Food and Chemical Toxicology 49 (2011) 1353–1360

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms

Mehmet Öztürk[a], Mehmet Emin Duru[a,*], Şeyda Kivrak[b], Nazime Mercan-Doğan[c], Aziz Türkoglu[d], Mehmet Ali Özler[e]

[a] Muğla University, Faculty of Sciences, Department of Chemistry, 48121 Muğla, Turkey
[b] Muğla University, Faculty of Engineering, Department of Metallurgy and Materials Engineering, 48121 Muğla, Turkey
[c] Pamukkale University, Faculty of Arts and Sciences, Department of Biology, 20020 Denizli, Turkey
[d] Nevsehir University, Faculty of Arts and Sciences, Department of Biology, 50300 Nevsehir, Turkey
[e] Ahmet Yesevi University, Faculty of Engineering and Pedagogy, Ecology-Chemistry Department, 160400 Kentav-Shymkent, Kazakhstan

ARTICLE INFO

Article history:
Received 12 December 2010
Accepted 14 March 2011
Available online 17 March 2011

Keywords:
Agaricus species
Fatty acids
Iron content
Antioxidant activity
Anticholinesterase activity
Antimicrobial activity

ABSTRACT

The fatty acids of *Agaricus essettei*, *Agaricus bitorquis* and *Agaricus bisporus* were investigated by using GC and GC–MS. The dominant fatty acids were found to be linoleic (61.82–67.29%) and palmitic (12.67–14.71%) acids among the 13 fatty acids detected in the oils. Total unsaturation for the oils was calculated as 77.50%, 77.44%, and 79.72%, respectively. *In vitro* antioxidant, anticholinesterase and antimicrobial activities were also studied. The ethyl acetate extract of *Agaricus bitorquis* showed the highest activity in β-carotene-linoleic acid, $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays, while the hexane extract of *Agaricus bisporus* exhibited the best metal chelating activity. The ethyl acetate and hexane extract of *Agaricus bitorquis* and the hexane extract of *Agaricus essettei* showed meaningful butyrylcholinesterase activity being close to that of galantamine. The extracts were found to be effective on Gram (+) bacteria, especially against *Micrococcus luteus*, *Micrococcus flavus, Bacillus subtilis* and *Bacillus cereus*. In conclusion, *Agaricus bitorquis* and *Agaricus essettei* demonstrated higher iron content, and better antioxidant, anticholinesterase and antimicrobial activities than those of *Agaricus bisporus* commonly consumed mushroom. Hence, *Agaricus* species, particularly *Agaricus bitorquis* might be useful as antioxidant agents and moderate anticholinesterase agents, and their extracts will probably be used for development of dietary foods, food products and additives.

1. Introduction

Edible mushrooms are valuable healthy foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus. They are also low in calories and fats (Leon-Guzman et al., 1997). Moreover, lectins, polysaccharides, polysaccharide-peptides, polysaccharide-protein complexes, lanostane-type triterpenoids, phenolic and flavonoid have been isolated from the some edible mushroom species (Tong et al., 2009; Zhang et al., 2007). Furthermore, in previous studies various biologic activities such as antioxidant, antibacterial, antifungal (Türkoğlu et al., 2007), immunomodulatory, antiviral (Moradali et al., 2007), antitumor (Tong et al., 2009; Zhang et al., 2007), anti-inflammatory (Komura et al., 2010; Regina et al., 2008), cytotoxic (Zhang et al., 2007), antiaromatase (Chen et al., 2006) and anticholesterole (Jeong et al., 2010) activities of these compounds and/or complexes were investigated.

Agaricus bisporus, the most cultivated mushroom in the world, exhibits a high proportion of fatty acids. Literature survey shows that palmitic, stearic, oleic and linoleic acids are the most abundant fatty acids in *Agaricus* species (Barros et al., 2007; Pedneault et al., 2008; Yilmaz et al., 2006). Polyunsaturated fatty acids such as linoleic acid and linolenic acid called essential fatty acids are essential for human's basal metabolism and have many beneficial effects on human health (Parikh et al., 2005). Lack of dietary essential fatty acids or their inefficient metabolism has been implicated in etiology of disease including cardiovascular disease and progression of it (Browni, 2005). Therefore, investigation of the fatty acid content in edible mushrooms has become a topic of great interest.

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ) are widely used in the food industry. However, the uses of these synthetic antioxidants are suspected that they are responsible for liver damage and carcinogenesis (Grice, 1988). Therefore, the investigation of the

* Corresponding author. Tel.: +90 252 2111494; fax: +90 252 2111472.
E-mail address: eminduru@yahoo.com (M.E. Duru).

0278-6915/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2011.03.019

antioxidants without any side effect from the food consuming safely by people have been become important. On the other hand, excess amount of free radical species, which causes oxidative stress, is associated with pathology of many diseases including Alzheimer's disease (AD). AD is a progressive neurologic disorder characterized by cognitive deficit and behavioral abnormalities in the patient (Soholm, 1998). Up to date, pathogenesis of Alzheimer's disease has not been fully clarified. The only known valid hypothesis being accepted is the lack of amount of acetylcholine, a neuromediator. Thus, the acetylcholinesterase inhibitory drugs were used for the treatment of Alzheimer's disease. However, most of these drugs have side effects. Thus, the development and utilization of more effective antioxidants of natural origin as well as anticholinesterase compounds are desired. In a report it is suggested that the usage of antioxidants may reduce the progression of Alzheimer's disease and minimize neuronal degeneration (Atta-ur-Rahman and Choudhary, 2001) by inhibiting acetylcholinesterase and butyrylcholinesterase which are chief enzymes in pathogenesis of Alzheimer's disease. It is an advantageous, particularly for a food, to have both antioxidant activity accompanied with acetylcholinesterase and butyrylcholinesterase inhibitory activity.

Nowadays, the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day-medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (Finch, 1998; Kunin, 1993). Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which have the potential of being sources of novel antimicrobial chemotherapeutic agents. There has been no much study on antimicrobial activity of *Agaricus* species to date. Thus, one of the aims of the study is to evaluate antimicrobial potential of *Agaricus* species against several Gram-positive and Gram-negative bacteria as well as against two yeast-like fungus, *Candida albicans* and *C. tropicalis*.

Agaricus bisporus was well investigated in many studies by various researchers. Especially, its fatty acid profile and its antioxidant activity was studied (Leon-Guzman et al., 1997; Barros et al., 2007; Yilmaz et al., 2006; Saiqa et al., 2008). However, *A. bitorquis* and *A. essettei* have not been investigated in detail. There is only a recent study on *A. bitorquis* (Saiqa et al., 2008). Furthermore, antioxidant and antimicrobial activities of the *A. bitorquis* and *A. essettei* and anticholinesterase activity of all *Agaricus* species tested were studied for the first time in this study.

Agaricus bisporus together with other *Agaricus* species are the most edible mushroom in the world, due to their high proportion of fatty acids and their nutritional value. Regarding to the consumption of *Agaricus* species in Turkey as well as in some other countries we aimed to investigate the fatty acid compositions, and iron contents of *A. bitorquis*, *A. essettei* and *A. bisporus* with antioxidant, anticholinesterase and antimicrobial activities by comparing with those of commercial antioxidants and that of galantamine. The objective of this study is also to make comparison of the fatty acid compositions, iron contents and the tested biologic activities of *A. bitorquis* and *A. essettei* with those of *A. bisporus*.

2. Materials and methods

2.1. Chemicals and spectral measurements

Quercetin, potassium persulfate, ferrous chloride, ferric chloride, pyrocatechol, quercetin, copper (II) chloride, ethylenediaminetetraacetic acid (EDTA) and boron trifluoride-methanol complex (BF_3:MeOH) were obtained from E. Merck (Darmstadt, Germany). β-Carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), Folin–Ciocalteu's reagent (FCR), 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferene), neocuproine and ammonium acetate butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$), Electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg), 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide and butyrylthiocholine chloride, galantamine were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Fluka Chemie (Fluka Chemie GmbH, Sternheim, Germany). All other chemicals and solvents were in analytical grade.

GC analyses were performed on a Shimadzu GC-17 AAF, V3, 230 V series gas chromatography (Japan), GC–MS analyses were carried out on Varian Saturn 2100 (USA), and Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384}, Molecular Devices (USA), at Department of Chemistry, Muğla University. The measurements and calculations of the activity results were evaluated by using Softmax PRO v5.2 software.

2.2. Mushroom materials and preparation of the extracts

Agaricus bisporus (J.E. Lange) Pilát, *Agaricus bitorquis* (Quél.) Sacc. and *Agaricus essettei* Bon. were identified by Dr. Aziz Türkoğlu and collected from Banaz-Uşak, Turkey in December 2007 Voucher specimens were deposited in the Herbarium of Department of Biology, University of Nevşehir and coded as Türkoğlu 4003, Türkoğlu 4004 and Türkoğlu 4005 Herbarium numbers, respectively.

Each *Agaricus* species were extracted separately with 2.5 L hexane for four times (24 h × 4) at room temperature (25 °C), filtered and evaporated to dryness *in vacuum*. The residue mushroom materials were similarly extracted, filtered and evaporated by using ethyl acetate and aqueous methanol solvents, successively. The yields of the extracts were given in Table 1.

Table 1
Yield percentages, total iron content, total phenolic and total flavonoid contents of the extracts of the three *Agaricus* species.[a]

Mushrooms extracts	Yields (%)	Fe content mg Fe^{3+}/kg mushroom	Phenolic contents μg PEs/mg extract [b]	Flavonoid contents μg QEs/mg extract [c]	Phenolic contents mg PEs/100 g mushroom [b]	Flavonoid contents mg QEs/100 g mushroom [b]
A. bisporus						
Hexane	0.68	206.20 ± 1.14	9.76 ± 1.00	5.12 ± 0.55	383.83 ± 2.36	544.27 ± 2.69
Ethyl acetate	0.65	42.38 ± 0.56	62.71 ± 0.23			
Methanol	5.84	59.87 ± 0.55	85.45 ± 0.36			
A. bitorquis						
Hexane	0.36	2964.54 ± 4.40	13.06 ± 0.46	4.34 ± 0.36	315.69 ± 3.56	378.90 ± 3.45
Ethyl acetate	0.92	56.21 ± 0.22	37.94 ± 0.12			
Methanol	10.33	25.10 ± 0.11	33.15 ± 0.10			
A. essettei						
Hexane	0.67	2618.46 ± 4.49	10.93 ± 0.59	7.09 ± 0.46	325.32 ± 3.00	668.68 ± 3.66
Ethyl acetate	0.72	23.49 ± 0.42	31.29 ± 0.31			
Methanol	12.00	27.11 ± 0.30	53.45 ± 0.20			

[a] Values expressed are means ± standard deviation of three parallel measurements ($p < 0.05$).
[b] PEs, pyrocatechol equivalents.
[c] QEs, quercetin equivalents.