LWT - Food Science and Technology 63 (2015) 799–806

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms

Sandrina A. Heleno [a], Lillian Barros [a], Anabela Martins [a], Patricia Morales [b], Virginia Fernández-Ruiz [b], Jasmina Glamoclija [c], Marina Sokovic [c], Isabel C.F.R. Ferreira [a, *]

[a] Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, apartado 1172, 5301-854 Bragança, Portugal

[b] Departamento de Nutrición y Bromatología II. Bromatología, Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Plaza Ramón y Cajal s/n, E-28040 Madrid, Spain

[c] University of Belgrade, Institute for Biological Research "Siniša Stanković", Department of Plant Physiology, Belgrade, Serbia

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form
10 April 2015
Accepted 11 April 2015
Available online 18 April 2015

Keywords:
Wild mushrooms
Chemical composition
Bioaccessibility
Antimicrobial activity

ABSTRACT

Wild mushrooms are important sources of nutrients and bioactive compounds, namely phenolic acids. After their ingestion, bioactive molecules have to be released from the matrix to be absorbed by the organism. In the present work, two wild edible mushrooms (*Volvopluteus gloiocephalus* and *Clitocybe subconnexa*) were studied for their nutritional value, detailed chemical composition and antimicrobial activity. Bioaccessibility studies were also performed using the *in vitro* digestion of the crude powder, phenolic extracts and individual phenolic acids identified in the samples. The studied species proved to be rich sources of nutrients, minerals and bioactive molecules such as phenolic acids. The *in vitro* digestion conducted to a decrease in antibacterial activity, but not in antifungal and demelanizing properties. Nevertheless, in most of the cases, the analyzed samples presented higher antibacterial and antifungal activities than the standards. The bioactive molecules (phenolic acids) were found in higher concentrations in the phenolic extracts before *in vitro* digestion, which is agreement with the highest antibacterial activity revealed by these extracts. It should be highlighted that the phenolic acids were still bioavailable after digestion.

1. Introduction

Mushrooms are known worldwide as being valuable health foods, not only for their unique and subtle flavor, but also for their nutritional properties. The richness in carbohydrates, proteins, fibers, vitamins, and minerals, the presence of unsaturated fatty acids, and the poorness in fat as turned mushrooms in an excellent food choice to include in low caloric diets (Heleno, Barros, Sousa, Martins, & Ferreira, 2010; Kalac, 2012; Reis, Barros, Martins, & Ferreira, 2012).

Furthermore, these organisms are described as functional foods and/or a source of nutraceuticals due to biologically and physiologically active substances such as phenolic acids (Ferreira, Barros, & Abreu, 2009). Mushroom extracts and their phenolic acids have been evaluated regarding antimicrobial activity, showing a very strong antibacterial, antifungal and also demelanizing properties; in several cases, even higher activity than the antibiotics/antifungals frequently used (Alves et al., 2012, 2013; Heleno et al., 2013). Thus, mushrooms could be introduced in our daily diet in order to help the organism in the prevention and combat against microbiological infections, taking advantage of the additive and synergistic effects of all their bioactive compounds (Alves et al., 2013).

Nevertheless, it is important to study the bioaccessibility of these extracts and compounds in order to guarantee the maintenance of the initial bioactivity, since the molecules can suffer structural modifications during the digestion and metabolism, before being absorbed by the intestine (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013). Several easy and cheap methodologies such as *in vitro* digestions were developed and are described as being capable of mimetize the *in vivo* conditions in order to analyze the digestive stability of the food constituents (Bouayed, Hoffmann, & Bohn, 2011).

In the present work, two wild and edible mushrooms (*Volvopluteus gloiocephalus* ((DC.) Vizzini, Contu & Justo) and *Clitocybe*

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

http://dx.doi.org/10.1016/j.lwt.2015.04.028
0023-6438/

subconnexa (Murril)) were studied for their nutritional value, detailed chemical composition and antimicrobial activity. To access their bioaccessibility, an *in vitro* digestion of the crude powder, phenolic extracts and individual phenolic acids identified in the two samples was carried out.

2. Materials and methods

2.1. Samples

Samples of *V. gloiocephalus* ((DC.) Vizzini, Contu & Justo) and *C. subconnexa* (Murril), two wild edible mushrooms, were collected in Bragança (Northeast Portugal) during November of 2013. After authentications by Dr. Anabela Martins (Polytechnic Institute of Bragança), voucher specimens were deposited at herbarium of School of Agriculture of Polytechnic Institute of Bragança, Portugal. The specimens (for each species, three fruiting bodies in the same maturity stage) were immediately lyophilised (FreeZone 4.5, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh), mixed to obtain an homogenate sample and kept at −20 °C until further analysis.

2.2. Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (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 and standards of sugars (D-(+)-mannitol, D-(+)-trehalose), tocopherols (α-, β-, and γ-isoforms), organic acids (oxalic and fumaric acids), phenolic compounds (gallic, *p*-hydroxybenzoic, protocatechuic, cinnamic and *p*-coumaric acids), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and dietary fiber enzyme kit (TDF-100A Kit), pepsin enzyme (P-7000), pancreatin enzyme (P-1750) and porcine bile (B-8631). Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, 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). Anthrone was obtained from Panreac Química (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Chemical composition

2.3.1. Proximate composition

The samples were analyzed for proteins, fat, carbohydrates and ash, using the AOAC procedures (AOAC, 2012). The crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by the extraction of a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash and mineral content was determined by incineration at 550 ± 15 °C.

Total available carbohydrate (TAC) assay was performed by the Anthrone method as described by Osborne and Voogt (1986) using 0.25 g of sample. The samples were pre-treated with 13 mL of $HClO_4$ (52:100, v/v) and kept for 18 h in the dark. After this period, distilled water was added, the sample was filtered and the volume of the filtrate was adjusted to 100 mL. Finally, the solution was further diluted to 10%, and 5 mL of 0.1% anthrone solution (in H_2SO_4 73:100, v/v) was added. Samples were kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green colour, and absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW ver. 1.2. The absorbance of the sample solution was compared to a 10–100 μg/mL concentration range standard glucose calibration curve. TAC values were expressed as g/100 g of dry weight.

AOAC enzymatic-gravimetric methods (993.19 and 991.42) were used for soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) analysis (Latimer, 2012). In brief, freeze-dried samples were treated with alpha-amylase (heat-stable), protease and amyloglucosidase. The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C. Total fiber is the sum of soluble and insoluble fiber fractions; both were expressed as g/100 g of dry weight.

Energy was calculated according to the following equation according to Regulation (EC) No. 1169/2011 of the European Parliament and of the Council, of 25 October 2011: Energy (kcal) = 4 × (g protein + g total available carbohydrate) + 2 × (g fiber) + 9 × (g fat).

2.3.2. Macro and microelements

Total mineral content (ashes) and mineral elements analysis were performed on dried samples. The method 930.05 of AOAC procedures was used; 500 mg of each sample were subject to dry-ash mineralization at 450 °C ± 15 °C. The residue of incineration was extracted with HCl (50% v/v) and HNO_3 (50% v/v) and made up to an appropriate volume with distilled water, where Fe, Cu, Mn and Zn were directly measured. An additional 1/10 (v/v) dilution of the sample extracts and standards was performed to avoid interferences between different elements in the atomic absorption spectroscopy: for Ca and Mg analysis in 1.16% La_2O_3/HCl (leading to $LaCl_2$); for Na and K analysis in 0.2% CsCl (Fernández-Ruiz, Olives Barba, Sanchez-Mata, Camara, & Torija, 2011; Ruiz-Rodríguez et al., 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 $Fe(NO_3)_3$, $Cu(NO_3)_2$, $Mn(NO_3)_2$, $Zn(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg band. Triplicate mineralization and extractions were carried out on the same material. The results were expressed in mg per 100 g of dry weight.

2.3.3. Free sugars

Free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisted 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 (RI detector Knauer Smartline 2300) as previously described by the authors (Heleno, Barros, Sousa, Martins, & Ferreira, 2009). Sugars were identified by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex, Prague, Czech Republic). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) 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.

2.3.4. Fatty acids

Fatty acids were determined after a transesterification procedure as described previously by the authors (Heleno et al., 2009), using a gas chromatographer (DANI 1000, Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detector (GC-FID). 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, Prague, Czech Republic). The results were expressed in relative percentage of each fatty acid.