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Antioxidant Properties of Edible Mushrooms

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ABSTRACT

Antioxidants or molecules with radical scavenger capacity gained attention a few years ago because of their potential protective effect against free radical damage. Epidemiological studies have shown that a higher intake of these compounds is associated with lower risk of mortality from cancer and coronary heart disease. Recent scientific studies confirmed that many edible mushrooms, described before by the traditional folklore of Asian culture as medicinal remedies towards a variety of disorders and diseases, indeed contained specific bioactive compounds. These were shown to lower cholesterol levels, to protect against tumours and other disorders, microbes and virus. Most of these properties were directly or indirectly related to the high antioxidant activity exhibited by specific compounds. The bioactive compounds are not yet well defined in all the species. Their antioxidant activity has been associated with minerals such as selenium and zinc. Also biomolecules such as ergothioneine, polysaccharide-protein complexes (β-D-glucans, etc.) phenolic compounds and, in lower quantities, peptides, vitamins A, C, and E (quantification depend on species) have been identified. However, flavonoids and related polyphenols are rare in mushrooms suggesting that these edible fungi might be an interesting source of new bioactive compounds different than plant antioxidants.

Keywords: *Agaricus*, fungi, *Lentinula*, *Pleurotus*
Abbreviations: **ABTS**, 2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid or $ABTS^{+\bullet}$; **DAA**, 6-deoxyascorbic acid; **L-Dopa**, 3,4-dihydroxyphenilalanine; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl or $DPPH^{\bullet}$; **EAA**, erythroascorbic acid; **ERT**, ergothioneine; **FRAP**, ferric reducing antioxidant power; **GDHB**, γ-L(+)-glutaminyl-3,4-dihydroxybencene; **GHB**, γ-L(+)-glutaminyl-4-hydroxybencene; **GRD**, gluthatione reductase; **GSH-PX**, glutathione peroxidase; **GST**, glutathione *S*-transferase; **HORAC**, hydroxyl radical averting capacity; **LDL**, low-density lipoprotein; **NORAC**, peroxynitrite radical averting capacity; **ORAC**, oxygen radical absorbance capacity; **SOD**, superoxide dismutase; **SORAC**, superoxide radical averting capacity; **TEAC**, trolox equivalent antioxidant capacity

CONTENTS

INTRODUCTION

There are approximately 12,000-20,000 species of macrofungi which produce fruiting bodies, most of them belonging to the Basidiomycetes class but there are also a few others from the Ascomycetes class. The life cycle of these fungi are complex and may involve a number of different morphological forms including mycelium and fruiting body stages. These macroscopic fruiting bodies are the so-called 'mushrooms' and they showed different degrees of edibility, some are palatable, even delicious and others deathly poisonous (Ooi 2000).

Actually, only a few of these mushrooms species can be cultivated since many of them need to form mycorrhizae with pines, oaks and other types of trees. The latter are usually harvested during autumn or spring depending on the

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place and specie from woods or shadowed locations. The common button mushroom (*Agaricus bisporus*) is the largest cultivated crop in the Western hemisphere covering more than 80% of the market followed by other species such as Oyster mushroom (*Pleutorus* spp.), and shiitake (*Lentinus edodes*). However, Asian countries had millenarian tradition on mushroom cultivation producing wider range of mushroom crops such as wood ear (*Auricularia* spp.), enokitake or winter mushroom (*Flammulina velutipes*), reishi o mannentake (*Ganoderma lucidum*), maitake (*Grifola frondosa*), pholiota or nameko (*Pholiota nameko*), white jelly or silver ear (*Tremella fuciformis*), straw mushroom (*Volvariella* spp.), etc.

Mushrooms have been traditionally utilized by medicine men as domestic remedies to cure diseases, improve health, to stimulate sexual reactions, hallucinations even to provoke death and harmful reactions. Scholars believed that the ancient Greek word 'agarikon' originates from a Scythian tribe called Agari who were well versed in the use of medicinal plants and employed a fungus called 'agarikon' (probably referring to *Fomitopsis officinalis*) as if it was the panacea, able to cure almost everything. It was so important to them that they took it as a totem and named themselves after the fungus (Stamets and Chilton 1983). The name of a fungal class: *Agaricales*, to which the common button mushroom (*Agaricus bisporus*) belongs, derivate from this word. At the present, numerous reports with a better scientific background have shown that mushrooms contain many bioactive compounds with significant medicinal properties such as immunomodulatory, anti-cancer, antioxidant, blood pressure-lowering, cholesterol lowering, liver protective, antifibrotic, anti-inflammatory, anti-diabetic, antiviral and antimicrobial activities, etc. (Lindequist *et al*. 2005).

Many plant crops have shown similar bioactivities. They contain compounds such as flavonoids (anthocyanins, isoflavones, etc.) and other polyphenols, glucosinolates, stilbenes, tannins, phytosterols, etc. pointed as the major metabolites responsible for the activities. However, although mushrooms are placed at the same location as vegetables in supermarkets, they belong to the fungal kingdom which is phyllogenetically far from plants and they share few biochemical and metabolic similarities. They contain many phenolic compounds but only a few of the potentially active molecules described for plants such as tocopherols, carotenoids, ascorbic acid or specific polysaccharides and many others still unidentified. Therefore, mushrooms are a potential source of new bioactive compounds.

ANTIOXIDANT PROPERTIES

The antioxidant properties of the most frequently consumed mushrooms (wild and cultivated) have been evaluated utilizing most of the standardized *in vitro* tests such as TEAC, β-carotene–linoleic acid method, conjugated diene method, scavenging ability on hydroxyl or DPPH radicals, chelating ability against ferrous ions, reducing power, inhibition of lipid oxidation, etc. and they showed intermediate values if compared to fruits and vegetables. For instance, cultivated mushrooms such as portabella and criminis (*A. bisporus*

Table 1 Edible mushroom species organized according to the scavenging effect of their extracts on 1,1-diphenyl-2-picrylhydrazyl radical and approx. EC_{50}.

Type of extract	EC_{50} or EC_{25} (mg/mL)	Order (from higher to lower DPPH scavenging capacity)	Reference
Ethanol	EC_{25} (1 - 2.5)	*Agaricus blazei* > *Agaricus cylindracea* > *Boletus edulis*	Tsai *et al*. 2007
Methanol	EC_{50} (0.01 - 0.02)	BHA > α-tocoferol > *Lepista nuda* > *Russula delica* > *Polyporus squamosus* > *Pleurotus ostreatus* > *Agaricus bisporus* > *Verpa conica* > *Boletus badius*	Elmastas *et al*. 2007
Methanol		*Leucopaxillus giganteus* > *Sarcodon imbricatus* > *Agaricus arvensis*	Barros *et al*. 2007
Methanol	EC_{50} (2 - 4.5)	*Dictyophora indusiata* > *Grifola frondosa* = *Hericium erinaceus* = *Tricholoma giganteum*	Mau *et al*. 2002
Methanol	EC_{25} (<0.25 - >1.5)	*B. edulis* > *Xerocomus chrysenteron* > *Suillus collitinus*	Sarikurkcu *et al*. 2008
Methanol 80%	EC_{25} (<0.4 - >0.8)	*A. bisporus* > *Hypsizigus marmoreus* > *Volvariella volvacea* > *Flammulina velutipes* > *Pleurotus eryngii* > *P. ostreatus* > *Lentinus edodes* > *H. erinaceus*	Fu and Shieh 2002
Ethanol 75%	EC_{50} (0.31 - 2.42)	*A. bisporus* > *V. volvacea* > *P. ostreatus* > *L. edodes* > *F. velutipes*	Lee *et al.* 2004
Methanol	EC_{25} (2 - 6)	*V. volvacea* > *L. edodes*	Cheung *et al*. 2003
Methanol	EC_{50} (4 - 8)	*P. ostreatus* > *L. edodes* (1) > *P. cystidiosus* > *F. velutipes* (1) > *L. edodes* (2) > *F. velutipes* (2)	Yang *et al*. 2002
Methanol	EC_{50} (0.1 - 3.5)	*Auricularia fuscosuccinea* (white strain) > *Auricularia mesenterica* > *Auricularia polytricha* > *Auricularia fuscosuccinea* (brown strain) > *Tremella fuciformis*	Mau *et al*. 2001
Methanol	EC_{50} (8.52 - 22.9)	*Lactarius deliciosus* > *Tricholoma portentosum*	Ferreira *et al*. 2007
Methanol	EC_{50} (6.95 - 33.7)	*Macrolepiota procera* > *Macrolepiota mastaidea* > *S. imbricatus* > *L. deliciosus*	Barros *et al*. 2007
Methanol	EC_{50} (1.24 - 14.5)	*Termitomyces tylerance* > *B. edulis* >*Morchella conica* = *Russula brevepis* = *Termitomyces microcarpus* > *Termitomyces shimperi* > *Cantharellus clavatus* = *Lentinus sajor-caju* > *Pleurotus djamor* > *Morchella anguisticeps* > *Pleurotus sajor-caju* > *Termitomyces heimii* > *Helvella crispa* > *Lactarius sanguifluus* = *Geastrum arinarius* > *Termitomyces mummiformis* = *Macrolepiota procera* > *Lentinus squarrulosus* > *Sparassis crispa* > *L. deliciosus* > *Cantharellus cibarius* > *Auricularia polytricha* > *Hydnum repandum*	Puttaraju *et al*. 2006
Methanol	EC_{50} (0.4 - 2.5)	*P. ostreatus* > *A. bisporus* = *M. esculenta* > *B. edulis* > *L. edodes*> *A. cesarea* > *C. cibarius* > *L. deliciosus*	Ramirez-Anguiano *et al*. 2007
Ethanol	EC_{50} (0.81 - 5.58)	*Clitocybe maxima* > *Pleurotus ferulae* > *P. ostreatus*	Tsai *et al*. 2009
Water (100ºC)	EC_{50} (1.10 - 10.9)	*T. heimii* > *T. mummiformis* > *B. edulis* > *C. clavatus* > *M. anguisticeps* > *L. squarrulosus* > *H. crispa* = *P. sajor-caju* = *R. brevepis* > *H. repandum* > *S. crispa* > *T. shimperi* = *L. deliciosus* > *L. sanguifluus* > *T. tylerance* > *T. microcarpus* > *P. djamor* > *G. arinarius* > *M. procera* > *M. conica* > *C. cibarius* > *A. polytricha* > *L. sajor-caju*	Puttaraju *et al*. 2006
Water (100ºC)	EC_{25}(0.12 - 1.0)	*Tricholomopsis rutilans* > *Suillus bellini* > *B. edulis* > *Suillus granulatus* > *Amanita rubescens* > *Suillus luteus* > *Hygrophorus agathosmus* > *Tricholoma ecuestre* > *Russula cyanoxantha*	Ribeiro *et al*. 2006
Water (100ºC)	EC_{25} (1 - 6)	*A. blazei* > *Agrocybe cylindracea* > *B. edulis*	Tsai *et al*. 2007
Water (100ºC)	EC_{25} (0.18 - 0.83)	*B. edulis* > *Suillus granulatus* > *Amanita rubescens* > *Russula cyanoxantha*	Ribeiro *et al*. 2008
Water (80ºC)	EC_{50} (0.88 - 3.5)	*A. bisporus* > *L. edodes* > *V. volvacea* > *P. ostratus* > *F.velutipes*	Lee *et al.* 2004
Water (100ºC)	EC_{50} (14.8 - 22.9)	*P. ostreatus* > *P. ferulae* > *C. maxima*	Tsai *et al*. 2009
Water (100ºC)	EC_{25} (2 - 1.2)	*L. edodes* > *V. volvacea*	Cheung *et al*. 2003

Table 2 Ergothioneine content in several mushroom species.

Common name	Strain	ERT (mg/g dw)	Reference	ERT (mg/kg fw)	Reference
White buttom	*A. bisporus*	0.21 - 0.47	Dubost *et al*. 2006, 2007	0.46	Ey *et al*. 2007
Crimini	*A. bisporus* (brown variety)	0.40 - 0.83	Dubost *et al*. 2006, 2007		
Portabella	*A. bisporus* (mature brown variety)	0.45 - 0.72	Dubost *et al*. 2006, 2007	0.93	Ey *et al*. 2007
Maitake	*G. frondosa*	1.13 - 1.84	Dubost *et al*. 2006, 2007		
Shiitake	*L. edodes*	1.98 - 2.09	Dubost *et al*. 2006, 2007	b.d.l.	Ey *et al*. 2007
Oyster	*P. ostreatus*	2.59 - 2.01	Dubost *et al*. 2006, 2007	118.91	Ey *et al*. 2007
King bolete	*B. edulis*	-		528.14	Ey *et al*. 2007
Chaterelle	*C. cibarius*	-		0.06	Ey *et al*. 2007
P. eryngii	*P. eryngii*	1.72	Dubost *et al*. 2006	-	
Winter (water extracts)	*F. velutipes*	3.03 (mg/mL)		-	Bao *et al*. 2008

b.d.l.: below detection level ; ERT: ergothioneine

varieties) showed radical scavenging activities (measured using the ORAC test) of respectively 9.7 and 9.5 μmoles trolox/g while carrots and green beans had an ORAC value of 5, red pepper 10 and broccoli 12 (Zhou and Lu 2006; Dubost *et al*. 2007). But, there are not many studies comparing plants, animals or food products with mushrooms, most of the publications were aimed to compare between different mushroom species.

The DPPH (2,2-diphenyl-1-picrylhydrazyl or $DPPH^{\bullet}$) scavenging activity was one of the *in vitro* tests more frequently utilized to evaluate the antioxidant activity of many mushroom species (**Table 1**). Results varied from author to author probably because of different environmental conditions, cultivation methodologies, development stage when they were harvested and genetic variation within strains. However, both methanol and water (or hot water) extracts showed antioxidant activities with EC_{50} values ranging from 0.01 to 34 mg/mL (similar to plants) and most of the commonly consumed mushrooms such as *Agaricus bisporus*, *Pleurotus ostreatus*, *Boletus edulis* and *Lentinus edodes* were generally highlighted as mushrooms with high antioxidant activities compared to other less common species.

ABTS (2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid or $ABTS^{+\bullet}$) is another radical often utilized to study the scavenging capacities of water-soluble compounds extracted from mushrooms. But as occurred with DPPH, results differed within the reports and it is hard to point to only one specific mushroom species as the best strain. For instance, *A. bisporus* showed the best ABTS scavenging capacity in a comparison carried out by Lee *et al.* (2004) with 5 other species, *Pleurotus eryngii* was highlighted by Choi *et al*. (2005) out of 8 other species and *B. edulis* and *Amanita cesaria* were the best of other different 8 species (Ramirez-Anguiano *et al*. 2007). Similarly when the reducing powder was analyzed *Termitomyces heimii* was identified as the best strain out of 23 species by Puttaraju *et al*. (2006), but according to the results of Elmastas *et al*. (2007) the methanolic extracts *of Russula delica* and *Verpa conica* were better than 5 other strains that were not included in the previous comparison. In addition, standard controls were not often included and results were differently expressed. Some authors calculated the antioxidant activities as their EC_{50} or EC_{25} values but others preferred to indicate them as effective percentages for a fixed concentration, etc. Therefore, it is hard to organize or classify all the analyzed mushroom species depending on their evaluated antioxidant properties.

ANTIOXIDANT COMPOUNDS IN MUSHROOMS

The compounds responsible for the described activities are still not completely identified. Many publications correlate the antioxidant activity with the total phenolic content (Mau *et al*. 2002; Cheung and Cheung 2005) but this does not apply to all species for instance for *Auricularia* sp. and *Tremella fuciformis* (Mau *et al*. 2001). No correlations were found with other compounds such as ascorbic acid, tocophenols, β-carotene, etc. However, these correlations were usually calculated by comparison of a few mushroom species which are phylogenetically located far from each other indicating that probably their metabolic pathways are different so they might have a completely different range of bioactive metabolites (it would be as if apples were compared with tomatoes because they both have phenolic compounds). It seems hard to believe that with such a wide amount of mushroom species, showing so different colors, textures, flavors and fruiting bodies shapes, their antioxidant properties were all due to a single or a couple of compound groups common for all the species.

Ergothioneine

One of the most powerful antioxidants found in some mushroom species is ergothioneine (ERT) and it is present in high amounts (**Table 2**) compared to other important sources such as liver (10.78–8.71), bean (13.49–4.52), garlic (3.11), egg yolk (0.68), trout (0.07 mg/kg fw), etc. *B. edulis* showed by far the highest ERT level among many food items, 528.14 mg/kg (Ey *et al*. 2007).

L-Ergothioneine (**Fig. 1**) is a water-soluble thiol compound (2-thioimidazole betaine) that has recently gained attention because it was identified as the biogenic key substrate of the organic cation transporter OCTN1. OCTN1 is implicated as a susceptibly factor in the etiopathology of autoimmune disorders such as rheumatoid arthritis and Crohn's disease (Ey *et al*. 2007). But this molecule showed many other biological functions including inhibition of metaloenzymes, modulation of the oxidative stress induced by ferric nitrilotriacetic acid sparing consumption of glutathione, modulating the potential toxicity of N-acetyl cysteine/H_2O_2 in neuronal cells and other immunomodulatory activities, as well as the *in vivo* functional ability to scavenge singlet oxygen, hydroxyl radicals and peroxyl radicals acting, thus, as a protective agent against oxidative stress (Misiti *et al.* 2001; Guijarro *et al.* 2002; Moncaster *et al.* 2002; Deiana *et al.* 2004; Colognato *et al*. 2006). Many publications singled out this compound as a potential nutraceutical compound to treat oxidative stress-induced pathologies (Aruoma *et al*. 1999; Franzoni *et al*. 2006).

Ergothioneine showed similar HOCl scavenging activity (EC_{50} = 70.68 μM) to other important phenolic compounds characteristic from plants such as flavonols (isoquercitrin EC_{50} = 77.88 μM), catechins (Epigallocatechin EC_{50} = 67.44 μM) and simple phenols (rosmarinic acid EC_{50} = 23.55 μM). It also showed similar TEAC values (0.87 mmol trolox/L) to myricitrin, higher than genistein

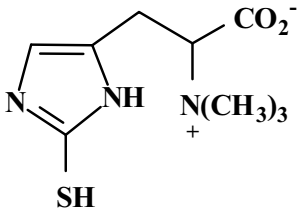

Fig. 1 Chemical structure of L-Ergothioneine.

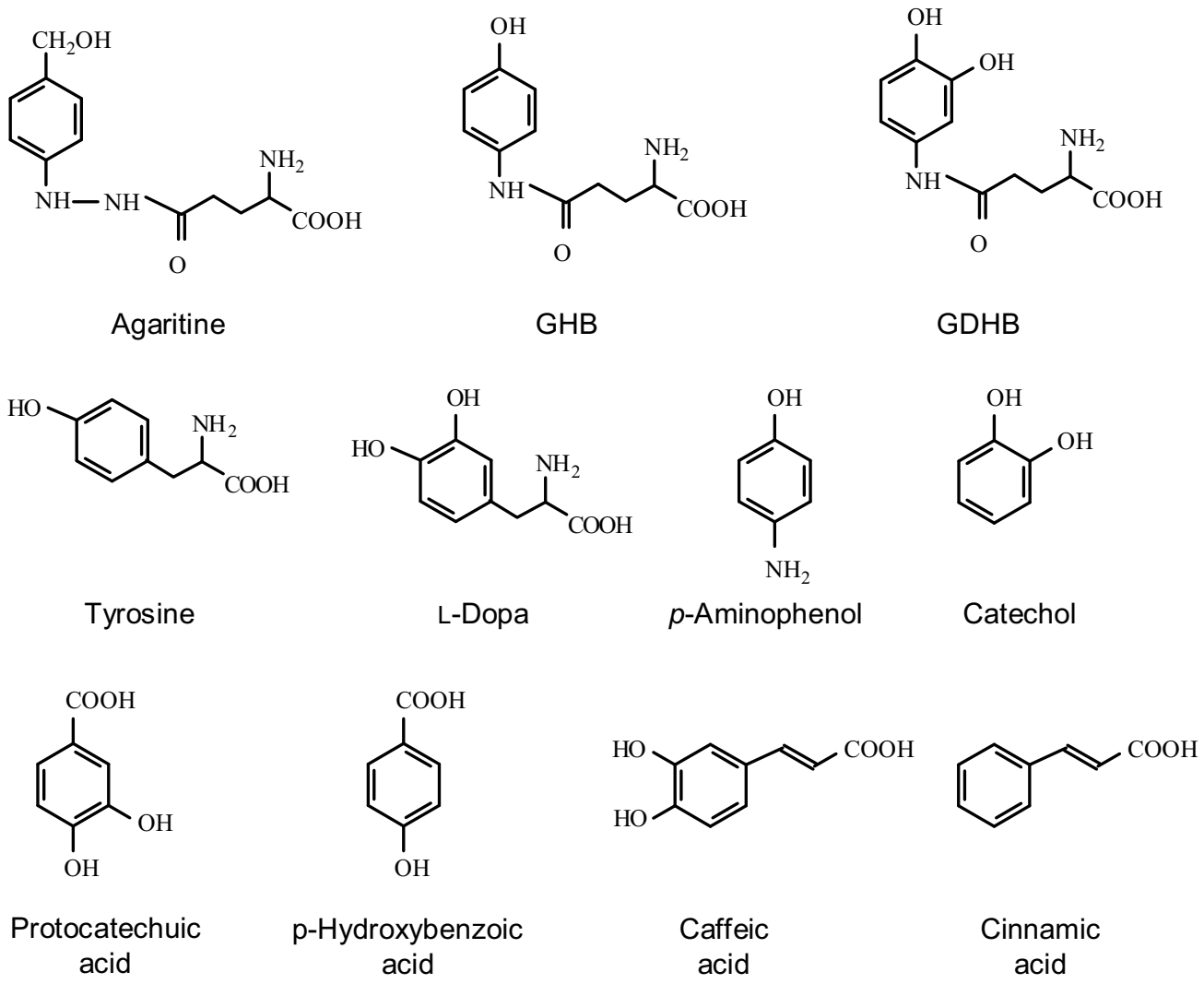

Fig. 2 Chemical structure of some phenolic compounds described in *Agaricus bisporus*.

but lower than quercetin, ellagic acid, etc. Its FRAP values (0.89 mmol Fe (II)/L) were lower than many flavonoids and phenols except for rhamnetin and isorhamnetin (Soobrattee *et al*. 2005).

This compound can also be easily extracted and concentrated, i.e. extractions from *Flammulina velutipes* fruiting bodies yielded a specific fraction containing 3.03 mg/mL ERT. However, the obtained extract showed higher DPPH scavenging activity and higher capacity to suppress lipid oxidation than authentic ergothioneine added at the same concentration suggesting that other water-soluble compounds might also be involved in the mushroom antioxidant properties (Bao *et al*. 2008).

Phenols and organic acids

Oxalic, citric, malic, quinic and fumaric acids are organic acids almost omnipresent in many mushroom species such as *Suillus bellini*, *Tricholomopsis rutilans*, *Hygrophorus agathosmus*, *Amanita rubescens*, *Russula cyanoxantha*, *Boletus edulis*, *Thricholoma equestre*, *Suillus luteus* and *Suillus granulatu.* Some of them also contained aconitic, ketoglutaric, succinic and shikimic acids. Quantification of the identified compounds indicated that malic and quinic acids were the main compounds in all analyzed species (35-84% of non-aromatic acids), usually followed by citric acid (9-10% of non-aromatic acids). However, no correlation was found between the total amount of organic acids and the antioxidant potential (as DPPH scavenging activities) (Ribeiro *et al*. 2006).

When the phenolic compounds were studied only *p*-hydroxybenzoic acid was identified in *A. rubescens*, *R. cyanoxantha* and *T. equestre* species. Tannic, gallic, protocatechuic, gentisic, vanillic, syringic, caffeic, coumaric, ferulic and cinnamic acids were detected in the water and methanolic extracts obtained from many species including common mushrooms such as *Boletus edulis*, *Lactarius deliciosus*, *Pleurotus sajor-caju*, *Cantharellus cibarius*, etc. The water extracts from *Termitomyces heimii* showed the highest amount of tannic acid (15.54 mg/g) while *Morchella conica* showed the highest levels of gallic acid (12.85 mg/g), *Helvella crispa* water extracts showed high values of protocatechuic (18.48 mg/g) and gentisic (4.89 mg/g) acids (Puttaraju *et al*. 2006; Ribeiro *et al*. 2006).

S. granulatus and *S. bellini* also showed high levels of phenolic compounds but they could not be identified. They were present in lower concentrations than organic acids and, according to Ribeiro *et al*. (2006), their total values did not correlate with their antioxidant activity.

Agaricus bisporus contained significant amounts of phenolic amino acids (tyrosine, γ-L(+)-glutaminyl-4-hydroxybencene (GHB), 3,4-dihydroxyphenilalanine and γ-L(+)-glutaminyl-3,4-dihydroxybencene (GDHB) (Soler-Rivas *et al*. 1998). Cinnamic, *p*-hydroxybenzoic, protocatechuic and caffeic acids were also detected (2.69, 0.51, 0.3, and 0.82 μg/g, respectively) (Mattila *et al*. 2001) (**Fig. 2**). They might be involved, perhaps together with ergothioneine, in the relatively high antioxidant activity observed for this strain. However, agaritine (γ-L(+)-glutamyl-4-hydroxymethylphenylhydrazine) was the phenolic compound present in higher concentration (on average 15.1 μmol/g in the skin of *A. bisporus* (**Fig. 3**) being higher in

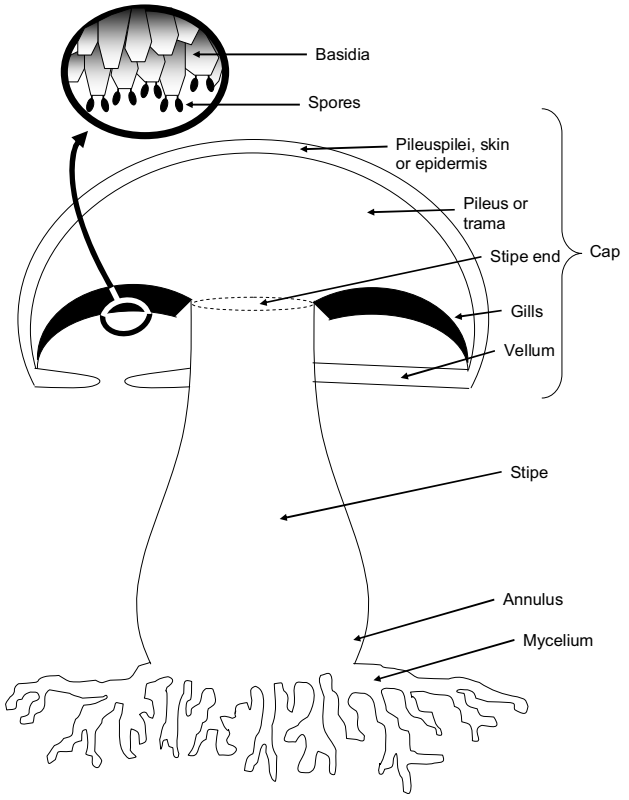

Fig. 3 Constitutive parts of *Agaricus bisporus* sporophores.

the gills depending on the strain) followed by free tyrosine (0.48 mg/g), GHB (0.70 mg/g) and its oxidation product GDHB (0.47 mg/g) and only some *A. bisporus* strains contained catechol and *p*-aminophenol (0.32 mg/g), although the latter is a highly unstable compound. L-Dopa (l-3,4-dihydroxyphenylalanine), the oxidation product of tyrosine was not detected (Jolivet *et al*. 1995). All these compounds are derived from the shikimate pathway as secondary metabolites (Soler-Rivas *et al.* 1998).

Unfortunately, most of the phenolic compounds from other mushroom species have not been individually quantified but only estimated as total phenolic content. A detailed quantification of these compounds was carried out using many mushroom fruiting bodies by Signore *et al*. (1997). Phenolic compounds from freshly collected fruiting bodies (of several basidiomycete genera) varied in the range 2.35–9.05 mg/g. Later, Yang *et al*. (2002) and Elmastas *et al*. (2007) found *Pleurotus* spp. and *Russula delica* mushrooms with even higher values (15.7–26 mg/g).

Some authors indicated similar phenolic concentrations in ethanol extracts from mushrooms such as *A. blazei*, *A. cylindracea* and *B. edulis* (5.8, 5.7, 5.73 mg/g, respectively) than for hot water extracts (5.67, 5.8 and 5.81 mg/g, respectively) (Tsai *et al*. 2007). However, other reports found significantly higher levels of phenolic compounds (sometimes more than 10-fold in some species) in water than in methanol extracts (Cheung *et al*. 2003; Puttaraju *et al*. 2006; Ramirez-Anguiano *et al*. 2007). Besides the high level of phenolic antioxidants, most of the mushrooms' water extracts also contained a high amount of oxidative enzymes. These peroxidases and polyphenol oxidases (laccases and tyrosinases) might use the phenols as substrates if they are activated, for instance, by a bacterial infection or tissue bruising, reducing the high antioxidant powder of the water extracts. However, the influence of the enzymes differed depending on the mushroom strain. The water extracts of mushrooms such as *L. edodes*, *Amanita cesarea* and *Pleurotus ostreatus* showed a remarkable reduction in their antioxidant activities if their oxidative enzymes were active while antioxidant levels remained similar if the enzymes were inhibited or separated from the phenolic substrates. Other species such as *Morchella esculenta* did not show any reduction suggesting that the high antioxidant activity found in the latter was due to other compounds which were not substrates of the oxidative enzymes (Ramirez-Anguiano *et al*. 2007).

Polysaccharides

At present, fungal polysaccharides are the subject of several studies because their specific carbohydrate composition and structure appears to confer important biological activities as antitumour and immumodulator agents. These polysaccharide fractions, usually bound to proteins forming specific complexes, showed antioxidant properties too. However, it is still not clear whether these important biological activities are or not related (Liu *et al*. 1997).

Polysaccharide extracts from *Ganoderma lucidum*, *Grifola umbellata*, followed by *Tricholoma lobayense*, *Tremella fuciformis* and *Volvariella volvacea* were reported to have scavenging effects on superoxide and hydroxyl radicals while lentinan (from *L. edodes*) and schizophyllan (polysaccharide extracts from *Schizophyllum commune*) had only negligible activity. Superoxide radicals could be quenched rapidly in the presence of PSK, a protein-bound polysaccharide from *Coriolus versicolor*, in a cell-free system consisting of hypoxanthine-xanthine oxidase. The superoxide radical scavenging activity of polysaccharide extracts appeared to depend on the amount of protein (peptide) present as polysaccharide-protein complexes. For example, lentinan and schizophyllan, which contained only a trace amount of protein in the polysaccharide samples, demonstrated almost no scavenging activities. The previously mentioned mushroom polysaccharides were not able to inhibit microsomal lipid peroxidation and on the contrary, lentinan and PSK significantly increased microsomal lipid peroxidation (Liu *et al*. 1997).

Chitosan, another polysaccharide extract obtained from *L. edodes* stipes showed interesting abilities such as scavenging of hydroxyl radicals and chelating of ferrous ions (Yen *et al*. 2007).

Besides their *in vitro* properties, mushroom polysaccharides were able to enhance the *in vivo* defense systems against oxidative damage. A polysaccharide-peptide complex (F22) extracted from *Pleurotus abalonus* fruiting bodies was able to increase activity and gene expression of antioxidant enzymes and reduced lipid peroxidation in senescence-accelerated mice (Li *et al*. 2007). Pleuran, another β-1,3-D-glucan extracted from *Pleurotus ostreatus* improved the rats' antioxidant status (increased superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) activity and glutathione reductase (GRD) activity in liver) and diminished the effect of dimethylhydrazine-induced precancerous lesions in rat colon (Bobek and Galbavy 2001) and a *Lentinus edodes* polysaccharide extract significantly raised activities of serum antioxidant enzymes and decreased levels of serum, mucosal interleukin-2 and tumor necrosis factor alpha in rats with oral ulceration (Yu *et al*. 2009).

Ergosterol and derivatives

The major fungal sterol, ergosterol, is abundant in all mushrooms species since it is a constitutive compound of the hyphae membranes and it is known as a vitamin D_2 (ergocalciferol) precursor. Ergosterol was shown to inhibit phorbol-12-myristate 1-acetate (TPA)-induced inflammatory ear edema in mice and vitamin D_2 has been shown to contribute to prevention of prostate and colon cancer. They both showed DPPH scavenging activities; in fact, part of the activity detected in methanol extracts was due to ergosterol-derivatives structures (Soler-Rivas *et al*. 2010). The peroxide of ergosterol, 5a,8aepidioxy-22E-ergosta-6, 22-dien-3b-ol (ergosterol peroxide), is also common in mushrooms and it was not only able to inhibit the growth of some cancer cells by inducing apoptosis, or inhibit inflammations and tumour in mice but was also able to decrease

lipid peroxidation of rat liver microsomes (Kobori *et al*. 2007).

Ergosterol in *B. edulis* was estimated between 9.61-4.89 mg/g depending on authors, *Chantharellus cibarius* 2.78-3.04, *A. bisporus*, *P. ostreatus* and *L. edodes* 7.8-4.4, *Suillus granulatus* showed 7.02 mg/g ergosterol and 0.8 mg/g fungisterol, other species such as *Russula cyanoxantha* and *Clitocybe nebularis* contained 1.28 and 1.04 mg/g fungisterol, too (Mattila *et al.* 2002; Teichmann *et al.* 2007; Kalac 2009).

Ergocalciferol levels from *C. cibarius* and *C. tubaeformis* ranged from 0.84 to 1.94 μg/g dw and these concentrations were rather stable to frying or freezing since, after processing, concentrations of 0.77-0.72 and 1.94 μg/g dw were still detectable (Mattila *et al*. 1999).

Vitamin C

According to a few publications, mushroom genera such as *Auricularia*, *Agaricus* and *Cantharellus* spp. showed moderate-high to low vitamin C contents. *Auricularia fuscosuccinea*, *A. polytricha* and *A. mesenterica* were the species with a higher level of vitamin C from all the analyzed samples ranging from 1.63 to 11.24 mg/g. Other more common species such as *Volvariella* species, *C. cibarius* and *Craterellus cornucopioides* contained 0.8-1.2 mg/g dw, *Flammulina velutipes*, *L. edodes* and *Calocybe gambosa* contained 0.4-0.6 mg/g followed by some *Pleurotus* (0.36-0.58 mg/g). No ascorbic acid was found in *B. edulis* and in the common button mushroom *Agaricus bisporus* although traces (0.03-0.04 mg/g) were described for other *Agaricales* spp (Mau *et al*. 2001; Fu and Shieh 2002; Barros *et al.* 2008a). However, in these works the ascorbic acid was quantified using a colorimetric method with 2,6-dichloroindophenol as reactive agent. This determination might be highly inaccurate depending on the analyzed matrix because many compounds such as tannins, sulphydryl compounds and metals are able to interfere oxidising the dye and leaving the ascorbic acid values overrated (Arya *et al.* 1998; Raghu *et al.* 2007).

HPLC determinations showed no ascorbic acid in mushrooms such as *Suillus* sp., *B. edulis*, *Hygrophorus agathosmus*, *Tricholoma equestre*, *Russula cyanoxantha*, etc. (Ribeiro *et al.* 2006).

However, Okamura (1994, 1998) in more detailed studies using an HPLC system specific for hydrazine-treated derivatives (osazones) detected ascorbic acid but in various forms, i.e. 6-deoxyascorbic acid (DAA), erythroascorbic acid (EAA) and their glycosides. Ascorbic acid compounds were analyzed in several mushroom species and *Pleurotus ostreatus* was found to contain all the previously mentioned forms as well as an unexpected analogue. The absorption spectrum of the analogue indicated it to be a glycoside, suspected from further investigation to be 5-*O*-(α-D-xylopyranosyl)-EAA. In *Lentinus edodes*, glycosides comprised 97% of total ascorbic acid, although small amounts of EAA and DAA were detected. In *Hypsizigus marmoreus*, *Flammulina velutipes* and *Agaricus bisporus*, single glycosides comprised >80% of total. In *Agrocybe cylindracea* and *Pholiota nameko*, DAA and EAA predominated. *Grifola frondosa* contained 78% DAA glycoside and 20% DAA.

Tocopherols

Many mushroom strains showed α-, γ- and δ-tocopherols, α-tocopherol usually being in higher concentrations. Concentrations ranged between 29.54 mg/g α-tocopherol in *Auricularia fuscosuccinea* and 2 mg/g in *A. blazei* and *A. cylindracea*. The latter showed high levels of γ- and δ-tocopherols, ranging from 1.6 to 0.7 mg/g (Mau *et al*. 2001; Tsai *et al*. 2007). β-Tocopherols were also described by Barros *et al*. (2008a) in mushroom strains such as *Agaricus* spp., *B. edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* (0.03–8.9 μg/g).

Other authors found total tocopherol concentrations ranging from 0.7 to 0.11 mg/g dw in *Clitocybe maxima*, *Pleurotus ferulae* and *P. ostreatus* (addition of α-, γ- and δ-tocopherols) and in *L. edodes*, *Pleurotus cystidiosus* and *P. ostreatus* but not in *Flammulina velutipes* (Yang *et al*. 2002). The tocopherol distribution within the sporophore tissues (**Fig. 3**) differed since higher γ-tocopherol concentration was found in *C. maxima* caps while δ-tocopherol was the dominant form detected in the stipe (Tsai *et al*. 2009).

Carotenoids

Mushrooms such as *L. giganteus*, *S. imbrictus* and *A. arvensis* showed very low β-carotene and lycopene concentrations: 1.88, 2.53 and 2.97 μg/g β-carotene and 0.69, 1.3 and 1 μg/g lycopene, respectively (Barros *et al*. 2007c) while other strains such as *Agaricus silvicola* (3.02 and 2.63 μg/g, respectively) and *Agaricus silvaticus* (5.42 and 2.63 μg/g, respectively) showed higher β-carotene and lycopene concentrations but the highest values were found in orange-colored mushrooms such as *Cantharellus cibarius* (13.56 and 5.06 μg/g) and *Clitocybe maxima.* The latter showed a higher β-carotene level in the cap (50 μg/g) than in the sporophore stipe (40 μg/g) (Barros *et al*. 2008a; Tsai *et al*. 2009).

Selenium

Mushrooms are also considered as an excellent source of selenium besides other minerals and vitamins such as copper, potassium, phosphorus, riboflavin (vitamin B2), pantothenic acid (vitamin B5) and niacin (vitamin B3). Selenium plays an important role in antioxidant systems throughout the human body by acting as cofactor of GSH-PX, enhancing α-tocopherol activities and helping the DNA repairing mechanisms. Relatively high selenium content was detected in *Boletus* mushrooms 1-5 mg/kg dw (*B. edulis*, *B. pinicola* and *B. aestivalis*) (Kalac 2009). However, mushrooms with lower Se levels such as *A. bisporus* might be fortified by adding sodium selenite to their cultivation substrate. Se-fortified mushrooms significantly increased both liver and mammary glutathione *S*-transferase (GST) activity and reduced total and anti-2,4-dihydrodiol-1,2-epoxide-deoxyguanosine adducts compared to feeding basal diet indicating that apart of enhancing the antioxidant status of rats, fortified mushrooms might be used as an effective method to retard chemically induced tumours (Spolar *et al*. 1999).

Flavonoids?

According to various authors mushroom species such as *B. edulis*, *Lactarius deterrimus*, *Suillus collitinus*, *Xerocomus chrysenteron*, *Laetiporus sulphurous*, *L. edodes*, *Agaricus* spp., *L. deliciosus*, *M. mastoidea*, *M. procera*, *Tricholoma matsutake* and *S. imbricatus* showed high levels of flavonoids (Choi *et al*. 2006; Barros *et al*. 2007c; Lim *et al*. 2007; Turkoglu *et al*. 2007; Barros *et al*. 2008a; Sarikurkcu *et al*. 2008). However, flavonoids were quantified in these publications as total flavonoid content using a colorimetric method which utilizes aluminium chloride ($AlCl_3$) as an agent apparently able to react with flavonoids showing a pink colour measured at 510 nm. This referred assay was used by Jia *et al*. (1999) to measure the flavonoid content in mulberry and it was, indeed, correlated with the flavonoid content evaluated by HPLC in these berries. However, $AlCl_3$ forms complexes with hydroxyls and neighbouring ketones and with ortho-dihydroxyl groups (Nikolovska-Coleska *et al*. 1995). Therefore, besides flavonoids, $AlCl_3$ might react with many of the endogenous phenolic compounds from mushrooms with structural similarities. Only one work detected by HPLC the presence of quercetin in a single sample of *S. luteus* (Ribeiro *et al*. 2006). However, this flavonoid might have been absorbed from closely located plants (perhaps forming mycorrhizae) because the fungal kingdom lacks the key enzymes to undergo the meta-

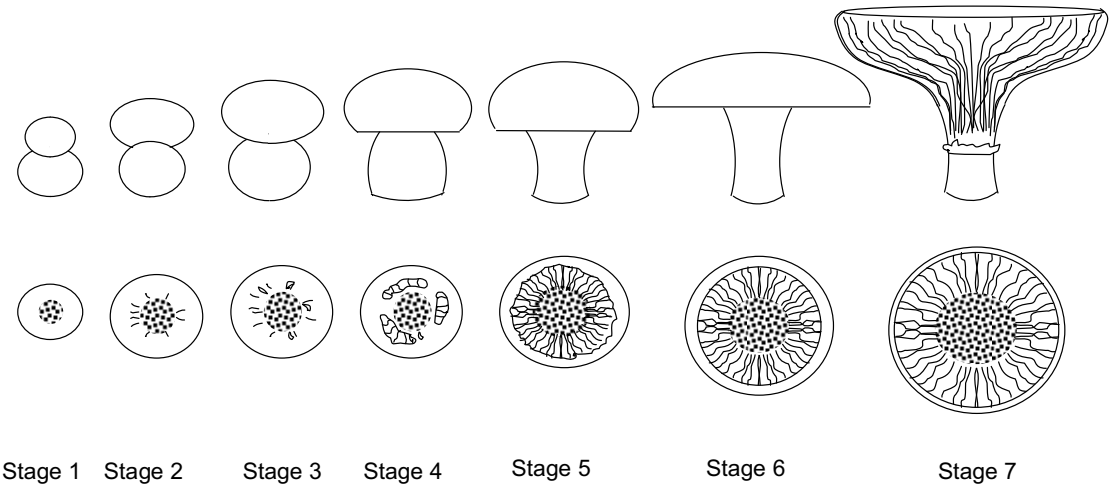

Fig. 4 ***Agaricus bisporus*** **developmental stages according to Hammond and Nichols (1975).**

bolic pathways to synthesize flavonoids from the phenolic compounds generated by the shiikimate pathway. According to the USDA, mushrooms are regarded as non-sources of flavonoids (Iwalokun *et al*. 2007).

FACTORS INFLUENCING THE MUSHROOM ANTIOXIDANT ACTIVITIES

As previously mentioned, many factors might influence the antioxidant capacities of edible mushrooms since they are cultivated or harvested from the woods until they are served as elaborated dishes ready to eat. Later on, mushroom antioxidants have to survive human digestion and pass through the intestinal barrier in order to exert their beneficial activities.

Influence of fruiting bodies development and culture conditions

A few reports indirectly described the influence of cultivation substrates or environmental conditions on mushroom antioxidants. For instance, mushrooms cultivated in the dark or wild mushrooms receiving day/night light cycles showed different Vitamin D_2 contents (Teichmann *et al*. 2007) being higher in the illuminated fruiting bodies. *A. bisporus*, a mushroom cultivated in the dark, showed Vitamin D_2 levels ranging from 0.3-0.6 µg/100g fw while wild mushrooms such as *B. edulis* and *Cantharellus* sp. showed 10.7-58.7 µg/100g fw. Vitamin D_2 is synthesized from ergosterol in the presence of light and the latter compound showed 19 fold higher activity as inhibitor of liposomal lipid peroxidation than vitamin D_2 (Wiseman 1993) therefore, higher vitamin D_2 content resulted in lower antioxidant properties.

Moreover, ethanol extracts from *A. bisporus* fruiting bodies harvested at different maturity stages (**Fig. 4**) showed effective antioxidant activities determined by the conjugated diene method but, fruiting bodies harvested at stages 1, 4 and 5 were more effective than those at stages 2 and 3. However, their reducing power and scavenging activities were not significantly different between the developmental stages (Tsai *et al*. 2008). *A. blazei* young and mature fruiting bodies showed similar antioxidant activities except for their chelating ability for ferrous ions, being higher in mature than young fruiting bodies. These differences were not due to their phenolic compounds but probably due to their variation on dicarboxylic acids levels (Soares *et al*. 2009). In *Lactarius piperatus* mushrooms, the highest antioxidant contents were obtained in the mature stage with immature spores (Barros *et al*. 2007a).

As previously indicated, some mushroom antioxidants seemed to be differently distributed within the sporophore constitutive tissues (**Fig. 3**). Methanol extracts obtained from gills showed higher DPPH and ABTS scavenging activities than the caps and stipes of *A. bisporus* fruiting bodies (Savoie *et al*. 2008) while caps appeared to contain higher reducing power and free radical scavenging capacity than their stipes in mushroom species such as *Lactarius deliciosus*, *Tricholoma portentosum*, *Russula cyanoxantha*, *B. edulis* and *Suillus granulatus* except for *Amanita rubescens*, which showed higher activity in the stipes. Differences seemed to correlate with variations in several constituents; for instance, phenolic compounds, organic acids and alkaloids, which were preferably fixed in the cap except for *B. edulis*, which showed equal alkaloid distribution, and *A. bisporus* stipes, which showed higher phenolic content (Ferreira *et al*. 2007; Ribeiro *et al*. 2008; Savoie *et al*. 2008).

Influence of industrial and domestic processing

The high antioxidant activities were usually measured in raw freshly harvested fruiting bodies, but an important part of the mushroom crops (approx. 35% for *A. bisporus*) are often submitted to industrial processes such as freezing, canning or drying to preserve the fruiting bodies during long transportation and storage. These treatments modified their chemical composition which means that their antioxidant properties might also change. However, not many reports describe their precise effect on antioxidant compounds or activities.

Freezing of *B. edulis* fruiting bodies reduced the ascorbic acid content (measured by the 2,6-dichloroindophenol method) from 0.18 to 0.16 mg/g due to the blanching pretreatment before cold storage. Afterwards, a 47% reduction was observed after 12 months storage at -25°C (Jaworska and Bernas 2009).

Canning of *A. bisporus* fruiting bodies in water, salt and citric acid or drying and re-hydrating *Boletus* mushrooms did not modify significantly their nutritional value only heating treatments seemed to decrease their level of dietary fibres (β-glucans) and phenolic compounds (Manzi *et al*. 2001, 2004). Heating was also the most detrimental process for DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition *of L. deliciosus*, *M. mastoidea*, *M. procera* and *S. imbricatus* fruiting bodies when compared with freezing or drying as preservative processes (Barros *et al*. 2007b).

Both fresh and processed mushrooms are usually submitted to culinary treatments before intake. Raw *A. bisporus* extracts showed higher DPPH and ABTS scavenging activities and higher total phenolic content than pickled mushrooms (prepared using a standardized traditional recipe). Both total phenolic content and antioxidants decreased in mushrooms fried in mustard oil prior to pickling, the decrease followed a strong negative correlation with increasing frying time but no further decrease in either total

phenolic or antioxidants was observed thereafter following pickling and storage of the mushrooms (Ganguli *et al*. 2006). Results demonstrated frying time to be a critical factor in the current traditional recipe for preparation of mushroom pickles. Other cooking treatments such as boiling or microwaving have been found more detrimental than frying (Soler-Rivas *et al*. 2009). The effect of different cooking methods was species dependent since *A. bisporus* water and methanol extracts were more resistant to heat treatments than *L. edodes* and *B. edulis* indicating the presence of different antioxidant compounds within the selected strains. Microwaving of *L. edodes* fruiting bodies increased their scavenging capacities during the first cooking minutes but later their EC_{50} increased. Results suggested that the thermal treatments at the beginning might improve antioxidants' extractability but later reduce their levels depending on the cooking time. Other reports (Manzi *et al*. 2004; Barros *et al*. 2007b) also proved that cooked mushroom showed lower nutrient concentration (including phenols) and lower antioxidant activity. Only the study by Choi *et al*. (2006) reported an increase in both phenol concentrations and antioxidant properties of *L. edodes* mushrooms. However, differences with respect to the first mentioned results could be due to unaccounted losses of moisture and soluble solids that concentrates the sample per unit weight and/or greater extractability depending on heating time or temperature.

Influence of human digestion and absorption

Culinary treatments seem to be more detrimental for mushrooms antioxidants than human digestion (Soler-Rivas *et al*. 2009). Grilled mushrooms were submitted to mastication, gastric and intestinal digestion following an *in vitro* digestion model and, depending on the mushroom species, mastication and gastric digestion reduced the ABTS and DPPH scavenging activities but later, the intestinal digestion step appeared to increase them to levels similar to grilled mushrooms, probably by liberating or generating new antioxidant compounds. Moreover, 47.6 and 33.4% of *A. bisporus* and *B. edulis* antioxidants, respectively were absorbed by Caco-2 monolayers suggesting that the observed antioxidant activity might be partially bioavailable. The scavenging capacity of the *L. edodes* bioavailable fraction was higher than that initially applied indicating that Caco-2 cells might transform original antioxidants from the digestates into other derivatives with higher antioxidant activity. Phenolic compounds and not proteins or digestion products from polysaccharides seemed to be related with the bioavailable antioxidant activity.

AGARICUS BISPORUS AND RELATIVES

The common buttom mushroom, *Agaricus bisporus* (J. Lange) Imbach, is the most widely cultivated species of edible mushrooms in the world. However, it has been considered less tasteful and nutritive that other wild species and with less bioactive properties than Asiatic species. However, recent studies indicated that this mushroom is an interesting strain because of the wide amount of biological properties that have been ascribed (Grube *et al*. 2001; Shi *et al*. 2002; Savoie *et al*. 2008).

Concerning the *A. bisporus* antioxidant activity, the ethanol extracts obtained from this species showed higher scavenging activity of DPPH, ABTS and H_2O_2 radicals than other cultivated mushroom crops such as *Volvariella volvacea*, *Flammulina velutipes*, *Pleurotus ostreatus* and *Lentinus edodes* and a higher effect on retarding emulsion oxidation on a corn oil-in-water system at 60°C and on retarding lard oxidation under heat treatment (130°C) than *Hypsizigus marmoreus*, *Volvariella volvacea*, *Flammulina velutipes*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Hericium erinaceus* and *Lentinula edodes* (Fu and Shieh 2002), although its extracts demonstrated the lowest scavenging activity against hydroxyl radicals. The aqueous and ethanol extracts of *A. bisporus* also effectively protected plasmid DNA from oxidative damage induced by peroxyl or hydroxyl radicals due to UV irradiation using the DNA strand nicking assay (Lee *et al.* 2004). The genoprotective effect was associated with tyrosinase, the enzyme that catalyzes the enzymatic browning that occurs when the mushroom tissue is infected or damaged. The effect was dependent upon the two catalytic activities of this enzyme using tyrosine as substrate. Tyrosinase catalyses the hydroxylation of substrates such as tyrosine (*mono*-phenol) to L-Dopa (the corresponding *di*-phenol) and the subsequent oxidation of L-Dopa to dopaquinone. These results were surprising since L-Dopa was normally associated with toxic pro-oxidant effects attributed to metabolic and autooxidative breakdown in the presence of molecular oxygen which produces highly unstable dopaquinones which spontaneously react rendering oxy radicals, H_2O_2 and other complex compounds. However, some authors also showed that L-Dopa stimulates cellular antioxidant defense mechanisms under certain conditions (Shi *et al*. 2002).

The most cultivated *Agaricus bisporus* variety is a white hybrid (usually the U1 strain developed at Host, The Netherlands) but there are many other strains with different cap colors including off-white to dark brown. Moreover, the fruiting bodies are usually harvested when their gills are not yet visible (stages 2-3, **Fig. 4**), but there are some brown varieties which are harvested when the caps are open (stages 4-5). The latter are commercialized under the name of Portobella mushrooms.

Within *A. bisporus* varieties, Portabellas showed higher antioxidant capacity (measured as HORAC, NORAC and SORAC tests related respectively to $HO^{\bullet}$, $ONOO^-$ and O_2^- radicals) compared to a white and a brown (crimini mushroom) strains and higher ergothioneine and total phenolic contents (Dubost *et al*. 2007). Differences were also significant between commercialized and wild *A. bisporus* strains such as X25 (white commercial), Bs0633 (brown wild) and Bs0118H (cream wild). The methanol extracts of the cream-colored mushrooms showed the highest radical scavenging activities and reducing power but the brown one showed higher catalase, GRDs and GSH-PXs activities (free radical-processing enzymes) in its water extracts (Savoie *et al*. 2008). When various species from the *Agaricales* genus were compared, *A. silvaticus* showed higher DPPH scavenging activities, antioxidant power (measured by electrochemical assays) and phenolic content than *A. arvensis*, *A. bisporus*, *A. romagnesii* and *A. silvicola* (Barros *et al*. 2008b).

The antioxidant properties of *Agaricus arvensis* were evaluated in detail using various methods (reducing power, DPPH radical-scavenging capacity, inhibition of erythrocyte hemolysis, and antioxidant activity using the β-carotene linoleate model system) and were compared with two wild edible mushroom species, *Leucopaxillus giganteus* and *Sarcodon imbricatus*. *A. arvensis* had the highest ascorbic acid and β-carotene concentrations of the 3 species but they all showed very low amounts. Only phenolic compounds were detected in high levels and according to authors, they might account for the good antioxidant properties found in the methanol extracts of the 3 analyzed species (Barros *et al*. 2007b).

Agaricus blazei (also called *Agaricus brasiliensis*) was also pointed out as an excellent source of ethanol-soluble (Oliveira *et al*. 2007) and thermostable antioxidants according to the results obtained in a particular system to screen for antioxidant activities. Cytosolic thioredoxin is a negative regulator for an oxidative stress responsive transcription factor, Yap1p (yeast AP-1-like transcription factor), i.e. this transcription factor is constitutively concentrated in the nucleus in the thioredoxin-deficient mutant (*trx1Δ/trx2Δ*) due to an impairment of the reactive O_2 species-scavenging activity of this mutant. Based on these findings, antioxidant activity was evaluated by monitoring the subcellular localization of Yap1p. As Yap1p is oxidized and accumulates in the nucleus in *trx1Δ trx2Δ* cells, antioxidant activity is easily identified by observing the localization of green fluo-

rescent protein (GFP)-tagged Yap1p. If exogenous substances taken in by *trx1Δ/trx2Δ* cells were able to function as antioxidants to reduce the oxidized form of Yap1p, GFP1-Yap1p would diffuse into the cytoplasm. *A. blazei* showed the highest activity of all the investigated mushroom strains (Izawa and Inoue 2004).

PLEUROTUS GENUS

Pleurotus spp. or oyster mushrooms are the second commercialized mushroom in the world after *A. bisporus*, but they are more commonly found in Western European supermarkets than in those in North America. Their cultivation conditions are less standardized than for the buttom mushroom but nowadays mushroom growers manage to obtain high yields with more or less controlled environments.

The methanol extracts obtained from *Pleurotus ostreatus* (3 different oyster varieties) showed the highest scavenging activities compared to others such as the white and yellow varieties of *Flammulina velutipes*, two *Lentinus edodes* strains and *Pleurotus cystidiosus*. These three oyster mushrooms showed a 54.3% scavenging of hydroxyl-free radicals while the other samples ranged from 29.2 to 36.6% at 40 mg/mL. However, all mushroom extracts demonstrated moderate to high antioxidant activity in the TBARS assay, with percentages of lipid peroxidation of 24.7-62.3 (at extract concentration 1.2 mg/mL), in comparison with a value of 66.1% with 10 mg/mL BHA. HPLC analysis revealed that the major antioxidant compounds in the mushrooms were phenols. According to the authors (Yang *et al*. 2002), ascorbic acid and β-carotene were not detected and tocopherols were only present in small amounts. The three oyster mushrooms also contained higher phenol content than the other samples.

In vivo experiments demonstrated that *P. ostreatus* extracts were effective agents to reduce the incidence and size of atherosclerotic plaques in rabbits (Lindequist *et al*. 2005) since the mushrooms extracts showed ability to inhibit lipid peroxidation and a pronounced hypocholesteremic effect because of the production of lovastatine, a powerful HMG-Co A reductase inhibitor (Bobek and Galbavy 1999). Both reactive oxygen species and increased levels of blood lipids are key elements in the pathogenesis of atherosclerosis. Moreover, other authors showed that the administration of *P. ostreatus* extracts to aged rats improved their antioxidant status during ageing and alleviated the hepatotoxicity induced by CCL_4 (Jayakumar *et al*. 2006, 2007).

Pleurotus citrinopileatus, a popular edible mushroom from Taiwan, also showed important physiological activities in both humans and animals. An *in vivo* study using hyperlipidaemic hamster rats indicated that powdered dry fruiting body, hot-water extract and 2 specific extracts significantly reduced serum triglycerides and total cholesterol levels as compared with control groups that received no mushroom additive. High-density lipoprotein levels in these experimental groups were also significantly higher than those in the negative control group. The rats that were fed with the extracts had higher serum GSH-PX, and SOD activities. The extracts showed *in vitro* DPPH free radical scavenging activities and ferric-reducing abilities and their major constituents were identified as ergosterol and nicotinic acid. Results suggested that *P. citrinopileatus* extracts might have *in vivo* antihyperlipidaemic and antioxidant activities (Hu *et al*. 2006).

Pleurotus eryngii was another mushroom with antioxidant activities similar to *Ganoderma lucidum*, a mushroom considered as medicinal because of its many biological activities tested *in vivo* (even with humans trials), including the antioxidant activities. Their ethanol extracts (compared with 8 edible mushrooms grown in Korea) showed the highest DPPH and ABTS scavenging activities. Authors found positive correlations between total phenols contents and these antioxidant activities (Choi *et al*. 2005).

The methanolic extract of *Pleurotus pulmonarius* fruiting bodies reduced carrageenan-induced and formalin-induced paw edema in mice. The activity was comparable to the reference diclofenac. The effect seemed to be related to the significant antioxidant activity of the extract since the EC_{50} value for hydroxyl-radical scavenging was 476 µg/mL and for lipid peroxidation inhibition 960 µg/mL (Lindequist *et al*. 2005).

Pleurotus florida extracts (water, methanol and ethyl acetate) showed hydroxyl radical scavenging activity and inhibition of lipid peroxidation and the methanol extracts also showed inhibition of tumor growth, still the mechanism of action is unknown but it seemed to be related to the protective effect of the extracts against DNA oxidation (Jose and Janardhanan 2000).

OTHER CULTIVATED MUSHROOMS CROPS

L. edodes is another cultivated mushroom which contains several therapeutic compounds with many biological activities including antioxidant properties (Ooi 2000). This mushroom and *Volvariella volvacea* were tested using methods to study their potential effect against lipid peroxidation of rat brain homogenate and against the oxidation of human low-density lipoprotein (LDL). Results indicated that both organic and aqueous extracts from those mushrooms showed high antioxidant activities with EC_{50} values of 0.11 and 1.05 mg/mL against lipid peroxidation (Cheung and Cheung 2005). Both mushrooms showed also the ability to inhibit haemolysis of rat erythrocyte induced by peroxyl radicals (Cheung *et al*. 2003). Because of these interesting properties Kitzberger *et al*. (2007) developed a method to produced food-grade *L. edodes* extracts using green technologies such as supercritical fluid extraction (SFE) to obtain antioxidant enriched fractions that could be used to prepare, for instance, functional foods with improved antioxidant properties.

As described for *L. edodes* and *V. volvacea*, the methanol extracts of *Agrocybe aegerita* showed high radical scavenging activities and inhibition of lipid peroxidation of rat brain homogenate, too. A sub-fractionation of the extract revealed that ethyl acetate fraction showed the most potent antioxidant activity and was further fractionated by Sephadex LH-20 column into 4 fractions. The third fraction showed very high radical scavenging activities and showed a similar extent of *in vitro* inhibition of human LDL oxidation to caffeic acid. Significant correlation was found between the total phenolic content and the activities (Lo and Cheung 2005).

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