ecology*. 15 (2004) 261–264.
17. M. Jamaluddin Ahmed, Roger M. Smith. Determination of stevioside by high-performance liquid chromatography with pulsed amperometric detection. *J Sepa Sci*. 25 (2002) 170-172.

18. Cang H, Shi W, Shao J, Xu Q. Study of Stevia rebaudiana leaves as green corrosion inhibitor for mild steel in sulphuric acid by electrochemical techniques. *International Journal of Electrochemical Science*. 7(4) (2012) 3726–36.
19. Singh A, Singh VK, Quraishi MA. Effect of fruit extracts of some environmentally benign green corrosion inhibitors on corrosion of mild steel in hydrochloric acid solution. *Journal of Materials and Environmental Science*. 1(3) (2010) 62–74.
20. AOAC," Official methods of analyses", Association of Official Analytical Chemists: Washington, (1990).
21. Pomeranz, Y., Clifton,M.E Food analysis: Theory and practice. 2nd edition Van Nostrand Reinold. New-York (1987) 797.
22. Sukker MY. Human Nutrition for Medical Studies and Applied Health Science. *Ithaca Thaca Press*, London (1985).
23. Goyal, S., Samsher et Goyal, R. stevia (*Stevia rebaudiana*) a bio-sweetener: A review. *Inte J Food Sci Nutr*. 61 (2010) 1–10.
24. Kaushik, R., Pradeep, N., Vamshi, V., Geetha, M., et Usha, A. Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. *J Food Sci Tech*. 47 (2010) 27–33.
25. Serio, L. La *Stevia rebaudiana*, une alternative au sucre. *Phytothérapie*. 8 (2010) 26–32.
26. Mishra, P., Singh, R., Kumar, U., Prakash, V. *Stevia rebaudiana* – A magical sweetener. *Global J Biotecnol Biochem*. 5 (2010) 62–74.
27. Varanuj Chatsudthipong, Chatchai Muanprasat. Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacol Therapeut*.121 (2009) 41–54
28. Martha Krieps. Le The: Origine, Actualité et Potentialités. Thèse de doctorat, Faculté de pharmacie de l'Université Henri Poincaré - Nancy 1 (2009) p 69.
29. Erbay, Z., Icier, F. Optimization of hot air drying of olive leaves using response surface methodology. *J Food Eng*. 91 (2009) 533-541.
30. Marie-Aude Caillaud. Etude de l'espese Origanum vulgare L. thèse de doctorat université de nantes UFR Sciences pharmaceutique et biologiques (2013) p 103.
31. Francine Draghi. L'ortie Dioique (*Urtica Dioica* L.): Etude Bibliographique. Thèse de doctorat, Faculté de pharmacie de l'Université Henri Poincaré - Nancy 1 (2005) p 45.
32. Erna Wati Ibnu Hajara. Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of *Stevia rebaudiana* Plant. *Procedia Environ Sci*. 20 (2014) 386 – 393.
33. Adotey, D., Serfor-Armah, Y., Fianko, J., & Yeboah, P. Essential elements content in core vegetables grown and consumed in Ghana by instrumental neutron activation analysis. *Afr J Food Sci*. 3 (2009) 243–249.
34. Choudhary, K., & Bandyopadhyay, N. Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. *J Ethnopharmacol*. 64 (1999) 179–184.
35. Jimoh F.O. and Oladiji A.T. Preliminary Studies on Piliostigma thonningii seeds: Proximate analysis, mineral composition and phytochemical screening. *Afr J Biotechnol*. Vol. 4. 12 (2005) 1439-1442.
36. Mohammed Abdalbasit A Gasmalla, Ruijin Yang, Issoufou Amadou, Xiao Hua. Nutritional Composition of *Stevia rebaudiana* Bertoni Leaf: Effect of Drying Method. *Trop J Pharm Res*. 13 (2014) 61-65.
37. Cardello, H.M.AB., MAPA Da Silva, M.H. Damasio and MAAB. De Silva. Time-intensity analysis of sweet and bitter tastes of stevia (*Stevia rebaudiana* Bertoni) leaf extract in sucrose equivalent sweetness. Ciencia *Tecnol Alime*. 19 (1999) 8-13.
38. Barriocanal, L. A., Palacios, M., Benitez, G., Benitez, S., Jimenez, J. T., Jimenez, N., *et al*. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. *Regul Toxicol Pharm*. 51 (2008) 37–41.
39. Joint Food and Agriculture Organization/World Health Expert Committee on Food Additives. Evaluation of certain food additives (Rep. No. 63) (2005). Geneva: World Health Organization.