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 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 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 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). Therefore, 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.

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

2. Material and Methods

2.1. Stevia samples and drying conditions

Organically produced Stevia rebaudiana leaves from Valencia (Spain) were used in this study. Four different drying conditions were used: shade drying at 20°C for 30 days, hot air...
drying at 100°C and 180°C for 3 minutes in a convective drier, and freeze drying at a vacuum
depression 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 40°C,
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 (N₂) 325°C 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 20°C and the working standard solution at 4°C.

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

2.5. Determination of total flavonoid content

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

2.6. Determination of total antioxidant capacity

The antioxidant activity (AA) was measured based on of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Germany) free radical as described by Shahidi, Liyana-Pathirana and Wall (2006), with some modifications. Accordingly, 0.1 mL of the infusion sample (diluted in methanol:water (80:20)) was mixed with 3.9 mL of a methanolic solution of DPPH (0.025mg/mL, prepared in methanol:water (80:20)). The 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 ($\alpha=99\%$). 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 100°C and 180°C, 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).
By far the most abundant steviol glycoside in fresh leaves was stevioside (81.2 ± 9.3 mg/g), followed by rebaudioside C (3.8 ± 0.3 mg/g), dulcoside A (2.8 ± 0.5 mg/g) and rebaudioside A (3.5 ± 0.3 mg/g) (Fig. 1).

With respect to the results obtained when the leaves were dehydrated, it can be observed that rebaudioside A and rebaudioside C showed very low concentration values in all the conditions applied, ranging from 0.5 ± 0.14 mg/g (in shade drying) to 6.1 ± 1.6 mg/g (in hot 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 shade drying), respectively. For these compounds, as Figure 1 shows, there were practically no differences between fresh and dehydrated leaves, even though the ANOVA analyses found different homogeneous groups. However, different behavior was observed in the case of stevioside and dulcoside A, for dehydrated samples. For both compounds, the highest values in the treated samples were obtained for shade drying. In the case of stevioside an important decrease occurred as a consequence of all the drying treatments applied, in comparison to the levels obtained in the fresh samples. For this compound there were no significant differences between shade drying (48±12 mg/g), hot air drying at 180ºC (37±6 mg/g) and freeze drying (35±8 mg/g). There is no information in the literature relating the behavior of steviosides and the air drying temperature. However, some authors reported that an increase in extraction temperature in combination with solvents results a higher yield of this compound. Specifically, Pól et al (2007) found that a temperature of 160ºC resulted in a 20% increase compared to 110ºC. Meanwhile, the behavior of dulcoside A was very different to the other three compounds showing a significant increase in yield as a consequence of the shade drying and the freeze drying treatments in comparison to the fresh sample, reaching 22.3±1.9 mg/g and 14.1±3.5 mg/g, respectively. The increase in the concentration as a consequence of using freeze drying and shade drying is not surprising as this is seen with other compounds such as phenols and flavonoids. This was observed in this study (section 3.2) and also by other

The research data reported by other authors about the concentration of the different steviol glycosides in dried *Stevia* leaves vary greatly, and in some occasions do not provide information about the drying method applied. One of the most recent works is by Woelver-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 mg/g and 42.8±2.9 mg/g of rebaudioside A, in *Stevia* dried leaves grown in two different types of soil, fertile sandy loam and light loamy soil, respectively. The values for stevioside are similar to those obtained in this work, however for rebaudioside A they are much higher. Moreover, Shafii, Vismeh, Beaudry, Warner and Jones (2012) found from 2 to 125 mg/g of stevioside, from 2.5 to 164 mg/g of rebaudioside A and from 1.5 to 125 mg/g of rebaudioside C in 1,100 *Stevia* leaf extracts. Gardana, Scaglianti and Simonetti (2010) reported 5.8 g of stevioside, 1.8 g of rebaudioside A, 1.3 g of rebaudioside C and 0.7 g of dulcoside A in 100g of *Stevia*.

3.3. Influence of drying method on the antioxidants.

The 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*) quantified in fresh, and dried *Stevia* leaves obtained applying the different drying methods, are shown in Fig. 2. The ANOVA homogenous groups are indicated by letters in this figure.

In fresh leaves the amount of phenols, flavonoids and antioxidants were: 44.40±1.04 mg gallic acid equivalent/g *Stevia* , 2.52±0.24 mg catechin equivalent/g *Stevia* and 52.92±0.84 mg Trolox equivalent/g *Stevia*, respectively. It is noteworthy that drying treatments caused an increase in the content of flavonoids and antioxidants when compared with fresh leaves.

In contrast to the steviol glycosides, phenols, flavonoids and antioxidants exhibited similar behaviour as a consequence of the application of the different drying conditions. The highest
values for the three parameters (total phenols, flavonoids and antioxidants) were found for hot air drying at 180°C (76.8, 45.1 and 126 mg equivalent/g), followed by shade drying (39.1, 20.3, 75.9 mg equivalent/g), hot air drying at 100°C (31.5, 17.2, 64.9 mg equivalent/g), and finally freeze drying (26.2, 9.9, 48.5 mg equivalent/g), respectively. This last treatment showed the lowest values, thus being the least suitable treatment for the extraction of antioxidants.

The high content of flavonoids is due to the presence of flavonols and flavones in Stevia leaves. Ghanta, Banerjee, Poddar and Chattopadhyay (2007) isolated 6 flavonoids (quercetin-3-O-β-D-arabinoside, quercetin-3-O-β-D-rhamnoside, kaempherol-3-O-rhamnoside, apigenin, apigenin-4-O-β-D-glycoside, luteolin) and Cacciola et al. (2011) 4 different ones (quercetin-3-O-glucoside, quercetin-3-O-rutinoside, apigenin-7-O-β-D-glycoside, luteolin-7-O-β-D-glycoside). In this work, the flavonoid content was higher for all drying methods applied in comparison to fresh leaves. This result could be related to an increase in the extractability of such compounds as a consequence of the matrix changes during the drying process. As observed in the present work, Hamrouni-Sellami et al. (2013) also obtained higher values of total flavonoids in dried leaves of *S. Officinalis* than in fresh plants. However, in contrast to the present study, Ferreira and Luthria (2010), obtained lower levels of antioxidant capacity (in dried *Artemisia annua* L. leaves) for shade drying than hot air drying. In the case of phenols, in this study, hot air drying at 180°C and fresh leaves showed the highest values, respectively. Capecka et al. (2005) also obtained lower levels of phenols for shade dried leaves (in Lemon balm leaves) than the fresh ones.

There are some works in the literature regarding the levels of total phenol, flavonoids and antioxidant activity in dried *Stevia* leaves, however, very few studies specify the drying method. For instance, in the case of phenols: 25.18 mg gallic acid/g (Tadhani, Patel, & Subhash, 2007); 56.74 mg gallic acid/g, obtained with air drying (Shukla et al. 2012); 0.86 mg gallic/mg with shade drying (Ghanta et al. 2007) and 130.67 mg catechin/g, air drying at 40°C.
In the case of total flavonoids: 21.73 mg gallic acid/g (Tadhani et al. 2007); 0.83 mg quercetin/mg (Ghanta et al. 2007); 15.64 mg quercetin/g (Kim et al. 2011) and 20.68 mg catechin/g drying room temperature (Muanda et al. 2011), and finally, for antioxidant activity: 38.24 mg trolox/g (Tadhani et al. 2007) and 8.72 mg gallic acid/g (Abou-Arab, Abou-Arab, & Abu-Salem, 2010).

### 3.4. Global behavior of antioxidants and steviol glycosides.

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

### 4. Conclusions

The drying conditions applied in fresh *Stevia* leaves have a great impact on the extraction of steviol glycosides and antioxidants. In general, the yield of these compounds was affected in different ways according to the drying conditions (hot air drying at 100ºC and 180ºC, freeze drying and shade drying). The drying conditions produced an important increase in antioxidant capacity but an important decrease in the principal steviol glycoside (stevioside).
which diminished with all treatments, especially with hot air at 100°C. For this compound, there were no significant differences between the other treatments, although shade drying produced the highest values of this compound. Dulcoside A increased only with the shade and freeze drying treatments. On the other hand, the levels of the less abundant glycosides (rebaudioside A and rebaudioside C) changed very little when comparing fresh and dehydrated leaves. Considering all the steviol glycosides, the least aggressive treatment was shade drying.

With respect to the antioxidant parameters (total phenols, flavonoids and total antioxidants), the most suitable drying method was hot air at 180°C, since it was able to substantially increase the level of all of them compared to the fresh Stevia leaves. Therefore, the optimum drying conditions for fresh Stevia leaves is determined by whether they are used for sweetening or for their antioxidant properties. Although, if one treatment had to be chosen, hot air drying at 180°C is the most recommendable overall.

As drying methods are known to be highly effective in the extraction of antioxidants, the profile of specific antioxidant compounds should be studied in greater depth.

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References


**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 100°C and 180°C, 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 100°C and 180°C, 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 ♦).