Journal of Food Composition and Analysis 67 (2018) 149–158

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original research article

Metal content and crude polysaccharide characterization of selected mushrooms growing in Romania

Daniela Elena Zavastin[a], Gabriela Biliuță[b], Gianina Dodi[c], Ana-Maria Macsim[b], Gabriela Lisa[c], Simona Petronela Gherman[a], Iuliana Gabriela Breabăn[d], Anca Miron[a], Sergiu Coseri[b,*]

[a] "Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Pharmacy, 16 University Street, 700115, Iasi, Romania
[b] "Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Street, 700487, Iasi, Romania
[c] "Gheorghe Asachi" Technical University, Faculty of Chemical Engineering and Environmental Protection, 73 Prof. dr. docent Dimitrie Mangeron Street, 700050, Iasi, Romania
[d] "Alexandru Ioan Cuza" University, Faculty of Geography and Geology, 20A Carol I Boulevard, 700505, Iasi, Romania

ARTICLE INFO

Keywords:
Food analysis
Food composition
Metal content
Crude polysaccharide
Antioxidant activity
Armillaria mellea
Boletus edulis
Cantharellus cibarius

ABSTRACT

Three selected mushrooms growing in Romania, *i.e. Armillaria mellea* (Vahl.: Fr.) P.Kumm., *Boletus edulis* Bull. and *Cantharellus cibarius* Fr., were analyzed in terms of metal (measured in fruiting bodies) and crude polysaccharide content. Inductively coupled plasma mass spectrometry (ICP-MS) technique was used to determine the metal contents, revealing the abundant presence of several microelements such as copper (varied from 15.8 mg/kg in *A. mellea* to 64.1 mg/kg in *C. cibarius*), iron (varied from 118 mg/kg in *B. edulis* to 221 mg/kg in *A. mellea*) and zinc (varied from 77.3 mg/kg in *A. mellea* to 188 mg/kg in *C. cibarius*). Selenium content varied from 0.2 mg/kg in *C. cibarius* to 4.18 mg/kg in *B. edulis*. The yield of the crude polysaccharides content in mushrooms varied from 3.6% in *Boletus edulis,* to almost 7% in *Armillaria mellea* and their characterization was employed by Nuclear Magnetic Resonance and Infra-Red Spectroscopy, Gel Permeation Chromatography and Thermogravimetric Analysis. The antioxidant activity of the detected polysaccharides was evaluated by means of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging and ferrous ion chelating assays. The best DPPH radical scavenging activity was found in the crude polysaccharides extracted from *A. mellea* (CE_{50} = 222 ± 3 μg/mL).

1. Introduction

Mushrooms belong to a large category of valuable nutritious foods, because they contain biologically active compounds that help maintain optimal physical and mental health of the population when consumed in a current diet (Bernaś et al., 2006; Chang and Miles, 2004). Mushrooms are low in calcium but instead contain phosphorus, copper and cobalt. Their composition varies from one species to another depending on the substrate they grow (Kalač, 2009, 2010; Bernaś et al., 2006; Wang et al., 2014a,b).

Carbohydrates are the main components of the fruiting body. In the human body, carbohydrates are used as energy source in the synthesis of glycoproteins and glycolipids (Kohlmeier, 2014). In the cell walls of fungi, chitin, a water insoluble polysaccharide, can be found in a proportion of 80–90% of their dry weight (Kalač, 2009). In mushrooms the carbohydrate content may vary between 12 and 75% dry weight (Kalač, 2009, 2010; Kohlmeier, 2014; Akata et al., 2012). In recent years, numerous polysaccharides with cytotoxic (Wu et al., 2012; Chang and Miles, 2004; Zhang et al., 2007), antioxidant (Lung and Chang, 2011), and anti-inflammatory effects have been isolated from various species of mushrooms and characterized. The polysaccharides isolated from mushrooms consist mainly of mannose, xylose, rhamnose, glucuronic acid, galacturonic acid, galactose and fucose (Wang et al., 2013; Luo et al., 2012; Zhang et al., 2011). Polysaccharides with immunomodulatory activity isolated from mushrooms are used today as adjuvants in the treatment of malignancies, and also for production of antibiotics and vaccines. For example, lentinan is used, since 1985, in Japan, as an adjuvant in the treatment of gastric cancer, being administered along with radiotherapy and chemotherapy (El Enshasy and Hatti-Kaul, 2013).

The bioavailability of mushroom polysaccharides depends on their degree of branching, molecular weights and conformation (triple helix, single helix or random coil structure) (Meng et al., 2016). Although human digestive enzymes are unable to hydrolyze the β-glucosidic bonds of mushroom β-glucans (Singdevsachan et al., 2016), some studies, conducted on rats, reported an increase in the antioxidant defense

* Corresponding author.
E-mail address: coseris@icmpp.ro (S. Coseri).

https://doi.org/10.1016/j.jfca.2018.01.011
Received 16 January 2017; Received in revised form 5 January 2018; Accepted 7 January 2018
Available online 10 January 2018
0889-1575/

Fig. 1. Fotographs of *Armillaria mellea* (*A. mellea*) left, *Boletus edulis* (*B. edulis*) center and *Cantharellus cibarius* (*C. cibarius*) right. Courtesy of http://ciupercomania.blogspot.ro/.

Table 1
Collection information of the studied edible mushrooms from natural ecosystems.

Species	Sample codification	Collection period	Collection area	Trophic status	Edibility
Armillaria mellea ((Vahl.: Fr.) P.Kumm.)	AM	October 2012	Dobrovat forest (Iasi county)	saprotrophic	edible with caution
Boletus edulis (Bull.)	BE	September 2011	Poiana Stampei area (Suceava county)	mycorrhizal	excellent
Cantharellus cibarius (Fr.)	CC	September 2011	Poiana Stampei area (Suceava county)	mycorrhizal	excellent

system after the administration of mushroom extracts (Rathore et al., 2017; Reis et al., 2017). The polysaccharides isolated from *Catathelasma ventricosum* (Liu et al., 2013), *Opuntia dillenii* (Zhao et al., 2011) or *Agaricus bisporus* (Jeong et al., 2010) caused a decrease in insulin levels and an improvement in total cholesterol in diabetic mice.

Mushrooms nutritional value is given, first, by the protein content. In mushrooms, proteins represent the second major component, with variations between 12 and 60% dry matter (Kalač, 2009). The protein content of mushrooms varies from one species to another, and also for the same species collected in different regions (Faure et al., 2014). Mushrooms have lower protein content than products originating from animals, but two times higher than asparagus, three times higher than rice and approx. equal to that of soybean (Chang and Miles, 2004).

Armillaria mellea (*A. mellea*), *Boletus edulis* (*B. edulis*) and *Cantharellus cibarius* (*C. cibarius*), Fig. 1, are one of the most appreciated mushrooms, their flavor and texture making them superior to other mushrooms.

Several studies in the last years have revealed that these mushrooms possess various biological properties including antitumor, antimicrobial, immunomodulatory as well as antioxidant activities (Akata et al., 2012; Wu et al., 2012; Lung and Chang, 2011; Chang et al., 2013; Wang et al., 2013; Luo et al., 2012; Zhang et al., 2011; Sun et al., 2009; Villares et al., 2013). Till now, few studies focusing on the possible biological effects of these mushrooms collected from Romania (Vamanu and Nita, 2014) have been done.

According to IFOAM EU GROUP (2012) Romania's main export products are cereals wild mushrooms, and berries. As mushrooms can be contaminated with toxic metals from different sources (from the environment, processing, degradation and spoilage, and food contact materials), it is important to evaluate their potential risk to human health (Niksic et al., 2016).

In this study, the metal contents of *A. mellea*, *B. edulis* and *C. cibarius* collected from Romania was investigated; in addition, the crude polysaccharides were isolated and characterized.

2. Material and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade. Nitric acid (HNO_3) (65%), acetone, potassium bromide, deuterium oxide (D_2O), sodium sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), catechin, ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemicals (Sigma-Aldrich Chemie GmbH, Steinheim, Germania). Ethanol 96% and absolute methanol were provided by Chemical Company (Chemical Company, Bucharest, Romania). Metranal® 3, Strawberry Leaf was purchased from Analytika (Analytika, Prague, Czech Republic), bovine serum albumin (BSA) from Fluka (St. Louis, USA), D-glucose from Lach-Ner (Lach-Ner, Neratovice, Czech Republic) and pullulan from Shodex Denko (Shodex Denko, Japan).

2.2. Mushroom samples

The study was conducted on three species of edible mushrooms from natural ecosystems (Table 1), collected from Dobrovat forest, Iasi County, (*A. mellea*,) and Poiana Stampei area, Suceava County, Romania (*B. edulis* and *C. cibarius*). The mushroom samples were identified by Prof. dr. Catalin Tanase, Laboratory of Mycology and Phytopathology, Faculty of Biology, "Alexandru Ioan Cuza" University Iasi, Romania.

Voucher specimens were deposited in the Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania.

After sampling, the fruiting bodies (approximately 2 kg of each mushroom species) were cleaned of soil and debris with a soft brush and a plastic knife without washing and air dried in shade and powdered.

2.3. Determination of metal content

The dried and powdered samples were dehydrated (105 °C for 24 h) and then digested as described by Ouzouni et al. (2007) with minor changes. Briefly, 1 g of mushroom powder was digested with 5 mL of HNO_3 (65%) and 1 mL of H_2O_2 and maintained for 4 h at 70 °C. After cooling, a volume of 20 mL of ultrapure water was added to each sample and the mixture was again heated at 70 °C for 6 h. This process was repeated twice in order to obtain clear solutions. After filtration through Whatman no. 42 filter paper, the samples were transferred to suitable containers. The glassware was pre-washed overnight in a 10% HNO_3 solution and then rinsed with ultrapure water.

The metals content was determined by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700× ICP-MS system. Metranal® 3, Strawberry leaf was used as quality control material. Metal concentrations were determined on a dry weight basis.

2.4. Crude polysaccharide isolation

Dried and powdered mushroom material was first extracted with 500 mL of 96% ethanol and then with methanol:water mixture (1:1, v:v) using the procedure described elsewhere (Zavastin et al., 2015).