Food Chemistry 157 (2014) 323–331

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms

Mustafa Özyürek*, Mustafa Bener, Kubilay Güçlü, Reşat Apak

Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 15 October 2013
Received in revised form 7 February 2014
Accepted 12 February 2014
Available online 22 February 2014

Keywords:
Wild edible mushrooms
Microwave-assisted extraction
Total phenolic content
Total antioxidant capacity
Antioxidant activity

ABSTRACT

A microwave-assisted extraction (MAE) process for polyphenols from three wild edible mushrooms was studied. The optimal extraction conditions were found to be methanol concentration of 80%, extraction temperature of 80 °C, and extraction time of 5 min. Different antioxidant assays (i.e., total antioxidant capacity (TAC) and total phenolic content (TPC)) were utilized to evaluate the antioxidant capacity of the methanolic extracts of *Terfezia boudieri* Chatin, *Boletus edulis*, and *Lactarius volemus*. The reactive species scavenging activities of these extracts were also investigated *in vitro*. High contents of phenolic and flavonoid compounds may be the major contributors to the observed high antioxidant activities of these extracts. *B. edulis* showed the higher TAC and TPC; highest inhibitory effect on DPPH and on other studied reactive oxygen species (ROS). MAE showed obvious advantages of high extraction efficiency with lower solvent consumption in terms of high antioxidant capacity/activity of extracts achieved within the shortest time.

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1. Introduction

'Oxidative stress' conditions emerge as a result of the generation of an unbalanced excess of reactive oxygen species concomitant with a change in cellular redox status, in which biological macromolecules (i.e., proteins, lipids and nucleic acids) can suffer oxidative damage causing tissue injury leading to various diseases (Halliwell & Gutteridge, 1999). Almost all organisms have established antioxidant defences to protect them against oxidative damage. Exogenous dietary antioxidants, which can scavenge free radicals and oxidants, contribute to the defence system as beneficial protecting agents for the human body (Mayakrishnan et al., 2013). As a result, the consumption of dietary antioxidants have been suggested for preventing serious diseases originating from oxidative stress.

The consumption of natural foods, such as fruits, vegetables and juices provides protection against various important diseases (Ames, Shigenaga, & Hagen, 1993). The search for new products with antioxidative properties is a very attractive field of research. Mushrooms have been used for many years as nutritional food and food flavouring materials in soups and sauces, due to their unique and subtle flavour. Mushrooms contain various polyphenolics recognized as excellent antioxidants due to their ability to scavenge free radicals (Hirano et al., 2001). Wild edible mushrooms are widely consumed in many countries, especially by local populations of lower income, and have been found to possess good antioxidant properties well correlated with their phenolic content (Barros, Ferreira, Queiros, Ferreira, & Baptista, 2007).

Anatolian Peninsula (i.e., the Asian part of Turkey) is rich in the diversity of mushrooms as well as medicinal plants. Turkish people have a tradition of using a number of wild edible mushrooms essentially for food rather than for medicinal purposes, e.g., treatment of infectious diseases (Akyuz, Onganer, Erecevit, & Kirbag, 2010). Anti-proliferative and anti-tumour effects, primarily in human cell lines, have been reported for polysaccharides, i.e., acidic and neutral compounds with different types of glycosidic linkages, as well as some that are bound to protein or peptide residues such as polysaccharide–protein complexes, extracted from various mushrooms; prevention of oncogenesis, enhancement of immune responses, and induction of apoptosis of tumour cells are possible mechanisms governing the antitumour and anti-proliferative effects of polysaccharides in mushrooms and their extracts (Wasser, 2002). *Terfezia boudieri* Chatin is a kind of desert truffles. Desert truffles are seasonal and socio-economically important fungi. These truffles are edible and grow wild in the southeast Anatolian part of Turkey. Desert truffles are a rich source of protein, amino acids, fatty acids, minerals and carbohydrates (Bokhary & Parvez, 1993). *Boletus edulis* is a delicious mushroom growing in many districts of Europe, North America, and Asia. Fresh and dried species

* Corresponding author. Tel.: +90 212 4737070; fax: +90 212 4737180.
E-mail address: mozyurek@istanbul.edu.tr (M. Özyürek).

http://dx.doi.org/10.1016/j.foodchem.2014.02.053
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are found in oriental restaurants, gourmet and health food stores. The flavour of dried *B. edulis* including odour and taste is marvellous-nutty, earthy, and meaty all at once (Tsai, Tsai, & Mau, 2007). *Lactarius volemus* is widely distributed in the warm temperate or northern districts of the Northern Hemisphere, and is known as an edible mushroom (Shimono, Hiroi, Iwase, & Takamatsu, 2007). The seasonal character of wild mushroom collection cause difficulties in the distribution and marketing as fresh products, and consequently, there is very little information concerning the antioxidant/antiradical activities of the species tested in this study, such as *B. edulis* (Fernandes et al., 2013), and *L. volemus* (Ozen, Darcan, Aktop, & Turkekul, 2011). To fill this literature gap, the antioxidant capacity of wild edible mushrooms was determined in this work by using the optical sensor-based CUPRAC method (Bener, Özyürek, Güçlü, & Apak, 2010). The chromogenic oxidizing reagent of the CUPRAC assay (Apak, Özyürek, Güçlü & Karademir, 2004), bis(2,9-dimethyl-1,10-phenanthroline)copper(II), is simple, diversely applicable to both hydrophilic and lipophilic antioxidants, stable and easily available at low cost. The CUPRAC method has been successfully applied to food plants (apricot, herbal teas, wild edible plants, herby cheese, etc.), and human serum for evaluating antioxidant properties. The main CUPRAC method was modified for measuring the hydroxyl radical scavenging activities of polyphenolics (Özyürek, Bektaşoğlu, Güçlü, & Apak, 2008), xanthine oxidase (XO) inhibition activity (Özyürek, Bektaşoğlu, Güçlü, & Apak, 2009), hydrogen peroxide scavenging activity of polyphenolics in the presence of Cu(II) catalyst (Özyürek, Bektaşoğlu, Güçlü, Güngör, & Apak, 2010), and development of a CUPRAC-based antioxidant sensor on a Nafion membrane (Bener et al., 2010). As another easy, rapid and low-cost assay, the DPPH free radical scavenging method (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998) was used for screening antiradical activity of sample extracts, based on decolorization of the DPPH radical upon single electron uptake from antioxidants (Koleva, Van Beek, Linssen, Groot, & Evstatieva, 2002). On the other hand, the measurement of the scavenging activity of reactive oxygen species (ROS) is also important for plant foods containing antioxidants. Although ROS cause adverse health effects such as lipid and protein oxidation, DNA strand break and base modification leading to tissue damage, they may have benign functions, including the activation of nuclear transcription factors, gene expression, cell signalling and regulation, and a defence mechanism to target tumour cells and microbial infections (Lee, Koo, & Min, 2004). Thus, in this work, the antioxidant/antiradical activities of the wild mushroom sample extracts were determined for the first time by a variety of protocols including the measurement of the scavenging activities of DPPH radical, hydroxyl radical, hydrogen peroxide, and superoxide anion radicals, the latter three ROS being the partially reduced and highly reactive metabolites of molecular oxygen.

Several extraction techniques and solvents are used for obtaining phenolic extracts from different food sources. Extraction of phenolics from mushrooms has been abundantly investigated in the last decades, focusing mainly on conventional solvent extraction. Interest in microwave-assisted extraction (MAE) has recently increased due to its special advantages (reduction in extraction time, solvent volume, and better extraction efficiency) over conventional solid–liquid extraction techniques (Ballard, Mallikarjunan, Zhou, & O'Keefe, 2010), because localized heating by microwaves increases the temperature of the solvent above its boiling point to enhance the extraction efficiency. This high temperature does not give rise to thermal degradation, as MAE with hexane–acetone (1:1, v/v) solvent mixture at 115 °C and for 10 min was shown earlier not to cause any degradation of the 14 tested phenols, recoveries ranging between 80% and 111% (Lopez-Avila & Young, 1994). In MAE, all parameters of the extraction can be achieved by a precise software-based control. The system is specially designed to operate at elevated temperature monitored by a fibre optic temperature probe. The effects on the composition and quantity of the phenolics of interest depend on many factors involved in MAE (Li et al., 2012). Due to the many factors such as microwave temperature, extraction time and solvent concentration that influence MAE, optimization of the extraction protocol is required. In the present study, a rapid and effective microwave-assisted method for extracting antioxidant compounds from wild edible mushroom species (*T. boudieri* Chatin, *B. edulis*, *L. volemus*) was optimized for the first time.

2. Materials and methods

2.1. Standards and reagents

The following chemical substances of analytical reagent grade were supplied from the corresponding sources: Neocuproine (Nc) (2,9-dimethyl-1,10-phenanthroline), DPPH (2,2-diphenyl-1-picrylhydrazyl), catalase from bovine liver (1340 U mg^{-1} solid), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), nitroblue tetrazolium chloride (NBT), methanol (MeOH), ethanol (EtOH) and the Folin–Ciocalteau reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA); Nafion® 115 perfluorinated membrane (thickness 0.005 in.) was purchased from Aldrich (Steinheim, Germany); Potassium sodium tartarate tetrahydrate, copper(II) sulphate, copper(II) chloride dihydrate, iron(II) chloride tetrahydrate, dimethyl sulfoxide (DMSO), hydrogen peroxide (30%, by wt.), $Na_2HPO_4{\cdot}2H_2O$, $NaH_2PO_4{\cdot}2H_2O$, ammonium acetate (NH_4Ac), sodium hydroxide, and sodium carbonate were purchased from E. Merck (Darmstadt, Germany); Disodium–EDTA, phenazine methosulphate (PMS) and sodium salicylate were purchased from Fluka (Buchs, Switzerland).

2.2. Samples

Wild edible mushrooms (*T. boudieri* Chatin, *B. edulis*, *L. volemus*):were purchased from local markets in different regions of Anatolia-Turkey. The mushrooms were lyophilized (Telstar LyoQuest, Terrassa, Spain), and kept at +4 °C in hermetically vacuum-sealed plastic bags prior to analysis. The yields of *T. boudieri* Chatin, *B. edulis* and *L. volemus* were 26.1%, 14.9% and 15.3%, respectively.

2.3. Sample preparation

The fine dried mushroom samples were extracted with the aid of a microwave-assisted extraction system (Milestone ETHOS ONE, Shelton, CT, USA). MAE was utilized in twelve Teflon closed vessels with an automatic fibre optic temperature control system, dried sample (0.2 g) was extracted with 20 mL methanol/water (80:20, v/v) at 80 °C for 5 min after 3 min temperature balancing time, and the microwave power (0–1500 W) was adjusted automatically according to temperature. The obtained methanolic extracts were filtered through a filter paper, then through 0.45 μm PTFE syringe filters (Whatman), and kept at +4 °C until use. All the following spectrophotometric assays on the extracts were carried out in $n = 5$ replicates.

2.4. Determination of total phenolic content (TPC)

Total phenolic content (TPC) of the methanolic extract was determined using the Folin–Ciocalteau (FC) method as described by Singleton, Orthofer, and Lamuela-Raventos (1999). The solutions used in the assay were prepared as follows: Lowry A: 2% aqueous Na_2CO_3 in 0.1 M NaOH; Lowry B: 0.5% $CuSO_4$ aqueous

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