Chemical composition, antioxidant activity and bioaccessibility studies in phenolic extracts of two *Hericium* wild edible species

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ABSTRACT

Mushrooms are rich sources of bioactive compounds such as phenolic acids. When ingested, these molecules have to be released from the matrix to be transformed/absorbed by the organism, so that they can exert their bioactivity. Several *in vitro* methodologies have been developed in order to evaluate the bioavailability of bioactive compounds. Herein, two *Hericium* species were analyzed for their chemical composition and antioxidant activity. Furthermore, an *in vitro* digestion of the mushrooms and mushroom phenolic extracts was performed, and the digested samples were also submitted to antioxidant activity evaluation in order to evaluate the bioaccessibility of the phenolic acids identified in the samples. *Hericium* species showed similar chemical profiles (except for tocopherols), varying only in the concentration of the compounds. The phenolic extracts revealed higher antioxidant activity than the *in vitro* digested samples, meaning that this process decrease the antioxidant properties of the extract/mushroom. Nevertheless, phenolic acids were found in the digested samples, meaning that those molecules are bioaccessible.

Keywords: Wild mushrooms; chemical composition; antioxidant activity and bioaccessibility; phenolic extracts

1. Introduction

Wild mushrooms have been extensively consumed since the primords of human civilization, due to their unique and delicate flavor, being rich sources of minerals and having high amounts of water, protein, fiber, and carbohydrates. Lipids are present in low values, which make them excellent to be included in low-caloric diets (Heleno, Barros, Sousa, Martins & Ferreira, 2009; Kalac, 2009; Kalac, 2012).

Beyond the nutritional characteristics, mushrooms have been also extensively studied for their medicinal properties, mainly due to their richness in bioactive compounds that presented antioxidant, anticancer and antimicrobial properties, among other bioactivities (Ferreira, Barros & Abreu, 2009; Ferreira, Vaz, Vasconcelos & Martins, 2010; Alves, Ferreira, Dias, Teixeira, Martins & Pintado, 2012).

Phenolic acids are the major low molecular weight bioactive components usually found in mushroom species, responsible for their antioxidant properties (Ferreira et al., 2009). Once ingested, these bioactive compounds have to be released from the matrix and modified in the gastrointestinal tract so that they become accessible to be absorbed in the intestine (Bouayed, Hoffmann & Bohn, 2011; Rodríguez-Roque, Rojas-Graü, Elez-Martínez & Martín-Belloso, 2013; Carbonell-Capella, Buniowska, Barba, Esteve & Frígola, 2014).

Bioaccessibility is usually evaluated by *in vitro* digestion procedures, generally simulating gastric and small intestinal digestion; sometimes followed by Caco-2 cells uptake to evaluate the bioavailability (Courraud, Berger, Cristol & Avallone, 2013). With the aim of evaluating if the compounds are bioaccessible, *in vitro* methodologies have been developed as an attempt to mimic the *in vivo* conditions. Those assays are described as being easy, cheap and reproducible, being possible to evaluate the digestive stability of the food constituents (Failla & Chitchumroonchokchai, 2005; Rodríguez-

Roque et al., 2013). Furthermore, in some cases, the use of *in vitro* models to study the bioaccessibility of food constituents have proven to be well correlated with results from human studies and animal models (Biehler & Bohn, 2010; Bouayed et al., 2011).

Our daily diet could be enriched with food rich in antioxidant such as fruits, vegetables and mushrooms in order to help the organism in the combat against oxidative stress, taking advantage of the additive and synergistic effects of all the antioxidant compounds present. Public health authorities consider prevention with nutraceuticals/functional foods as a powerful instrument in maintaining and promoting health, longevity and life quality (Ferreira et al., 2009). Nevertheless, it is important to guarantee the bioaccessibility and bioavailability of the ingested compounds.

Therefore, in the present work, two wild edible mushroom species originated from our region (Northeast Portugal) (*Hericium erinaceus* (Bull.) Persoon and *Hericium coralloides* (Scop.) Pers., were analyzed for their nutritional value, detailed chemical composition and antioxidant properties. Furthermore, in order to evaluate the bioaccessibility of the compounds responsible for the mushrooms antioxidant properties, a digestion of the dry powder and extracts enriched in phenolic acids was performed under *in vitro* conditions.

2. Materials and methods

2.1. Samples and samples preparation

Specimens of *Hericium erinaceus* (Bull.) Persoon and *Hericium coralloides* (Scop.) Pers., two edible mushrooms belonging to the Hericiaceae family, were collected in Bragança (Northeast Portugal) during November of 2013. These species are saprobic and can also be parasitic, typically fruiting from fallen hardwoods branches and stumps; they are widely distributed and common. After authentication, voucher specimens were

deposited at herbarium of School of Agriculture of Polytechnic Institute of Bragança, Portugal. The specimens 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 ºC until further analysis.

For phenolic extracts preparation, 1.5 g of each dry mushroom powder was twice extracted with a mixture of methanol (800 mL/L) and water (200 mL/L) at -20 ºC for 1.5 h; after a sonication step for 15 min, the extract was filtered through Whatman No. 4 paper (Barros et al., 2009). The combined extracts were evaporated under reduced pressure at 30ºC to remove methanol.

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 well as other individual fatty acid isomers and standards of sugars (L-(+)-arabinose, D-(+)-mannitol, D-(+)-trehalose), tocopherols (α-, β-, δ- and γ-isoforms), organic acids (malic, oxalic and fumaric acids), phenolic compounds (gallic, *p*-hydroxybenzoic, and *p*-coumaric acids), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), dietary fiber enzyme kit (TDF-100A Kit), pepsin (P-7000), pancreatin (P-1750) and porcine bile (B-8631). 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). 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 (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 analysed for moisture, proteins, fat, carbohydrates and ash, using the AOAC procedures (AOAC, 1995). The crude protein content (N × 4.38) 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 available carbohydrate (TAC) assay was carried out by the anthrone method as described by Osborne & Voogt (1986) using 0.25 g of sample. The samples were pre-treated with 13 mL of 52 mL/100 mL $HClO_4$ 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 mL/100 mL, and 5 mL of 0.1 mL/100 mL anthrone solution in 73 mL/100 mL H_2SO_4 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 0.1 mL of heat-stable α-amylase in 50 mL

phosphate buffer (pH 6) and incubated in a water bath at 100ºC, during 15 min, after cooling the pH was adjusted to 7.5 with NaOH (0.275 N) 5 mg of protease was added and incubated at 60ºC during 30 min. Finally, 0.3 mL of amyloglucosidase was added after the pH was adjusted to 4.5 with HCl (0.325 N), and incubated at 60ºC during 30 min; all the incubations was performed under continuous agitation. The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C. Total fiber was calculated as the sum of soluble and insoluble fiber, both were expressed as g/100 g of dry weight.

Energy content was calculated according to the following equation according to Regulation (EC) No. 1169/ 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. AOAC method No. 930.05 was used; 500 mg of each sample was subject to dry-ash mineralization at 450°C. The incineration residue was extracted with HCl 0.5 mL/mL and HNO_3 0.5 mL/mL and made up to a suitable volume of distilled water to which were directly weighed Fe, Cu, Mn and Zn. The other elements were determined after dilutions (Fernández-Ruiz et al., 2004; 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, supplied by Merck (Darmstadt, Germany) and Panreac Química (Barcelona, Spain).

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 et al., 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.

2.3.5. Tocopherols. Tocopherols were determined by following a previously described procedure (Heleno, Barros, Sousa, Martins & Ferreira, 2010). Analysis was performed by HPLC (equipment described above), and with fluorescence detector (excitation 290 nm, emission 330 nm). The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence

signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in μg per 100 g of dry weight.

2.3.6. Organic acids. Organic acids were determined by following previously described procedure (Reis et al., 2013). Analysis was performed by ultra-fast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Detection was carried out in a PDA, using 215 nm and 245 as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

2.4. In vitro gastrointestinal model (dialysis) for bioaccessibility studies

Bioaccessibility was determined using 25 mL of aqueous solutions prepared from dry mushroom powder (20 mg/mL) or lyophilized phenolic extract (50 mg/mL) as decribed by (Ramírez-Moreno, Marquês, Sánchez-Mata & Goñi, 2011).

The gastric digestion was simulated, adjusting the pH of each sample to 2, adding 150 mL of a pepsin solution (40 mg/mL of HCl 0.1 mol/L), and incubating the mixture in a water bath at 37 ºC for 2 h with stirring (60 osc/min). The intestinal digestion was then simulated, adding to the mentioned mixture a pancreatin/bile solution (5/25mg of pancreatin/bile per 1 mL of $NaHCO_3$ 0.1 mol/L) for 3 hours. The mixture was then transferred to dialysis membranes (Medicell 7000/2, width 34 mm, 7000 Mw cut off), previously boiled in distilled water for 15 min. The dialysis membranes/mixture was then placed in to a flask containing 250 mL of $NaHCO_3$ pH 7.5 and incubated in a water

bath at room temperature for 3 h with stirring (60 osc/min). After dialysis, the obtained final solution of $NaHCO_3$ pH 7.5 was frozen and lyophilized for evaluation of antioxidant activity and phenolic compounds analysis.

2.5. Antioxidant activity

2.5.1. General. Successive dilutions of the stock solution of the phenolic extract, *in vitro* digested mushroom powder and *in vitro* digested phenolic extract, were made and submitted to *in vitro* assays already described by the authors (Heleno et al., 2009) to evaluate the antioxidant activity of the samples. The sample 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/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. In the Folin-Ciocalteu assay, the results were expressed as mg of gallic acid equivalents (GAE) per g of extract. The commercial standard Trolox was used as positive control.

2.5.2. Reducing power. Two different procedures were used to evaluate the reducing power: A). The first methodology, the ferricyanide Prussian blue assay, was performed following the method first reported by Oyaizu et al., 1986 with some modifications, using a Microplate Reader ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). 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 (10 g/L, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (100 g/L, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (1 g/L, 0.16 mL), and the absorbance was measured at 690 nm. B) The second

methodology followed the Folin-Ciocalteu assay first described by Wolfe et al., 2003. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted ten folds with water) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for colour development. Absorbance was then measured at 765 nm.

2.5.3. DPPH scavenging activity assay. This methodology was performed following Hatano et al. (1988) first description with some modifications, by using the Microplate Reader mentioned above. The reaction mixture on 96 wells plate consisted of a solution by well of the extract solutions with different concentrations (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 30 min in the dark, and the absorption was measured 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 containing the sample, and A_{DPPH} is the absorbance of the DPPH solution.

2.5.4. Lipid peroxidation inhibition. Lipid peroxidation inhibition was assessed by using two different approaches: The first methodology was β-carotene/linoleate assay by following the methodology first described by Burda and Oleszek, 2001 with some modifications: 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. The chloroform was removed at 40ºC under vacuum and 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 test tubes containing extract solutions with different concentrations (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. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The second methodology was TBARS (thiobarbituric acid reactive species) assay following Kishida et al. (1993) with some modifications: Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mmol/L, pH 7.4) to produce a 5g/L brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 µL) of the supernatant was incubated with the different concentrations of the samples solutions (200 µL) in the presence of $FeSO_4$ (10 mmol/L; 100 µL) and ascorbic acid (0.1 mmol/L; 100 µL) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (280 g/L, 500 µL), followed by thiobarbituric acid (TBA, 20 g/L, w/v, 380 µL), and the mixture was then heated at 80ºC for 20 min. After centrifugation at 3000*g* 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] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

2.6. Analysis of phenolic compounds

The phenolic extracts previously described in section 2.1. were submitted to a liquid–liquid extraction with diethyl ether (2 x 20 mL) and ethyl acetate (2 x 20 mL). Anhydrous sodium sulphate was added to the combined organic phases and the extracts were filtrated through Whatman No. 4 paper, evaporated to dryness and re-dissolved in a mixture of methanol (200 mL/L) and water (800 mL/L) (Barros et al., 2009). The

extracts (1 ml) were filtered through a 0.22 µm nylon disposable LC filter disk. Phenolic acids determination was performed using a Shimadzu 20A series ultra-fast liquid chromatograph (UFLC, Shimadzu, equipment described above) as previously described by Reis et al. (2013). Detection was carried out in a photodiode array detector (PDA), using 280 nm as the preferred wavelength. The phenolic compounds were quantified by comparison of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in µg per 100 g of dry phenolic extract or 100 g of dry mushroom.

2.7. Statistical analysis

Three specimens of each mushroom species were used, and all the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD), and further 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. In the case of proximate composition, free sugars, fatty acids, tocopherols and organic acids a student´s *t*-test was used because there were less than three groups. This treatment was carried out using SPSS v. 22.0 program.

3. Results and Discussion

3.1. Chemical composition

The results of *Hericium erinaceus* and *H. coralloides* proximate composition, free sugars and fatty acids contents are shown in **Table 1**. Carbohydrates, total available carbohydrates (TAC) and fiber fraction, were the most abundant macronutrients, and among them fiber provided a higher contribution than TAC and free sugars. Insoluble dietary fiber (IDF) content was higher than soluble dietary fiber (SDF); this tendency

and content values are in agreement with the ones reported in literature for mushrooms in general (Kalac, 2012). *H. erinaceus* presented the double content of proteins (which contribute to a higher energetic value), but contents slightly lower of TAC, fibers and fat than *H. coralloides*.

Arabinose, mannitol and trehalose were found in both samples, but arabinose was the most abundant sugar, followed by mannitol and trehalose (**Table 1**). *H. erinaceus* gave the highest amount of total free sugars due to the highest concentration of arabinose and mannitol. In literature, arabinose has been reported as one of the minor sugars present in mushrooms (Kalac, 2012), but for the species studied herein, arabinose was the major sugar. High levels of arabinose seem to be a characteristic of the genus *Hericinum* (Keong, Rashid, Ing & Ismail, 2007; Han, Ye & Wang, 2013). Regarding fatty acids composition of *H. erinaceus*, saturated fatty acids (SFA) predominated over polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids, due to the high contents of palmitic (C16:0) and stearic (C18:0) acids (**Table 1**). For *H. coralloides* the contents of SFA, PUFA and MUFA were similar. Oleic (C18:1n9c) and linoleic (C18:2n6c) acids were also found in higher percentages, mainly in *H. coralloides*.

Mushrooms have been reported in literature as sources of mineral elements (Mattila et al., 2001; Kalac, 2012). In the present study, the macroelements Ca, Mg, Na and K, and the microelements Fe, Cu, Mn and Zn were identified in the two samples (**Table 2**). K was the macroelement present in higher levels, followed by Na, Mg and Ca in both samples; Fe was the microelement detected in higher amounts, followed by Zn, Cu and Mn, also, in both samples. Several authors described K as the major macroelement in mushrooms (Kalac, 2013), which is in agreement with our results. Nevertheless, calcium and sodium have been described in literature as the less abundant ones (Kalac, 2013). *H. coralloides* showed higher contents of macro and microelements, mostly Fe,

probably due to the environmental conditions of the mushroom growth that have influence on the concentration of metal absorption by mushrooms (Gençcelep et al., 2009).

Regarding tocopherols, α-isoform was found in both species, but in higher amounts in *H. erinaceus*, while β- and γ-isoforms were only identified in *H. erinaceus*, and δ-isoform only in *H. coralloides* (**Table 3**). The content in total tocopherols was similar in both species. Despite not having β- and γ-tocopherols in its composition, *H. coralloides* gave a significant content of δ-isoform. Concerning to organic acids, oxalic, malic and fumaric acids were also quantified in both species (**Table 3**), but malic and fumaric acids were the most abundant ones. *H. erinaceus* presented the highest total organic acids content, due to the high contribution of malic acid.

3.2. Bioaccessibility studies and antioxidant potential

The results of antioxidant activity (reducing power, free radicals scavenging activity and lipid peroxidation inhibition) of the phenolic extracts, *in vitro* digested fruiting bodies and *in vitro* digested phenolic extracts are expressed in **Table 4**.

In the literature it is possible to find some reports on the antioxidant activity of *H. erinaceus*. Mau, Lin & Song (2002) reported EC_{50} values of 5.06 mg/mL and 4.46 mg/mL in the DPPH and ferrycianide Prussian blue assays, respectively. Otherwise, Unekwu, Audu, Makun & Chidi (2014) also studied the antioxidant activity of *H. erinaceus* and reported a weak activity in the mentioned assays. The value described by Mau et al. (2002) in Folin-Ciocalteu assay (12.05 mg GAE/g extract) was similar to the one obtained in the present study. As far as we know, there are no previous reports on the antioxidant activity of *H. coralloides*.

For almost all the antioxidant activity assays, the phenolic extracts showed the highest potential, presenting the lowest EC_{50} values and the highest concentration in phenolic acids (**Table 4**). Nevertheless, in the Ferricyanide/Prussian blue assay, *in vitro* digested phenolic extracts presented the highest reducing power.

The phenolic extracts revealed the highest antioxidant activity for almost all the assays, followed by the *in vitro* digested phenolic extracts and the *in vitro* digested mushrooms. The decrease in the antioxidant activity after gastrointestinal digestion is related with the decrease in the phenolic content present in those samples. During gastrointestinal digestion, phenolic acids may undergo structural modifications caused by drastic pH variations (mainly alkaline pH conditions), by the action of the enzymes used in the digestion that can alter the molecules by changing the bioactive groups such as the loss of hydrogens that results in a significant loss of the antioxidant activity, and to the presence of phenolic acids linked to other molecules or to the food matrix that difficults the cross through dialysis membrane (Bermúdez-Soto, Tomás-Barberán & García-Conesa, 2007; Rodríguez-Roque et al., 2013).

Phenolic acids are described as being important compounds that contribute to the antioxidant activity of mushrooms due to the presence of OH groups in their structures that are known for their ability to scavenge free radicals (Heleno et al., 2012). Thus, a significant loss or change of those OH groups during the gastrointestinal digestion can result in a decrease in their antioxidant capacity.

In the present study, we can attribute the loss of antioxidant activity to the decrease in phenolic acids (gallic, *p*-hydroxybenzoic and *p*-coumaric acids) concentration observed for *in vitro* digested phenolic extracts (**Figure 1**) and, mostly, in mushroom fruiting bodies.

The results obtained are in agreement with other reports in the same subject; Rodríguez-Roque et al. (2013) studied the changes in phenolic compounds (isoflavones) and antioxidant activity of soymilk after *in vitro* gastrointestinal digestion and also verified that gastric digestion significantly influenced the release of bioactive substances from the soymilk matrix. The authors concluded that after *in vitro* gastrointestinal digestion the concentration of those bioactive molecules was significantly lower than in the non-digested ones, as also described by Bouayed et al. (2011) that studied the presence of phenolics, flavonoids, anthocyanins and the antioxidant activity of apples at different phases of simulated digestion using an extended *in vitro* model. These authors described that after *in vitro* digestion the concentration of these compounds was significantly lower as well as the antioxidant activity.

It should be highlighted that after *in vitro* digestion, the mushrooms kept antioxidant capacity (antioxidant activity and phenolic acids), but in lower levels, being the percentage of dialyzed phenolic acids of 9.65% and 7.89% for *H. erinaceus* and *H. coralloides*, respectively. Although being less active after gastrointestinal digestion, the bioactive molecules are still bioaccessible.

4. Conclusion

As far as we know, this is the first report on the chemical composition of *H. erinaceus* and *H. coralloides*, studying also their antioxidant properties after *in vitro* gastrointestinal digestion. The wild samples of *H. erinaceus and H. coralloides* are low caloric foods (low fat contents) and rich sources of carbohydrates (namely, fibers and free sugars), proteins, minerals and also bioactive compounds (namely, phenolic compounds and tocopherols). The phenolic extracts presented the highest antioxidant activity that is in agreement with the highest concentration in phenolic acids found in

those samples before *in vitro* digestion, meaning that after *in vitro* gastrointestinal digestion, the bioactive compounds can suffer structural changes (losing the OH groups responsible for the antioxidant activity) that decreased the antioxidant properties. Nevertheless, they are bioaccessible and can have different bioactivities.

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Table 1. Proximate composition, free sugars and fatty acids in the two wild edible mushroom fruiting bodies.

	Hericium erinaceus	*Hericium coralloides*	Student´s *t*-test *p*-value
Proximate composition (g/100 g dw)			
Ash	3.49±0.20	9.31±0.47	<0.001
Total carbohydrates	79.36±0.32	81.06±0.34	0.003
Total available carbohydrates	31.29±0.92	38.04±0.24	0.001
Total Dietary Fiber	41.32±1.46	44.28±0.94	0.008
Insoluble Dietary Fiber	38.66±1.88	40.06±0.77	0.346
Soluble Dietary Fiber	2.66±0.29	4.33±0.42	0.001
Total Free Sugars (g/100 g dw)	23.63±0.94	10.79±0.51	<0.001
Arabinose	17.46±0.70	6.25±0.32	<0.001
Mannitol	5.63±0.22	3.86±0.17	<0.001
Trehalose	0.54±0.07	0.68±0.03	0.032
Proteins	15.40±0.38	7.25±0.15	<0.001
Fat	1.75±0.27	2.38±0.14	0.007
Energy (kcal/100 g dw)	394.79±0.95	374.67±1.84	<0.001
Fatty acids (relative percentage)			
C16:0	37.57±2.23	23.34±0.66	<0.001
C18:0	7.61±0.72	6.78±0.48	0.079
C18:1n9c	26.11±1.25	33.74±0.39	<0.001
C18:2n6c	25.10±0.35	30.90±0.20	<0.001
SFA	47.57±1.47	33.51±0.13	<0.001
MUFA	26.80±1.19	34.59±0.39	<0.001
PUFA	25.63±0.28	31.90±0.22	<0.001

dw- dry weight; Palmitic acid (C16:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c). SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids. The difference to 100% corresponds to other 20 less abundant fatty acids (data not shown). In each row, different letters mean significant differences between samples (p<0.05). The number of replications was n=9.

Table 2. Macro and microelements in the two wild edible mushroom fruiting bodies.

	Hericium erinaceum	*Hericium coralloides*	Student´s *t*-test *p*-value
Macroelements (mg/100 g dw)			
Ca	44.35 ± 3.58	83.75 ± 3.24	<0.001
Mg	85.57 ± 3.49	134.00 ± 3.80	<0.001
Na	586.78 ± 2.20	678.04 ± 11.32	<0.001
K	1188.05 ± 13.14	1778.47 ± 139.37	<0.001
Microelements (mg/100 g dw)			
Fe	6.77 ± 0.06	77.96 ± 3.64	<0.001
Cu	0.22 ± 0.02	0.72 ± 0.07	<0.001
Mn	0.09 ± 0.01	0.31 ± 0.03	<0.001
Zn	2.11 ± 0.12	4.76 ± 0.48	<0.001

dw- dry weight. In each row, different letters mean significant differences between samples (p<0.05). The number of replications was n=9.

Table 4. Bioaccessibility studies and antioxidant potential of phenolic extracts and fruiting bodies of the two wild edible mushrooms.

		Hericium erinaceus			*Hericium coralloides*		
		Phenolic extract	*In vitro* digested mushroom	*In vitro* digested phenolic extract	Phenolic extract	*In vitro* digested mushroom	*In vitro* digested phenolic extract
Antioxidant activity							
Reducing power	Folin-ciocalteu (mg GAE/g extract)	13.41±0.41^{a}	2.76±0.01^{c}	5.61±0.03^{b}	16.29±0.15^{a}	3.00±0.04^{c}	8.41±0.15^{b}
	Ferricyanide/Prussian blue (EC_{50}; mg/mL)	3.47±0.07^{b}	9.40±0.22^{a}	2.43±0.03^{c}	1.63±0.12^{b}	4.10±0.02^{a}	1.23±0.00^{c}
Scavenging activity	DPPH scavenging activity (EC_{50}; mg/mL)	24.53±0.77^{c}	60.14±3.78^{a}	43.94±1.96^{b}	22.53±0.49^{c}	64.45±2.09^{a}	56.31±2.37^{b}
Lipid peroxidation inhibition	β-carotene/linoleate (EC_{50}; mg/mL)	0.27±0.00^{c}	21.51±1.83^{a}	4.42±0.11^{b}	0.28±0.00^{c}	16.37±0.45^{a}	8.73±0.67^{b}
	TBARS (EC_{50}; mg/mL)	1.37±0.03^{c}	4.93±0.40^{b}	7.81±0.99^{a}	0.74±0.09^{c}	3.70±0.03^{b}	5.68±0.19^{a}
Phenolic compounds							
Gallic acid	(µg/100 g dw)	76.25±0.04^{a}	8.01±0.40^{c}	15.63±0.38^{b}	133.16±0.75^{a}	9.92±0.12^{c}	22.03±0.20^{b}
p-Hydroxybenzoic acid	(µg/100 g dw)	73.99±2.43^{a}	tr	1.10±0.14^{b}	94.59±1.40^{a}	tr	2.10±0.03^{b}
p-Coumaric acid	(µg/100 g dw)	138.02±0.08^{a}	19.81±0.03^{c}	39.74±0.16^{b}	146.96±1.77^{a}	19.64±0.01^{c}	39.36±0.05^{b}
Total phenolic acids	(µg/100 g dw)	288.25±2.48^{a}	27.82±0.37^{c}	55.37±0.54^{b}	374.70±1.12^{a}	29.55±0.13^{c}	61.39±0.25^{b}

tr- Traces; Concerning the Folin-Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}- Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay. DPPH- 2,2-diphenyl-1-picrylhydrazyl radical; GAE- Gallic acid equivalents; TBARS- Thiobarbituric acid reactive substances. Trolox (commercial standard) was used as positive control ($EC_{50} \leq 0.04$ mg/mL). In each row, and for each mushroom species, different letters imply significant differences ($p<0.05$). The number of replications was n=9.

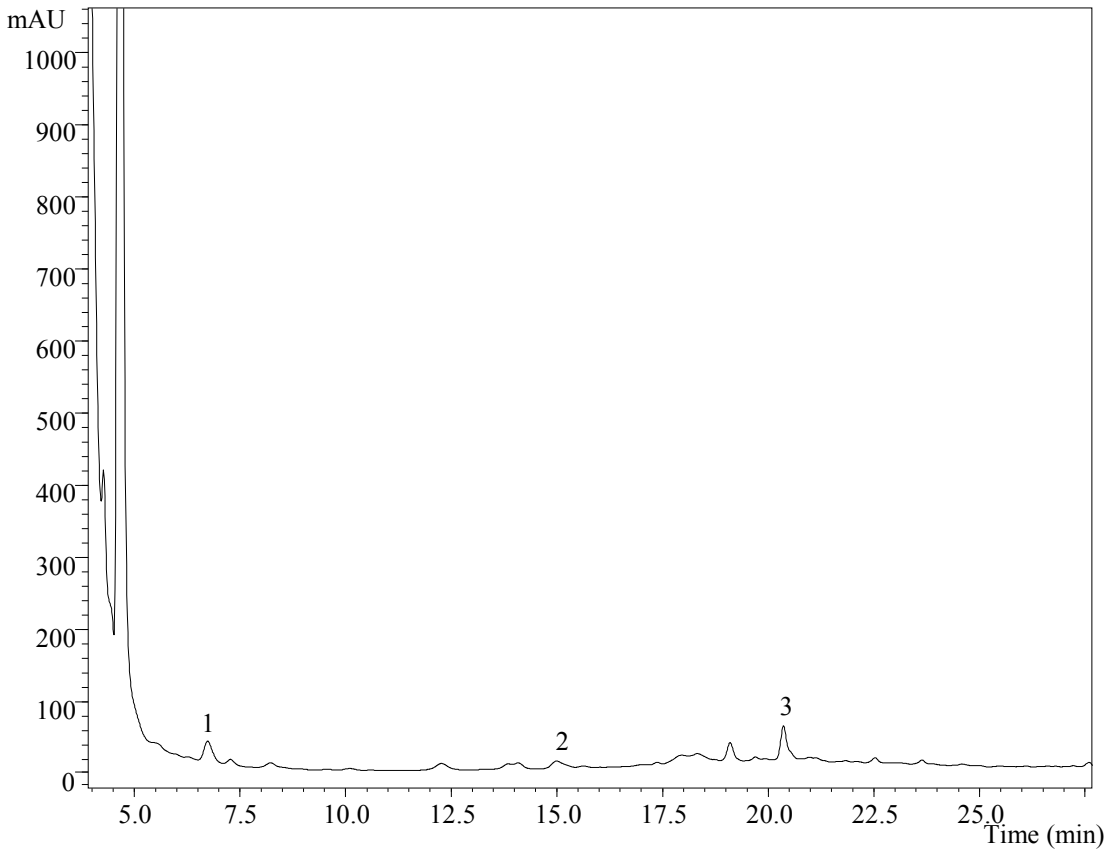

Figure 1. Chromatographic profile of *Hericium erinaceus in vitro* digested phenolic extract recorded at 280 nm. 1-Gallic acid; 2- *p*-Hydroxybenzoic acid; 3- *p*-Coumaric acid.

Table 3. Tocopherols and organic acids in the two wild edible mushroom fruiting bodies.

	Hericium erinaceus	*Hericium Coralloides*	Student´s *t*-test *p*-value
Tocopherols (μg/100 g dw)			
α-tocopherol	46.10±1.30	30.10±0.76	<0.001
β-tocopherol	54.90±1.82	nd	-
γ-tocopherol	11.28±1.52	nd	-
δ-tocopherol	nd	80.87±7.42	-
Total tocopherols	112.28±1.00	110.97±6.65	0.658
Organic acids (g/100 g dw)			
Oxalic acid	0.06±0.00	0.09±0.00	<0.001
Malic acid	3.09±0.04	0.47±0.05	<0.001
Fumaric acid	0.60±0.01	0.37±0.00	<0.001
Total organic acids	3.75±0.03	0.93±0.05	<0.001

dw- dry weight; nd- not detected. In each row, different letters mean significant differences between samples (p<0.05). The number of replications was n=9.