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Assessment of Quality Attributes and Steviosides of *Stevia rebaudiana* Leaves Subjected to Different Drying Methods

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Abstract *Stevia* is a plant of great scientific interest due to its high sweetening power and its health benefits giving birth many researches related to changes of this quality by applying a conservation process. Therefore, the objective of this study was to evaluate the influence of different drying techniques (convection, vacuum, microwave, infrared, shade and freeze drying) on proximal analysis, vitamins C and E, fatty acid and amino acid profiles and steviosides from *Stevia* leaves. Stevioside was the main sweetener found in fresh sample and showed significant increase in all treatments, although convective was the less aggressive treatment (7.45% DM) followed by vacuum (7.02% DM). *Stevia* leaves may be used as a nutritional complement besides its sweetening power.

Keywords: *Stevia leaves, drying treatments, color, aminoacids, antioxidant, natural sweeteners*

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1. Introduction

Stevia rebaudiana Bertoni (Family-Asteraceae) possessing a 250 - 300 times higher sweetening property than sucrose [1], and they also used in treatment of patients suffering from carbohydrate metabolic diseases such as diabetes mellitus, obesity, hypertension and stimulates cell regeneration [2,3,4]. Thus the steviol glycosides are also widely used in food, beverage, medicine, wine making cosmetics and other food/chemical industry [5,6].

Drying is a very common preservation method used in foodstuffs and the quality of the final products is strongly dependent on the technique and the process variables used [7]. Like many foods, *Stevia* leaves contain between 75-80% water and these levels must decrease to less than 15% when drying is applied for conservation and consumption purposes [8]. In addition, the drying reduces water activity by decreasing moisture content, thus, it prevents deterioration and contamination during storage period [7]. Many a drying process has been used to dehydrate plants or herbs, where solar drying one of the first and most simple operations applied to *Stevia* leaves; however, this drying type has several disadvantages associated with the lack of process control and final quality of dried product. That is why there has recently been a searching for new techniques (drying methods) to remove the water from plants or herbs in order to affect as little as possible the quality properties of the final product and to find a different alternative to the traditional drying types currently applied in plants or leaves.

Microwave drying is an alternative method to the conventional drying. It is rapid, more uniform, energy efficient, space utilization, prevents food decomposition and appears to have a high potential for the agricultural products processing [9]. The great interest in this technology is due to the high capacity of penetration of these waves, that heat not only on the surface but also inside the food. This speed up the drying process and can improve the quality of the final product (Contreras et al., 2008).

Periche et al. [5] evaluated that the different drying conditions (hot air drying at 100°C and 180°C, freeze drying and shade drying) on steviol glycosides (stevioside, dulcoside A, rebaudioside A and rebaudioside C) and antioxidants in *Stevia* leaves. Stevioside, the major glycoside found in fresh leaves (81.2 mg/g), suffered an important reduction in all cases, although shade drying was the least aggressive treatment. Considering the antioxidant parameters (total phenols, flavonoids and total antioxidants), the most suitable drying method was hot air at 180°C, since it substantially increased all of them (76.8 mg gallic acid, 45.1 mg catechin and 126 mg Trolox, all equivalent/g Stevia, respectively), with respect to those present in fresh leaves (44.4, 2.5 and 52.9 mg equivalent/g). This way, these authors [5] reported that the ideal method for drying Stevia leaves depends on their final use (sweetener or antioxidant), although, hot air at 180°C is the most recommendable if only one treatment has to be chosen.

Chan *et al.* [10] studied freeze-drying process on ginger leaves. Starting from this study, they showed that freeze-drying was superior to other drying methods to preserve antioxidant properties. Pinela *et al.* [11] also showed higher values of antioxidants obtained by freeze drying as compared to shade drying on plants belonging to the tribe Genisteae (Fabaceae), whilst Hossain *et al.* [12] studied aromatic herbs (rosemary, oregano, marjoram, sage, basil and thyme) and such a research yielded less antioxidant content by freeze drying when comparing against hot air drying process.

Freeze-drying would be the best method of water removal with high quality final product compared to other drying methods. Some studies reported that freeze-drying increases the extraction of bioactive compounds of different products in comparison to air drying [13,14]. Freeze-drying has been recognized as the most expensive process for manufacturing a dehydrated products and its application depends on the uses of the final product.

Moreover, there are few studies related to the influence of different drying methods as to quality attributes such as physicochemical composition and sweeteners content of *Stevia* leaves. For this reason, the aim of this study was to apply several drying methods (freeze-drying, convective-drying, vacuum-drying, microwave-drying, infrared-drying, sun-drying and shade-drying) and evaluate how these techniques affect to the proximal analysis, C and E vitamins, dietary fiber, fatty acids, amino acids and sweeteners in *Stevia* leaves.

2. Material and Methods

2.1. Raw Material

Fresh Stevia leaves were obtained from a greenhouse located in Elqui Valley (Coquimbo, Chile) and chosen according to their uniform size, and color. Further on, samples were kept in polyethylene bags in cooling conditions (5ºC) after applying the drying process. The moisture content of the fresh leaves was immediately determined according to the A.O.A.C. method [15]. Green leaves were manually separated from stem plant, washed with water and then drained and left to dry on a cheesecloth for 15 min at room temperature (25 ± 2°C).

2.2. Drying Treatments

Fresh samples were dehydrated by seven different drying techniques: **Freeze-drying (FD)**: Fresh samples were frozen overnight into glass lyophilizer cylinders at -18°C for 10 h, after this period the cylinders were connected to the lyophilizer starting its process at 0.125 mbar and -50ºC (Chan *et al.*, 2009). The process was performed on a cylinder lyophilizing equipment (Virtis, Benchtop 3L, Gardiner, NY, USA) and took about 10 h depending on the amount of samples. **Convective drying** (CD): This process was performed in a hot air dryer (designed and built at the Food Engineering Department, Universidad de La Serena, Chile) operated by convection being heated by electric resistors that own a control unit to adjust both air velocity (0.5-3.0 m/s) and the temperature (40-80°C). The leaves were dried at 60 ºC, at an air velocity of 1.5 m/s [16] taking about 2-3 h. **Vacuum-drying (VD):** This drying was also carried out under steady temperature (60°C) and vacuum (15 kPa) conditions [17,18]. As it was said before the drying time ranges from 3-5 h. **Microwave-drying (MW):** Fresh samples were dried in a microwave oven (IRT, Model MWM2812W, Santiago, Chile) at an average power level (800 W) using small amounts of samples (50-80 g) in a period ranging between 4-8 min [16,17,18,19]; **Infrared-drying (IR):** Fresh samples were dehydrated by an electric infrared radiation dryer (Teka, HT490 model, Germany) at 60ºC [16]; the equipment includes tubular heaters inside the oven (upper part) emitting radiation. Leaves were softly shaken each 30 min. The drying time ranged 2-4 h. **Sun-drying (SD):** Fresh samples were kept in a glass vessel (90×60×40 cm) [20]. Dehydration takes place by the sun radiation transmitted through the glass. There are holes near the base and the upper part to let air flow out by a natural convection. Samples were dried for five days [19] and about 8 h of daylight at 50ºC, and an approximate humidity of 30-40%. **Shade-drying (SH):** Fresh samples were placed on the glass vessel and were dried in a special room where the sun rays did not reach directly onto the samples [21]. The sample weight to different drying methods was controlled at the beginning and at the end of each treatment until the sample reached a constant weight (equilibrium condition). The drying time required to reach the equilibrium moisture content.

2.3. Proximal Analysis

The methods were performed according to the A.O.A.C. methodologies [20]. Moisture content was determined by the gravimetric method by a vacuum stove (OVA-031-MA1, Germany) at 10 mmHg of pressure, a temperature at 70 ºC for 72 h, a suitable time for the samples to reach a steady weight. The crude protein was determined by using the Kjeldahl method with a conversion factor of 6.25. The lipid content was gravimetrically determined by a Soxhlet extraction method by using fuel oil ether (Merck, p.a) as an extractor solvent. The crude fiber was determined by an acid/alkaline hydrolysis of insoluble residues and total ash were determined by an organic matter calcination at 550ºC in a muffle oven (FE-341, Jalisco, México). Carbohydrates were calculated by difference. The soluble and insoluble dietetic fiber was determined by a gravimetric-enzymatic method suggested by the official method analysis. Total dietetic fiber was obtained by the addition of soluble and insoluble fibers. The water activity (a_w) of samples was measured at 25ºC by an AQUA LAB equipment (4 TE, Pullman, WA, USA) with an accuracy of 0.01 g. All the analysis mentioned above were performed in triplicate and expressed in g/100 g dry matter (DM).

2.4. Vitamin Determinations

Vitamin C content was analytically determined according to the method described by Chebrolu *et al.* [22] with some modifications. It was used meta-phosphoric acid (MPA) to the extraction. The supernatant was recovered from the extract and subjected to a chromatographic analysis by injecting samples to the HPLC (Agilent 1200 model, Palo Alto, CA, USA) system being equipped with a high pressure pump, an automatic injector and a detector of diode UV-visible array controlled by software ChemStation. Total ascorbic acid (vitamin C) was assessed after dehydroascorbic acid

reduction (DHA) with TCEP (tris (2-carboxy ethyl) phosphine chlorhydrate) after 30 min. On the other hand, the extraction for determining the vitamin E content (α-tocopherol) was performed by a cold extraction aiming at obtaining the lipid extraction according to the method described by Bahloul *et al.* [23] with some modifications. Vitamin E content was determined by a HPLC analysis by using a fluorescence detector Varian (Walnut Creek, USA) using a column Varian Microsob 100-5 µ Si 250×4.6 mm. In addition, α-tocopherol was identified and quantized by using α- tocopherol standard. All the analyses were made in triplicate. Vitamins C and E content results were expressed as mg ascorbic acid/100 g DM and mg tocopherol/100 g DM, respectively.

2.5. Surface Color Measurement and Total Chlorophyll Determination

The surface color of samples was measured by using a color-meter Hunter Lab (HunterLab, MiniScan XE Plus, Virginia, USA), including an opening 2.5 cm diameter where light goes through. Upon the measurements, the equipment calibration was performed by using standard plates, a black-colored plate and a white one. The measurements were expressed by Hunter Lab units L*, a*, and b*values which represents light–dark spectrum with a range from 0 (black) to 100 (white), the green–red spectrum with a range from -60 (green) to +60 (red), and the blue–yellow spectrum with a range from -60 (blue) to +60 (yellow) dimensions, respectively. As of the coordinates results L*, a*, b*, the chroma difference was calculated (ΔC*), i.e., dullness (-ΔC*) or vividness (+ΔC*) of a color, equation (1), and Δ*E* from dried samples and standard. Δ*E* stands for the total variation as of its initial color (fresh leaf) according to the equation (2). A numerical chroma difference (ΔC*), total color difference (ΔE) were calculated according to Vega-Gálvez et al. [24] as follows: Ten replicates measurements were performed and results were averaged. Total chlorophyll content was assessed by a spectrophotometric method described by Meir *et al.* [25] with some modifications. Chloroplast extracts were obtained by using 80% ethanol after incubation for 30 min in a stirring bath at 80 ºC. Next, the absorbance from each sample was measured on a spectrophotometer (Spectronic R20 Genesys, Illinois, USA). The antioxidant capacity was expressed as) both at 645 and 663 nm successively. Total chlorophyll content was calculated by equation (3).

$$\Delta C^{*} = C_{t}^{*} - C_{o}^{*} \quad (1)$$

$$\Delta E^{*} = \sqrt{\left(a_{t}^{*} - a_{o}^{*}\right)^{2} + \left(b_{t}^{*} - b_{o}^{*}\right)^{2} + \left(L_{t}^{*} - L_{o}^{*}\right)^{2}} \quad (2)$$

$$\text{Total Chlorophill}\left(g/100\text{g DM}\right) = \left(20.2{*}\text{OD}_{645}\right) + \left(8.02{*}\text{OD}_{663}\right) \quad (3)$$

Where t is dried sample regarding to drying method to use, and o is control sample (untreated).

2.6. Fatty Acids and Amino Acid Compositions

A heatless extraction was carried out to obtain lipid extracts according to the method described by Bahloul *et al.* [23] with some modifications. The fresh sample was used as the control prior preparation of methylic esters according to Spanish standards by using NaOH for saponification, Isooctane as a solvent and boron trifluoride in methanol (BF_3 – CH_3OH) as an esterifying agent. A gaseous chromatograph (Perkin-Elmer AutoSystem XL, Norwalk, CT, USA) was used to fatty acid methylic esters analysis (FAME), it was also used a flame ionization detector (FID) and Helium as a carrying gas. Fatty acid methylic esters were identified based in the retention times to identify the fatty acids. On the other hand, the amino acid profile of *Stevia* samples was performed using modified PICO-TAG method [26] carried out in a HPLC system (UV detector). Samples were hydrolyzed in 6 N HCl for 24 h at 110 °C under vacuum conditions and then, the hydrolysate was derivatized using the PITC method and injected on the HPLC using a sodium acetate/triethylamine/acetonitrile elution method. Each sample analysis was made in triplicate and expressed in g/100 g DM.

2.7. Sweeteners Content

Individual steviosides were performed according to Compendium of Specifications on Food Additive from Joint expert committee FAO/WHO [27]. Individual steviosides were determined by using HPLC (Agilent 1200 model, Palo Alto, CA, USA) analysis with a mixture mobile phase of acetonitrile and water (80:20) at pH 3.0, filtered through 0.22 µm Millipore filter. The standard solutions were prepared from the stevioside (purity>99.0%) and rebaudioside A (purity >97.0%) standards and were weighed 50 mg of standard previously dried (105 °C, 2 h) in a 100 mL volumetric flask, dissolved in the mobile phase and then diluted to volume with the mobile phase; procedure described above was performed for each standard individually. Regarding to samples, it was accurately weighed between 50-100 mg of previously dried sample (105°C, 2 h) in a 100 mL volumetric flask and dissolved in the mobile phase and diluted to volume with the mobile phase. The chromatographic conditions used were: Column Supelcosil LC-NH2 (length 15-30 cm; inner diameter: 3.9-4.6 mm) and column temperature at 40 °C. The injection volume was adjusted between 5-10 µL to a retention time of about 21 min for rebaudioside A. For detection, a spectrophotometer at 210 nm was used. The chromatograms of sample solution and standard solution were automatically registered by equipment. The individual steviosides were identified based on their retention times by using the reference standard mixture, and the peak areas were measured. Each solution was injected in triplicate and the percentage of each of the seven steviosides, X, in the sample was calculated from the following equation (4):

$$\%X = \left[W_{s} - W\right].\left[F_{X}.A_{X} / A_{S}\right].100 \quad (4)$$

Where W_S is the amount (mg) of stevioside in the standard solution; W is the amount (mg) of sample in the sample solution; A_S is the peak area for stevioside from the standard solution; A_X is the peak area for *X* of the sample solution; F_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 for stevioside, 1.20 for rebaudioside A, 1.18 for rebaudioside C, 1.40 for

rebaudioside D and 0.98 for dulcoside A. The content of total sweetener was calculated as the content of the five steviol glycosides (steviosides).

2.8. Statistical Analysis

The differences among the drying methods for each quality parameter were obtained by variance ANOVA analysis by using the software Statgraphics Plus® 5.1 (Statistical Graphics Corp., Herndon, VA, USA). Comparison and differences among means values were analyzed using LSD (Least Significant Difference) at 5% of significance level and confidence interval of 95% ($p<0.05$). Furthermore, multiple range test (MRT) was performed to identify homogeneous groups within each parameter.

3. Results and Discussion

3.1. Effect of Drying Methods on Physicochemical Composition

Proximate compositions of fresh and dried stevia leaves by seven different drying methods (freeze-drying (FD), convective drying (CD), vacuum-drying (VD), microwave-drying (MW), infrared-drying (IR), sun-drying (SD) and shade-drying (SH)) are presented in Table 1. The fresh sample contain 4.23 % fat, 7.36 % ashes, 14.2 % protein, 19.8 % crude fiber, 73.6 % carbohydrate, and 74.7 % moisture. The moisture obtained from *Stevia* fresh leaves is similar to the water content in fresh herbs that usually bear between 75 to 80% of water (fresh sample) [28]. Similar values have been obtained by other studies on *Stevia* leaves reporting a moisture value at 77% [29]. The water activity (a_w) of the fresh sample showed a value of 0.993 [1]. Furthermore, the content of vitamin C and E to the fresh sample was 23.5 mg of ascorbic acid and 0.67 mg to α-tocopherol per 100 g fresh sample for both vitamins, respectively. As to protein, fat, fiber and ash content similar results were found for fresh samples that were similar to the ones obtained by Mishra *et al.* [6] in *Stevia* leaves (fresh sample) having 10, 3, 18 and 11 g/100 g DM, respectively. A clear methods influence was seen on definite parameters as to the fresh samples ($p<0.05$). As regards as moisture contents from the drying types sun drying and shade drying the highest values of moisture were gotten as compared to the SD and SH, likewise no difference were found between this two drying methods that might be owing to the fact that both methods dehydrated such a sample under heat intensity under sun very closed moisture were obtained. Generally, the results for this parameter are similar was found by other authors, between 5-7 g/100 g DM [30]. The relationship between moisture and water activity is directly proportional between each other. For the water activity results the higher reduction pointed to FD, CD and VD samples (drying temperature at 60 °C for the two latter ones) having values of 0.320, 0.308 and 0.333, respectively. For all drying processes, the value of water activity varied between two values (0.308-0.450), in such a ranged water is found moderately bound and it is characterized by its a availability as a dissolver for low molecular weight solutes and for some biochemical reactions [29]. According to literature, it can be pointed out that *Stevia* dried leaves are a good source of carbohydrates (35-62 g/100 g DM), proteins (10-20 g/100 g DM) that at very important to keep human health [5,31,32]. The high ash content (6-13 g/100 g DM) shows that *Stevia* is a good source of minerals (K, Ca, Na, Mg, Fe, etc). According to Table 1, it can be said to that *Stevia* leaves have abundant total dietary fiber, bearing values between 33.93-44.16 g/100 g DM (33.55-42.33 g/100 g sample), similar values were found by Tanongkankit *et al.* [33] on fresh cabbage leaves both for total dietary fiber (40.89 g/100 g DM) as for the insoluble dietary fiber (33.54 g/100 g DM). Insoluble dietary fiber is the main constituent of total dietary fiber, whose values are between 29.48-38.35 g/100 g DM, the relatively high percentage mentioned for insoluble dietary fiber suggests possible applications on physiological dietetic products [33]. The ingestion of this kind of fiber is linked to satiety sensation since the fibers absorbs the water that takes up the spaces in the stomach and diminishes the need of food consumption. It also increases the volume and weight of fecal skittle enhancing digestive system function and also prevents diseases like constipation and colon cancer [34]. Of all the treatments studied, vacuum-drying yielded the highest insoluble and soluble dietary fiber contents. On the other hand, shade-drying yielded the lowest total dietary fiber and insoluble dietary fiber values, this is possibly due to the treatment requires long drying times (days) to dehydrate the sample at such a moderate temperature (30-40°C), this long period might significantly damage the samples ($p<0.05$). IR samples yielded values closer to VD samples at 39.30 g/100 g DM. in total and insoluble dietary fibers, respectively, there are references pointing out that this kind of drying doesn't affect the final quality all products obtained, showing that parameters as color, texture and chemical composition in foods did not evidence any significant modification during infrared assisted drying.

3.2. Vitamin C and E Content

Table 1 also shows vitamin C and E values for dried *Stevia* samples including the seven drying treatments. As to FD, VD and MW dried samples (116.3, 122.7 and 117.2 g/100 g DM, respectively) showed a vitamin C higher values as compared against others drying treatments, this apparent increase of some quality parameters level, in this case vitamin C, might be attributed to the fact that drying brings forth a cellular wall breaking allowing the extraction during crushing and homogenization stages, therefore this broken cells cut liberated more compounds when it is shaken during extraction [12]. Another possibility is that fresh samples have not been correctly homogenized preventing cellular wall breaking and consequently surface exposed of cells is reduced and that could be a hindrance for solvent extraction [22,24]. Meaningful lower levels were obtained for CD and SH treatments, even for SD and IR treatments vitamin C content was not detected that might be due to the heat intensity that the samples received during both drying treatments, besides according to theoretical bibliographic sun-drying deprives control over operating conditions. The drying conditions for each treatment that are different from each other owing to the vitamin C content in the shade-drying is the most affected because long periods are required to dry the sample. In researching

about parsley leaves, some authors have suggested that the most suitable drying process is one that reaches equilibrium moisture content in less time as it achieves a lower impact on the decrease in vitamin C [22]. Dehydrated samples by VD treatment showed the lowest loss of vitamin C followed by MW treatment. Lisiewska *et al.* [35] found higher values of vitamin C in dill leaves (186 ± 7 g/100 g fresh sample). In addition, differences can be attributed to natural variation of ascorbic acid content between specimens and/or cultivars (genetic factors), preservation and postharvest factors (maturity stage, environmental practices, cultivar and storage conditions) [22]. Vitamin E content obtained in fresh leaves was significantly lower than vitamin E content by drying methods (2.65 ± 0.15 mg/100 g DM). This may be because the fresh sample has high water content (75%) and if it is mixed with hexane solvent (non-polar solvent used in the extraction) this hydrophobic vitamin is not removed effectively (Ref??). FD treatment had a significant increase showing the highest value of α-tocopherol (15.3 ± 0.27 mg/100 g DM), while the SH treatment had the lowest vitamin E content (1.22 ± 0.04 mg/100 g DM). Increases or decreases in vitamin E content may be attributed to the extraction process of α-tocopherol, which it might be a variability source for extracting other compounds (waxes or others) that are soluble in the chosen solvent (in this case hexane) [23].

Table 1. Moisture content, proximal analysis, dietary fiber, and Vitamin C and E of *Stevia* leaves dehydrated by different drying techniques

		Drying methods						
Parameters g/100g DM	Fresh	FD	CD	VD	MW	IR	SD	SH
Moisture	74.7^{a} ±0.19	3.95^{b} ±0.16	3.06^{c} ±0.22	4.14^{bd} ±0.09	4.35^{d} ±0.12	6.31^{e} ±0.22	5.69^{f} ±0.12	5.81^{f} ±0.10
Fat	1.07^{a} ±0.02	10.5^{b} ±0.45	8.25^{c} ±0.28	9.98^{d} ±0.24	3.58^{e} ±0.09	5.00^{f} ±0.08	6.77^{g} ±0.26	3.77^{e} ±0.08
Ash	1.86^{a} ±0.07	9.86^{be} ±0.00	9.75^{bc} ±0.18	9.99^{be} ±0.10	9.09^{d} ±0.03	10.13^{e}±0.00	9.54^{c} ±0.04	8.92^{d} ±0.10
Crude protein	3.59^{a} ±0.05	13.8^{b} ±0.14	12.5^{c} ±0.03	13.4^{bd} ±0.29	13.1^{d} ±0.17	13.2^{d} ±0.26	14.7^{e} ±0.09	13.4^{bd} ±0.13
Crude fiber	5.03^{a} ±0.24	9.80^{b} ±0.15	15.2^{c} ±0.27	14.2^{c} ±0.02	12.9^{d} ±0.08	19.3^{e} ±0.61	12.2^{d} ±0.04	15.2^{c} ±0.18
Carbohydrates*	18.6^{a} ±0.04	66.5^{b} ±0.54	70.1^{c} ±0.72	66.0^{b} ±0.55	73.9^{d} ±0.28	71.6^{e} ±0.12	68.8^{f} ±0.51	74.6^{d} ±0.77
Water activity **	0.993^{a} ±0.002	0.321^{b} ±0.017	0.308^{b} ±0.002	0.336^{b} ±0.012	0.387^{c} ±0.012	0.374^{c} ±0.019	0.452^{c} ±0.034	0.381^{c} ±0.010
Dietary fiber Insoluble dietary fiber		31.4^{a} ±0.89	31.5^{a} ±0.02	38.3^{b} ±0.70	29.8^{ac} ±0.10	34.7^{d} ±1.40	31.2^{a} ±0.46	29.5^{c} ±0.30
Soluble dietary fiber		3.55^{ad} ±0.04	4.25^{acd} ±0.08	5.81^{b} ±0.18	4.94^{bc} ±0.10	4.61^{cd} ±1.06	3.35^{a} ±0.50	4.07^{acd} ±0.61
Total dietary fiber		34.9^{a} ±0.94	35.7^{a} ±0.09	44.2^{b} ±0.52	34.7^{a} ±0.20	39.3^{c} ±2.46	34.6^{a} ±0.96	33.5^{a} ±0.31
Vitamin C***	23.5^{a} ±2.04	116.3^{b} ±11.6	70.1^{c} ±3.70	122.7^{b} ±7.43	117.2^{b} ±7.11	ND	ND	39.3^{d} ±1.26
Vitamin E ****	0.67^{a} ± 0.04	15.3^{b} ±0.27	5.96^{c} ±0.09	6.80^{d} ±0.55	5.97^{c} ±0.19	2.06^{e} ±0.02	4.30^{f} ±0.07	1.22^{g} ±0.04

Mean values ± SD *By difference; **Measured at 25 °C; ***mg ascorbic acid (AA)/100g dry matter; ****mg α-tocopherol/100g dry matter. ND: Not detected. FD: Freeze-drying; CD: Convective-drying; VD: Vacuum-drying; MW: Microwave-drying; IR: Infrared-drying; SD: Sun-drying; SH: Shade-drying. Same letters in the same row indicate values are not significantly different ($p<0.05$).

3.3. Changes Produced by Drying Methods on Surface Color and Total Chlorophyll

Colorimetric coordinates of fresh samples were 33.48, -6.99 and 17.39 to *L**, *a** and *b**, respectively. As of Table 2, it can be said that the bright or product luminosity *L** from darkness (0) to lightness (100) increased its value during process showing the highest value in FD samples. The same trend was obtained by Youssef and Mokhtar [36] by applying different drying methods (Convective drying at 50, 60 and 70 °C, microwave-drying at 360, 900 and 1250 W and freezing drying) on edible verdolaga leaves (*Portulaca oleracea* L.) obtained higher *L** values on dehydrated a samples as compared to the fresh ones. Regarding to the coordinates *a** negative values are related to the green color (-*a**) and positive values to the red one (+*a**). As far as to this aspect all the drying treatments yielded lower green values (-6.99 ± 0.15) in comparison to the fresh sample where IR was the most affected treatment with a value of -0.11 ± 0.07, whereas FD sample was the nearest to the fresh value with the value of -6.96 ± 0.15. On the other hand *b** positive values are related to the yellow color (+*b**) and the negative ones to the blue color (-*b**), according to this chromatic parameter results, all of the treatments being evaluated increased their value in comparison to the fresh sample, although is significant increase was shown in the FD sample. On the other hand as to the Chroma difference (Δ*C**), a lighter color was obtained in FD samples showing a more positive chroma value (+Δ*C**) in comparison to the other samples, whereas at the drying by IR treatment yielded a more negative chroma value (-Δ*C**), being the dullest samples of all. Since the difference of total color (Δ*C*) is a function of the three coordinates CIE (*L* a* b**), when analyzing Δ*E* values the highest change was observed in the FD treatment. As of these results, the CD treatment yielded a dry leaf undergoing less color changes. When analyzing chlorophyll results, it was observed that the values obtained in all the treatments increases in comparison to the fresh sample. However chlorophylls are very labile, they degrade by heat and by the action of chlorophylase enzyme from the vegetal tissue itself besides, in a habitat slightly acid they easily became in pheophytin *a* and *b* that are substances of brownish olive color where magnesium has been substituted by two hydrogen atoms [37]. Therefore, this apparent increase in chlorophyll quantifying when measuring might be due to the reason that the incoming of this degraded compounds was quantify (pheophytins) that are mainly made up during the cooking or food processing [38]. Reyes-Santamaría *et al.* [38] reported similar values of total chlorophyll 0.141 and 0.165 g/100g sample in tangerine and Valencia orange leaves, respectively. Of all the treatments studied IR showed the lowest total chlorophyll values at 0.402 g/100 g DM, whereas FD treatment showed the highest value with 0.646 g/100 g DM. Foidl *et al.* [39] reported total chlorophyll values similar to the values obtained in this research (0.689 g/100 g DM) in

leaves powder of *Moringa oleífera*. The results obtained could be related to the changes in the pigments of leaves owing to a thermic treatment [39]. Furthermore, in the dehydration processes there are a changes and color loss since food surface features change and thus there color and reflectancy, also under go changes [24]. Likewise enzymatic brownish that is caused by the polyphenol oxidase brings about a fast darkening mainly on the outer surface of the leaves [10,24]. On the other reason why there is a color change is the photo-oxidation of pigments by the light effect that combined with the oxygen causes a serious discoloration [12].

Table 2. Changes in chromatic coordinates (CIE *L*a* b), Δ *C*, Δ*E* and total chlorophyll content of fresh and dehydrated *Stevia* leaves**

Item	Fresh	Drying method						
		FD	CD	VD	MW	IR	SD	SH
CIE L*a*b* coordinates *L**	33.48^{a} ±0.23	50.21^{b} ±0.18	40.88^{c} ±0.02	40.84^{c} ±0.03	40.15^{d} ±0.06	39.81^{e} ±0.02	44.52^{f} ±0.12	43.31^{g} ±0.06
a*	-6.99^{a} ±0.15	-6.96^{a} ±0.09	-4.28^{b} ±0.06	-3.44^{c} ±0.11	-4.20^{b} ±0.10	-0.11^{d} ±0.07	-3.71^{e} ±0.11	-3.03^{f} ±0.04
b*	17.39^{a} ±0.18	24.5^{b} ±0.28	20.5^{c} ±0.07	21.70^{d} ±0.11	23.72^{e} ±0.21	17.89^{f} ±0.06	20.43^{c} ±0.04	18.45^{g} ±0.23
ΔC*	-	8.02^{a} ±0.15	2.42^{b} ±0.08	3.43^{c} ±0.12	5.54^{d} ±0.21	-0.46^{e} ±0.06	2.24^{b} ±0.04	-0.35^{e} ±0.18
ΔE*	-	18.92^{a} ±0.26	8.93^{b} ±0.04	9.35^{c} ±0.02	9.86^{d} ±0.02	9.90^{d} ±0.02	11.45^{e} ±0.18	11.26^{e} ±0.06
Total chlorophyll**	0.402^{a} ±0.00	0.646^{b} ±0.01	0.572^{cf} ±0.03	0.617^{bd} ±0.02	0.564^{cf} ±0.01	0.459^{e} ±0.01	0.554^{c} ±0.01	0.594^{df} ±0.00

Mean values ± SD. **Values expressed as g/100g dry matter. FD: Freeze-drying; CD: Convective-drying; VD: Vacuum-drying; MW: Microwave-drying; IR: Infrared-drying; SD: Sun-drying; SH: Shade-drying. Same letters in the same row indicate values are not significantly different ($p<0.05$).

3.4. Fatty Acids Composition

Fatty acids compounds from oils and/or saturated-unsaturated fatty acids ratio (proportion) are both medicinal and nutritional important [40]. Mean values and standard deviations of fatty acid profile from *Stevia* leaf oil is summed up in Table 3. Fatty acids were identified by comparison to the standards of fatty acid methylic esters. Miristic, palmitic, stearic, oleic, linoleic, linolenic were quantized in the oil of fresh and dehydrated *Stevia* leaf by the seven drying treatments. Unsaturated fatty acids ($C_{18:2}$, $C_{18:3}$) were the prevailing compounds in fresh and dehydrated *Stevia* leaf oil. The fresh sample showed a 2.27% (DM) of saturated fatty acids (SFA), 0.50% (DM) of monounsaturated fatty acids (MUFA) and 2.73% (DM) of polyunsaturated fatty acids (PUFA) as compared to drying treatment where the values detected ranged from 0.58-1.97% (DM) of SFA, 0.45-1.39% (DM) of MUFA and 2.16-8.18% (DM) of PUFA. The main fatty acid detected was the linolenic acid, showing the highest percentage of all the analyzed samples in the case of fresh samples, this acid reached a 30.2 % from the total fatty acids detected (saturated and unsaturated), whereas in dehydrated samples, total fatty acids exceeded a 50 %, the SH treatment highlighted because it exceed the 80 % from the total. Rezeng *et al.* [41] obtained similar results where linoleic, linolenic and palmitic where the main components in the *Rubus amabilis* leaves. It must be pointed out that the linoleic and linolenic acid belong to the essential acids that human beings and other animals are not able to synthesize and must obtain them from other food sources [16]. These essential acids also have a crucial task both in life and in death of cardiac cells. Besides, they are the principal unsaturated fatty acids from vegetable lipids that benefit human health [41]. Some of the fatty acids being found in control samples and the dehydrated leaves, report meaningful differences in their content ($p<0.05$). However, this analysis also slow that other fatty acids don't evidence such a statistical difference ($p>0.05$) between treatments and control sample.

Table 3. Effect of drying methods on SFA (Saturated Fatty Acids), MUFA (Monounsaturated Fatty Acids fatty acids) and PUFA (Polyunsaturated Fatty Acids) in *Stevia* leaves

		Drying methods						
Fatty acids	**Fresh**	**FD**	**CD**	**VD**	**MW**	**IR**	**SD**	**SH**
Miristic acid (C14:0)	ND	0.17^{a} ±0.04	ND	ND	0.08^{b} ±0.00	0.06^{b} ±0.00	ND	ND
Palmitic acid (C16:0)	1.27^{a} ±0.02	1.33^{a} ±0.08	1.05^{b} ±0.01	1.47^{c} ±0.01	0.46^{d} ±0.01	0.59^{e} ±0.00	ND	0.58^{e} ±0.13
Stearic acid (C18:0)	ND	0.47^{a} ±0.01	ND	ND	0.23^{b} ±0.02	0.26^{c} ±0.00	ND	ND
Total SFA	1.27^{a} ±0.02	1.97^{b} ±0.03	1.05^{c} ±0.01	1.47^{d} ±0.01	0.77^{e} ±0.03	0.91^{f} ±0.00	ND	0.58^{g} ±0.13
Oleic acid (C18:1)	0.50^{a} ±0.04	1.39^{b} ±0.03	1.05^{c} ±0.01	0.65^{d} ±0.03	0.62^{d} ±0.06	1.00^{c} ±0.01	0.45^{a} ±0.02	ND
Total MUFA	0.50^{a} ±0.04	1.39^{b} ±0.03	1.05^{c} ±0.01	0.65^{d} ±0.03	0.62^{d} ±0.06	1.00^{c} ±0.01	0.45^{a} ±0.02	ND
Linoleic acid(C18:2)	1.37^{a} ±0.02	0.17^{b} ±0.00	1.47^{c} ±0.02	2.22^{d} ±0.03	0.08^{e} ±0.01	0.09^{e} ±0.00	1.50^{c} ±0.06	0.71^{f} ±0.05
Linolenic acid (C18:3)	1.36^{a} ±0.03	5.78^{b} ±0.14	4.34^{c} ±0.07	5.96^{d} ±0.06	2.08^{e} ±0.00	2.72^{f} ±0.00	4.08^{g} ±0.14	2.14^{e} ±0.18
Total PUFA	2.73^{a} ±0.05	5.95^{b} ±0.15	5.82^{b} ±0.05	8.18^{c} ±0.04	2.16^{d} ±0.01	2.81^{a} ±0.00	5.57^{e} ±0.18	2.85^{a} ±0.22

Mean values ± SD. Values expressed as g/100g dry matter. ND: Not detected. FD: Freeze-drying; CD: Convective-drying; VD: Vacuum-drying; MW: Microwave-drying; IR: Infrared-drying; SD: Sun-drying; SH: Shade-drying. Same letters in the same row indicate values are not significantly different ($p<0.05$).

3.5. Amino Acids Profile

The mean values and standard deviations of amino acid profile of fresh and dried samples are presented in Table 4. Within essential amino acids, lysine and arginine showed the highest values in fresh sample and for all drying methods. In fresh sample, lysine value represented a 26%, whereas the arginine represented a 14 % of total essential amino acids quantified. In drying methods, lysine values reached between 22-45 % and the arginine stood for between 9-16 % of total essential amino acids. Moreover, the glutamic acid reached between 21-23% of non-essential amino acids. Similar values were found by Korus [42] who applied hot air drying and freeze drying to a variety of kale (*Brassica oleracea* L. var. Acephala) pretreated (Bblanched) and showed that the predominant amino acids were glutamic acid (2.19- 2.51 g/100 g DM), aspartic acid (1.76-1.90 g/100 g DM) and proline (1.77-1.94 g/100 g DM). Other studies have suggested that

amino acid composition of plants might be modified depending on the structural composition of each plant [43]. The amino acids composition in *Stevia* leaves, only it have been performed to fresh leaves, where it were found a high content of methionine (1.45 g/100g DM), histidine and valine (1.13 g/ 100 g DM) [31]. The differences among results obtained by other studies might be due to complexity of drying systems that generated different types of reactions during the process, which hinders prediction of some amino acids' final content [31]. Outstanding lysine levels in *Stevia* leaves might be useful due to the amino acid lack in foods known to be limited in lysine (corn or wheat); this low lysine content may be overcome by using of *Stevia* leaves as a supplement that containing more quantity of this limited amino acid and thereby to reduce protein deficiency due to lysine lack [31,43].

Table 4. Changes on Amino acid profile of dehydrated *Stevia* leaves by drying methods

Essential amino acid*	Drying method							
	Fresh	FD	CD	VD	MW	IR	SD	SH
Arginine	1.06^{a} ±0.04	1.12^{a} ±0.02	0.81^{bc} ±0.07	0.87^{bc} ±0.03	0.97^{ac} ±0.02	0.75^{b} ±0.05	0.87^{bc} ±0.13	0.96^{ac} ±0.12
Phenyl alanine	0.69^{a} ±0.06	0.60^{ab} ±0.04	0.56^{b} ±0.05	0.61^{ab} ±0.02	0.55^{b} ±0.04	0.54^{b} ±0.03	0.54^{b} ±0.03	0.58^{b} ±0.07
Histidine	0.62^{a} ±0.02	0.51^{a} ±0.04	0.98^{a} ±0.38	0.73^{a} ±0.01	0.70^{a} ±0.17	0.62^{a} ±0.08	0.88^{a} ±0.43	0.50^{a} ±0.02
Isoleucine	0.62^{a} ±0.04	0.57^{abc} ±0.05	0.53^{b} ±0.02	0.60^{ac} ±0.01	0.54^{bc} ±0.03	0.52^{b} ±0.03	0.53^{bc} ±0.03	0.55^{abc} ±0.05
Leucine	1.01^{a} ±0.03	1.11^{b} ±0.06	0.73^{c} ±0.04	0.87^{de} ±0.00	0.87^{de} ±0.02	0.73^{c} ±0.03	0.81^{cd} ±0.03	0.94^{ae} ±0.07
Lysine	1.94^{ab} ±0.04	1.54^{a} ±0.32	2.57^{b} ±0.20	2.57^{b} ±0.05	2.26^{ab} ±0.07	3.67^{c} ±0.08	3.51^{c} ±0.59	2.02^{ab} ±0.63
Methionine	0.36^{a} ±0.04	0.21^{b} ±0.01	0.36^{a} ±0.00	0.39^{a} ±0.01	0.27^{c} ±0.03	0.35^{a} ±0.02	0.29^{c} ±0.03	0.23^{bc} ±0.02
Threonine	0.65^{a} ±0.05	0.51^{bc} ±0.02	0.50^{b} ±0.03	0.58^{ac} ±0.02	0.51^{bc} ±0.00	0.50^{b} ±0.02	0.58^{ac} ±0.05	0.56^{bc} ±0.02
Valine	0.66^{ab} ±0.02	0.73^{b} ±0.02	0.53^{cd} ±0.05	0.59^{acd} ±0.03	0.56^{acd} ±0.05	0.51^{c} ±0.04	0.56^{acd} ±0.02	0.63^{abd} ±0.09
Total essential amino acids	7.60^{abc} ±0.24	6.90^{ab} ±0.19	7.57^{abc}±0.31	7.81^{bcd}±0.01	7.22^{ab}±0.42	8.20^{cd} ±0.19	8.58^{d} ±0.76	6.98^{a} ±0.30
Non-essential amino acid*								
Aspartate	1.21^{ae} ±0.03	1.49^{b} ±0.04	0.88^{c} ±0.02	0.96^{c}±0.01	1.17^{ad} ±0.02	0.78^{c} ±0.02	1.10^{d} ±0.02	1.27^{e} ±0.09
Serine	0.88^{ab} ±0.13	0.72^{ab} ±0.01	0.64^{a} ±0.04	0.79^{ab} ±0.01	0.81^{ab} ±0.16	0.70^{ab} ±0.10	0.80^{ab} ±0.20	0.90^{b} ±0.03
Glutamic	1.39^{a} ±0.10	1.66^{b} ±0.00	1.02^{c} ±0.01	1.16^{c} ±0.05	1.36^{a} ±0.12	1.04^{c} ±0.08	1.39^{a} ±0.16	1.50^{ab} ±0.01
Proline	0.73^{abd} ±0.05	0.75^{bd} ±0.04	0.57^{c} ±0.09	0.61^{abc} ±0.06	0.66^{abc} ±0.09	0.60^{ac} ±0.06	0.83^{d} ±0.05	0.65^{abc} ±0.04
Glycine	0.80^{a} ±0.19	0.68^{a} ±0.05	0.55^{a} ±0.03	0.75^{a} ±0.06	0.68^{a} ±0.28	0.66^{a} ±0.08	0.73^{a} ±0.17	0.83^{a} ±0.01
Alanine	0.87^{ab} ±0.09	1.04^{b} ±0.04	0.66^{ac} ±0.08	0.73^{ac} ±0.03	0.77^{ac} ±0.02	0.63^{c} ±0.02	0.74^{ac} ±0.17	0.80^{ac} ±0.16
Cysteine	ND	ND	ND	ND	ND	ND	ND	ND
Tyrosine	0.70^{a} ±0.01	0.55^{bc} ±0.00	0.58^{cd} ±0.03	0.64^{d} ±0.02	0.54^{bc} ±0.02	0.58^{c} ±0.03	0.54^{bc} ±0.02	0.52^{b} ±0.04
Total non-essential amino acids	6.58^{ab} ±0.24	6.88^{b} ±0.19	4.90^{c} ±0.31	5.63^{cd} ±0.01	6.00^{ad} ±0.42	4.98^{c} ±0.19	6.13^{abd} ±0.76	6.45^{ab} ±0.30

*Mean values ± SD. Values expressed as g/100g dry matter. ND: Not Detected. FD: Freeze-drying; CD: Convective-drying; VD: Vacuum-drying; MW: Microwave-drying; IR: Infrared-drying; SD: Sun-drying; SH: Shade-drying. Same letters in the same row indicate values are not significantly different ($p < 0.05$).

Table 5. Effect of different drying techniques on steviol glycosides profile of *Stevia* leaves

Steviol glicosides*	Drying method							
	Fresh	FD	CD	VD	MW	IR	SD	SH
Stevioside	1.40^{a} ±0.03	6.45^{b} ±0.02	7.45^{c} ±0.05	7.02^{cd} ±0.55	6.94^{cde} ±0.24	5.91^{b} ±0.25	6.79^{de} ±0.07	7.15^{cd} ±0.20
Rebaudioside A	0.67^{a} ±0.00	2.50^{b} ±0.20	2.80^{b} ±0.12	2.55^{b} ±0.04	2.54^{b} ±0.37	2.38^{b} ±0.30	2.62^{b} ±0.28	2.43^{b} ±0.05
Rebaudioside C	0.26^{a} ±0.03	0.66^{bc} ±0.03	0.77^{c} ±0.08	0.75^{c} ±0.02	0.65^{bc} ±0.09	0.60^{b} ±0.03	0.72^{bc} ±0.05	1.20^{d} ±0.09
Rebaudioside D	0.16^{a} ±0.00	1.20^{be} ±0.18	1.11^{b} ±0.05	0.92^{b} ±0.01	0.61^{c} ±0.04	0.28^{a} ±0.02	1.50^{d} ±0.25	1.44^{de} ±0.09
Rebaudioside F	ND	ND	ND	ND	ND	ND	ND	ND
Rubusoside	ND	ND	ND	ND	ND	ND	ND	ND
Dulcoside A	0.12^{a} ±0.00	0.28^{b} ±0.01	1.04^{c} ±0.07	0.71^{d} ±0.10	0.42^{e} ±0.07	0.46^{e} ±0.01	1.04^{c} ±0.06	0.38^{be} ±0.02
Steviolbioside	ND	ND	ND	ND	ND	ND	ND	ND
Total steviosides content	2.61^{a} ±0.06	11.1^{b} ±0.01	13.2^{c} ±0.20	12.0^{be} ±0.71	11.2^{b} ±0.11	9.63^{d} ±0.51	12.7^{ce} ±0.60	12.6^{ce} ±0.23

Mean values ± SD. *Values expressed as g/100g dry matter. ND: Not Detected. FD: Freeze-drying; CD: Convective-drying; VD: Vacuum-drying; MW: Microwave-drying; IR:Infrared-drying; SD: Sun-drying; SH: Shade-drying. Same letters in the same row indicate values are not significantly different ($p < 0.05$).

3.6. Impact of Drying Methods on Steviosides Content

Table 5 shows the mean value and standard deviation of steviol glycosides found in both fresh and dehydrated *Stevia* leaves by the different methods. Stevioside and rebaudioside A were the prevailing components in fresh *Stevia* leaves at a 53% and 25%, respectively. The remaining percentage (22 %) was obtained by the summary of the other three glycosides was obtained by quantized, standing from a 53 and 61 %. CD was the treatment obtaining the highest stevioside content reaching a 7.45 g/100 g DM. Rebaudioside A was the second steviol glycoside highlighted yielding percentages that ranges from 19 to 25 % of total steviosides. CD treatment once again, turned out to be the best methods to retain this stevioside reaching a value of 2.80 % (DM). In the case of rebaudioside C and D, the highest values of the samples treated by different methods were yielded by CD and SD treatments (1.04 g/100 g DM by both). The IR treatment yielded the lowest values in most of steviol glycosides; therefore, it is the least adequate treatment to stevioside extraction. According to the bibliography, *Stevia* leaves mainly contain stevioside (4-13 % w/w) followed by rebaudioside A (2-4 % w/w), rebaudioside C (1-2 % w/w) and dulcoside A (0.4-0.7 % w/w) [44,45,46]. Other authors suggest that steviol glycosides content in *Stevia* leaves depends on cultivar conditions [47] and agricultural techniques being applied [48]. Likewise, Tavarini and Angelini [49] stated that steviol glycoside content is

affected by edaphoclimatic conditions (ground characteristics, climatic and geographical factors) the plant age and its growing phase. The statistical analysis showed meaningful differences between the fresh and dehydrated sample ($p<0.05$). There existing a clear influence on all the steviol glycosides being quantized. Recently, some researches related to sweeteners content in *Stevia* leaves have yielded results lower than those obtained by this research in stevioside content where stevioside was main component in all drying treatments values that were applied in this study that it shows values to 4.8, 3.7 and 3.5 g/100 g DM (48 ± 12; 37 ± 6 and 35 ± 8 mg/g DM) for shade drying, hot air drying (180°C) and freeze drying, respectively [1].

4. Conclusions

VD preserved the most important effects of drying methods in *Stevia* leaves were found on insoluble dietary fiber content that was the most abundant fiber and was retained mainly by VD treatment, as well as, vitamins C and E. Color and chlorophyll content were closely related, so that color is affected in different drying treatments, and as it already noted, chlorophyll content experienced changes during the process what led to appear color disrupting compounds. CD treatment managed to keep leaf color as close to the initial condition (fresh sample) however, FD treatment maintained green color closest to fresh sample. Regarding the fatty acid and amino acid profiles, VD is the most recommended process to maintain polyunsaturated fatty acids (α-linolenic acid) and SD treatment to conserve essential amino acids, but VD treatment was not significantly different to SD treatment, but this latter drying implied long drying times and little process control. *Stevia* sweeteners are the most attractive components of its leaves, especially stevioside and rebaudioside A. The drying conditions applied to *Stevia* leaves had a major impact on the sweetener extraction, these conditions generated an increase in these stevioside, especially in CD treatment where it was not significantly different to SH and VD treatments, but SH treatment entailed long drying times and accordingly, a longer exposure of product to environment and less efficiency. Therefore, optimum drying conditions (drying type) for *Stevia* leaves depend on quality property being desired to take advantage of whether as a diet supplement or as a sweetener. VD (60°C) is the most recommended treatment for keeping to most insoluble dietary fiber, C and E vitamins, polyunsaturated fatty acids and stevioside content.

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