Nutritional parameters of infusions and decoctions obtained from *Fragaria vesca* L. roots and vegetative parts

Maria Inês Dias[a,b,c], Lillian Barros[a], Patricia Morales[c], María Cortes Sánchez-Mata[c], M. Beatriz P.P. Oliveira[b], Isabel C.F.R. Ferreira[a,*]

[a]*Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5301-855 Bragança, Portugal.*

[b]*REQUIMTE, Science Chemical Department, Faculty of Pharmacy of University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.*

[c]*Dpto. Nutrición y Bromatología II, Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Pza Ramón y Cajal, s/n, E-28040 Madrid, Spain*

*Corresponding author. Tel.+351 273 303219; fax +351 273 325405. *E-mail address*: iferreira@ipb.pt (I.C.F.R. Ferreira)

Abstract

Fragaria vesca L. (wild strawberry) roots and vegetative parts are commonly used in infusions and decoctions for different medicinal purposes. The composition in non-nutrients (mainly phenolic compounds) has previously been reported, but the contribution in nutritional compounds has not been researched. Therefore, chemical parameters with nutritional role, namely macronutrients, mineral components, some vitamins (ascorbic acid, folate and tocopherols), as well as, fatty acids, soluble sugars and organic acids, present in *F. vesca* roots and vegetative parts were evaluated using commercial and wild samples. Furthermore, their infusions and decoctions were also fully characterized; as well as the percentages of vitamins and minerals released for the aqueous preparations. The processing steps, the collection region and also the physiological state in which the samples were collected could influence the differences found between commercial and wild samples. The infusion and decoction preparations showed significantly high released percentages of folate and minerals, and also allowed the detection of xylose, proving to be more effective for soluble sugars extraction. Roots and vegetative parts of *F. vesca*, normally consumed as infusions and decoctions, can be sources of macro and micronutrients.

Keywords: Wild strawberry; Wild/commercial samples; Macronutrients; Minerals; Vitamins

1. Introduction

Fragaria vesca L. (Rosaceae), commonly known as wild strawberry, grows spontaneously in low mountain zones such as forests, slopes and roadsides. It is spread across Europe, being also found in Korea, Japan, North America and Canada (Castroviejo et al., 1998). The leaves of wild strawberry have been traditional used in decoctions against hypertension, presenting also diuretic, antidiarrheal and anticoagulant activity. Decoctions and infusions prepared from the roots are also used to treat urinary tract infections, skin problems, haemorrhoids and cough symptoms (Pawlaczyk, Czerchawski, Pilecki, Lamer-Zarawska & Gancarz, 2009; Camejo-Rodrigues, Ascensão, Bonet & Vallès, 2003; Özüdogru, Akaydın, Erika & Yesila, 2011; Savo, Giulia, Maria & David, 2011). Furthermore, the consumption of roots and vegetative parts (leaves and stems) of *F. vesca* is also believed to increase haematopoiesis, and to have some anti-dysenteric, tonic, antiseptic and detoxifying properties (Neves, Matos, Moutinho, Queiroz & Gomez, 2009; Sõukand & Kalle, 2013).

F. vesca roots and vegetative parts have been reported as sources of non-nutrient compounds, such as procyanidins, ellagic acid and hydroxycinnamic derivatives (Simirgiotis & Schmeda-Hirschmann, 2010; Dias et al., 2014). Nevertheless, to the author's knowledge, there are no reports on nutrients composition of the mentioned parts of *F. vesca,* as well as, their infusions and decoctions. Only the fruits were studied regarding sugars and organic acids (Doumett et al., 2011; Ornelas-Paz et al., 2013), as also the fruits of the hybrid *Fragaria* x *ananassa* Duch. (Hakala, Lapvetelainen, Huopalahti, Kallio & Tahvonen, 2003; Ekholm et al., 2007) concerning minerals content.

A balanced diet containing micronutrients such as vitamins, namely ascorbic acid, folate and tocopherols, and antioxidant compounds is an increasingly central issue for the maintenance of human health and against certain pathologies, such as hypertension and cardiovascular diseases (Houston, 2005). Mineral elements have a very important role in the human health, regarding their physiological functions and requirements. From a nutritional point of view, mineral elements have been classified into two main groups: macroelements, which are needed in higher amounts for physiological function (*e.g.*, potassium, sodium, calcium, magnesium or phosphor), and microelements, in which most of them may be essential to maintain the body functions (*e.g.*, iron, zinc or manganese) (Mahan et al., 2013; Özcan, 2004; Leśniewicz et al., 2006).

The present work intends to improve the knowledge on chemical parameters with nutritional role of *F. vesca* roots and vegetative parts, which have been scarcely studied. Commercial and wild samples were used to prepare infusions and decoctions in order to compare their chemical and nutritional composition with the initial plant matrix, and to determine the percentages of vitamins and minerals released from them to the aqueous preparations (infusions and decoctions).

2. Materials and methods

2.1. Standards and Reagents

Acetonitrile (99.9%), n-hexane (95%) and ethyl acetate (99.8%) were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as well as other individual Fatty Acid Methyl Ester isomers, L-ascorbic acid, tocopherol, sugar, organic acid standards, nitric acid and hydrochloric acid. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA). Micro

(Fe, Cu, Mn and Zn) and macroelements (Ca, Mg, Na and K) standards (> 99% purity), as well $LaCl_2$ and CsCl (> 99% purity) were purchased from Merck (Darmstadt, Germany). Standars of 5-CH_3-H_4folate monoglutamate (ref. 16252; Schircks laboratories, Jona, Switzerland) and pteroyl diglutamic acid (ref. 16235; Schircks laboratories, Jona, Switzerland), pancreatic chicken homogenate (Pel Freeze, Rogers, Arkansas), rat serum, $NaBH_4$, formaldehyde and octanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile fluorescence grade was bought from Fisher Scientific (Madrid, Spain). All other general laboratory reagents were purchased from Panreac Química S.L.U. (Barcelona, Spain).

2.2. Samples and preparation of infusions and decoctions

The commercial samples of *Fragaria vesca* L. vegetative parts and roots were purchased separately in a local supermarket. The wild samples were collected in Serra da Nogueira, Bragança, North-eastern Portugal, in July 2013, and transported to the laboratory in paper bags properly identified. Voucher specimens of the wild samples are deposited in the School of Agriculture Herbarium (BRESA). The vegetative parts and roots were then separated. All the samples were freeze-dried immediately after collection (FreeZone 4.5, Labconco, Kansas, MO, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenate samples.

For infusions preparations, each sample (1 g) was added to 200 mL of boiling distilled water (pH 6.6) at 100ºC and left to stand at room temperature for 5 min; then filtered under reduced pressure (0.22μm). For decoction preparation, each sample (1 g) was added to 200 mL of distilled water (pH 6.6), heated (heating plate, VELP scientific, Keyland Court, NY, USA) and boiled for 5 min at 100^{o}C, in a closed recipient to prevent evaporation. The mixture was left to stand for 5 min and then filtered under

reduced pressure (0.22μm). The obtained infusions and decoctions were frozen at -20°C and freeze-dried.

2.3. Proximate composition

The samples were analyzed for proteins, fat, carbohydrates and ash according to the AOAC procedures (AOAC, 2005). The crude protein content (N×6.25) 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 550±15°C. Total carbohydrates were calculated by difference.

2.4. Minerals composition

Mineral elements analysis was performed according to the method 930.05 of AOAC procedures and following the methodology previously described by the authors (Fernández-Ruiz, Olives, Cámara, Sánchez-Mata & Torija, 2011; Ruiz-Rodríguez et al., 2011). Mineral element analysis was performed on freeze-dried samples. After dry-ash mineralization at 450°C the minerals were extracted in an acid mixture (2 mL HCl 0.5 mL/mL+2 mL HNO_3 0.5 mL/mL) and made up to 50 mL of distilled water. For Ca and Mg determination, a dilution with La_2O_3 (58.6 mg/L acidified deionized water) was performed in order to avoid interferences. All measurements were performed in atomic absorption spectroscopy (AAS) with air/acetylene flame in Analyst 200 Perkin Elmer equipment (Perkin Elmer, Waltham, MA, USA), comparing absorbance responses with > 99.9% purity analytical standard solutions for AAS made with $Fe(NO_3)_3$, $Cu(NO_3)_2$, Mn $(NO_3)_2$, Zn $(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg band. The released percentage of

minerals to infusion and decoction preparations was calculated considering the amount of minerals found in the dry samples as 100%.

2.5. Soluble sugars

Soluble sugars were determined by high performance liquid chromatography system consisting of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco, Easton, MD, USA), coupled to a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany), as previously described by the authors (Pereira et al., 2014). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (5 mm, 250 mm × 4.6 mm i.d., Knauer) operating at 35 ºC (7971 R Grace oven). The mobile phase was acetonitrile (700 mL/L)/deionized water (300 mL/L), at a flow rate of 1 mL/min. The identification was carried out by chromatographic comparisons of the relative retention times of sample peaks with authentic standards, while the quantification was performed using the internal standard (melezitose) method and by using calibration curves obtained from the commercial standards of each compounds.

The results were expressed in g per 100 g of dry weight for dry plants and in mg per 100 mL for infusion and decoction preparations.

2.6. Fatty acids

Fatty acids were determined, after a trans-esterification process as previously described by the authors (Pereira et al., 2014). The fatty acid profile was analysed using a gas-liquid chromatographer (DANI model GC 1000 instrument, Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detection (GC-FID, 260

ºC) and a Macherey–Nagel (Düren, Germany) column (0.5 g/kg cyanopropyl-methyl-0.5 g/kg phenylmethylpolysiloxane, 30 m × 0.32 mm i.d. × 0.25 µm df). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/ min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (61 kPa), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC). The identification was made by comparing the relative retention times of FAME (Fatty Acid Methyl Esters) peaks of the samples with commercial standards. The results were recorded and processed using Clarity 4.0.1.7 Software (DataApex, Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

2.7. Vitamin C (ascorbic acid) and organic acids

Vitamin C and other organic acids were determined by ultra-fast liquid chromatography coupled to photodiode array detection (UFLC-PDA; Shimadzu Coperation, Kyoto, Japan) and following a procedure previously described by the authors (Pereira et al., 2014). Separation was achieved on a SphereClone (Phenomenex) reverse phase C_{18} column (5 mm, 250 mm × 4.6 mm i.d) thermostatted at 35 ºC. The elution was performed with sulphuric acid 3.6 mmol/L using a flow rate of 0.8 mL/min. The quantification was performed by comparison of the area of the peaks recorded at 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths with calibration curves obtained from commercial standards of each compound: oxalic acid (y=9x10^6x + 377946, R^2=0.994); quinic acid (y =612327x + 16563, R^2=1); malic acid (y =863548x + 55571, R^2=0.999); ascorbic acid (y =10^8x + 751815, R^2=0.998); shikimic acid (y =9x10^7x - 95244, R^2=0.999); citric acid (y =10^6x + 16276, R^2=1); fumaric acid (y

=148083x + 96092, R^2=1). The results were expressed in g per 100 g of dry weight for dry plants and in mg per 100 mL for infusion and decoction preparations.

2.8. Folate and tocopherols

Folate content was determined according to the methodology previously described by Morales et al., 2014, using HPLC-FL system, consisted of a Beta 10 (Ecom, Prague, Czech Republic) gradient pump with Gastorr Degasser HPLC Four Channel BR-14 (Triad Scientific, New Jersey, USA) as degassing device, joined to an AS-1555 automatic injector (Jasco, Easton, MD, USA), and to a FP-2020 Plus Fluorescence detector (Jasco, Easton, MD, USA) with RP 18 endcapped Lichrospher 100 column (Merck, Darmstadt, Germany; 250 × 5 mm; 5 μm). The quantification results were obtained from the comparison of the area of the recorded peaks with calibration curves obtained from commercial standards (5-CH_3-H_4folate in both mono and diglutamate forms), and expressed as total folate (from the sum of both compounds). The results were expressed in μg per 100 g of dry weight for dry plants and in μg per 100 mL for infusion and decoction preparations. The released percentage of folate to infusion and decoction preparations was calculated considering the amount of folate found in the dry samples as 100%.

The four isoforms of tocopherols were determined following a procedure previously described by the authors (Pereira et al., 2014), using HPLC coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (5 mm, 250 mm × 4.6 mm i.d., YMC Waters), operating at 35 °C. 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 identification was performed by chromatographic

comparisons with authentic standards, while the quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight for dry plants and in mg per 100 mL for infusion and decoction preparations.

2.9. Statistical analysis

In each assay, three samples were used and all the analyses were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). Results 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. 22.0 program (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Chemical characterization of F. vesca roots and vegetative parts

Results regarding chemical characterization of roots and vegetative parts of *F. vesca* commercial and wild samples are described in **Table 1**. The commercial vegetative parts revealed the highest contents in proteins and fat, while the corresponding wild samples gave the highest ash content.

Regarding minerals composition, the wild roots and vegetative parts gave very high amount of iron and manganese microelements, while commercial vegetative parts and wild roots gave the highest amount of copper and zinc, respectively. In terms of macroelements, the highest levels of calcium and magnesium were found in wild vegetative parts, while the highest potassium concentration was observed in commercial vegetative parts.

The soluble sugars detected in the four studied samples presented some similarities; raffinose was only detected in wild vegetative parts (**Table 1**). The highest total soluble sugars content was observed in wild roots sample (20.66 g/100 g), mainly due to the presence of sucrose (13.53 g/100 g), which was also present in high concentration in the wild vegetative parts of *F. vesca* (1.76 g/100 g). Commercial roots and vegetative parts samples presented fructose and glucose as the major ones, followed by sucrose and trehalose.

Regarding fatty acids profile, 22 compounds were identified; the most abundant ones in the four studied samples are presented in **Table 1**. Linoleic acid (C18:2n6) was the major fatty acid found in commercial and wild roots samples (30.97 and 45.16%, respectively) followed by palmitic acid (C16:0; 26.93 and 15.82%, respectively). Contrarily, in commercial vegetative parts, palmitic acid (C16:0) was the major acid, while linoleic acid (C18:3n3) was the most abundant in wild vegetative parts. Eicosapentaenoic acid (C20:5n3) was not detected in root samples. The highest concentration of polyunsaturated fatty acids (PUFA; 60.91%) was observed in the wild roots sample. Saturated fatty acids (SFA) are also present in high concentrations followed by monounsaturated fatty acids (MUFA) in all samples.

Folate was found in higher amounts in wild roots sample (253.3 μg/100 g), followed by commercial roots and wild and commercial vegetative parts. Regarding tocopherols content, the wild roots sample also presented the highest concentration mainly due to α-tocopherol (65 mg/100 g). Commercial roots and vegetative parts samples showed only the presence of α- and γ-tocopherols. Both vitamins are highly degradable molecules and, therefore, these results can be explained by the less processing steps to which wild samples were submitted: freeze drying immediately after collection, which preserves ascorbic acid by means of freezing temperatures and oxygen absence (Davey et al.,

2000); some authors proved the effects of blanching in the stability of folate describing a decrease of half of the folate content in vegetables (Puupponen-Pimia et al., 2003); as also the effects of temperature on tocopherols content in vegetables, reporting that cooking and baking process lead to a decrease in tocopherols availability (Knecht et al., 2015). Only trace amounts of ascorbic acid were found in the wild root sample, being not detected in commercial samples, which could be explained by the degradation caused by processing steps (Allwood & Martin, 2000).

The organic acids profile varied depending on the plant material analysed; these compounds are normally found in higher amounts in aerial parts, where their biosynthesis is increased. Furthermore, its content is highly influenced by the environmental conditions (López-Bucio et al., 2003). As expected, organic acids profile was very different between samples, due to the different plant material analysed. Vegetative parts revealed the presence of more organic acids, revealing commercial sample the highest amount (5.48 g/100 g). Wild roots presented only oxalic acid, while commercial roots gave malic acid as the major organic acid.

3.2. Chemical and nutritional characterization of infusions and decoctions prepared from F. vesca roots and vegetative parts

The results of chemical and nutritional characterization in infusions and decoctions prepared from roots of *F. vesca* commercial and wild samples are provided in **Table 2**. In general, micro and macroelements were found in higher amounts in the infusions. Iron and zinc were more abundant in commercial roots infusion (0.04 and 0.08 mg/100 mL), while cooper and manganese predominated in wild roots infusion (0.03 and 0.06 mg/100 mL); copper was not detected in wild roots decoction sample. Calcium,

magnesium and potassium were found in commercial roots infusion in the highest concentrations (5.82, 3.48 and 4.12 mg/100 mL, respectively).

The soluble sugars profile is very similar among all the samples; the highest sum was found in commercial roots infusions and decoctions samples (35.97 and 36.51 mg/100 mL, respectively), mainly due to the presence of high concentrations of glucose and fructose. For wild roots samples, the decoction presented the highest level of sugars (14.26 mg/100 mL), being also found xylose.

Folate content was higher in wild roots decoction and infusion sample (26.37 and 28.06 µg/100 mL, respectively), while α-tocopherol was the only isoform of tocopherols identified in all the analysed samples, presenting commercial roots infusion the highest amount (0.32 µg/100 mL). The level of ascorbic acid present in the plant samples was very low (traces amounts), which might explain the fact of not being detected in the infusions and decoctions. Besides, it is known that this compound decreases with increasing temperature (Lester, 2006).

Organic acids were also present in higher amounts in the commercial roots samples, mainly due to the contribution of malic acid (infusion and decoction, 5.37 and 4.93 mg/100 mL, respectively). The profiles were very different in the studied samples; oxalic and malic acids were only identified in the wild samples infusion and decoction, being the last one presented in traces amount; this was also observed in the wild root sample, while commercial roots, commercial vegetative parts and wild vegetative parts samples presented it as the second major compound.

Regarding *F. vesca* commercial and wild vegetative part samples (**Table 3**), the infusion of commercial vegetative parts presented the highest levels of macro and microelements; copper was not detected in the wild samples infusion and decoction.

Similarly to root samples, it is in the commercial vegetative parts samples (infusion and decoction) that sugars and organic acids were found in the highest amounts. In the case of sugars, fructose and glucose were once more found in the highest concentrations in commercial vegetative part infusions and decoctions (40.44 and 39.86 mg/100 mL, respectively); xylose was also found in the infusions and decoctions of vegetative parts. The presence of xylose (free sugar) in the aqueous extracts can be explained by a higher extractability capacity in the process of infusions and decoctions preparation.

In terms of organic acids, the highest amounts were found in commercial vegetative parts infusions and decoctions (58.79 and 68.0 mg/100 mL, respectively), mainly due to citric acid, which is in accordance with the content found in the vegetative parts (**Table 1**); shikimic and fumaric acids were not detected in the wild samples, while fumaric acid was only detected in traces amount in commercial samples. In the decoctions of wild vegetative parts, higher amounts of folate (13.99 μg/100 mL) and α-tocopherol (0.33 μg/100 mL) were found; different results were obtained for root samples.

As mentioned before, some highly thermal sensible vitamins, as folate and tocopherols (Puupponen-Pimia et al., 2003; Knecht et al., 2015), were characterized in decoctions and infusions of *F. vesca* samples. Furthermore, their release percentage from plant matrix was calculated and showed in **Figure 1A**. The highest folate release percentage was found in commercial vegetative part infusions and decoctions (13.59% and 16.82%, respectively) and in wild vegetative part decoctions (12.22%). Moreover, after thermal treatment the release percentage of tocopherols was also higher in the infusions than in decoctions but in all cases, lower than 2% (data not shown), mainly due to the lipophilic character of vitamin E.

Infusion and decoction minerals release percentage was calculated, being illustrated in **Figure 1B**. The vegetative parts of *F. vesca* provided higher deliver percentages of

micro and macroelements to the infusions and decoctions. Copper (with the exception to wild vegetative parts infusions and decoctions, in which copper was not detected) and magnesium represented the micro and macroelements with the highest released percentages for the infusions and decoctions. The maximal released percentage for copper observed in commercial vegetative parts decoction sample (~69%), while for magnesium was observed in wild vegetative parts infusion (~91%). The commercial vegetative parts infusion sample presented also the highest released percentage for iron (~46%), manganese (~41%), zinc (~29%) and calcium (40%). Otherwise, potassium reached the maximal released percentage in wild roots decoction sample (~36%).

In general, the amount of each nutrient found in the infusion or decoction liquid, would be the result of the balance between extraction rate, and non-diffusion to water. Both are expected to be higher in decoctions, where boiling temperatures are maintained during 5 min, with respect to infusions where temperature decreases during this time. As a result, lipophilic compounds (such as tocopherols) are not expected to be extracted in a high extent into the liquid (aqueous environment) being also highly prone to thermal degradation; hydrophilic substances would behave in a different way depending on their thermal stability: mineral elements, highly stable, are in many cases more extracted into decoction liquids (higher exposition time at boiling temperature), while folate could suffer some degradation in these conditions.

Iron, manganese, zinc and calcium also showed lower released percentages when compared to the results obtained for our samples. Herbal infusion mixtures containing several plants were also studied for their content in macro and microelements in comparison with the dry plant; the authors obtained good results in the amount of minerals that are released to the infusion, however, unlike the herein observed, Mn was the more soluble component. In the present study, Cu, Zn and Na were the elements

released in the highest amounts to the infusions (Aldars-García, Zapata-Revilla & Tenorio-Sanz, 2013). Łozak, Sołtyk, Ostapczuk & Fijałek (2002) also studied the percentage of released minerals from plant to infusions of *Menthae piperitae folium*. (mint) and *Urticae folium* (nettle), describing much lower values for Mg (38 and 25% for mint and nettle, respectively) and Cu (25 and 33% for mint and nettle, respectively) in comparison with the herein studied sample commercial vegetative parts decoction.

Overall, fruits are the most commonly studied part of *F. vesca*. However, and despite the various ethnobotanical uses reported for vegetative parts and roots, their nutritional characterization has been discarded. The present study proved that *F. vesca* roots and vegetative parts (either commercial or wild samples) are sources of nutrients and molecules with high physiological and nutritional importance, such as tocopherols (α-tocopherol), folate, mineral elements, soluble sugars and organic acids. Moreover, according to the regulation of the European Parliament the reference daily intake (RDA) of folate is 200 μg/day (Regulation (EC) No 1169/2011), and some of the studied samples (wild roots) presented a release of folate to infusions and decoctions higher than 14% towards providing this RDA.

Even though some nutrients losses were observed during infusions and decoctions preparation, the release percentages of folate and minerals in the aqueous extracts are significantly high. Tocopherols almost disappear after infusion and decoction elaboration, which was expectable due to their lipophilic properties and its low thermal stability. Infusion and decoction preparations proved to be also effective for soluble sugars extraction allowing the detection of xylose.

The qualitative differences found in some chemical profiles of commercial and wild samples can be explained by several factors such as the processing steps, the collection region, as also the physiological state of the samples (Tiwari & Cummins, 2013).

The present work shows the huge potential of roots and vegetative parts of *F. vesca*, normally consumed as infusions and decoctions, in order to provide different macro and micronutrients.

Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT, Portugal) for financial support to CIMO (strategic project PEst-OE/AGR/UI0690/2011), REQUIMTE (PEst-C/EQB/LA0006/2011) and ALIMNOVA research group (UCM-GR35/10A). M.I. Dias thanks to FCT for the grant (SFRH/BD/84485/2012) and L. Barros contract under "Programa Compromisso com a Ciência-2008".

References

Aldars-García, L., Zapata-Revilla, M.A., & Tenorio-Sanz, M.D. (2013). Characterization and study of the essential mineral components of Spanish commercial herbal products and their infusions. *Journal of Food and Nutrition Research*, *52*, 172-180.

Allwood, M.C., & Martin, H.J. (2000). The photodegradation of vitamins A and E in parenteral nutrition mixtures during infusion. *Clinical Nutrition*, 19, 339-342.

AOAC. (2005). Official methods of analysis of AOAC international. In W. Horwitz, & G. Latimer (Eds.) (18th ed.). Gaithersburg, MD: AOAC International.

Camejo-Rodrigues, J., Ascensão, L., Bonet, M. À., & Vallès, J. (2003). An ethnobotanical study of medicinal and aromatic plants in the Natural Park of "Serra de São Mamede" (Portugal). *Journal of Ethnopharmacology*, *89*, 199-209.

Castroviejo, S., Aedo, C., Cirujano, S., Laínz, M., Montserrat, P., Morales, R., Muñoz Garmendia, F., Navarro, C., Paiva, J. & Soriano, C. (eds.). (1998). Flora Ibérica 6. Real Jardín Botánico, CSIC, Madrid.

Davey, M.W., Montagu, M.V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., & Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, *80*, 825-860.

Dias, M.I., Barros. L., Oliveira, M.B.P.P., Santos-Buelga, C., & Ferreira, I.C.F.R. (2014). Phenolic profile and antioxidant properties of commercial and wild *Fragaria* vesca L. roots: A comparison between hydromethanolic and aqueous extracts. *Industrial Crops and Products*, *63*, 125-132.

Doumett, S,. Fibbi, D., Cincinelli, A., Giordani, E., Nin, S., & Bubba M.D. (2011). Comparison of nutritional and nutraceutical properties in cultivated fruits of *Fragaria vesca* L. produced in Italy. *Food Research International*, *44*, 1209-1216.

Ekholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellström, J., & Ovaskainen, M. (2007). Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *Journal of Food Composition and Analysis*, *20*, 487-495.

Fernández-Ruiz, V., Olives, A.I., Cámara, M., Sánchez-Mata, M.C., & Torija, E. (2011). Mineral and trace elements content in 30 accessions of tomato fruits (*Solanum lycopersicum* L.,) and wild relatives (*Solanum pimpinellifolium* L.,

Solanum cheesmaniae L. Riley, and *Solanum habrochaites* S. Knapp & D.M. Spooner). *Biological Trace Element Research*, *141*, 329-339.

Hakala, M., Lapvetelainen, A., Huopalahti, R., Kallio, H., & Tahvonen, R. (2003). Effects of varieties and cultivation conditions on the composition of strawberries. *Journal of Food Composition and Analysis*, *16*, 67-80.

Houston, M.C. (2005). Nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. *Progress in Cardiovascular Diseases*, *47*, 396-449.

Knecht, K., Sandfuchs. K., Kulling, S.E., & Bunzel, D. (2015) Tocopherol and tocotrienol analysis in raw and cooked vegetables: A validated method with emphasis on sample preparation. *Food Chemistry*, *169*, 20-27.

López-Bucio, J., Cruz-Ramírez, A., & Herrera-Estrella, L. (2003) The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology*, *6*, 280-287.

Leśniewicz, A., Jaworska, K., & Żyrnicki W. (2006). Macro- and micro-nutrients and their bioavailability in polish herbal medicaments. *Food Chemistry*, *99*, 670-679.

Lester, G.E. (2006). Environmental regulation of human health nutrients (ascorbic acid, β-carotene, and folic acid) in fruits and vegetables. *HortScience*, *41*, 59-64.

Łozak, A., Sołtyk, K., Ostapczuk, P., & Fijałek, Z. (2002) Determination of selected trace elements in herbs and their infusions, Science of The Total Environment, *289*, 33-40.

Mahan & Escott-Stump (2013). Krause's Food & the Nutrition Care Process, 13ed. Elsevier, USA.

Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.C., Cámara, M., & Tardío, J. (2014). Optimization and application of FL-HPLC for folates analysis in 20 species of mediterranean Wild vegetables. *Food Analytical Methods*, *8*, 302-311.

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

Ornelas-Paz, J.J., Yahia, E.M., Ramírez-Bustamante, N., Pérez-Martínez, J.D., Escalante-Minakata, M.P., Ibarra-Junquera, V., Acosta-Muñiz, C., Guerrero-Prieto, V., & Ochoa-Reyes, E. (2013). Physical attributes and chemical composition of organic strawberry fruit (*Fragaria x ananassa* Duch, *Cv*. Albion) at six stages of ripening. *Food Chemistry*, *138*, 372-381.

Özcan, M. (2004). Mineral contents of some plants used as condiments in Turkey. *Food Chemistry*, *84*, 437-440.

Özüdogru, B., Akaydın, G., Erika, S., & Yesila, S. (2011). Inferences from an ethnobotanical field expedition in the selected locations of Sivas and Yozgat provinces (Turkey). *Journal of Ethnopharmacology*, *137,* 85-98.

Pawlaczyk, I., Czerchawski, L., Pilecki, W., Lamer-Zarawska, E., & Gancarz, R. (2009). Polyphenolic-polysaccharide compounds from selected medicinal plants of Asteraceae and Rosaceae families: Chemical characterization and blood anticoagulant activity. *Carbohydrate Polymers*, *77*, 568-575.

Pereira, E., Barros, L., Calhelha, R.C., Dueñas, M., Carvalho, A.M., Santos-Buelga, C., & Ferreira, I.C.F.R. (2014). Bioactivity and phytochemical characterization of Arenaria montana L.. *Food & Function*, *5*, 1848 -55.

Puupponen-Pimia, R., Hakkinen, S.T., Aarni, M., Suortti, T., Lampi, A., Eurola, M., Piironen, V., Nuutila A.M., & Oksman-Caldentey, K. (2003) Blanching and long-

term freezing affect various bioactive compounds of vegetables in different ways. *Journal of the Science of Food and Agriculture*, *83*, 1389-1402.

EC. (2011). Regulation (EC) No 1169/2011 of the European Parliament and of the Council, of 25 October 2011, on the provision of food information to consumers. *Official Journal of the European Union*. *L304*, 18- 63.

Ruiz-Rodríguez, B., Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.C.; Cámara, M., Díez-Marqués, C., Santayana, M.P., Molina, M., & Tardío, J. (2011). Valorization of wild strawberry tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. *Food Research International*, *44*, 1244-1253.

Savo, V., Giulia, C., Maria, G.P., & David, R. (2011). Folk phytotherapy of the Amalfi Coast (Campania, Southern Italy). *Journal of Ethnopharmacology*, *135*, 376-392.

Simirgiotis, M.J., & Schmeda-Hirschmann, G. (2010). Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. *chiloensis* form *chiloensis*) using HPLC-DAD–ESI-MS and free radical quenching techniques. *Journal of Food Composition and Analysis*, *23*, 545-553.

Sõukand, R., & Kalle R. (2013). Where does the border lie: Locally grown plants used for making tea for recreation and/or healing, 1970s–1990s Estonia. *Journal of Ethnopharmacology, 150,* 162-174.

Tiwari, U., & Cummins, E. (2013). Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. *Food Research International*, *50*, 497-506

Table 1. Nutritional value, minerals, soluble sugars, fatty acids, vitamins and organic acids in roots and vegetative parts of *Fragaria vesca* L. commercial and wild samples (mean ± SD; results expressed on dry weight basis).

	Roots		Vegetative parts	
	Commercial	Wild	Commercial	Wild
Nutritional value				
Fat (g/100 g)	1.62 ± 0.01[c]	1.1 ± 0.1[d]	2.87 ± 0.05[a]	2.2 ± 0.1[b]
Proteins (g/100 g)	3.91 ± 0.01[b]	4.02 ± 0.02[b]	6.4 ± 0.5[a]	2.21 ± 0.08[c]
Ash (g/100 g)	5.85 ± 0.04[d]	6.50 ± 0.04[c]	7.5 ± 0.2[b]	8.21 ± 0.04[a]
Carbohydrates (g/100 g)	88.63 ± 0.05[a]	88.4 ± 0.2[a]	83.2 ± 0.4[c]	87.33 ± 0.08[b]
Microelements (mg/100 g)				
Fe	5.2 ± 0.3[c]	57 ± 6[a]	3.8 ± 0.3[c]	45.3 ± 0.3[b]
Cu	0.38 ± 0.05[d]	0.99 ± 0.06[b]	1.12 ± 0.02[a]	0.44 ± 0.04[c]
Mn	0.53 ± 0.04[d]	14.0 ± 0.8[b]	7.4 ± 0.8[c]	18.3 ± 0.8[a]
Zn	14 ± 1[a]	8.4 ± 0.3[b]	4.2 ± 0.3[c]	3.3 ± 0.1[d]
Macroelements (mg/100 g)				
Ca	816 ± 27[c]	929 ± 85[b]	883 ± 21[b]	1272 ± 36[a]
Mg	224 ± 3[b]	170 ± 5[d]	230 ± 3[c]	235.9 ± 0.7[a]
K	965 ± 17[b]	192 ± 8[d]	1700 ± 28[a]	674 ± 13[c]
Soluble sugars (g/100 g)				
Fructose	4.2 ± 0.3[a]	2.08 ± 0.06[b]	1.7 ± 0.2[c]	1.63 ± 0.04[c]
Glucose	4.0 ± 0.2[a]	2.44 ± 0.03[c]	3.76 ± 0.08[b]	1.71 ± 0.09[d]
Sucrose	0.20 ± 0.02[d]	13.5 ± 0.1[a]	0.40 ± 0.01[c]	1.76 ± 0.08[b]
Trehalose	0.23 ± 0.01[d]	2.62 ± 0.08[a]	0.5 ± 0.1[c]	0.69 ± 0.02[b]
Raffinose	nd	nd	nd	0.29 ± 0.03
Sum	8.7 ± 0.5[b]	20.66 ± 0.06[a]	6.4 ± 0.2[c]	6.08 ± 0.03[c]
Fatty acids (relative percentage)				
C16:0	26.9 ± 0.4	15.8 ± 0.2	21.6 ± 0.8	16 ± 2
C18:0	8.91 ± 0.04	3.9 ± 0.1	6.41 ± 0.04	5.3 ± 0.6
C18:1n9	10.5 ± 0.1	7.9 ± 0.2	8.0 ± 0.4	5.1 ± 0.3
C18:2n6	31.0 ± 0.1	45.2 ± 0.2	18.06 ± 0.04	7.8 ± 0.2
C18:3n3	11.4 ± 0.5	15.32 ± 0.08	21.4 ± 0.3	24.8 ± 0.7
C20:0	2.33 ± 0.01	2.5 ± 0.2	3.7 ± 0.3	7.7 ± 0.6
C20:5n3	nd	nd	3.4 ± 0.9	8 ± 2
C22:0	2.01 ± 0.03	2.8 ± 0.2	4.4 ± 0.5	9 ± 1
C24:0	1.35 ± 0.03	2.69 ± 0.04	3.6 ± 0.5	8 ± 1
SFA	45.9 ± 0.6[b]	30.7 ± 0.4[c]	45.6 ± 0.2[b]	53 ± 3[a]
MUFA	11.38 ± 0.03[a]	8.3 ± 0.1[c]	10.5 ± 0.5[b]	5.6 ± 0.2[d]
PUFA	42.7 ± 0.6[c]	60.9 ± 0.3[a]	43.9 ± 0.3[b]	41 ± 3[d]
Vitamin C (Ascorbic acid, mg/100 mg)	nd	tr	nd	tr
Vitamin B_9 (Folate, µg/100 g)	149 ± 3[b]	253 ± 20[a]	62.6 ± 0.3[d]	115 ± 3[c]
Vitamin E (Tocopherols, mg/100 g)				
α-Tocopherol	1.36 ± 0.01[d]	65.00 ± 0.01[a]	2.9 ± 0.3[c]	3.3 ± 0.3[b]
β-Tocopherol	nd	1.61 ± 0.01[a]	nd	0.38 ± 0.04[b]
γ-Tocopherol	0.15 ± 0.01[d]	2.52 ± 0.01[a]	0.29 ± 0.01[c]	1.0 ± 0.1[b]
δ-Tocopherol	nd	2.42 ± 0.01[a]	nd	1.3 ± 0.2[b]
Sum	1.50 ± 0.02[d]	71.56 ± 0.01[a]	3.2 ± 0.3[c]	6.5 ± 0.6[b]
Organic acids (g/100 g)				
Oxalic acid	1.26 ± 0.03[a]	0.26 ± 0.01[c]	0.59 ± 0.01[b]	0.26 ± 0.04[c]
Quinic acid	nd	nd	0.85 ± 0.17[a]	0.24 ± 0.02[b]
Malic acid	2.1 ± 0.3[a]	tr	1.13 ± 0.16[b]	0.54 ± 0.07[c]
Shikimic acid	0.01 ± 0.00[b]	nd	0.04 ± 0.00[a]	nd
Citric acid	nd	nd	2.86 ± 0.07[b]	3.44 ± 0.16[a]
Fumaric acid	0.002 ± 0.00[b]	nd	0.01 ± 0.00[a]	nd

Sum	3.4 ± 0.3^{c}	0.26 ± 0.01^{d}	5.48 ± 0.07^{a}	4.5 ± 0.3^{b}

nd- not detected; tr- traces; Fe- iron Cu- cooper, Mn- manganese, Zn- zinc, Ca- calcium, Mg- magnesium, K- potassium; C16:0- palmitic acid, C18:0- stearic acid, C18:1n9- oleic acid, C18:2n6- linoleic acid, C18:3n3- linolenic acid, C20:0- arachidic acid, C20:5n3- *cis*-5,8,11,14,17-eicosapentaenoic acid, C22:0- behenic acid, C24:0- lignoceric acid; SFA- saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA- polyunsaturated fatty acids. In each row different letters mean significant differences between samples ($p<0.05$), where "a"and "d" correspond to the highest and lowest values, respectively.

Table 2. Minerals, soluble sugars, vitamins and organic acids in infusions and decoctions prepared from roots of *Fragaria vesca* L. commercial and wild samples (mean ± SD).

	Commercial Roots		Wild Roots	
	Infusion	Decoction	Infusion	Decoction
Ash content (g/100 mL)	0.04 ± 0.01^{d}	0.38 ± 0.06^{a}	0.14 ± 0.02^{b}	0.11 ± 0.02^{c}
Microelements (μg/100 mL)				
Fe	40 ± 1^{a}	20 ± 1^{b}	20 ± 1^{b}	20 ± 1^{b}
Cu	10 ± 1^{b}	2.0 ± 0.5^{c}	30 ± 1^{a}	nd
Mn	2.0 ± 0.5^{c}	4.0 ± 0.5^{c}	60 ± 1^{a}	30 ± 1^{b}
Zn	80 ± 1^{a}	60 ± 1^{b}	20 ± 1^{c}	10 ± 1^{d}
Macroelements (mg/100 mL)				
Ca	5.8 ± 0.6^{a}	5.3 ± 0.3^{b}	3.65 ± 0.06^{c}	3.24 ± 0.06^{c}
Mg	3.5 ± 0.4^{a}	3.2 ± 0.1^{b}	1.52 ± 0.01^{c}	0.72 ± 0.02^{d}
K	4.12 ± 0.09^{a}	2.43 ± 0.06^{b}	0.27 ± 0.01^{d}	1.4 ± 0.1^{c}
Soluble sugars (mg/100 mL)				
Xylose	0.59 ± 0.08^{b}	0.58 ± 0.06^{b}	0.34 ± 0.07^{c}	0.88 ± 0.05^{a}
Fructose	18.0 ± 0.3^{a}	17.77 ± 0.17^{b}	1.66 ± 0.13^{d}	4.58 ± 0.09^{c}
Glucose	13.6 ± 0.1^{a}	14.18 ± 0.02^{b}	1.53 ± 0.17^{d}	4.25 ± 0.03^{c}
Sucrose	2.3 ± 0.4^{c}	2.75 ± 0.00^{b}	1.81 ± 0.25^{d}	3.75 ± 0.09^{a}
Trehalose	1.3 ± 0.2^{a}	1.22 ± 0.06^{b}	0.25 ± 0.02^{d}	0.80 ± 0.05^{c}
Sum	36.0 ± 0.9^{a}	36.5 ± 0.3^{a}	5.6 ± 0.6^{c}	14.3 ± 0.2^{b}
Vitamin C (Ascorbic acid, mg/100 mL)	nd	nd	nd	nd
Vitamin B_9 (Folate, μg/100 mL)	10 ± 1^{c}	10.6 ± 0.1^{d}	28.1 ± 0.7^{a}	26 ± 3^{b}
α-Tocopherol (μg/100 mL)	0.32 ± 0.01^{a}	0.20 ± 0.03^{b}	0.04 ± 0.01^{c}	0.19 ± 0.01^{b}
Organic acids (mg/100 mL)				
Oxalic acid	4.15 ± 0.05^{b}	4.48 ± 0.04^{a}	0.25 ± 0.01^{d}	1.35 ± 0.06^{c}
Malic acid	5.4 ± 0.4^{a}	4.9 ± 0.8^{b}	tr	tr
Shikimic acid	0.06 ± 0.01^{a}	0.05 ± 0.01^{a}	nd	nd
Fumaric acid	tr	tr	nd	nd
Sum	9.6 ± 0.4^{a}	9.5 ± 0.8^{a}	0.25 ± 0.01^{c}	1.35 ± 0.06^{b}

nd- not detected; tr- traces; Fe- iron Cu- cooper, Mn- manganese, Zn- zinc, Ca- calcium, Mg- magnesium, K- potassium. In each row different letters mean significant differences between samples ($p<0.05$), where "a"and "d" correspond to the highest and lowest values, respectively.

Table 3. Minerals, soluble sugars, vitamins and organic acids in infusions and decoctions prepared from vegetative parts of *Fragaria vesca* L. commercial and wild samples (mean ± SD).

	Commercial vegetative parts		Wild vegetative parts	
	Infusion	Decoction	Infusion	Decoction
Ash content (g/100 mL)	0.24 ± 0.03^{b}	0.24 ± 0.01^{b}	0.24 ± 0.03^{b}	0.37 ± 0.04^{a}
Microelements (μg/100 mL)				
Fe	70 ± 1^{a}	30 ± 1^{b}	10 ± 1^{d}	20 ± 1^{c}
Cu	20 ± 1	20 ± 1	nd	nd
Mn	130 ± 1^{a}	60 ± 1^{c}	70 ± 1^{b}	60 ± 1^{c}
Zn	50 ± 1^{a}	20 ± 1^{b}	10 ± 1^{c}	20 ± 1^{b}
Macroelements (mg/100 mL)				
Ca	14 ± 2^{a}	8.47 ± 0.06^{b}	6.5 ± 0.2^{c}	5.29 ± 0.01^{d}
Mg	7.3 ± 0.7^{a}	4.65 ± 0.01^{b}	4.2 ± 0.2^{b}	2.32 ± 0.02^{c}
K	11.4 ± 0.1^{a}	4.79 ± 0.07^{b}	1.26 ± 0.03^{c}	0.46 ± 0.01^{d}
Soluble sugars (mg/100 mL)				
Xylose	2.1 ± 0.1^{c}	1.76 ± 0.03^{d}	5.82 ± 0.07^{a}	3.31 ± 0.03^{b}
Fructose	11.7 ± 0.2^{a}	11.7 ± 0.6^{a}	6.4 ± 0.1^{b}	4.19 ± 0.09^{c}
Glucose	16.29 ± 0.09^{b}	17.7 ± 0.6^{a}	7.42 ± 0.01^{c}	4.8 ± 0.2^{d}
Sucrose	7.1 ± 0.3^{b}	6.0 ± 0.2^{c}	8.53 ± 0.05^{a}	2.91 ± 0.03^{d}
Trehalose	3.2 ± 0.3^{b}	2.7 ± 0.3^{c}	3.56 ± 0.02^{a}	1.77 ± 0.06^{d}
Sum	40.4 ± 0.4^{a}	39.9 ± 0.5^{b}	31.7 ± 0.2^{c}	17.0 ± 0.2^{d}
Vitamin C (Ascorbic acid, mg/100 mL)	nd	nd	nd	nd
Vitamin B_9 (Folate, μg/100 mL)	8.5 ± 0.5^{b}	10.5 ± 0.6^{d}	11.7 ± 0.7^{c}	13.9 ± 0.2^{a}
α-Tocopherol (μg/100 mL)	0.10 ± 0.01^{d}	0.33 ± 0.02^{a}	0.22 ± 0.01^{b}	0.20 ± 0.01^{c}
Organic acids (mg/100 mL)				
Oxalic acid	1.18 ± 0.09^{b}	2.4 ± 0.5^{a}	2.51 ± 0.01^{a}	0.74 ± 0.07^{c}
Quinic acid	1.2 ± 0.2^{c}	1.5 ± 0.1^{b}	4.56 ± 0.08^{a}	4.5 ± 0.1^{a}
Malic acid	2.0 ± 0.1^{c}	2.8 ± 0.4^{b}	1.8 ± 0.1^{c}	27.2 ± 0.5^{a}
Shikimic acid	0.13 ± 0.01^{b}	0.20 ± 0.01^{a}	nd	nd
Citric acid	54 ± 6^{b}	61 ± 4^{a}	1.08 ± 0.06^{c}	0.56 ± 0.07^{c}
Fumaric acid	tr	tr	nd	nd
Sum	59 ± 6^{b}	68 ± 3^{a}	9.99 ± 0.03^{d}	33.1 ± 0.2^{c}

nd- not detected; tr- traces; Fe- iron Cu- cooper, Mn- manganese, Zn- zinc, Ca- calcium, Mg- magnesium, K- potassium. In each row different letters mean significant differences between samples (p<0.05), where "a"and "d" correspond to the highest and lowest values, respectively.