Food Bioscience 13 (2016) 9–14

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Minerals and vitamin B_9 in dried plants *vs.* infusions: Assessing absorption dynamics of minerals by membrane dialysis tandem *in vitro* digestion

Maria Inês Dias [a,b,c], Patricia Morales [c,*], João C.M. Barreira [a,b], M. Beatriz P.P. Oliveira [b], Mª Cortes Sánchez-Mata [c], 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*

A R T I C L E I N F O

Article history:
Received 30 July 2015
Received in revised form
3 November 2015
Accepted 10 November 2015
Available online 12 November 2015

Keywords:
Vitamin B_9
Minerals
Infusions
Wild plants

A B S T R A C T

Vitamins and mineral elements are among the most important phytochemicals due to their important role in the maintenance of human health. Despite these components had already been studied in different plant species, their full characterization in several wild species is still scarce. In addition, the knowledge regarding the *in vivo* effects of phytochemicals, particularly their bioaccessibility, is still scarce. Accordingly, a membrane dialysis process was used to simulate gastrointestinal conditions in order to assess the potential bioaccessibility of mineral elements in different preparations of *Achillea millefolium* (yarrow), *Laurus nobilis* (laurel) and *Taraxacum* sect. *Ruderalia* (dandelion). The retention/passage dynamics was evaluated using a cellulose membrane with 34 mm pore. Dandelion showed the highest levels of all studied mineral elements (except zinc) independently of the used formulations (dried plant or infusion), but yarrow was the only species yielding minerals after the dialysis step, either in dried form, or as infusion. In fact, the ability of each evaluated element to cross the dialysis membrane showed significant differences, being also highly dependent on the plant species. Regarding the potential use of these plants as complementary vitamin B_9 sources, the detected values were much lower in the infusions, most likely due to the thermolability effect.

1. Introduction

The interest for traditionally used plants is rising, since they are considered a valuable and reliable source of natural compounds with recognised health effects. Among those compounds, the study of vitamins and mineral elements is crucial, due to their important role in the maintenance of human health; in fact, the lack of vitamins can cause a number of diseases, and mineral trace elements have essential biochemical functions such as the activation of chemical components present in the organism (Rihawy, Bakraji, Aref & Shaban, 2010). The possible applications of plants should be complemented by a complete chemical characterization (Leśniewicz, Jaworska & Żyrnicki, 2006). Despite the high number of scientific publications profiling chemical compounds in plants, some wild species are still lacking for comprehensive studies. *Achillea millefolium* L. (yarrow, Asteraceae), *Laurus nobilis* L. (bay leaves, Laureacea) and *Taraxacum* sect. *Ruderalia* (dandelion, Asteraceae) were scarcely studied for their mineral profile and vitamin B_9 composition, making them good candidates for this type of profiling studies.

Vitamin B_9 (folic acid/folates) is an important cofactor of many biochemical reactions in cells. The absence of this vitamin would lead to non-cell division, anaemia, cardiovascular disease and neural tube defects in infants. Common food sources of vitamin B_9 are vegetables, bread and cereals, which may contain various forms of folate depending on food processing and storage. In food, folates are naturally presented as polyglutamates (PteGlun), mainly as mono-, penta- and hexaglutamates (Scott, Rébeillé & Fletcher, 2000), being the monoglutamate form absorbed in the intestinal tube (Scott, 1999) and further converted to tetrahydrofolate (the most bioactive form of this vitamin) (Bailey & Ayling, 2009).

Microelements such as iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) represent a group correlated with the prevention of cardiovascular diseases, and some of them display also important biological functions such as osmoprotection (Fe), mitochondrial

* Corresponding authors.
E-mail addresses: patricia.morales@farm.ucm.es (P. Morales), iferreira@ipb.pt (I.C.F.R. Ferreira).

http://dx.doi.org/10.1016/j.fbio.2015.11.004
2212-4292/

respiration (Cu), and energy production and maintenance of structural integrity of biomembranes (Zn) (Hänsch & Mendel, 2009). These elements, which are required by the body in low amounts, can be obtained (together with numerous organic compounds) in the infusions of medicinal plants, subsequently leading to different physiologic functions, toxicity and absorption rates (Mutaftchiev, 2001; Özcan, 2004). Macroelements such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K) and sodium (Na) serve as structural elements of the tissues and modulate the metabolism and acid-base balance, being present in the body in higher amounts than microelements (Leśniewicz et al., 2006; Özcan, 2004). Within the same species, the concentration of micro and macroelements in plants is conditioned by geochemical characteristics, rainfall and agricultural practices (Łozak, Sołtyk, Ostapczuk & Fijałek, 2002; Konieczyński & Wesołowski, 2007).

Many exogenous (food matrix and compound structure) and endogenous (active transport, metabolism and excretion in the human body) factors affect the entrance of compounds in the lumen and therefore its bioavailability. As a part of the concept of bioavailability, bioaccessibility is defined as the amount of a food constituent that is present in the gut as a consequence of its release from the solid food matrix, and may be able to pass through the intestinal barrier and be potentially bioavailable (Saura-Calixto, Serrano & Goñi, 2007). *In vitro* gastrointestinal models provide a very useful methodology to screen food ingredients (*e.g.*, minerals, vitamins, phenolic compounds, among others) for their bioavailability. These system provide a great amount of results in a short period of time, allowing the study of matrices with different compositions and structures, simultaneously overcoming the complexity of *in vivo* studies (Hur, Lim, Decker & McClements, 2011).

The content of mineral elements was already determined by atomic absorption spectroscopy methods in *A. millefolium* (Chizzola, Michitsch & Franz, 2003; Konieczyński & Wesołowski, 2007; Divrikli, Horzum, Soylak & Elci, 2006), *L. nobilis* (Özcan, 2004; Divrikli et al., 2006; Sekeroglu, Ozkutlu, Kara & Ozguven, 2008; Zengin, Özcan, Çetin & Gezgin, 2008) and *Taraxacum obovatum* (Willd.) DC. basal leaves (García-Herrera et al., 2014) samples from different locations. Nevertheless, to our knowledge, there are no reports of the content of vitamin B_9 in yarrow or bay leaves. A particular species of dandelion, *Taraxacum obovatum* (Willd.) DC., was previously studied for the vitamin B_9 content in its basal leaves (Morales, Fernández-Ruiz, Sánchez-Mata, Cámara, & Tardío, 2015). Nevertheless, to our knowledge, there are no studies on the vitamin B_9 content of yarrow and laurel, nor on the *in vitro* bioaccessibility of mineral elements from the plants studied herein. Therefore, the main objective of the present work was to characterize vitamin B_9 and minerals profile in dried material and infusions of wild samples of *A. millefolium*, *L. nobilis* and *Taraxacum* sect. *Ruderalia*. Furthermore, an *in vitro* gastrointestinal model was applied to provide a preliminary study of mineral elements bioaccessibility in these food matrices.

2. Materials and methods

2.1. Samples and infusions preparation

The wild samples of yarrow (inflorescences and upper leaves), laurel (leaves; before flowering) and the vegetative parts of wild *Taraxacum* sect. Ruderalia were collected in Bragança (Portugal). Voucher specimens of yarrow (no. 9623 BRESA), laurel (no. 9634 BRESA) and dandelion (no. 9686) were deposited at the Herbarium of the Escola Superior Agrária de Bragança (BRESA) (Dias et al., 2013; Dias, Alves, Oliveira, Santos-Buelga & Ferreira, 2014a; Dias et al., 2014b). Morphological key characters from the Flora Iberica (Castroviejo, 1986–2012) were used for plant identification. The wild samples were lyophilized (FreeZone 4.5, Labconco, Kansas, USA) and stored at 4 °C until analysis.

The infusions were prepared according to the traditional procedure used to prepare tea (1 bag with ~1 g dry material, and 1 teapot with ~200 mL); therefore, each sample (1 g) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure. The obtained infusions were frozen, lyophilized and stored at −6 °C until analysis.

2.2. Standards and reagents

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). Standards 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, 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). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Vitamin B_9 (folic acid/folates)

Vitamin B_9 content was determined according to the methodology previously described by Morales et al. (2015), 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). Quantification was performed by comparison of the area of the peaks recorded with calibration curves obtained from commercial standards (5-CH_3-H_4folate mono and diglutamate), and expressed as total folates (from the sum of both compounds) per 100 g plant (dw) or per 100 mL infusion.

Chromatographic parameters, namely limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, repeatability and reproducibility were accepted as previously assessed (Morales et al., 2015).

2.4. Mineral and trace elements content

Mineral elements analysis was performed according to the method 930.05 of AOAC procedures for ash obtention, and then following the methodology previously described by Fernández-Ruiz, Olives, Cámara, Sánchez-Mata & Torija (2011). 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(NO3)$_3$, $Cu(NO_3)_2$, Mn $(NO_3)_2$, Zn $(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg band. Limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, repeatability and reproducibility were accepted as previously assessed (Sanchez-Mata, 2000).

2.5. In vitro gastrointestinal model (dialysis)

The *in vitro* model applied consisted of an initial simulation