Influence of the drying method in the antioxidant potential and chemical composition of four shrubby flowering plants from the tribe Genisteae (Fabaceae)

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Abstract

Flowers from several common Mediterranean shrubs, such as those from the *Cytisus* genus and *Genista* genus (tribe Genisteae/Fabaceae) have long been used for medicinal purposes and seasoning in the northeastern Portuguese region. Despite, the shade-drying traditionally used to process these plants, freeze-drying is claimed to better preserve the quality of medicinal plants. Herein, the effects of drying process in the antioxidants composition and properties of *Cytisus multiflorus*, *Cytisus scoparius*, *Cytisus striatus* and *Pterospartum tridentatum* were evaluated. Freeze-dried *P. tridentatum* revealed the highest antioxidant properties (EC_{50} values ≤ 0.15 mg/ml). Freeze-drying benefits were confirmed showing, the samples submitted to this process, higher antioxidant activity and higher concentrations of hydrophilic (phenolics, ascorbic acid and sugars) and lipophilic (tocopherols, chlorophylls and lycopene) compounds. This process could be applied in scale-up treatments of the studied plants for cosmetic or pharmaceutical applications.

Keywords: Genisteae; MAP; Antioxidants; Energetic value; Drying process

1. Introduction

The World Health Organization estimates that 80% of the world's population uses medicinal plants in primary health needs (WHO, 2009). Medicinal plants are often dried and stored for a long time before use in manufacturing various types of products. Drying is an effective method that increases the shelf life of the final product by slowing the growth of microorganisms and preventing certain biochemical reactions that may alter the organoleptic characteristics. Furthermore, it reduces the weight and bulk of plants for cheaper transport and storage (Lin et al., 2011; Sellami et al., 2011).

Dehydration of plants can be performed using different methods. Natural drying (drying in the shade) and hot air drying are still most widely used methods because of their lower cost. Nevertheless, natural drying has many disadvantages due to the inability to handle the large quantities and to achieve consistent quality standards (Soysal and Öztekin, 2001). Freeze-drying is generally better in preserving the quality of medicinal plants during processing (Abascal et al., 2005), but the drying cost is considerably higher than hot air-drying (Lin et al., 2011).

Cytisus multiflorus, *Cytisus scoparius*, *Cytisus striatus* and *Pterospartum tridentatum* are four species from the Tribe Genisteae (Fam. Fabaceae, subFam. Faboideae) commonly encountered in the Portuguese wild flora. These species are frequent in the Iberian Peninsula, growing spontaneously in thermo-Mediterranean conditions and widely used in local folk medicine and sometimes as condiment (Blanco and Diez, 2005; Carvalho, 2010; Cunha et al., 2007; Gallego and Gallego, 2008; Pardo de Santayana, 2008).

In Portugal, these Fabaceae have been used for centuries and are considered to have anti-inflammatory properties (Camejo et al., 2003; Carvalho, 2010; Cunha et al., 2007;

Novais, et al., 2004; Neves et al., 2009; Salgueiro, 2004). Medicinal infusions, decoctions and tonics are prepared with fresh or shade-dried flowers, and largely used for several respiratory, gastrointestinal and skin affections and to control diabetes and metabolic system disorders, such as cholesterol and gout. *P. tridentatum* flowers in short racemes are traditionally used for seasoning (Carvalho, 2010; Pardo de Santayana et al., 2007). Furthermore, our research group recently reported antioxidant properties of one of those species- *Cytisus multiflorus* (Barros et al., 2011), remaining the knowledge about antioxidant potential of the other three species very scanty.

Many species of the Fabaceae family are known as poisonous and injurious plants. Especially, bark, pods and seeds of several genus are noxious and reveal potential toxins (Cooper and Johnson, 1984; Rivera and Obón de Castro, 1991; Nelson et al., 2007). Empirical traditional knowledge and local healers assume that consuming oral dosage forms from these plants can lead to some risks especially due to excessive doses and long-term treatments. However, folk medicine avoids these kinds of risks recommending specific dosages and controlling the periods of intake with ritual healing practices.

According to growing demand for medicinal species, artificial drying has been one of the most important needs of the pharmaceutical industry, which does not have structure to use fresh plants in the quantities required for industrial production. Also, increasing usage of drying procedures within various technological lines in the food industry and biotechnology has made studies of the drying process of important practical interest (Sellami et al., 2011). Moreover, the control of herbs processing is a priority in order to prevent contamination, adulteration and to ensure a good quality of herbal products. Therefore, the aim of this study was to evaluate the effects of drying process in the

antioxidants composition and chemical composition of four shrubby flowering plants from the tribe Genisteae (Fabaceae) widely consumed in folk medicine.

2. Materials and methods

2.1. Samples

Flowers fully opened and functional of the four species were gathered in 2010 spring (April and May, depending on each species), in the territory of Trás-os-Montes, North-eastern Portugal, considering the most favorable stage according local medicinal uses (**Table 1**) mentioned by healers and selected consumers. Therefore sites, and gathering season and practices took into account local criteria and preferences for herbal dosages forms, such as infusion and decoction.

The samples were submitted to two different drying processes: 1) Freeze-drying (Ly-8-FM-ULE freeze-dryer) immediately after being collected; 2) Shade-drying, being stored in a dark, dry place and at room temperature for 30 days, simulating informants' general conditions of use. The samples were reduced to power and kept in the best conditions for subsequent use.

Voucher specimens are deposited in the Herbário da Escola Superior Agrária de Bragança (BRESA).

2.2. Standards and Reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as

also other individual fatty acid isomers, L-ascorbic acid, tocopherols (α-, β-, γ-, and δ-isoforms) and sugars (D(-)-fructose, D(+)-glucose anhydrous, D(+)-melezitose hydrate, D(+)-sucrose, and D(+)-trehalose) standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), caffeic acid, chlorogenic acid, and quercetin dehydrate. Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. In vitro antioxidant properties

2.3.1. General. A fine dried powder (20 mesh; ~1g) stirring with 50 ml of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 50 ml portion of methanol. The combined methanolic extracts were evaporated at 35ºC under reduced pressure (rotary evaporator Büchi R-210) and re-dissolved in methanol at 5 mg/ml (stock solution). Different concentrations of the extracts (prepared from the stock solution) were submitted to *in vitro* assays (Barros et al., 2011) to evaluate their antioxidant properties. The extract concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene bleaching and TBARS assays) or absorbance at 690 nm (reducing power assay) against extract concentrations. Trolox was used as standard.

2.3.2. DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μl) and methanolic solution (270 μl) containing DPPH radicals (6×10^{-5} mol/l). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

2.3.3. Reducing power. This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 ml) were mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The mixture (0.8 ml) was poured in the 48-wells, as also deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance was measured at 690 nm.

2.3.4. Inhibition of β-carotene bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing different concentrations of

the extracts (0.2 ml). The tubes were shaken and incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (spectrophotometer AnalytikJena). β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100.

2.3.5. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000*g* (Centorion K24OR refrigerated centrifuge) for 10 min. An aliquot (0.1 ml) of the supernatant was incubated with the different concentrations of the extracts (0.2 ml) in the presence of $FeSO_4$ (10 μM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively.

2.3.6. Phenolic compounds. The extract sample concentrated at 0.625 mg/ml (250 μl) was mixed with HCl 0.1% in 95% etanol (250 μl) and HCl 2% (4550 μl). After 15 min

the absorbance was measured at 280, 320 and 360 nm. The absorbance (A) at 280 nm was used to estimate total phenolic content, $A_{320\ nm}$ was used to estimate tartaric esters and $A_{360\ nm}$ was used to estimate flavonols (Mazza et al., 1999). Chlorogenic acid was used to calculate the standard curve (0.2-3.2 mM) and the results were expressed as mg of chlorogenic acid equivalents (ClAE) per g of extract. Caffeic acid was used to calculate the standard curve (0.2-3.6 mM) and the results were expressed as mg of caffeic acid equivalents (CAE) per g of extract. Quercetin was used to calculate the standard curve (0.2-3.2 mM) and the results were expressed as mg of quercetin equivalents (QE) per g of extract.

2.4. Chemical composition in hydrophilic compounds

2.4.1. Vitamin C. A fine powder (20 mesh) of sample (500 mg) was extracted with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through Whatman Nº 4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichloroindophenol (9 ml) and the absorbance was measured after 30 min at 515 nm (Klein and Perry, 1982). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (6×10^{-3}-0.1 mg/ml), and the results were expressed as g of ascorbic acid per 100 g of dry weight (dw).

2.4.2. Sugars. Dried sample powder (1.0 g) was spiked with the melezitose as internal standard (IS, 5 mg/ml), and was extracted with 40 ml of 80% aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged (Centorion K24OR- 2003 refrigerated centrifuge) at 15,000*g* for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted three times with 10 ml of ethyl ether,

successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 ml and filtered through 0.2 µm nylon filters from Whatman. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by the authors in previous reports (Batista et al., 2011). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

2.5. Chemical composition in lipophilic compounds

2.5.1. Tocopherols. BHT solution in hexane (10 mg/ml; 100 µl) and IS solution in hexane (tocol; 50 µg/ml; 400 µl) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 ml) by vortex mixing (1 min). Subsequently, hexane (4 ml) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 ml) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000*g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 ml of

n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22 µm disposable LC filter disk, transferred into a dark injection vial and analysed by the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm (Barros et al., 2010). The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols contents were further expressed in mg per 100 g of dry weight (dw).

2.5.2. Pigments. β-carotene, lycopene, chlorophylls a and b were determined following a procedure previously described by Nagata and Yamashita (1992). A fine dried powder (150 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Content of β-carotene was calculated according to the following equation: β-carotene (mg/100 ml) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Lycopene (mg/100 ml) = $-0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Chlorophyll a (mg/100 ml) = $0.999 \times A_{663} - 0.0989 \times A_{645}$; Chlorophyll b (mg/100 ml) = $-0.328 \times A_{663} + 1.77 \times A_{645}$, and further expressed in mg per 100 g of dry weight (dw).

2.5.3. Fatty Acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Batista et al., 2011), and after methylation of the fatty acids (obtained after Soxhlet extraction) with 5 ml of methanol:sulphuric acid:toluene 2:1:1 (v:v:v), during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 ml of deionised water were added, to obtain phase separation; the FAME were recovered with 3 ml of diethyl ether by shaking in vortex , and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Whatman. The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30ºC/min ramp to 125 ºC, 5ºC/min ramp to 160 ºC, 20ºC/min ramp to 180 ºC, 3ºC/min ramp to 200 ºC, 20ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.6. Energetic value. The samples were analysed for moisture, proteins, fats, carbohydrates and ash using the AOAC procedures (AOAC, 1995). The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the

crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations: Energy (kcal) = 4 × (g proteins +g carbohydrates) + 9 × (g fats).

2.7. Statistical analysis

The results are expressed as mean values and standard deviation (SD). The results of each drying method were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

3.1. Antioxidant properties

The composition in phenolic molecules (hydrophilic antioxidants) and antioxidant activity of the studied Fabaceae species is shown in **Table 2**. *P. tridentatum* submitted to freeze- or shade-drying proved to be the species with highest antioxidant capacity in all the assayed methods (lowest EC_{50} values), which is in agreement to its highest levels of phenolics (~500 mg ClAE/g extract). Beside the phenolic groups (flavonols and tartaric esters) quantified in the present work, this plant was already reported as a source of isoflavones such as 5,5-dihydroxy-3-metoxi-isoflavone-7-*O*-glucoside, prunetin, genistin and sissotrin (Vitor et al., 2004). Curiously, *P. tridentatum* was the only species showing similar results for antioxidant activity (unless for DPPH assay) after freeze- or shade-drying. *C. striatus* and *P. tridentatum* revealed the highest levels of flavonols and

tartaric esters after freeze-drying and shade-drying, respectively. In general, shade-drying seemed to significantly decrease the antioxidant capacity, increasing the corresponding EC_{50} values, and also decrease the phenolic content.

Among the studied species, only *C. scoparius* was reported to protect liver from oxidative stress induced by CCl_4 in rats (Raja et al., 2007), and no other reports on antioxidant potential of the four species are available. Therefore, besides the novelty of the results, the antioxidant effects herein reported for the four Fabaceae species support local recommendations of these plants. Furthermore, considering the involvement of oxidative stress in inflammation, the present results could explain the empirical use of these species as anti-inflammatory (**Table 1**).

3.2. Chemical composition

Other hydrophilic compounds such as ascorbic acid and sugars (mono and disaccharides) were also quantified and results are given in **Table 3**. Freeze- and shade-drying *C. scoparius* and *C. striatus* reveled the highest concentration of ascorbic acid (~0.2 g/100 g dw) and total sugars (~19 and 10 g/100 g dw), respectively. Fructose, glucose and sucrose were found in all the studied species, while trehalose was not found in *C. striatus* and *P. tridentatum*. Shade-dried samples revealed slightly lower ascorbic acid contents and significantly lower sugars concentrations than freeze-dried samples. This effect can be observed in **Figure 1** for the example of *C. multiflorus*.

Lipophilic compounds including tocopherols, carotenoids and chlorophylls were also determined in these shrubby plants, and the results are given in **Table 4**. The four isoforms of tocopherols were detected, being α-tocopherol the major compound in all the species. *C. striatus* (~23 mg/100 g dw) and *C. scoparius* (~12 mg/100 g dw)

presented the highest content of tocopherols for freeze- and shade-dried samples, respectively. *C. scoparius* samples also gave the highest levels of lycopene, and the shade-dried sample revealed the highest chlorophylls content. Among the freeze-dried samples, the highest levels of chlorophyll a and b were found in *P. tridentatum* and *C. striatus*, respectively.

The shade-drying process significantly decreased tocopherols content in *C. striatus* and *P. tridentatum* as can be observed in **Figure 2** for the first species. Particularly, δ-tocopherol was not detected in three of the four analysed shade-dried samples. This process also decreased the levels of chlorophylls and lycopene. Otherwise, shade-drying seemed to preserve more β-carotene than freeze-drying, unless for *P. tridentatum*.

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 of the studied Fabaceae species are shown in **Table 5**. The drying process did not have influence in fatty acids profile. α-Linolenic acid (C18:3n3) was the main fatty acid in all the studied samples, followed by palmitic acid (C16:0) or linoleic acid (C18:2n6). Both samples submitted to freeze- or shade-drying gave PUFA/SFA ratios higher than 0.45, and n-6/n-3 fatty acids ratios lower than 4.0 as recommended for "good nutritional quality", including health beneficial effects (Guil et al., 1996).

As the studied species are sometimes used as condiment, and as medicinal oral dosage forms (**Table 1**), their energetic value was also evaluated using protein, fat and carbohydrates contents (**Table 6**). Moisture was the most abundant macronutrient; freeze and shade-drying were efficient processes to remove water, as drying matter was similar after both drying processes. Carbohydrates were also abundant macronutrients

and the highest levels were found in freeze-dried *P. tridentatum* and shade-dried *C. striatus* (~79 g/100 g). The latter species gave the highest ash contents, while *C. scoparius* showed the highest protein contents (~22 or 23 g/100g dw for freeze and shade-dried samples, respectively), similar values to the ones reported by other authors: 18.9 g/100 g dw in a sample from UK (Tolera et al., 1997) and 21.6 g/100 g dw in a sample from Spain (Ammar et al., 2004). The highest levels of fat were found in *C. scoriapus* and *C. striatus* without significant statistical differences (~3 or 4 g/100g dw). In general, fat levels decreased in shade-dried samples. Nevertheless, the drying process did not have significantly influence in energetic contribution (values ranging from 386 and 404 kcal/100g dw).

Concerning the particular case of *C. multiflorus*, previous research of our team (Barros et al., 2011) has already assess its phytochemical composition and antioxidant activity using plant material gathered in May 2009, in the same sites. In vitro assays, based on chemical, biochemical and electrochemical methods were performed and the results obtained for antioxidant phytochemicals, such as phenolics, flavonoids, ascorbic acid and tocopherols presented a better performance in 2009 fresh frozen samples than those existing on 2010 also fresh frozen samples. This discrepancy may be explained by slightly differences in flower development (e.g. flowers unfertilized *versus* already fertilized already including pod and seed primordia) and by annual variation of climatic conditions affecting blossoms.

Despite all the information given herein, it should be pointed out that there are some toxicity reports on the studied species under long-term use (**Table 1**).

As far as we know this is the first detailed characterization in antioxidant properties and chemical composition of Genisteae shrubs flowers, *C. multiflorus*, *C. scoparius*, *C.*

striatus and *P. tridentatum* after freeze and shade-dried treatments. The obtained results give scientific and technical support to the traditional uses of these plants in folk medicine as anti-inflammatory, highlighting these species as a source of bioactive compounds (e.g. antioxidants) to be incorporated in functional beverages or other formulations. Moreover, the drying process to be used in industrial applications is very important, since freeze-drying proved to preserve more the quality of the studied medicinal plants, including antioxidant activity, phenolics, ascorbic acid, sugars, tocopherols, chlorophylls, and lycopene contents.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support to the research centre CIMO. L. Barros thanks to FCT, POPH-QREN and FSE for her grant (SFRH/BPD/4609/2008).

References

Abascal, K., Ganora, L., Yarnell, E. 2005. The effect of freeze drying and its implications for botanical medicines: a review. Phytother. Res. 19, 655–660.

Ammar, H., López, S., González, J.S., Ranilla, M.J. 2004. Seasonal variations in the chemical composition and in vitro digestibility of some Spanish leguminous shrub species. Animal Feed Sci. Technol. 115, 327–340.

AOAC 1995. Official methods of analysis (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Barros, L., Cabrita, L., Vilas Boas, Miguel, Carvalho, A.M., Ferreira, I.C.F.R. 2011. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chem. 127, 1600-1608.

Barros, L., Carvalho, A.M., Ferreira, I.C.F.R. 2010. Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: A comparative study of the nutraceutical potential and composition. Food Chem. Toxicol. 48, 1466–1472.

Batista, C., Barros, L., Carvalho, A.M., Ferreira, I.C.F.R. 2011. Nutritional and nutraceutical potential of rape (*Brassica napus* var napus) and "tronchuda" cabbage (*Brassica oleraceae* var costata) inflorescences. Food Chem. Toxicol. 49, 1208-1214.

Blanco, E., Díez, J. 2005. Guia de la Flora de Sanabria, La Carballeda y Los Valles. Catálogo de etnoflora selecta. Zamora: Adisac-La Voz.

Camejo-Rodrigues, J.S., Ascensão, L., Bonet, M.À., Vallès, J. 2003. An ethnobotanical study of medicinal and aromatic plants in the Natural Park of Serra de S. Mamede (Portugal). J. Ethnopharmacol. 89, 199–209.

Carvalho, A.M. 2010. Plantas y sabiduría popular del Parque Natural de Montesinho. Un estudio etnobotánico en Portugal. Biblioteca de Ciencias nº 35. Madrid: Consejo Superior de Investigaciones Científicas.

Cooper, M., Johnson, A. 1984. Poisonous plants in Britain and their effects on animals and man. H.M.S.O., London.

Cunha, A.P., Ribeiro, J.A., Roque, O.R. 2007. Plantas aromáticas em Portugal. Caracterização e utilizações. Lisboa: Fundação Calouste Gulbenkian.

Dias, C. 2005. Guia das Plantas Aromáticas e Medicinais do Parque Natural do Internacional. ICN-PNDI.

Gallego, E.C., Gallego, A.C. 2008. Usos, tradiciones y conocimientos de las plantas por las gentes de Sayago. Zamora: ADERISA.

Guil, J.L., Torija, M.E., Giménez, J.J., Rodriguez, I. 1996. Identification of fatty acids in edible wild plants by gas chromatography. J. Chromat. A, 719, 229-235.

González, J.A., García-Barriuso, M., Amich, F. 2010. Ethnobotanical study of medicinal plants traditionally used in the Arribes del Duero, western Spain. J. Ethnopharmacol. 131, 343–355.

Klein, B.P., Perry, A.K. 1982. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. J. Food Sci. 47, 941–945.

Lin, S.-D., Sung, J.-M., Chen, C.-L. 2011. Effect of drying and storage conditions on caffeic acid derivatives and total phenolics of Echinacea Purpurea grown in Taiwan. Food Chem. 125, 226–231.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., Ewert, B. 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot noir wines from British Columbia. J. Agric. Food Chem. 47, 4009-4017.

Nagata, M., Yamashita, I. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 39, 925–928.

Nelson L.S., Shih R.D., Balick M.J. 2007. Hand book of poisonous and injurious plants. The New York Botanical Garden and Springer.

Neves, J.M., Matosa, C., Moutinho, C., Queiroz, G., Gomes, L.R. 2009. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J. Ethnopharmacol. 124, 270–283.

Novais, M.H., Santos, I., Mendes, S., Pinto-Gomes, C. 2004. Studies on pharmaceutical ethnobotany in Arrábida Natural Park (Portugal). J. Ethnopharmacol. 93, 183-195.

Pardo de Santayana, M. 2008. Estudios etnobotánicos en Campoo (Cantábria). Biblioteca de Ciencias nº 32. Madrid: Consejo Superior de Investigaciones Científicas.

Pardo de Santayana, M., Tardio, J., Blanco, E., Carvalho, A.M., Lastra, J.J., San Miguel, E., Morales, R. 2007. Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. J. Ethnobiol. Ethnomed. 3, 27.

Raja, S., Ahameda, K.F.H.N., Kumara, V., Mukherjee, K., Bandyopadhyay, A., Mukherjee, P.K. 2007. J. Ethnopharmacol. 109, 41–47.

Rivera, D., Obón de Castro, C. 1991. La guia Incafo de las planta utiles de la Península Ibérica y Baleares. Incafo, Madrid.

Salgueiro, J. 2004. Ervas, usos e saberes. Lisboa: MARCA, Associação de Desenvolvimento Local.

Sellami, I.H., Wannes, W.A., Bettaieb, I., Berrima, S., Chahed, T., Marzouk, B., Limam, F. 2011. Qualitative and quantitative changes in the essential oil of Laurus nobilis L. leaves as affected by different drying methods. Food Chem. 126, 691–697.

Soysal, Y., Öztekin, S. 2001. Technical and economic performance of a tray dryer for medicinal and aromatic plants. J. Agric. Eng. Res. 79, 73–79.

Tolera, A., Khazaal, K., Orskov, E.R. 1997. Nutritive evaluation of some browse species. Animal Feed Sci. Technol. 67, 181-195.

Vitor, R.F., Mota-Filipe, H., Teixeira, G., Borges, C., Rodrigues, A.I., Teixeira, A., Paulo. 2004. Flavonoids of an extract of Pterospartum tridentatum showing endothelial protection against oxidative injury. J. Ethnopharmacol. 93, 363–370.

World Health Organization. Regional Office for South-East Asia. 2009. The use of herbal medicines in primary health care. Report of the Regional Meeting 10-12 March, Yangon, Myanmar.

Table 1. Medicinal uses of the studied flowers from Fabaceae species reported in ethnobotanical surveys recently carried out in the Iberian Peninsula.

Scientific name	English name	Local name	Traditional uses and indications	Preparation	Toxicity and particular caution according references
Cytisus multiflorus (L'Hér.) Sweet	White Spanish broom	Giesta branca	Diabetes. Blood pressure. Cholesterol. Headache and migraine. Heart failure. Rheumatoid arthritis. Sores and cutaneous eruptions and inflammations. Acne Anti-inflammatory, blood depurative, hypocholesterolemia, vasodilator	Oral dosage form: flower infusions or strained decoctions Topical fumigate: exposure to fumes from burned flowering stems	Recommended herbal doses and duration of use to be strictly applied. Long-term use not acceptable (Carvalho, 2010). Not reported (Dias, 2005; Gallego and Gallego, 2008; Neves et al., 2009).
Cytisus scoparius (L.) Link	Common broom	Giesta negral	Cholesterol. Diabetes. Gout and rheumatic disorders. Hypotension. Heart failure and tachycardia. Join and muscles pain. Poor liver function. Renal failure. Inflammations and diseases of the skin Anti-haemorrhagic, anti-inflammatory, cardiotonic, cholagogue, diuretic, hypocholesterolemia, vasopressor	Oral dosage form: flower infusion and maceration of flowers in wine. Topical application: poultices from flowers boiled in milk, applied while still hot	Considered poisonous (Salgueiro, 2004). Recommended caution with use frequency and dosage approach (Dias, 2005). Not reported (Cunha et al, 2008; Neves et al, 2009; González et al., 2010).
Cytisus striatus (Hill) Rothm.	Portuguese broom	Giesta amarela	Gout and rheumatic disorders. Hypotension. Heart failure. Join and muscles pain. Poor liver function Anti-inflammatory, cardiotonic, cholagogue, diuretic, depurative, vasopressor	Oral dosage form: flower infusion Topical application: flower decoction	Precaution recommended (Carvalho, 2010)
Pterospartum tridentatum (L.) Willk	unknown	Carqueja	Acute rheumatic pain. Cholesterol. Diabetes. Digestive and respiratory systems. Headache and migraine. Liver and biliary system. Nervous system. Urinary system. Flu. Skin inflammation and infection Anti-asthenia, anti-inflammatory, anxiolytic, carminative; depurative, diaphoretics, diuretic, laxative, stomachic, vasodilator, vulnerary	Oral dosage form: Flower infusions Topical application, baths, poultices: flower decoction,	Recommended herbal doses and applications to be strictly used. Long-term use not acceptable (Carvalho, 2010). Precaution recommended (Blanco and Diez, 2005). Not mentioned (Dias, 2005; Neves et al, 2009; González et al, 2010).

Table 2. Antioxidant properties (EC_{50} values) and phenolic compounds of the shrubby flowering plants submitted to different drying processes.

	Freeze-drying				Shade-drying			
	Cytisus multiflorus	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*	*Cytisus multiflorus*	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*
DPPH scavenging activity (mg/ml)	0.50 ± 0.02^{a}	0.39 ± 0.00^{b}	0.51 ± 0.04^{a}	0.15 ± 0.01^{c}	1.78 ± 0.01^{a}	1.06 ± 0.05^{b}	1.03 ± 0.02^{b}	0.76 ± 0.03^{c}
Reducing power (mg/ml)	0.41 ± 0.01^{a}	0.36 ± 0.00^{c}	0.38 ± 0.00^{b}	0.13 ± 0.00^{d}	0.44 ± 0.01^{b}	0.39 ± 0.00^{c}	0.63 ± 0.00^{a}	0.13 ± 0.00^{d}
β-carotene bleaching inhibition (mg/ml)	0.70 ± 0.05^{a}	0.28 ± 0.02^{c}	0.38 ± 0.08^{b}	0.14 ± 0.02^{d}	1.68 ± 0.02^{a}	0.47 ± 0.09^{c}	1.05 ± 0.12^{b}	0.13 ± 0.01^{d}
TBARS inhibition (mg/ml)	0.18 ± 0.04^{a}	0.13 ± 0.05^{a}	0.16 ± 0.01^{a}	0.12 ± 0.02^{a}	0.41 ± 0.01^{a}	0.34 ± 0.01^{ba}	0.42 ± 0.09^{a}	0.13 ± 0.04^{b}
Phenolics (mg ClAE/g extract)	313.87 ± 36.27^{c}	427.10 ± 60.55^{b}	389.04 ± 9.29^{b}	523.42 ± 36.09^{a}	198.88 ± 26.31^{c}	282.73 ± 25.14^{c}	232.92 ± 9.16^{b}	519.81 ± 40.24^{a}
Tartaric esters (mg CAE/g extract)	60.08 ± 7.26^{ba}	50.03 ± 7.76^{c}	70.45 ± 4.61^{a}	65.18 ± 7.07^{b}	48.76 ± 6.48^{b}	55.11 ± 5.48^{b}	41.54 ± 3.79^{b}	89.41 ± 8.97^{a}
Flavonols (mg QE/g extract)	72.64 ± 7.08^{b}	45.55 ± 7.36^{d}	86.98 ± 4.24^{a}	58.12 ± 5.78^{c}	67.84 ± 5.48^{ba}	47.35 ± 3.56^{c}	57.62 ± 2.27^{bc}	85.58 ± 5.60^{a}

In each row and for each drying process, different letters mean significant differences ($p<0.05$); n=9.

Table 3. Hydrophilic compounds (vitamin C and sugars) of the shrubby flowering plants submitted to different drying processes.

	Freeze-drying				Shade-drying			
	Cytisus multiflorus	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*	*Cytisus multiflorus*	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*
Vitamin C	0.05 ± 0.00^{d}	0.25 ± 0.01^{a}	0.13 ± 0.01^{b}	0.08 ± 0.01^{c}	0.04 ± 0.00^{b}	0.23 ± 0.00^{a}	0.05 ± 0.00^{b}	0.03 ± 0.00^{b}
Fructose	4.03 ± 0.27^{c}	10.04 ± 1.02^{a}	8.07 ± 0.35^{b}	3.49 ± 0.11^{c}	3.81 ± 0.51^{ba}	3.86 ± 0.90^{ba}	4.94 ± 0.51^{a}	2.58 ± 0.63^{b}
Glucose	4.86 ± 0.28^{b}	6.06 ± 1.06^{b}	9.36 ± 0.20^{a}	1.19 ± 0.05^{c}	1.45 ± 0.29^{ba}	1.89 ± 0.30^{a}	1.64 ± 0.15^{a}	0.84 ± 0.16^{b}
Sucrose	1.27 ± 0.23^{a}	1.27 ± 0.06^{a}	1.24 ± 0.25^{a}	0.58 ± 0.03^{b}	1.10 ± 0.34^{b}	3.45 ± 0.71^{a}	2.96 ± 0.29^{a}	1.54 ± 0.25^{b}
Trehalose	0.27 ± 0.09^{a}	0.35 ± 0.01^{a}	nd	nd	0.17 ± 0.04^{b}	0.27 ± 0.04^{a}	nd	nd
Total Sugars	10.43 ± 0.15^{b}	17.72 ± 2.15^{a}	18.67 ± 0.79^{a}	5.27 ± 0.09^{c}	6.53 ± 0.53^{ba}	9.47 ± 1.95^{a}	9.54 ± 0.95^{a}	4.96 ± 1.04^{b}

The results are expressed in g/100 g dw. In each row and for each drying process, different letters mean significant differences ($p<0.05$); n=9.

Table 4. Lipophilic compounds (tocopherols, carotenoids and chlorophylls) of the shrubby flowering plants submitted to different drying processes.

	Freeze-drying				Shade-drying			
	Cytisus multiflorus	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*	*Cytisus multiflorus*	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*
α-tocopherol	0.75 ± 0.04^{c}	10.71 ± 0.87^{b}	19.97 ± 1.20^{a}	9.13 ± 0.49^{b}	1.08 ± 0.01^{c}	11.65 ± 0.34^{a}	9.88 ± 0.64^{b}	0.70 ± 0.02^{c}
β-tocopherol	0.02 ± 0.01^{d}	0.21 ± 0.02^{c}	0.48 ± 0.04^{a}	0.14 ± 0.01^{d}	0.04 ± 0.01^{d}	0.37 ± 0.02^{a}	0.20 ± 0.02^{b}	0.13 ± 0.01^{c}
γ-tocopherol	0.26 ± 0.02^{c}	0.42 ± 0.04^{b}	2.12 ± 0.08^{a}	0.27 ± 0.02^{c}	0.35 ± 0.00^{b}	0.32 ± 0.03^{b}	0.76 ± 0.05^{a}	0.05 ± 0.01^{c}
δ-tocopherol	0.05 ± 0.01^{d}	0.32 ± 0.01^{b}	0.57 ± 0.02^{a}	0.10 ± 0.01^{c}	nd	0.12 ± 0.01^{a}	nd	nd
Total tocopherols	1.08 ± 0.07^{c}	11.66 ± 0.94^{b}	23.14 ± 1.29^{a}	9.64 ± 0.51^{b}	1.47 ± 0.01^{c}	12.46 ± 0.37^{a}	10.84 ± 0.70^{b}	0.88 ± 0.03^{c}
β-carotene	0.16 ± 0.03^{c}	1.56 ± 0.04^{b}	1.55 ± 0.09^{b}	2.05 ± 0.07^{a}	0.21 ± 0.00^{d}	1.82 ± 0.05^{b}	2.15 ± 0.04^{a}	0.92 ± 0.02^{c}
Lycopene	0.04 ± 0.00^{c}	0.38 ± 0.03^{a}	0.24 ± 0.03^{b}	nd	0.03 ± 0.00^{b}	0.16 ± 0.05^{a}	nd	nd
Chlorophyll a	0.65 ± 0.05^{d}	1.00 ± 0.04^{c}	1.09 ± 0.01^{b}	1.20 ± 0.12^{a}	0.37 ± 0.00^{c}	0.82 ± 0.04^{a}	0.65 ± 0.09^{b}	0.19 ± 0.02^{d}
Chlorophyll b	0.27 ± 0.01^{d}	0.48 ± 0.04^{b}	0.70 ± 0.10^{a}	0.38 ± 0.07^{c}	0.15 ± 0.01^{c}	0.35 ± 0.04^{a}	0.18 ± 0.02^{b}	0.03 ± 0.00^{d}

nd- not detected. The results are expressed in mg/100 g dw. In each row and for each different drying process, different letters mean significant differences (p<0.05); n=9.

Table 5. Fatty acids of the shrubby flowering plants submitted to different drying processes.

	Freeze-drying				Shade-drying			
	Cytisus Multiflorus	*Cytisus scoparius*	*Cytisus Striatus*	*Pterospartum tridentatum*	*Cytisus multiflorus*	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*
C6:0	0.66 ± 0.08	0.04 ± 0.00	0.06 ± 0.01	0.35 ± 0.06	0.20 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.25 ± 0.08
C8:0	0.08 ± 0.01	0.05 ± 0.00	0.09 ± 0.00	0.65 ± 0.13	0.06 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.13 ± 0.01
C10:0	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.28 ± 0.07	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.22 ± 0.00
C12:0	0.37 ± 0.01	0.72 ± 0.01	0.86 ± 0.03	3.08 ± 0.20	0.37 ± 0.02	0.97 ± 0.07	0.97 ± 0.01	2.83 ± 0.01
C13:0	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
C14:0	0.54 ± 0.04	2.24 ± 0.02	2.44 ± 0.09	2.30 ± 0.01	0.54 ± 0.03	2.55 ± 0.13	2.86 ± 0.00	2.56 ± 0.04
C14:1	0.15 ± 0.02	0.09 ± 0.01	0.10 ± 0.03	0.08 ± 0.01	0.13 ± 0.01	0.10 ± 0.00	0.25 ± 0.01	0.06 ± 0.00
C15:0	0.24 ± 0.01	0.09 ± 0.02	0.10 ± 0.01	0.19 ± 0.00	0.20 ± 0.01	0.07 ± 0.01	0.13 ± 0.01	0.22 ± 0.01
C16:0	17.09 ± 0.07	18.51 ± 0.10	22.53 ± 0.78	14.84 ± 0.83	13.01 ± 0.06	18.33 ± 0.22	20.15 ± 0.09	15.23 ± 0.22
C16:1	0.59 ± 0.13	0.12 ± 0.02	0.10 ± 0.01	0.34 ± 0.07	0.30 ± 0.02	0.04 ± 0.00	0.06 ± 0.01	0.51 ± 0.02
C17:0	0.56 ± 0.02	0.26 ± 0.00	0.29 ± 0.01	0.42 ± 0.02	0.54 ± 0.02	0.29 ± 0.01	0.35 ± 0.00	0.47 ± 0.03
C18:0	7.96 ± 0.82	4.19 ± 0.01	4.59 ± 0.05	3.97 ± 0.16	7.21 ± 0.12	4.89 ± 0.07	4.82 ± 0.03	5.71 ± 0.03
C18:1n9	7.96 ± 0.01	8.01 ± 0.05	4.40 ± 0.22	9.22 ± 1.09	7.26 ± 0.12	7.11 ± 0.01	4.46 ± 0.06	9.44 ± 0.17
C18:2n6	18.00 ± 0.03	12.82 ± 0.10	11.06 ± 0.08	19.59 ± 0.67	16.93 ± 0.32	10.49 ± 0.13	10.19 ± 0.25	18.00 ± 0.06
C18:3n6	nd	0.09 ± 0.00	0.04 ± 0.01	nd	nd	0.08 ± 0.01	0.08 ± 0.00	nd
C18:3n3	18.97 ± 0.43	41.40 ± 0.62	40.68 ± 0.08	29.50 ± 1.98	22.76 ± 0.33	39.79 ± 0.51	39.89 ± 0.64	27.98 ± 0.81
C20:0	5.56 ± 0.03	0.77 ± 0.03	1.69 ± 0.06	4.87 ± 0.03	5.74 ± 0.16	1.00 ± 0.01	1.91 ± 0.18	4.63 ± 0.23
C20:1	0.49 ± 0.04	0.27 ± 0.01	0.17 ± 0.00	0.34 ± 0.02	0.39 ± 0.02	0.29 ± 0.03	0.26 ± 0.01	0.46 ± 0.02
C20:2	0.11 ± 0.01	0.10 ± 0.00	0.11 ± 0.01	0.21 ± 0.07	0.33 ± 0.06	0.05 ± 0.01	0.02 ± 0.00	0.12 ± 0.03
C20:3n3+C21:0	0.45 ± 0.05	0.19 ± 0.00	0.22 ± 0.01	0.23 ± 0.03	0.54 ± 0.07	0.27 ± 0.04	0.35 ± 0.02	0.22 ± 0.02
C20:5n3	0.21 ± 0.04	0.05 ± 0.00	0.10 ± 0.01	nd	0.14 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	nd
C22:0	2.92 ± 0.07	1.26 ± 0.00	1.45 ± 0.04	2.26 ± 0.01	3.16 ± 0.02	1.45 ± 0.01	1.66 ± 0.05	2.82 ± 0.02
C23:0	14.35 ± 0.25	7.21 ± 0.51	7.35 ± 0.59	5.06 ± 0.50	16.56 ± 0.85	10.21 ± 0.18	9.83 ± 0.13	4.75 ± 0.17
C22:6n3	0.12 ± 0.02	0.04 ± 0.00	0.06 ± 0.01	0.11 ± 0.03	0.14 ± 0.05	0.03 ± 0.00	0.03 ± 0.01	0.86 ± 0.08
C24:0	2.53 ± 0.07	1.46 ± 0.07	1.49 ± 0.14	2.06 ± 0.09	3.42 ± 0.08	1.85 ± 0.04	1.58 ± 0.06	2.47 ± 0.24
Total SFA	52.95 ± 0.44^{a}	36.83 ± 0.80^{d}	42.96 ± 0.16^{b}	40.38 ± 1.54^{c}	51.08 ± 0.85^{a}	41.75 ± 0.64^{c}	44.39 ± 0.34^{b}	42.35 ± 0.89^{c}
Total MUFA	9.18 ± 0.12^{ba}	8.49 ± 0.09^{b}	4.78 ± 0.19^{c}	9.98 ± 1.18^{a}	8.08 ± 0.15^{b}	7.53 ± 0.04^{c}	5.03 ± 0.07^{d}	10.47 ± 0.17^{a}
Total PUFA	37.86 ± 0.56^{c}	54.69 ± 0.71^{a}	52.27 ± 0.03^{ba}	49.64 ± 2.72^{b}	40.84 ± 0.70^{c}	50.72 ± 0.69^{a}	50.58 ± 0.41^{a}	47.18 ± 0.72^{b}

PUFA/SFA	0.72 ± 0.02^{c}	1.49 ± 0.05^{a}	1.22 ± 0.00^{b}	1.23 ± 0.11^{b}	0.80 ± 0.03^{c}	1.22 ± 0.04^{a}	1.14 ± 0.02^{b}	1.11 ± 0.04^{b}
n-6/n-3	0.92 ± 0.02^{a}	0.31 ± 0.00^{c}	0.27 ± 0.00^{d}	0.66 ± 0.02^{b}	0.72 ± 0.00^{a}	0.26 ± 0.00^{c}	0.25 ± 0.01^{c}	0.62 ± 0.02^{b}

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3); Behenic acid (C22:0); Tricosanoic acid (C23:0) and Lignoceric acid (C24:0).
The results are expressed in percentage. In each row and for each drying process, different letters mean significant differences (p<0.05); n=9.

Table 6. Energetic value of the shrubby flowering plants submitted to different drying processes.

	Freeze-drying				Shade-drying			
	Cytisus multiflorus	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*	*Cytisus multiflorus*	*Cytisus scoparius*	*Cytisus striatus*	*Pterospartum tridentatum*
Moisture	73.33 ± 0.34	78.18 ± 0.65	79.85 ± 1.08	60.80 ± 0.16	74.69 ± 2.19	79.05 ± 0.50	80.15 ± 0.21	60.90 ± 0.17
Ash	4.57 ± 0.14^{b}	4.58 ± 0.20^{b}	5.12 ± 0.06^{a}	2.36 ± 0.00^{c}	4.42 ± 0.03^{a}	4.08 ± 0.00^{b}	4.36 ± 0.28^{ba}	2.72 ± 0.11^{c}
Proteins	17.36 ± 0.39^{c}	22.12 ± 0.11^{a}	21.04 ± 0.79^{b}	15.92 ± 0.60^{d}	21.31 ±0.58^{b}	22.80 ± 0.10^{a}	13.96 ± 0.09^{d}	18.99 ± 0.38^{c}
Fat	1.03 ± 0.14^{c}	4.17 ± 0.33^{a}	3.29 ± 0.57^{ba}	2.69 ± 0.51^{b}	1.29 ± 0.16^{b}	2.56 ± 0.21^{a}	2.79 ± 0.72^{a}	1.40 ± 0.28^{b}
Carbohydrates	77.04 ± 0.11^{b}	69.13 ± 0.09^{d}	70.55 ± 0.34^{c}	79.03 ± 0.74^{a}	72.98 ± 0.49^{c}	70.56 ± 0.10^{d}	78.89 ± 0.31^{a}	76.89 ± 0.53^{b}
Energy	386.85 ± 2.68 ^{c}	402.53 ± 1.71^{ba}	395.95 ± 2.19^{b}	404.01 ± 4.05^{a}	388.77 ± 0.63^{b}	396.46 ± 0.73^{a}	396.51 ± 3.35^{a}	396.10 ± 0.66^{a}

The results are expressed in g/100 g fw for moisture and g/100 g dw for all the other nutrients. In each row and for each drying process, different letters mean significant differences (p<0.05); n=9.

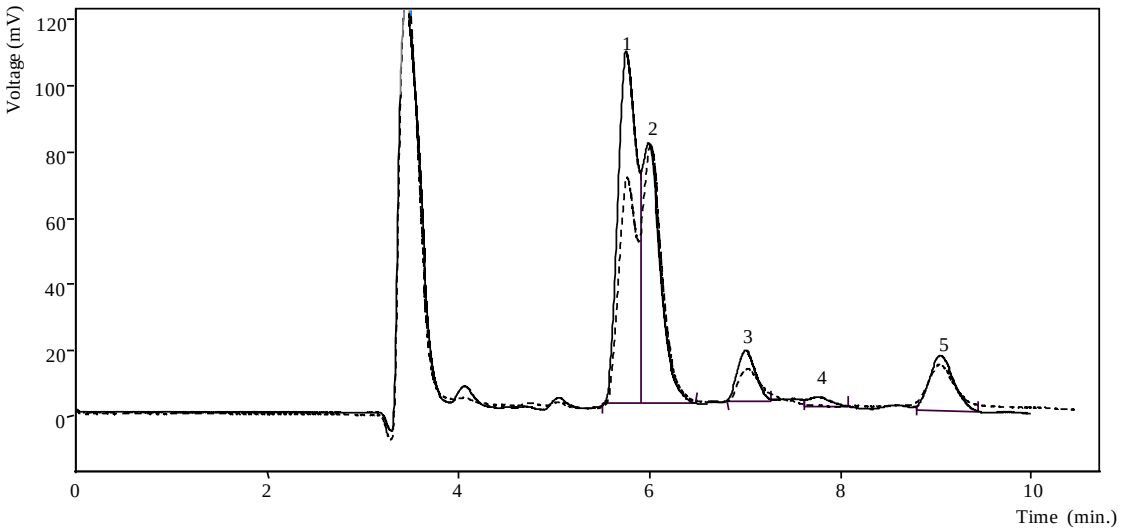

Figure 1. Sugars profile of *Cytisus multiflorus* freeze-drying (——) and *Cytisus multiflorus* shade-drying (- - - -). 1- Fructose; 2-glucose; 3-sucrose; 4- trehalose and 5-melezitose (IS).

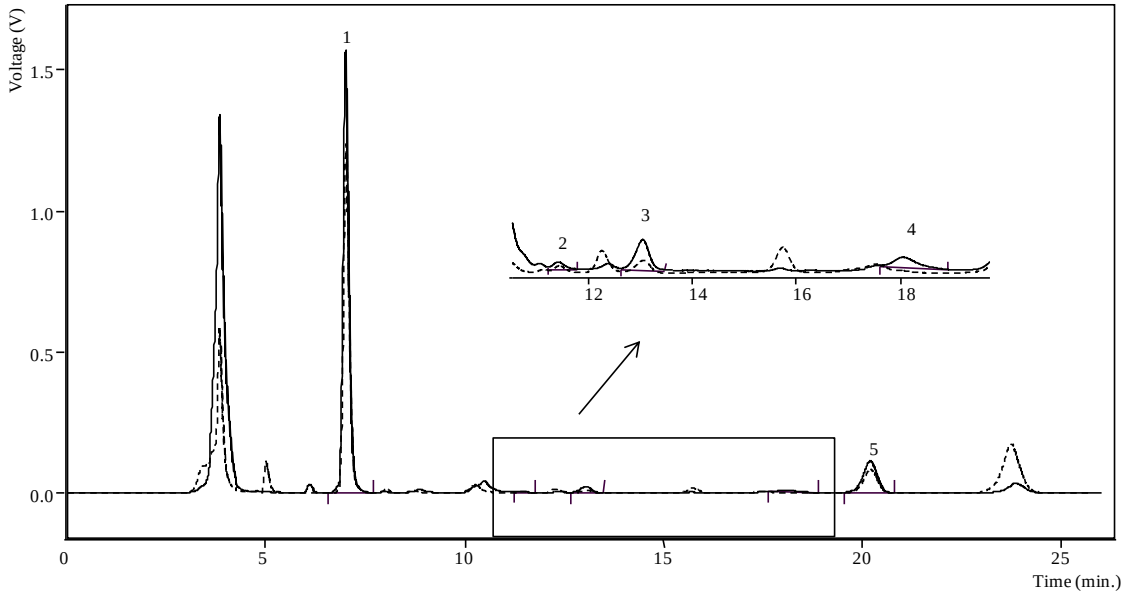

Figure 2. Tocopherols profile of *Cytisus striatus* freeze-drying (——) and *Cytisus striatus* shade-drying (- - - -) . 1- α-Tocopherol; 2-β-tocopherol; 3-γ- tocopherol; 4- δ-tocopherol and 5-tocol (IS).