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Influence of Drying Method on Steviol Glycosides and Antioxidants in *Stevia Rebaudiana* Leaves

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Abstract

The application of different drying conditions (hot air drying at 100ºC and 180ºC, freeze drying and shade drying) on steviol glycosides (stevioside, dulcoside A, rebaudioside A and rebaudioside C) and antioxidants in *Stevia* leaves was evaluated. 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 180ºC, 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). Therefore, the ideal method for drying *Stevia* leaves depends on their final use (sweetener or antioxidant), although, hot air at 180ºC is the most recommendable if only one treatment has to be chosen.

Keywords: steviol glycosides, antioxidants, total phenols, total flavonoids, freeze drying, shade drying, hot air drying.

1.Introduction

The food industry is increasingly interested in replacing artificial sweeteners with other natural sugars in order to offer the consumer a wider range of choice, and to satisfy the

requirements of a segment of the population that does not want to or cannot eat sucrose. *Stevia* leaves (*Stevia rebaudiana*) have been used as a sweetener in South America for centuries, and nowadays its consumption all over the world. In fact, it is 300 times sweeter than sucrose, with the additional advantages of having: zero calories, zero carbohydrates, and not causing spikes in blood sugar levels. The sweetness of this plant is due to the presence of diterpenes such as steviol glycosides: stevioside (4-13%), rebaudioside A (2-4%), rebaudioside C (1-2%), dulcoside A (0.4-0.7%), and other less abundant types such as steviolmonoside, rubusoside, steviolbioside, rebaudioside B and rebaudioside F (Lemus-Moncada, Vega-Gálvez, Zura-Bravo, & Ah-Hen, 2012). The acceptable daily intake (ADI) for these compounds is 4 mg per kg bodyweight per day (JECFA 2008). The European Food Safety Authority recognized the safety of *Stevia* leaf extracts for alimentary use in November 2011(EFSA 2011).

Recently there has been an upsurge of interest in the therapeutic potential of plants, as antioxidants in reducing free radical induced tissue injury (Shukla, Mehta, Menta, & Bajpai, 2012). *Stevia* leaves are increasingly consumed as infusions due to their antioxidant properties, which stem from their high levels of flavonoids and phenolic compounds. Muanda, Soulimani, Diop and Dicko (2011) identified 18 phenolic compounds which demonstrated the high antioxidant capacity of *Stevia* leaves. Periche, Koutsidis, and Escriche (2014) found high levels of total phenols and flavonoids in *Stevia* infusions. Carbonell-Capella, Barba, Esteve and Frígola (2013) incorporated extracts of *Stevia* as a natural source of antioxidants to obtain low-calorie fruit extracts with antioxidant and antimicrobial activity.

Like other kinds of herbal teas, *Stevia* leaves need to be dried for conservation and consumption purposes. Thanks to the drying process two goals are reached, on one hand the growth of microorganisms is prevented and on the other hand storage and transportation is facilitated (Lin, Sung, & Chen, 2011). Dehydration of plants can be carried out using different methods. Capecka, Mareczek and Leja (2005) demonstrated the efficacy of shade drying (the

53 simplest and cheapest method) for leaves of the Lamiaceae species. Chan et al. (2009) used
54 hot air to accelerate the process of drying leaves for ginger species, while Pinela, Barros,
55 Carvalho and Ferreira (2011) did the same for Fabaceae species.
56 A newer technique using freeze drying (Lin et al. 2011) has been proved to better preserve the
57 quality of medicinal plants (Abascal, Ganora, & Yarnell, 2005) although the cost is
58 considerably higher than hot air drying.
59 It is important to highlight that the different drying techniques can influence the composition
60 of some characteristic compounds present in different herbal teas. In this respect, Lin et al.
61 (2011) obtained better results for the antioxidant capacity and total phenol values when the
62 leaves of *Echinacea purpurea* were freeze dried, than when they were dehydrated with hot
63 air. Pinela et al. (2011) also obtained larger amounts of antioxidants when leaves of the
64 *Genista* sp. were freeze dried, in comparison with shade drying. On the contrary, Hossain,
65 Barry-Ryan, Martin-Diana and Brunton (2010) obtained less antioxidants from leaves of the
66 Lamiaceae family applying freeze drying than hot air drying.
67 Clearly, there is a great discrepancy about the extraction of active compounds from herbal
68 teas according to the different drying techniques applied (Lewicki, 2006). Moreover, as far as
69 the authors know, there is no research related to the influence of different drying methods on
70 the antioxidants and steviol glycosides of *Stevia* leaves. For this reason, the aim of this study
71 was to evaluate how the drying method (shade drying, hot air drying and freeze drying)
72 affects steviol glycosides and antioxidants (total phenols, flavonoids and antioxidant capacity)
73 in *Stevia* leaves.

74 **2.Material and Methods**

75 *2.1.Stevia samples and drying conditions*

76 Organically produced *Stevia rebaudiana* leaves from Valencia (Spain) were used in this
77 study. Four different drying conditions were used: shade drying at 20ºC for 30 days, hot air

drying at 100ºC and 180ºC for 3 minutes in a convective drier, and freeze drying at a vacuum pressure of $9.5x10^{-1}$ mm Hg for 24 hours.

2.2.Steviol glycosides analysis

2.2.1.Steviol glycoside extraction procedure

The *Stevia* leaves (fresh or dried leaves) were ground in a grinding mill (A11 Basic, IKA, Germany), and 100 mg of *Stevia* leaves were shaken in 10 mL of ethanol/water (6:4 v/v) for 5 minutes. The mixture was sonicated for 10 minutes and then centrifuged at 5000 x g for 5 minutes. An aliquot of 0.5 mL of the alcoholic extract was diluted with water (2.5 mL). This solution was loaded on a 3 mL Strata SPE cartridge (500 mg, 55 μm, 70 Å, StrataC18-E Phenomenex, Torrance, CA) pre-activated with methanol (3 mL) and washed with water (3 mL). Then, the SPE cartridge was washed with 3 mL of water, followed by 3 mL of acetonitrile in water (2:8 v/v); and then air dried for 2 minutes. Finally, the steviol glycosides were eluted from the cartridge with 5 mL of 80% acetonitrile in water (Woelwer-Rieck, Lankes, Wawrzun, & Wüst 2010). The eluate was subjected to LC-MS-MS analysis.

2.2.2.Methodology

A LC-MS-MS method (HPLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer, Agilent Technologies Inc., CA, USA) was used in this study for the analysis of the steviol glycosides. Chromatographic separation was carried out in gradient mode by Zorbax SB-C18 column (50mm x 2.1mm, 1.8 μm). The temperature was maintained at 40ºC, with a mobile phase of 10 mM aqueous ammonium acetate (A) and acetonitrile (B). Binary gradient conditions were used: starting with, 7% B, held for 0.2 min: linear gradient to 20% B at 0.3 min and then to 48% B at 5 min; increased to 100% B at 5.1 min and held until 7 min; followed by a linear gradient to initial conditions at 7.1 min and a final hold at this composition until 9 min. The flow-rate and injection volume were 0.4 mL/min. and 5 μL, respectively. The electrospray was in negative ion mode. Choi et al. (2002) stated that negative ion mode is 10 times more sensitive than positive ion mode. The ionization source

conditions were: temperature of the drying gas (N_2) 325ºC to 11L/min, nebulizer pressure of 50 psi and capillary voltage of 4000 V. Identification and quantification of steviol glycosides in the samples and the standards were performed using the multiple reaction monitoring mode (MRM).

The stock standard solutions of steviol glycosides (stevioside, steviolbioside, rebaudioside A, rebaudioside C, dulcoside A standards (purity > 98%), Chromadex (CA, USA) were prepared by weighing the appropriate amount of the pure standard and diluting it with methanol to obtain a final concentration of 1 mg/mL. The working standard solution had a concentration of 0.01 mg/mL in water. The stock standard solution was stored at 20ºC and the working standard solution at 4ºC.

Quantification was carried out by means of calibration curves obtained from standard solutions (0.5-10 μg/mL). Samples were spiked in order to verify the absence of a matrix effect in the analysis. To ensure the quality of the results and evaluate the stability of the proposed method, an internal quality control (a standard solution) was injected as a first step before each batch of the sample.

2.3.Validation of the steviol glycosides analysis method

The validation of the steviol glycosides analytical methodology was carried out according to the guidelines established by EU Commission Decision (2002). To this end, the parameters: linearity, accuracy and precision (repeatability and reproducibility) were studied. The accuracy of the method was established through recovery studies and the precision was verified by intraday precision or repeatability (RSD_r) and interday precision or reproducibility (RSD_R). Limit of detection (LOD) and limit of quantification (LOQ) were defined as the amount of analyte for which signal-to-noise ratios (S/N) were higher than 3 and 10 respectively.

2.4.Determination of total phenolic content

129 Total phenolic determination was realized with spectrophotometry (JASCO V-630) using the
130 modified Folin-Ciocalteu method (Sakanaka, Tachibana, & Okada, 2004). Distilled water (0.5
131 mL), 0.125 mL of the infusion sample and 0.125 mL of Folin-Ciocalteu reagent (Sigma-
132 Aldrich, Germany) were mixed and shaking. After six minutes, 1.25 mL of a 7% sodium
133 carbonate solution and 1 mL of distilled water were added. After 90 min, the absorbance was
134 measured at 760 nm. A blank was considered in this analysis. The quantification was carried
135 out considering a standard curve of gallic acid, expressing the results as mg of gallic acid
136 equivalent per gram of dry matter. The fresh weight of the all fresh samples was converted
137 into dry weight, on the basis of their respective moisture contents and then the dry weight was
138 used for calculation.

139 *2.5.Determination of total flavonoid content*

140 Total flavonoid content was analyzed with colorimetry as described by Dewanto, Wu, Adom
141 and Liu (2002). The infusion sample (0.25 mL), distilled water (1 mL) and sodium nitrite
142 solution at 5% (0.075 mL) were mixed in a cuvette. After 6 min, a 10% aluminum chloride
143 solution (0.15 mL) and 1M sodium hydroxide solution (0.5 mL) was mixed and left to settle
144 for 5 min. Finally, distilled water (2 mL) was added and the absorbance was measured at 510
145 nm straightaway. A blank was considered in this analysis. The quantification was carried out
146 considering a standard curve of (+)-catechin (Sigma-Aldrich, Germany) and the results were
147 expressed as mg of (+)-catechin equivalent per gram of dry matter, as was explained above.

148 *2.6.Determination of total antioxidant capacity*

149 The antioxidant activity (AA) was measured based on of the scavenging activities of the
150 stable 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Germany) free radical as described by
151 Shahidi, Liyana-Pathirana and Wall (2006), with some modifications. Accordingly, 0.1 mL of
152 the infusion sample (diluted in methanol:water (80:20)) was mixed with 3.9 mL of a
153 methanolic solution of DPPH (0.025mg/mL, prepared in methanol:water (80:20)). The
154 solution was shaken, after 30 min the absorbance of the samples were measured at 515 nm

using methanol as a blank. The quantification was calculated with a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as mg of Trolox equivalent per gram of dry matter, as explained previously.

2.7.Statistical analysis

An ANOVA (Statgraphics Centurion) was used to study the influence of the treatments on the steviol glycosides, antioxidants, phenols and flavonoids. In this analysis, the homogenous groups indicate statistical differences between types of treatment (α=99%). A Principal Component Analysis (PCA) was also performed using the software Unscrambler X.10 to describe the relationships between the treatments and the variables analysed.

3.Results and Discussion

3.1.Validation of the steviol glycosides analytical methodology.

An external standard calibration curve was made using standard solutions with final concentration levels of: 0.5, 1, 2, 5, 7 and 10 μg/mL, with the aim of obtaining the linearity value. For each level, six replicates were made. The linearity response from 0.5 to 10 μg/mL was $R^2 \geq 0.995$.

The recovery studies were carried out by adding known quantities of steviol glycosides to a sample (1, 5 and 10 μg/g). Six replicates of all the spiked sample levels were analyzed. The method used permitted recovery of steviol glycosides between 70.5% (for steviolbioside at 10 μg/g level) and 105.6 % (for rebaudioside A at 5 μg/g level) for the concentration range studied. The standard deviation corresponding to recovery values was less than 20% in all cases (ranging from 4.0 to 18), proving that the analytical method was accurate.

Repeatability or Intra-day precision (RSD_r) (carried out by the same operator on the same day) was evaluated by performing the assay (on six replicates of fortified *Stevia* samples) at three levels: 1, 5 and 10 μg/g. These values ranged from 1.7% for dulcoside A to 14.6% for steviolvioside. Reproducibility or inter-day precision (RSD_R) (carried out by 2 different operators on 3 consecutive days) ranged from 5.2% for dulcoside A to 16.5% for

steviolbioside. These RSD values are in total agreement with EU Commission Decision (2002) requirements, since they were always lower than 20% for all the concentration levels assayed.

The limits of detection (LOD) were: 0.05 μg/g (dulcoside A), 0.11 μg/g (rebaudioside A), 0.09 μg/g (rebaudioside C), 0.04 μg/g (stevioside) and 0.14 μg/g (steviolbioside); and the limits of quantification (LOQ) were: 0.15 μg/g (dulcoside A), 0.32 μg/g (rebaudioside A), 0.31 μg/g (rebaudioside C), 0.15 μg/g (stevioside) and 0.49 μg/g (steviolbioside).

From the results of these validation parameters, it can be concluded that the methodology applied in this work is appropriate to guarantee the quantitative values of steviol glycosides obtained in the *Stevia* leaves analyzed.

3.2.Influence of drying method on the steviol glycosides.

Figure 1 shows the average values and the standard deviation of the 4 steviol glycosides (dulcoside A, rebaudioside A, rebaudioside C and stevioside) identified and quantified in fresh, and dried *Stevia* leaves obtained applying different drying conditions (hot air drying at 100ºC and 180ºC, freeze drying and shade drying). All values are expressed in mg of compounds per gram of dry matter. Additionally, this figure shows the homogenous groups of the ANOVA carried out for the factor "drying method" for every compound. The F-ratio values were: 49.84, 5.31, 7.22 and 87.52 for dulcoside A, rebaudioside A, rebaudioside C and stevioside, respectively. These values reflect the greater influence of the drying method on dulcoside A and stevioside than the other two compounds.

In contrast to other studies (Cacciola, Delmonte, Jaworska, Dugo, Mondello & Rader, 2011), steviolbioside was not found in any sample in this work. In fact, this is logical since this compound, like rebaudioside B, is not a native constituent of *Stevia rebaudiana*, however, in some cases they may appear as artifacts during the extraction process (Kennelly 2002; Prakash, Dubois, Clos, Wilkens & Fosdick, 2008).

206 By far the most abundant steviol glycoside in fresh leaves was stevioside (81.2± 9.3 mg/g),
207 followed by rebaudioside C (3.8± 0.3 mg/g), dulcoside A (2.8 ± 0.5 mg/g) and rebaudioside A
208 (3.5± 0.3 mg/g) (Fig. 1).
209 With respect to the results obtained when the leaves were dehydrated, it can be observed that
210 rebaudioside A and rebaudioside C showed very low concentration values in all the
211 conditions applied, ranging from 0.5 ± 0.14 mg/g (in shade drying) to 6.1 ± 1.6 mg/g (in hot
212 air to 180ºC drying), and from 2.1 ± 0.6 mg/g (hot air to 100ºC drying) to 3.6 ± 0.7 mg/g (in
213 shade drying), respectively. For these compounds, as Figure 1 shows, there were practically
214 no differences between fresh and dehydrated leaves, even though the ANOVA analyses found
215 different homogeneous groups. However, different behavior was observed in the case of
216 stevioside and dulcoside A, for dehydrated samples. For both compounds, the highest values
217 in the treated samples were obtained for shade drying. In the case of stevioside an important
218 decrease occurred as a consequence of all the drying treatments applied, in comparison to the
219 levels obtained in the fresh samples. For this compound there were no significant differences
220 between shade drying (48±12 mg/g), hot air drying at 180ºC (37±6 mg/g) and freeze drying
221 (35±8 mg/g). There is no information in the literature relating the behavior of steviosides and
222 the air drying temperature. However, some authors reported that an increase in extraction
223 temperature in combination with solvents results a higher yield of this compound.
224 Specifically, Pól et al (2007) found that a temperature of 160ºC resulted in a 20% increase
225 compared to 110ºC. Meanwhile, the behavior of dulcoside A was very different to the other
226 three compounds showing a significant increase in yield as a consequence of the shade drying
227 and the freeze drying treatments in comparison to the fresh sample, reaching 22.3±1.9 mg/g
228 and 14.1±3.5 mg/g, respectively. The increase in the concentration as a consequence of using
229 freeze drying and shade drying is not surprising as this is seen with other compounds such as
230 phenols and flavonoids. This was observed in this study (section 3.2) and also by other

231 authors (Chan et al. 2009; Hossain et al. 2010; Hamrouni-Sellami, Rahali, Rebey, Bourgou,
232 Limam, & Marzouk, 2013).
233 The research data reported by other authors about the concentration of the different steviol
234 glycosides in dried *Stevia* leaves vary greatly, and in some occasions do not provide
235 information about the drying method applied. One of the most recent works is by Woelver-
236 Rieck et al. (2010) who obtained 79±2.9 mg/g and 77.8±6.1 mg/g of stevioside and 49.3±4.4
237 mg/g and 42.8±2.9 mg/g of rebaudioside A, in *Stevia* dried leaves grown in two different
238 types of soil, fertile sandy loam and light loamy soil, respectively. The values for stevioside
239 are similar to those obtained in this work, however for rebaudioside A they are much higher.
240 Moreover, Shafii, Vismeh, Beaudry, Warner and Jones (2012) found from 2 to 125 mg/g of
241 stevioside, from 2.5 to 164 mg/g of rebaudioside A and from 1.5 to 125 mg/g of rebaudioside
242 C in 1,100 *Stevia* leaf extracts. Gardana, Scaglianti and Simonetti (2010) reported 5.8 g of
243 stevioside, 1.8 g of rebaudioside A, 1.3 g of rebaudioside C and 0.7 g of dulcoside A in 100g
244 of *Stevia*.
245 *3.3.Influence of drying method on the antioxidants.*
246 The average values and the standard deviation of total phenols (mg gallic acid equivalent/g
247 *Stevia*), flavonoids (mg of catechin equivalent/g *Stevia*) and total antioxidants (mg Trolox
248 equivalent/g *Stevia*) quantified in fresh, and dried *Stevia* leaves obtained applying the
249 different drying methods, are shown in Fig. 2. The ANOVA homogenous groups are indicated
250 by letters in this figure.
251 In fresh leaves the amount of phenols, flavonoids and antioxidants were: 44.40±1.04 mg
252 gallic acid equivalent/g *Stevia*, 2.52±0.24 mg catechin equivalent/g *Stevia* and 52.92±0.84 mg
253 Trolox equivalent/g *Stevia*, respectively. It is noteworthy that drying treatments caused an
254 increase in the content of flavonoids and antioxidants when compared with fresh leaves.
255 In contrast to the steviol glycosides, phenols, flavonoids and antioxidants exhibited similar
256 behaviour as a consequence of the application of the different drying conditions. The highest

257 values for the three parameters (total phenols, flavonoids and antioxidants) were found for hot
258 air drying at 180ºC (76.8, 45.1 and 126 mg equivalent/g), followed by shade drying (39.1,
259 20.3, 75.9 mg equivalent/g), hot air drying at 100ºC (31.5, 17.2, 64.9 mg equivalent/g), and
260 finally freeze drying (26.2, 9.9, 48.5 mg equivalent/g), respectively. This last treatment
261 showed the lowest values, thus being the least suitable treatment for the extraction of
262 antioxidants.
263 The high content of flavonoids is due to the presence of flavonols and flavones in Stevia
264 leaves. Ghanta, Banerjee, Poddar and Chattopadhyay (2007) isolated 6 flavonoids (quercetin-
265 3-O-β-D-arabinoside, quercetin-3-O-β-D-rhamnoside, kaempherol-3-O-rhamnoside, apigenin,
266 apigenin-4-O-β-D-glycoside, luteolin) and Cacciola et al. (2011) 4 different ones (quercetin-
267 3-O-glucoside, quercetin-3-O-rutinoside, apigenin-7-O-β-D-glycoside, luteolin-7-O-β-D-
268 glycoside). In this work, the flavonoid content was higher for all drying methods applied in
269 comparison to fresh leaves. This result could be related to an increase in the extractability of
270 such compounds as a consequence of the matrix changes during the drying process. As
271 observed in the present work, Hamrouni-Sellami et al. (2013) also obtained higher values of
272 total flavonoids in dried leaves of *S. Officinalis* than in fresh plants. However, in contrast to
273 the present study, Ferreira and Luthria (2010), obtained lower levels of antioxidant capacity
274 (in dried *Artemisia annua* L. leaves) for shade drying than hot air drying. In the case of
275 phenols, in this study, hot air drying at 180ºC and fresh leaves showed the highest values,
276 respectively. Capecka et al. (2005) also obtained lower levels of phenols for shade dried
277 leaves (in Lemon balm leaves) than the fresh ones.
278 There are some works in the literature regarding the levels of total phenol, flavonoids and
279 antioxidant activity in dried *Stevia* leaves, however, very few studies specify the drying
280 method. For instance, in the case of phenols: 25.18 mg gallic acid/g (Tadhani, Patel, &
281 Subhash, 2007); 56.74 mg gallic acid/g, obtained with air drying (Shukla et al. 2012); 0.86 mg
282 gallic/mg with shade drying (Ghanta et al. 2007) and 130.67 mg catechin/g, air drying at 40ºC

283 for 12h (Kim, Yang, Lee & Kang, 2011). In the case of total flavonoids: 21.73 mg gallic
284 acid/g (Tadhani et al. 2007); 0.83 mg quercetin/mg (Ghanta et al. 2007); 15.64 mg quercetin/g
285 (Kim et al. 2011) and 20.68 mg catechin/g drying room temperature (Muanda et al. 2011),
286 and finally, for antioxidant activity: 38.24 mg trolox/g (Tadhani et al. 2007) and 8.72mg gallic
287 acid/g (Abou-Arab, Abou-Arab, & Abu-Salem, 2010).

288 *3.4.Global behavior of antioxidants and steviol glycosides.*

289 A PCA was applied in order to appreciate the overall effect that the drying method had on
290 steviol glycosides and antioxidants together. The corresponding bi-plot obtained (scores
291 "treatments" and loading "variables") is shown in Fig. 3 (PC1 explained 46 % of the total
292 variance and PC2, 25 %). The proximity between variables indicates the correlation between
293 them, and in the case of drying treatments similar behavior. This figure shows more clearly
294 that the two groups of variables (antioxidants and glycosides of steviol) show in general
295 opposing behavior with respect to the effect of the drying treatments applied. That is to say,
296 the hot air drying treatment at 180ºC is placed at the far end of the right axis in the figure,
297 which corresponds to the highest values of the three antioxidant parameters (total phenols,
298 flavonoids and total antioxidants) and the lowest of the steviol glycosides. On the contrary,
299 fresh and shade drying are placed on the opposite side (left axis), which corresponds to the
300 highest content of steviol glycosides (especially dulcoside A, rebaudioside C and stevioside)
301 and the lowest level of all the antioxidant parameters. As it can been observed, not a single
302 drying treatment permits the maximum extraction of all the compounds together.

303 **4.Conclusions**

304 The drying conditions applied in fresh *Stevia* leaves have a great impact on the extraction of
305 steviol glycosides and antioxidants. In general, the yield of these compounds was affected in
306 different ways according to the drying conditions (hot air drying at 100ºC and 180ºC, freeze
307 drying and shade drying). The drying conditions produced an important increase in
308 antioxidant capacity but an important decrease in the principal steviol glycoside (stevioside)

309 which diminished with all treatments, especially with hot air at 100ºC. For this compound,
310 there were no significant differences between the other treatments, although shade drying
311 produced the highest values of this compound. Dulcoside A increased only with the shade and
312 freeze drying treatments. On the other hand, the levels of the less abundant glycosides
313 (rebaudioside A and rebaudioside C) changed very little when comparing fresh and
314 dehydrated leaves. Considering all the steviol glycosides, the least aggressive treatment was
315 shade drying.
316 With respect to the antioxidant parameters (total phenols, flavonoids and total antioxidants),
317 the most suitable drying method was hot air at 180ºC, since it was able to substantially
318 increase the level of all of them compared to the fresh *Stevia* leaves.
319 Therefore, the optimum drying conditions for fresh *Stevia* leaves is determined by whether
320 they are used for sweetening or for their antioxidant properties. Although, if one treatment
321 had to be chosen, hot air drying at 180ºC is the most recommendable overall.
322 As drying methods are known to be highly effective in the extraction of antioxidants, the
323 profile of specific antioxidant compounds should be studied in greater depth.

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Figure captions

Figure. 1 Average values and the standard deviation of the 4 steviol glycosides (dulcoside A, rebaudioside A, rebaudioside C and stevioside) in fresh and dried *Stevia* leaves obtained applying different drying conditions (hot air drying at 100ºC and 180ºC, freeze drying and shade drying). The ANOVA homogenous groups are indicated by letters

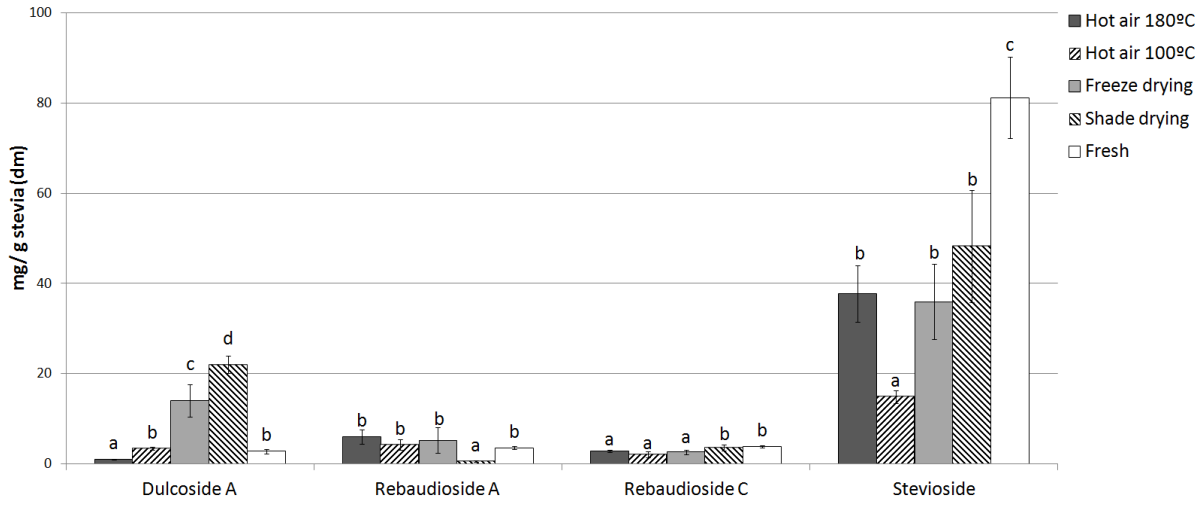

Figure. 2 Average values and the standard deviation of total phenols (mg gallic acid equivalent/g *Stevia*), flavonoids (mg of catechin equivalent/g *Stevia*) and total antioxidants (mg Trolox equivalent/g *Stevia*) in fresh and dried *Stevia* leaves obtained applying the different drying methods (hot air drying at 100ºC and 180ºC, freeze drying and shade drying). The ANOVA homogenous groups are indicated by letters.

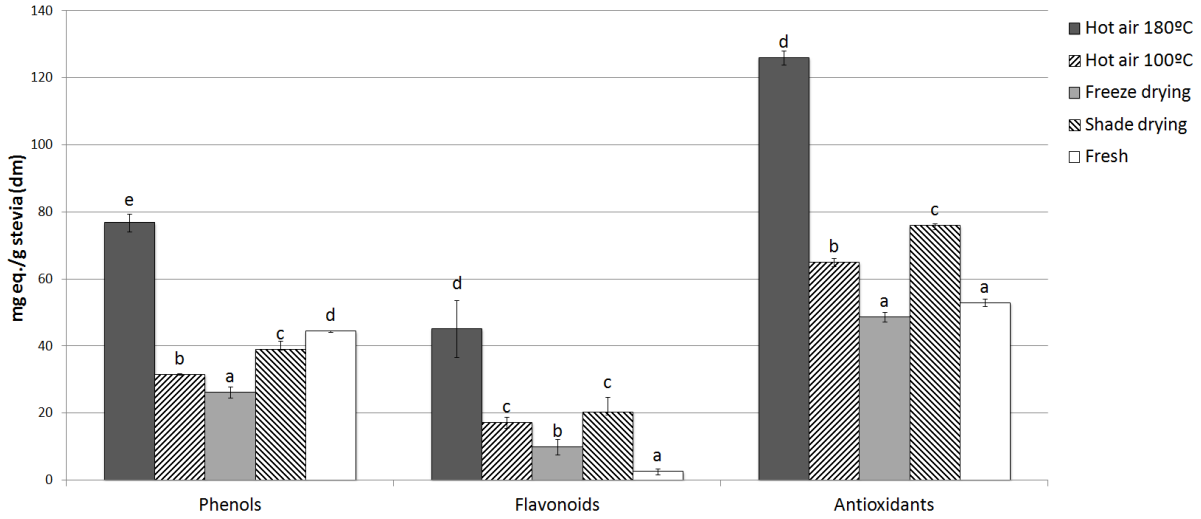

Figure. 3 Bi-plot of Principal Components Analysis for the drying treatments (white diamond ◊) and the analysed variables: steviol glycosides and antioxidant parameters (total phenols, flavonoids and antioxidant activity) (black diamond ♦).