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Agaricus bisporus and its by-products as a source of valuable extracts and bioactive compounds

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Abstract

Edible mushrooms constitute an appreciated nutritional source for humans due to their low caloric intake and their high content in carbohydrates, proteins, dietary fibre, phenolic compounds, polyunsaturated fatty acids, vitamins and minerals. It has been also demonstrated that mushrooms have health-promoting benefits. Cultivation of mushrooms, especially of the most common species *Agaricus bisporus*, represents an increasingly important food industry in Europe, but with a direct consequence in the increasing amount of by-products from their industrial production. This review focuses on collecting and critically investigating the current data on the bioactive properties of *Agaricus bisporus* as well as the recent research for the extraction of valuable functional molecules from this species and its by-products obtained after industrial processing. The state of the art regarding the antimicrobial, antioxidant, anti-allergenic and dietary compounds will be discussed for novel applications such as nutraceuticals, additives for food or cleaning products.

Keywords: *Agaricus Bisporus*; by-products; valorisation; extraction techniques; bioactive compounds

1. Introduction

Mushrooms are the fruiting bodies of macroscopic filamentous fungi and play essential roles in the contribution of nutrients to both, plant and animal kingdoms (Feeney, Miller, & Roupas, 2014). While the term "mushroom" is not itself a taxonomic category, it is used to refer to the fruits of the *Basidiomycota* and some *Ascomycota* phylums from the *Dikarya* fungi sub-kingdom (Ganeshpurkar, Rai, & Jain, 2010). The *Ascomycota* phylum is the largest group of fungi with approximately 33,000 species described across three subphyla; *Taphrimomycotina, Saccharomycotina* and *Pezizomycotina* with not all of them producing a fruiting body. *Basidiomycota* represents the second largest phylum with 30,000 identified species and three main lineages recognised in subphyla; *Ustilaginomycotina, Pucciniomycotina* and *Agaricomycotina*. The subphylum of *Agaricomycotina* produces basidiospores in various types of fruiting bodies, and they are recognisable due to conventional 'mushroom' characteristics (Carris, Little, & Stiles, 2012).

Fungi are distinctive from the plant kingdoms due to their lack of chlorophyll and inability to utilise photosynthesis in their metabolism. Alternatively, they are reliant on symbiosis, saprophytism, and parasitism; using several biochemical processes to exchange, degrade, and liberate organic matter. Many mushroom species are environmentally dependent, and factors such as temperature and relative humidity become major considerations in the successful cultivation of mushrooms for commercial purposes. Optimisation and regulation of temperature, growth substrate, air, and humidity have led to successful mushroom farms.

The global production of cultivated edible mushrooms has grown dramatically since the late 1990s thanks to appropriate conditions in farms and their recognition of their high nutritional value. In fact, mushroom production worldwide increased to 10, 378, 163 metric tons in 2016 (FAO, 2016), while the average *per-capita* consumption grown significantly in the last decades. *Lentinus edodes* and four other genera (*Pleurotus, Auricularia, Agaricus*, and *Flammulina*) account for 85 % of the world's total supply of cultivated edible mushrooms, being China the leading producer of cultivated edible mushrooms (Royse, Baars, & Tan, 2017). In particular, *Agaricus bisporus* (*A.bisporus*) is popular in the global food market, accounting for 15 % of total worldwide mushroom production.

However, a large amount of mushroom by-products are generated during the industrial production and their disposal could become a problem for mushroom producers due to difficulties in storing or re-using them. According to Schimpf and Schultz (2016) for every kg of edible processed mushroom, industry produces 5 kg of spent mycelium substrate, by-products of mushroom production, spent growth substrate or waste mushroom medium. In this context in which edible mushrooms show their high content in biologically active compounds, future practical applications based on their by-products valorisation into high valuable compounds might be developed by integrating the potential of these bioactive compounds and their high availability (Buruleanu, et al., 2018). The nutritional qualities of edible mushrooms and the health-beneficial effects of the bioactive compounds they contain have been recognised since ancient times due to their high protein, carbohydrate, fibre, vitamins, and essential amino acids contents (Fernandes et al, 2016). From the functional perspective, mushrooms are low in fat and cholesterol (Buruleanu, et al., 2018). The bioactivity exhibited by these compounds is also linked with the presence of phenolic compounds,

polyketides, terpenes, steroids, beta-carotenes, and some vitamins, such as A and C, all of them related with antimicrobial, antiviral, antioxidant or anti-inflammatory activities.

Although a few reviews on the composition and biological properties of edible mushrooms are available (Atila, Owaid, & Shariati, 2017; Muszyńska, Kała, Rojowski, Grzywacz, & Opoka, 2017), none of them have reported the detailed interrelationships among bioactive compounds and valorisation of mushroom by-products, in particular those from *A.Bisporus*. Therefore, this review aims to present an overview in the valorisation and the main compounds extracted from this mushroom species.

2. Morphology and nutritional composition of *Agaricus bisporus*

A.bisporus is an edible species of *Basidiomycota* that is extensively cultivated throughout Europe and North America (Ganeshpurkar, Rai, & Jain, 2010), contributing 35-45 % of total worldwide edible mushroom production (Rezaeian & Pourianfar, 2016). The consumption of *A.Bisporus* is second only to the baker's yeast *Saccharomyces cerevisiae* as the most commonly ingested fungal species.

Mushrooms share an innate anatomical structure but are also diversified in their evolutionary histories, being often used to characterise the species variation. Mushrooms structure consists of a cap/pileus structure, often textured externally with scales to protect the internal structure known as the gills/lamellae, functioning in spore dissemination when mushrooms are fully mature. The stape/stem of mushrooms connects the cap to the mycelial threads for nutrient transfer and consists of additional structures; the ring/annulus and cup/colva. The inferior portion of the mushroom (structures lower than the ring/annulus) is essential in the distribution of nutrients, chemical feedback and the viability of the reproductive lamellae. While sharing the innate mushroom structure, the pileus of the wild *A.Bisporus* species is pale grey-brown, with hemispherical broad flat scales fading towards the margins through maturity, its diameter ranging from 5 to 10 cm. The narrow and crowded lamellae are initially pink and darken to brown with a whitish edge as the mushroom matures. The spores are oval to round and measure approximately 4.5-5.5 x 5-7.5 μm^2. A distinguishing microscopic feature of this species is the atypical bearing of two spores from its basidia unlike the usual four from other *Agaricus* species. The cylindrical stipe is up to 8 cm long and 3 cm wide, bearing a narrow annulus, which is often streaked on the upper side (Mata, Medel, Callac, Billette, & Garibay-Orijel, 2016).

As an agricultural commodity, *A.Bisporus* is cultivated for commercial purposes and subsequently regulated to different grades and standards to ensure organoleptic qualities and consumer satisfaction. Depending on the size of the mushroom farm, the amount of waste ranges between 5-20 % of the production volume and results in the removal of mushrooms with excess stipe lengths, discolouration, and gross size variances. Despite these physical deformations, the constitution of the mushroom is innately similar, and the waste streams from larger mushroom farms could be valorised as a source of bioactive components.

2.1. Genomics

Commercial mushroom cultivation relies on the production of healthy *A.bisporus* species with favourable and reproducible characteristics. Improved composting techniques, optimised environmental conditions

and breeding selectivity have led to substantially improved yields and conforming organoleptic standards. Typical agronomic traits of mushrooms include yield, growth rates, maturation, size, colour, shape, firmness, aroma, and shelf life (Gao et al, 2016). These traits often have a complex genetic basis associated with quantitative gene loci and thus Genetically Modified Organisms (GMO's) are only viable if they meet the standards of the existing commercial strains. A large number of agronomic traits have been assigned to major loci on chromosomes 6 and 10, demonstrating their importance during cultivation. Since November 2011, genomic data concerning fungal species have been continually reported and updated within the 1000 Fungal Genomes project (Stajich, 2016). The resulting phylogenetic maps were compiled to demonstrate orthology since nearly 83 % of the predicted *A.bisporus* genes had homology with those in the public and JGI Mycocosm databases and conserved protein families within several fungal species.

2.2. Chemical composition and nutritional value

Many species of edible mushrooms grown widely in forests, while about 35 species are commercially cultivated, and 20 of them are produced at the industrial scale. *A.bisporus* is the most cultivated and consumed mushroom species worldwide, and consequently recent studies have focused on the nutritional value and increase of shelf-life in this particular species (Cardoso et al, 2019). Edible mushrooms are known to possess good quality protein, high fibre, high quantity of vitamins and minerals, lectins and also bioactive compounds like protein-polysaccharide complexes, β-glucans and polyphenols (Rezaeian & Pourianfar, 2016). Dry matter (DM) of different cultivated and wild-growing mushrooms is ranging between 83 and 285 g/kg, denoting their high water content that influences significantly in the fast ageing process of fresh mushrooms (Zsigmond et al, 2018). Secondary metabolites, such as phenolic compounds have been identified on a wide variety of mushrooms, and their nutritional and antioxidant activities have been reported (Boonsong, Klaypradit, & Wilaipun, 2016; Gasecka, Magdziak, Siwulski, & Mleczek, 2018). Most species of mushrooms are high in ergosterol. It is found within cell membranes of fungi in high concentrations and is a pro-vitamin form of vitamin D_2, the temporary exposure of mushrooms under UV radiation can encourage the production of vitamin D reducing the quantity needed for recommended daily intake levels (Feeney, Miller & Roupas, 2014). However, appreciable differences in the composition have been found among species and also within them, which can be partly due to the different development degree of mushrooms.

Regarding the polysaccharide fraction in the fruiting bodies of edible mushrooms, D-glucans are the main compounds (mainly β-D-glucans with small amounts of α-D-glucans); with chitin and other heteropolysaccharides in minor amounts. These polysaccharide components conform a tight polymeric network where the different D-glucan and chitin macromolecules may be either physically or chemically crosslinked to each other. Minor amounts of other polysaccharides, including galactans and mannans, can be also found in different mushroom species (Ruthes, Smiderle, & Iacomini, 2016). Structural proteins are also found in variable amounts in the fruiting bodies of edible mushrooms, and are commonly complexed with the polysaccharide and lipid components.

D-glucans comprise a heterogeneous family of homopolysaccharides, with D-glucopyranose as the unique

building block (monomer) that can be linked by α- or β- glycosidic bonds. Despite this simple monomeric structure, D-glucans can create very complex macromolecular architectures due to the stereochemistry of the β- and α-glycosidic linkages and the possibility to create substituted and hyperbranched structures. In particular, mushroom α-D-glucans exhibit mainly (1→3) and (1→6) glycosidic linkages, with trace amounts of the common (1→4) linkages found in cellulose and other plant polysaccharides (Ruthes, Smiderle, & Iacomini, 2015). These β-(1→4) linkages have been reported to covalently-link the β-D-glucans with the chitin microfibrils in some fungal species (Zhu, Du, Bian, & Xu, 2015). The nature of the glycosidic linkage in the backbone, the degree of branching, the length of the branches, and the molar mass distribution determine the molecular structure of each particular type of β-D-glucan, which varies depending on the particular fungal species and their development. The β-D-glucans in mushrooms exhibit similar molecular structure as glycogen, with (1→4) and (1→6) linkages and different branching structures and molar masses depending on the particular mushroom species. (1→3) and (1→6)-linked D-glucans have been also reported (Ruthes, Smiderle, & Iacomini, 2015). Table 1 summarises the structural characteristics of the most common D-glucans found in different edible mushroom species.

The cell walls contain a mixture of fibers and matrix components that are rich in chitin (a crosslinked polymer of N-acetylglucosamine). Chitin is a widespread polysaccharide in fungal species specially found in their cell wall, where it contributes to the structural stability in the form of microfibrils. Chitin is a linear homopolymer of 2-acetamido-2-deoxy-D-glucopyranose (D-GlcNAc) linked by β-(1→4) glycosidic linkages, which allows the formation of fibrillar structures through inter- and intramolecular hydrogen bonding. Chitin has therefore a similar molecular structure as the plant structural counterpart (cellulose), which explains their biological role as structural support of the cell wall.

On the other hand, proteins are usually complexed with polysaccharides and lipids in the fruiting bodies of edible mushrooms. Regarding the amino acid content, aspartic acid, histidine, glutamic acid, lysine, serine, are the major amino acids found in *A.bisporus* (Pei et al. 2014). Fungal species have also been identified as a source of natural enzymes, in particular, tyrosinases: type 3 copper proteins involved in the initial step of melanin synthesis. These enzymes were first characterised in *A.bisporus* because of unfavourable enzymatic browning during post-harvest storage, but also play a role in the formation/stability of spores, and defense/virulence mechanisms. Some authors have recently reported some measures to improve shelf-life of *A.Bisporus* by slowing the enzymatic degradation (Wu et al, 2019). Furthermore, mushrooms are also known to be an excellent accumulator of minerals from the environment in which they grow. High amounts of zinc, iron and manganese have been found, indicating that mushrooms could be used in well-balanced diets (Table 2) (Ghahremani-Majd & Dashti, 2015).

Some authors have considered mushrooms as a good source of vitamins since *A.Bisporus* (white) is a source of the B-group vitamins (B1, B2, B3, niacin, folates, B12), vitamin D2 and ergosterol. However, the content of these biologically-active substances varies depending on growing conditions (including environmental factors) (Muszyńska, Kała, Rojowski, Grzywacz, & Opoka, 2017) (Table 2).

Other valuable molecules such as polyphenols, lipids and terpenoids are present in edible mushrooms in lower quantities (2-5 %), but enough to provide antioxidant and flavour properties. In this sense, some

authors detected the presence of phenolic acids, such as gallic acid (8.4 ± 0.5 mg/100 g DW), *trans*-cinnamic acid (9.4 ± 0.3 mg/100 g DW), and chlorogenic acid (5.8 ± 0.3 mg/100 g DW) in *A.Bisporus* (white), denoting its valuable potential as source of bioactive compounds (Gasecka, Magdziak, Siwulski, & Mleczek, 2018).

A.Bisporus by-products were identified as a productive source of ergosterol since it is the most abundant mycosterol (90 % of the sterols fraction, 556 mg/g mushroom by-products), which has been related with different bioactive properties. In addition, ergosterol might be transformed by irradiation into vitamin D as a dietary supplement and food additive (Heleno et al, 2016b). Other authors showed the high content of L-ergothioneine (1.2-1.4 mg/g DM) on *A.Bisporus*, which can play a physiologic effect in antioxidant cytoprotection (Ghahremani-Majd & Dashti, 2015).

The presence of fat in *A.Bisporus* is low. However, the dominants in their composition were found to be linoleic acid (61.8-67.3 %) and palmitic acid (12.7-14.7 %), and the total unsaturated fatty acid percentages were found to range between 77.4 % and 79.7 % of the total fatty acids (Öztürk et al., 2011).

208 **Table 1.** Structural characteristics of D-glucans and heteropolysaccharides of edible mushrooms from Agaricales species (adapted from (Ruthes, Smiderle, & Iacomini, 2015) (Ruthes,
209 Smiderle, & Iacomini, 2016))

Polysaccharide	Source	Main Chain Residue	Substitution position	Branch Residue	Reference
D-glucans	*Agaricus bisporus* *Agaricus brasiliensis (=blazei)* *Coprinus comatus*	→6)-β-D-Glc*p*-(1→	-	linear	(Smiderle et al., 2013) (Li, Dobruchowska, Gerwig, Dijkhuizen, & Kamerling, 2013)
	Calocybe indica		O-4	α-D-Glc*p*	(Mandal, et al., 2010)
	Entoloma lividoalbum			→3)-β-D-Glc*p*-(1→	(Maity, et al., 2014)
	Macrolepiota dolichaula *Pleurotus citrinopileatus*		O-3	β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→ β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	(Samanta, et al., 2013) (Liu, et al., 2012)
	Termitomyces heimii			→3)-β-D-Glc*p*-(1→	(Manna, et al., 2015)
	Calocybe indica	→3)-β-D-Glc*p*-(1→	O-4	β-D-Glc*p*	(Mandal, et al., 2010)
	Flammulina velutipes		O-6		(Smiderle, Carbonero, Mellinger, Sassaki, Gorin, & Iacomini, 2006)
	Pleurotus eryngii				(Carbonero, et al., 2006)
	Pleurotus florida				(Santos-Neves, et al., 2008)
	Pleurotus geestanus				(Zhang, 2010)
	Pleurotus ostreatoroseus				(Carbonero, et al., 2006)
	Pleurotus ostreatus				(Giavasis, 2014; Lam & Chi-Keung Cheung, 2013; Palacios, García-Lafuente, Guillamón, & Villares, 2012)
	Pleurotus pulmonarius				(Smiderle, et al., 2008b)
	Schizophyllum commune				(Lam, et al., 2013; Zhang, Kong, Fang, Nishinari, & Phillips, 2013)
	Laetiporus sulphureus		-	linear	(Alquini, Carbonero, Rosado, Cosentino, & Iacomini, 2004)
	Termitomyces eurhizus				(Chakraborty, Mondal, Rout, & Islam, 2006)
	Agaricus bisporus	→4)-α-D-Glc*p*-(1→	O-6	α-D-Glc*p*	(Smiderle, et al., 2010)
	Coprinus comatus			α-D-Glc*p* or →6)-α-D-Glc*p*-(1→	(Li, et al., 2013)
	Pleurotus ostreatus		-	linear	(Palacios, García-Lafuente, Guillamón, & Villares, 2012)

	Hericium erinaceus	→3)-α-D-Glc*p*-(1→	O-4	α-D-Glc*p* or →3)-α-D-Glc*p*-(1→	(Wiater, et al., 2016)
	Pleurotus florida		O-6	β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	(Santos-Neves, et al., 2008)
Heterogalactans	*Agaricus brasiliensis*	→6)-α-D-Gal*p*-(1→	O-2	α-L-Fuc*p*	(Komura, et al., 2010)
	Coprinus comatus				(Li, et al., 2013)
	Flammuluna velutipes			3-*O*-D-Man*p*-L-Fuc*p,* β-D-Man*p* and α-L-Fuc*p*	(Smiderle, Carbonero, Sassaki, Gorin, & Iacomini, 2008a)
				Fuc*p* and Glc*p*	(Zhang, Xiao, Deng, He, & Sun, 2012)
				α-L-Fuc*p,* Glc and 3-*O*-Me-Rha	(Zhang, Fu, Xu, Sun, & Zhang, 2012)
	Hericium erinaceus			Glc (1→6)-linked, Glc (1→3)-linked and Fuc (1→4)-linked	(Li, et al., 2016)
				α-L-Fucp	(Li, et al., 2016)
	Laetiporus sulphureus			3-*O*-D-Man*p*-L-Fuc*p,* β-D-Man*p* and α-L-Fuc*p*	(Alquini, Carbonero, Rosado, Cosentino & Iacomini., 2004)
	Pleurotus florida			α-L-Glc*p* and β-D-Man*p*	(Rout, Mondal, Chakraborty, & Islam, 2006)
	Pleurotus ostreatus			β-L-Glc*p*	(Sun & Liu, 2009)
	Agaricus bisporus	→6)-α-D-Gal*p*-(1→ and →6)-3-*O*-Me-α-D-Gal*p*-(1→		β-D-Gal*p* and α-L-Fuc*p*	(Ruthes, Rattmann, Carbonero, Gorin, & Iacomini, 2012)
	Agaricus bisporus var. hortensis			α-L-Fuc*p* and β-D-Gal*p*	(Ruthes, et al., 2013)
				α-L-Fuc*p* and β-D-Gal*p*	(Komura, et al., 2010)
	Pleurotus eryngii			□-D-Man*p* linked to →6)-α-D-Gal*p*-(1→ and α-3-*O*-Me-D-Gal*p* linked to →6)-3-*O*-Me-α-D-Gal*p*-(1→	(Zhang, Zhang, Yang, & Sun, 2013)
	Pleurotus geesteranus			β-D-Man*p*	(Zhang, Xu, Fu, & Sun, 2013)
	Pleurotus pulmonarius				(Smiderle, et al., 2008b)
Heteroglucans	*Hericium erinaceus*	→6)- β -D-Glc*p*-(1→	O-3	β-D-Glc*p* or β-D-Glc*p*-(1→6)-β-D-Gal*p*-(1→ or β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→	(Li, et al., 2016)
	Tricholoma crassum	→6)- β -D-Glc*p*-(1→ and →6)-α-D-Glc*p*-(1→		β-D-Man*p*-(1→4)-α-D-Gal*p*-(1→	(Patra, et al., 2012)

	Lepista sordida	→6)-α-D-Glc*p*-(1→	O-2	α-D-Gal*p*	(Luo, Sun, Wu, & Yang, 2012)
	Pleurotus ostreatus	→3)- β -D-Glc*p*-(1→ and →6)-β-D-Glc*p*-(1→	-	α-D-Gal*p* or α-D-Glc*p*	(Maity, et al., 2011)
	Tricholoma matsutake	→4)- β -D-Glc*p*-(1→	O-6	α-D-Xyl*p*-(1→3)-α-D-Gal*p*-(1→	(Ding, et al., 2010)
Heteromanan	*Flammulina velutipes*	→3)-α-D-Man*p*-(1→	O-4	□-D-Xyl*p* or →3)-□-D-Xyl*p*-(1→	(Smiderle, et al., 2006)
Heterogeneous main chain	*Agaricus brasiliensis* (=*blazei*)	→6)-α-D-Gal*p*-(1→ and →2,6)-α-D-Glc*p*-(1→		α-D-Glc*p*	(Liu, et al., 2011)
	Armillaria mellea	→6)-α-D-Glc*p*-(1→, →2,6)-α-D-Glc*p*-(1→ and →6)-α-D-Gal*p*-(1→	O-2	β-D-Glc*p*	(Sun & Liu, 2009)
	Pleurotus sajor-caju	→6)-α-D-Gal*p*-(1→ and →2,4)-α-D-Glc*p*-(1→		β -D-Man*p*	(Pramanik, Mondal, Chakraborty, Rout, & Islam, 2005)
	Calocybe indica	→3,6)-α-D-Gal*p*-(1→, →4)-β-D-Glc*p*-(1→ and →6)-β-D-Glc*p*-(1→		α-L-Fuc*p*	(Mandal, et al., 2011)
	Termitomyces robustus	→3)- β -D-Glc*p*-(1→, →3,6)-β-D-Glc*p*-(1→, →6)-α-D-Glc*p*-(1→ and →3)-α-L-Fuc*p*-(1→	O-6	β -D-Glc*p*	(Mondal, et al., 2008)
	Volvariella bombycina	→6)- β -D-Glc*p*-(1→, →4,6)-α-D-Man*p*-(1→ and →6)-α-D-Glc*p*-(1→	O-4	α-D-Gal*p*	(Das, et al., 2008)
	Volvariella diplasia	→4)-α-D-Glc*p*-(1→, →2,4,6)-β-D-Glc*p*-(1→ and →6)-α-D-Man*p*-(1→	O-2 and O-4	α-D-Gal*p* and β -D-Glc*p*	(Ghosh, et al., 2008)

Table 2. Typical nutritional composition of the raw white mushroom from *A.Bisporus* (Reis, Barros, Martins, & Ferreira, 2012a)**,** [% values denote the percentage of daily recommended intake levels].

Typical Values		**Nutritional value per 100 g**	
Energy	Energy	93 kJ	
	Calories	22 kcal	
Carbohydrates	Total Carbohydrate	3.26 g	
	Sugars	1.98 g	
	Dietary Fibre	1 g	
Fat	Total Fat	0.34 g	
Protein	Total Protein	3.09 g	
Vitamins	Thiamine (B_1)	0.081 mg	[7 %]
	Riboflavin (B_2)	0.402 mg	[34 %]
	Niacin (B_3)	3.607 mg	[24 %]
	Pantothenic Acid (B_5)	1.497 mg	[30 %]
	Vitamin B_6	0.104 mg	[8 %]
	Folate (B_9)	17 µg	[4 %]
	Vitamin B_{12}	0.04 µg	[2 %]
	Vitamin C	2.1 mg	[3 %]
	Vitamin D	0.2 µg	[1 %]
Trace Metals	Iron	0.5 mg	[4 %]
	Magnesium	9 mg	[3 %]
	Phosphorus	86 mg	[12 %]
	Potassium	318 mg	[7 %]
	Sodium	3 mg	[0 %]
	Zinc	0.52 mg	[0.5 %]
Other	Water	92.45 g	

3. Strategies to obtain valuable compounds

Extraction and isolation of bioactive compounds from natural sources are well-established processes: exhaustive extraction, such as maceration, hydro-distillation, pressing, infusion, percolation and Soxhlet extraction are just some examples. Sometimes, the addition of chemicals is necessary in order to isolate target compounds in a purifed form. The extraction with organic solvents, such as ethanol, methanol or chloroform, is common since the majority of bioactive compounds are not soluble in water. In addition, these methods are time-consuming, while the large amount of solvents required for an efficient extraction as well as the high temperatures may cause degradation in target molecules with partial loss of volatiles (Cvjetko Bubalo, Vidović, Radojčić Redovniković, & Jokić, 2018). Consequently, the development of faster and more efficient extraction techniques should take into consideration the use of non-toxic solvents, such as water, carbon dioxide, and ethanol. The most innovative and promising extraction techniques are: microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE) and subcritical water extraction (SWE), also called pressurised hot water extraction (PHWE) (Roselló-Soto, et al., 2016). The application of conventional extraction techniques to obtain valuable extracts rich in bioactive compounds from *A.bisporus* or other mushroom species has been widely reported (Boonsong, Klaypradit, & Wilaipun., 2016; Kaewnarin, Suwannarach, Kumla, & Lumyong, 2016; Ruthes, Smiderle, & Iacomini, 2015). For example, a protein-rich extract was obtained by using conventional techniques with the aim to produce mushroom protein hydrolysates (MPHs) for functional foods as well as natural antioxidants for lipid food systems (Kimatu, et al., 2017). Chitin was extracted by alkaline treatment of *A.Bisporus* fruit bodies in 1 M NaOH solution at 80 °C for 2 h selecting

the optimum conditions of the alkaline medium, temperature and time (Hassainia, Satha, & Boufi, 2018).

Innovative extraction techniques based on green chemistry concepts have been reported by several authors to extract bioactive compounds from mushrooms. Özyürek et al. applied MAE for polyphenols extraction from three wild edible mushrooms (*Terfezia boudieri Chatin, Boletus edulis,* and *Lactarius volemus*) (Özyürek, Bener, Güçlü, & Apak, 2014). They found that the highest antioxidant capacity, determined by the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method, was found in methanolic extracts of *Boletus edulis* and can be associated to the higher content in phenolic and flavonoid compounds. In this way, MAE has proved as a powerful technique for the extraction of bioactive compounds from different mushroom species, showing the reduction in both, extraction time and solvent consumption, while increasing extraction rates and yields compared to conventional extraction methods. They also determined the profile and concentration of polyphenols in eight species of edible mushrooms, reporting the presence of free homogentisic acid, myricetin and chatechin in all samples, although its concentration varied significantly among different species.

Phenolic-rich extracts were obtained by applying MAE to *Coriolus versicolor* mushrooms. Experimental conditions were optimised by applying a central composite experimental design with three MAE parameters: extraction time, ethanol concentration and microwave power taking the extraction yield, total phenolic content and antioxidant activity as response variables (Maeng, Muhammad Shahbaz, Ameer, Jo, & Kwon, 2016).

MAE and PHWE were compared as powerful extraction techniques to obtain polysaccharides (particularly β-glucans) from *Pleurotus ostreatus* and *Ganoderma lucidum* fruiting bodies. Both techniques showed to be easy, fast and efficient approaches to extract mushroom β-glucans. However, all of them showed disadvantages in the post-extraction treatments, since some residues remained after extraction and further separation steps were required (Smiderle, et al., 2017).

UAE of polysaccharides from *A.Bisporus* was used by application of a central composite design with ultrasonic power, extraction temperature, extraction time and water/mushroom ratio as variables. The polysaccharides-rich extract was only composed of D-glucose with β-type glycosidic bonds (Tian, et al., 2012). In a recent study, Francisco et al. used UAE to obtain *A.Bisporus* extracts to be applied as food additive in yoghurts as a good source of polysaccharides with nutraceutical interest (Francisco, et al., 2018). It is known that mushroom's by-products are a rich source of vitamins, fibre, amino acids, proteins and other molecules with high nutritional value. For this reason, new approaches leading to valorisation of these by-products by using green and sustainable extraction technologies have been proposed. *A.Bisporus* discarded by-products obtained from local mushroom producers were used to obtain ergosterol. (Heleno, et al., 2016b). They reported that similar amounts of ergosterol were achieved by applying MAE in comparison with the classical Soxhlet extraction, resulting in a significant decrease in extraction time and solvents consumption.

Hot water and alkali extraction followed by precipitation with ethanol are the most common techniques used for polysaccharides, including β-glucans. However, due to their requirements of long times and high temperatures, UAE offers an inexpensive, environmentally-friendly, less time consuming and efficient

alternative to these conventional extraction techniques (Aguiló-Aguayo, Walton, Viñas, & Tiwari, 2017). UAE was also used to obtain mycosterol with ethanol at 375 W for 15 min, yielding 671.5 ± 0.5 mg ergosterol/100 g DW (Heleno, et al., 2016a).

Other technologies, such as molecularly imprinted polymers (MIP) have been recently proposed for the selective extraction of specific chemicals with the desired functionalities from mushrooms. Molecular imprinting permits the selective generation of macro-porous polymeric materials that are able to host specific molecules and protect them from the environment until they are designed to reach the intended biological or chemical molecular targets. The application of this technology to the specific extraction of ergosterol from *Ganoderma tsugae* mushroom species has been reported (Hashim, et al., 2016). A clear increase in the ergosterol amount extracted from this mushroom species was reported resulting in 217 mg/g DW, much higher than the amounts extracted by other conventional extraction techniques. These amounts of ergosterol extracted from *A.Bisporus* through MIPs can be considered as adequate for antioxidant/antimicrobial functionalities and consequently these strategies are highly promising for the selective extraction of chemicals. MIPs have been also proposed to selectively remove heavy metals (Dahaghin, Zavvar Mousavi & Maryam Sajjadi, 2017) and illegal whitening agents from mushrooms (Ding, Wang, Wang & Ji, 2018), showing the high potential of MIPs as specific extracting agents to be used in mushroom species to get tailor-made extracts with the desired functionalities.

4. *Agaricus bisporus* as a source of valuable extracts and their bioactivity

4.1. Antimicrobial activity

Many food products are perishable and also require protection from microbial spoilage during preparation, storage and distribution for acceptable shelf-life and organoleptic characteristics (Stojkovic, et al., 2014). However, a concern of many bacterial-control food studies is their ability to translate successfully to a dynamic *in-vivo* environment while not obscuring the appealing characteristics of foodstuff. This has led to an interest in natural-based preservatives from plants and fungi.

Extracts based on fungal species and their associated fruiting bodies have been studied for their activities against several bacteria, including *Agaricus, Boletus, Canthraellus, Clitocybe, Cortinarius, Ganoderma, Pycnoporus, Hygrophorous. Hypholoma, Lactarius, Tricholoma, and Lentinus* species (Rezaeian & Pourianfar, 2016). Specifically, these authors associated the bacteriostatic and bactericidal functions with the isolation of low molecular weight secondary metabolites, such as terpenes/sesquiterpenes, steroids, benzoic acid derivatives, anthraquinonoids and quinolones, as well as high molecular weight compounds such as peptides and proteins. In addition, phenolic compounds and sterols have been reported in fungal extracts (Sinanoglou, et al., 2015; Taofiq, et al., 2016). The antimicrobial activity of some species of wild and commercial mushrooms is summarised in Table 3 in terms of their minimum inhibitory concentration (MIC), which indicates the lowest concentration that prevents the visible growth of a bacterium.

Table 3. Antimicrobial activity of wild and commercial mushrooms: MIC values reported in µg/mL. (-) No antimicrobial activity; (+) Slight antimicrobial activity; (++) Moderate antimicrobial activity; (+++) High antimicrobial activity; (++++); Strong antimicrobial activity

	MIC (µg mL^{-1})					
Samples	*B.cereus*	*B.subtilus*	*S.Aureus*	*P.Aeroginosa*	*E.coli*	*K.Pneumoniae*
Agaricus bisporus	500(-)	5(++++)	500(-)	500(-)	500 (-)	500 (-)
Agaricus silvaticus	500(-)	500(-)	500(-)	500(-)	500 (-)	500 (-)
Agaricus silvicola	5(++++)	5(++++)	5(++++)	500(-)	500 (-)	500 (-)
Boletus edulis	500(-)	500(-)	5(++++)	500(-)	500 (-)	500 (-)
Calocybe gambosa	500(-)	500(-)	500 (-)	500(-)	500 (-)	500 (-)
Cantharellus cibarius	500(-)	5(++++)	50(++++)	500(-)	500 (-)	500 (-)
Craterellus-cornucopiodes	500(-)	500(-)	500 (-)	500(-)	500 (-)	500 (-)
Marasmius oreades	500(-)	500(-)	500(-)	500(-)	500(-)	500(-)
Ampicillin	3.13(++++)	12.5(++++)	6.25(++++)	6.25(++++)	6.25(++++)	6.25(++++)

The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.)* are multidrug-resistant bacteria identified as the leading cause of nosocomial infections throughout the world (Santajit & Indrawattana, 2016). Their unique resistance mechanisms are genetically derived and acquired through horizontal or vertical gene transfer, often as the result of selective pressure post-antibiotic exposure. With the impending resistance of bacterial species and the exhaustion of existing antimicrobial compounds, many natural-based chemicals are being investigated as potential sources of new and variants of existing antibiotic classes. The potential of *A.Bisporus* as an antimicrobial/fungal agent is high, but deep studies in extraction, broad testing of organisms for antimicrobial sensitivity, elucidation of the bioactive compounds and their associated mechanism of action are lacking and should be considered.

Crude extracts obtained from *A.Bisporus* showed to have antibacterial activity against Gram-positive (*S.aureus, B.cereus, M.flavus* and *L.monocytogenes*) and Gram-negative bacteria (*P.aeruginosa, S.typhimurium, E.coli,* and *E.cloacae*) (Stojkovic, et al., 2014). However, the noted efficacy was largely attributed to the solvent-based extraction methods, which demonstrated high variance in yields and antibacterial and antifungal activities. The majority of the bacterial assays employed for such purpose were either the agar-well diffusion method (AWD) or variations of the quantitative broth microdilution (BMD) method. Whilst the AWD assay is appropriate for screening by its ability to type resistance phenotypes, the standard BMD method, in contrast, is readily repeatable, able to determine MIC values and can be modified using colourimetric dye reagents for higher sensitivities. The research's shift from the AWD to the BMD method is progressive but the accurate quantification of MIC values against a wider range of microorganisms would complement the existing reporting (Balouiri, Sadiki, & Ibnsouda, 2016). Additionally, research into commercially-cultivated strains of *A.Bisporus* showed increased levels of inhibition, but sparse data have been recorded for the antibacterial potency of wild strains in different countries (Rezaeian & Pourianfar, 2016).

Three concentrations of methanolic extracts from a Nigerian wild strain of *A.Bisporus* reported bacterial

susceptibility associated with the total phenolic activity, as IC_{50} values decreased in relation to the free-radical scavenger activity (Abah & Abah, 2010). Methanolic preparates from commercially cultivated sources also demonstrated comparable levels of activity against Gram-positive organisms but no significant zone of inhibition was noted at concentrations lower than 50 mg/mL (Parashare, Pal, & Bhandari, 2013). Ethanolic extracts of laboratory-cultivated (Jagadish, Krishnan, Shenbhagaraman, & Kaviyarasan, 2009) and Eurasian-wild (Akyuz, Onganer, Erecevit, & Kirbag, 2010) strains of *A.Bisporus* continued to report higher levels of inhibition against Gram-positive bacteria, but results were not comparable to the activities and concentration ranges of the established control antibiotics.

Antibacterial activities of methanol and acetone extracts from a range of concentrations of *A.Bisporus* against *E.coli* and *S.aureus* continued to associate methanolic extractions with higher levels of growth inhibition (Sharma, 2015). Contradictingly, the presence and growth of the Gram-negative spectrum was favoured, particularly for *E.coli, P.aeruginosa,* and *K.pneumoniae* (Stojkovic, et al., 2014). In a similar way, a methanolic extract of a wild strain of *A.Bisporus* isolated from the Northeast of Portugal and analysed using a BMD assay exerted significant antibacterial activity (MIC of 5 µg/mL) against *B.subtillis,* but found that none of the Gram-negative species tested were affected (Alves, et al., 2013). Similar extracts from *A.Bisporus* wild strains collected from both Turkey (Öztürk, et al., 2011) and China (Shang et al., 2013) also showed inhibition of several Gram-positive species more effectively than Gram-negative bacteria. The continued susceptibility of Gram-positive bacteria could suggest its mode of action to include some cell wall peptidoglycan specificity. Its reduced efficacy in the Gram-negative spectrum could be also indicative of permeability of the outer lipopolysaccharide membrane and characteristically lower peptidoglycan content. Other authors found that the MIC values for methanolic extracts obtained from *A.Bisporus* collected in Nastik (India) was 100 mg/mL against *E.coli, Proteus sp, P.aeruginosa* and *S.aureus* (Parashare, Pal, & Bhandari, 2013), supporting that antibacterial activity of *A.Bisporus* species is highly dependent on their origin.

Fractionation of a methanol-dichloromethane (1:1) extract from a cultivated strain of *A.bisporus* eluted by ethyl acetate and ethyl acetate/methanol (1:1) showed quantifiable and dose-dependent antibacterial activity. The extract obtained from the cultivated strain of *A.bisporus* showed MIC values 880 µg/mL, 18 µg/mL and 1.25 mg/mL against Gram-positive species *B.cereus, S.aureus* and *E.faecalis* respectively, but MIC values were not reached in Gram-negative species (Soltanian, Rezaeian, Shakeri, Janpoor & Pourianfar, 2016). Antibacterial sensitivity of *L.monocytogenes* with an ethanolic extract from *A.bisporus* showed activity in a broth BMD assay but its correlation to an *in-situ* yoghurt infusion-based assay was dissimilar and was attributed to the complexity of the live culture based in yoghurt/growth media (Stojkovic, et al., 2014). The presence of *L.monocytogenes* in foodstuff is hard to control since it has the ability to survive in a broad range of adverse conditions (acidic pH, low temperatures and high sodium chloride concentrations). Similar studies were reported based on the antifungal activity of *A.Bisporus* extracts with inhibitory and fungicidal concentration ranges (Table 4).

Houshdar Tehrani et al. carried out studies based on aqueous total protein extracts from *A.bisporus,* showing significant antibacterial activity against *S.aureus* (Houshdar Tehrani, Fakhrehoseini, Kamali Nejad,

Mehregan, & Hakemi-Vala, 2012). They performed the protein fractionation using a DEAE-A50 ion exchange column and a stepwise salt gradient elution to produce three fractions. One of them reported antibacterial activity and produced a pure protein band at 22.5 kDa. Purification of this peptide showed MIC values of 100 µg/mL against both bacterial species.

Some scientific results on the antimicrobial activity against Gram-negative and Gram-positive bacteria of the individual phenolic compounds present in *A.bisporus* extracts are summarised in Table 5. The most common phenolic compounds identified in *A.bisporus* are benzoic acid derivatives (p-hydroxybenzoic acid, protocatechuic acid and gallic acid) and cinnamic acid derivatives (cinnamic acid, p-coumaric acid, ferulic acid and chlorogenic acid). Furthermore, these compounds show relevant antimicrobial activity (MIC > 1 mg/mL) against a wide variety of Gram-positive and Gram-negative bacteria. Specifically, the phenolic compound 2,4-dihydroxybenzoic/protocatechuic acid, previously isolated from several wild species of mushrooms including *A.bisporus*, exhibited MIC values around 1 mg/mL against clinical isolates of some Gram-negative bacteria including *E.coli*, *Pasteurella multocida* and *Neisseiria gonorrhoea*. 2,4-dihydroxybenzoic acid has also high levels of activity against *Methicillin-susceptible S.aureus* species. Structure-Activity Relationship (SAR) analysis suggests that the presence of carboxylic acid (COOH) and two hydroxyl (OH) groups in *para* and *ortho* positions of the benzene ring and the methoxyl (OCH_3) group in the *meta* position plays an important role in phenolic compound's against *Methicillin-resistant S.aureus* activity. The presence of some flavonoids with activity against some bacteria (such as catechin, myricetin and pyrogallol) and sterols like ergosterol provide additional biocidal effect to *A.bisporus* mushroom species (Alves, et al., 2013).

Table 4. Antifungal activity of the methanolic and ethanolic extracts of *A.bisporus* (mean values of MIC/MFC (Minimum Fungicidal Concentration) in mg/mL) (adapted from Stojkovic et al., 2014)

Fungi	Methanolic extract	Ethanolic extract
Aspergillus fumigatus	1.15 ± 0.10 / 9.4 ± 1.7	2.35 ± 0.10 / 9.4 ± 1.7
Aspergillus versicolor	0.15 ± 0.03 / 1.15 ± 0.10	0.15 ± 0.06 / 4.7 ± 0.1
Aspergillus ochraceus	0.30 ± 0.03 / 0.6 ± 0.02	1.13 ± 0.01 / 2.35 ± 0.10
Aspergillus niger	0.60 ± 0.06 / 1.15 ± 0.10	2.35 ± 0.06 / 4.7 ± 0.1
Trichoderma viride	0.30 ± 0.03 / 0.60 ± 0.01	0.15 ± 0.03 / 2.35 ± 0.01
Penicillum funiculosum	0.60 ± 0.01 / 1.15 ± 0.13	1.13 ± 0.10 / 3.25 ± 0.13
Penicillum ochrochloron	0.60 ± 0.06 / 1.15 ± 0.10	0.60 ± 0.06 / 2.35 ± 0.10
Penicillum verrucosum	0.60 ± 0.01 / 1.15 ± 0.10	2.35 ± 0.01 / 9.4 ± 1.7

Table 5. MIC values (mg/mL) of *A.bisporus* phenolic compounds against clinical isolates of Gram-negative and Gram-positive bacteria (adapted from Alves et al. 2013)

Phenolic compounds	**Gram-negative bacteria**					**Gram-positive bacteria**					
	Escherichia coli	*Proteus mirabilis*	*Morganella morganni*	*Pasteurella multocida*	*Neisseria gonorrhoeae*	*MSSA*	*MRSA*	*Staphylococcus epidermidis*	*Enterococcus faecalis*	*Listeria monocytogenes*	*Streptococcus agalactiae*
p-Hydroxybenzoic acid	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1
Protocatechuic acid	1	> 1	> 1	1	1	1	1	> 1	> 1	1	1
Gallic acid	> 1	> 1	> 1	1	1	> 1	> 1	> 1	> 1	> 1	> 1
Cinnamic acid	> 1	> 1	> 1	> 1	1	> 1	> 1	> 1	> 1	> 1	0.5
p-Coumaric acid	1	> 1	> 1	1	1	> 1	1	> 1	> 1	> 1	> 1
Ferulic acid	> 1	> 1	> 1	1	1	1	0.5	1	> 1	> 1	1
Chlorogenic acid	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	1	> 1
Catechin (Taylor, Hamilton-Miller, & Stapleton, 2005; Zhang, Jung, & Zhao, 2016)	0.3	-	-	-	-	-	> 1	*Listeria innocua* (MIC: 0.1 mg/mL)			
Myricetin (Cetin-Karaca & Newman, 2015; Rodríguez-Pérez et al., 2016; Semwal, Semwal, Combrinck, & Viljoen, 2016)	0.1	0.5	Salmonella species (MIC < 1)			-	> 1	> 1	> 1	-	-
Ergosterol (Taofiq, et al., 2016)	*Salmonella entérica* (MIC: 0.1 mg/mL)					*Staphylococcus aureus* (MIC: 0.1 mg/mL)					
Pyrogallol (Kocaçalişkan, Talan, & Terzi, 2006; Lima, et al., 2016)	*Pseudomonas putida; pseudomonas pyocyanea*					*Staphylococcus aureus* (MIC: 1 mg/mL)					

MSSA, Methicillin-susceptible *Staphylococcus aureus*; MRSA, Methicillin-resistant *Staphylococcus aureus.*
Values of MIC (mg/mL) < 1 indicate non-relevant antibacterial activity

4.2. Antioxidant activity

In the last years, extensive research has focused on the antioxidant potential of wild-growing and cultivated mushrooms, mainly associated to the presence of phenolic compounds in their composition. Table 6 summarises some results in the evaluation of the antioxidant activity of different mushroom species. In general terms, phenolic compounds are divided in two main groups, based on the hydroxyl derivatives of benzoic acid and trans-cinnamic acid respectively. Within the first group, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid and syringic acid have been detected in different mushroom species, while p-coumaric acid, ferulic and caffeic acid belong to the second group (Yildiz, Can, Laghari, Şahin, & Malkoç, 2015).

Table 6. Antioxidant activities of different mushroom species

Mushroom specie	Extraction process	Reducing power		Scavenging activity		Ref.
		TPC*	Ferricyanide assay	DPPH*	FRAP*	
Agaricus bisporus (white)	Methanolic extracts from lyophilized powder (25 °C, 150 rpm, 1 h)	23.34 ± 0.36 mg GAE/g extract	1.80 ± 0.03 EC_{50} mg/mL	3.13 ± 0.09 EC_{50} mg/mL	> 1	(Reis, Martins, Barros, & Ferreira, 2012b)
Coriolus versicolor	MAE (2 g dried poder, 3.8 min, 40 % etanol, 125 W)	470 mg GAE/100 g DM		773 mMTE/g DM	1,710 mM TE/g DM	(Maeng, Muhammad Shahbaz, Ameer, Jo, & Kwon, 2016)
Lentinus edodes	10 dried powder extracted with 50 % ethanol (150 rpm, room T, 24 h)		80 % (500 mg/mL)	64.3 % (500 µg/mL)		(Boonsong, Klaypradit, & Wilaipun, 2016)
Boletus edulis	MAE (0.2 g lyophilized powder, 80 % methanol, 80 ºC, 5 min)			2.9 ± 0.1 EC_{50} mg/mL		(Özyürek, Bener, Güçlü, & Apak, 2014)
Agaricus bisporus	Methanolic extracts from lyophilized powder (0.2 g, 65 °C, 1 h)	3-4 mg GAE/g DM				(Palacios, et al., 2011)
Ganodermal ucidum	Ultrasonic bath (60ºC, 3 h) with methanol	26.40 mg GAE/g DM		1.76 ± 0.38 EC_{50} mg/mL	240 ± 13 µM TE/100 g DM	(Yildiz, et al., 2015)

*DPPH (2,2-diphenyl-1-picrylhydrazyl method); FRAP: (Ferric Reducing Antioxidant Power); TPC (total phenolic content); TE (trolox equivalent); GAE (galic acid equivalent)

Not only the different compounds present in a particular mushroom species, but also the extraction method and the solvent used influence their antioxidant potential. In this sense, antioxidant properties and phenolic profiles of four cultivated mushrooms species (*A.bisporus, Pleurotus ostreatus, Pleurotus eryngii* and *Lentinula edodes*) were compared to their mycelia, but no correlation was found between *in vitro* and *in vivo* behaviour, probably due to the different growing conditions that affected their chemical composition (Reis, Martins, Barros, & Ferreira, 2012b). These authors found that the extracts from *A.bisporus* showed the highest antioxidant activity, with the lowest concentration of EC_{50} values (sample concentration that provides 50 %

of antioxidant activity). Tian et al. monitored the change in concentration of *A.bisporus* polysaccharide extracts to evaluate the antioxidant ability through the DPPH scavenging activity test. Results demonstrated that at 250 μg/mL, the extract showed 86.1 % free radical-scavenging activity significantly higher ($p < 0.01$) than BHT (83 %) (Tian, et al., 2012).

4.3. Valuable compounds

Polysaccharide hot water extracts from *A.bisporus* showed a total D-glucan content of 63.8 g/100 g DW, from which 5.6 g corresponded to α-D-glucans and 58.2 g to β-D-glucans (Kozarski, et al., 2011). These extracts were used to evaluate and compare immunomodulatory effects with hot water extracts from other mushroom species. *A.bisporus* polysaccharide extracts obtained by using UAE showed high purity in D-glucose with estimated molecular weight of 158 kDa after the extract purification by ion chromatography using DEAE-Cellulose-52 column and Sepharose G-100 column (Tian, et al., 2012).

The chitin content is variable depending on the mushroom species and *A.bisporus* shows 6-9 % of the total dry weight (Hassainia, Satha, & Boufi, 2018). Some authors have worked to obtain chitin and its deacetylated derivative chitosan from fungal species since their use presents some advantages due to the seasonal independence in the biotechnological usage of yeasts (in industrial fermentations) and the absence of demineralization treatments necessary in their shellfish counterparts (Erdogan, Kaya, & Akata, 2017). Hassainia et al. obtained chitin from *A.bisporus* fruit body parts by alkaline treatment (Hassainia, Satha, & Boufi, 2018). Results showed that the extracted chitin was in the alpha form, with a 63 % crystalline index and degree of N-acetylation of 70 %.

Fungal species have been also identified as source of natural enzymes, in particular, tyrosinases: type 3 copper proteins involved in the initial steps of the melanin synthesis. These enzymes were first characterised in *A.bisporus* because of the unfavourable enzymatic browning during post-harvest storage, but also have their role in the formation/stability of spores, and defense/virulence mechanisms. These enzymes catalyse both the *o*-hydroxylation of monophenols and the subsequent oxidation of the resulting *o*-diphenols into reactive *o*-quinones, which evolve spontaneously to produce intermediates associated to dark brown pigments.

5. Applications of mushroom extracts.

This section aims to give an overview of the scarcely-reported but interesting applicability of the extracts obtained from mushrooms or their by-products. Just a few mushroom extracts are currently used in functional foods, nutraceuticals, food supplements, medicine or pharmacological applications (Rathore, Prasad, & Sharma, 2017; Reis, Martins, Vasconcelos, Morales, & Ferreira, 2017; Ruthes, et al., 2013). However, they are also used as natural biocontrol agents in plant protection (acting as insecticides, fungicides, bactericides, etc) and in cosmetics

due to their film-forming ability, antioxidant, antiallergic or antibacterial activities, and stimulation of collagen activity, among others. In general terms, microorganisms such as *B.cereus*, *E.coli*, *Shigella spp*., *S.aureus*, *Listeria spp*. and *Vibrio spp.* can be deposited on household surfaces to form biofilms and may constitute grounds for potential disease sources (Bridier et al, 2015). In this sense, the antimicrobial compounds extracted from mushrooms fruiting bodies and/or by-products should be able to inactivate these bacteria by the presence of different functional groups (Ashraf et al, 2014). Ethanolic extracts prepared from *A.bisporus*, *Pleurotus ostreatus*, and *Lentinula edodes* rich in ergosterol showed excellent properties for application in a base cream for cosmetics, since ergosterol, cinnamic acid and other polyphenols showed good stability after their incorporation to the base products. In addition, the formulation with *A.bisporus* extracts maintained good antioxidant and anti-inflammatory activities by inhibition of NO production and melanin by suppression of tyrosinase activity (Taofiq, et al., 2016).

Many results about the *in vitro* antioxidant and antimicrobial potential of mushroom extracts have been reported, but the number of *in vivo* studies is much lower. Until now, not much extensive research has been reported about the great potential benefits of mushroom extracts as food additives with antimicrobial and/or antioxidant activity to meet the increasing demands for food quality and safety avoiding spoilage deterioration (Shen, Shao, Chen, & Zhou, 2017). A successful application of *Agaricus bohusii Bon* extracts to inhibit *Penicillium verrucosum var. cyclopiumin* (Reis, et al., 2012c) and methanolic *Agrocybe aegerita* extracts (Petrović, et al., 2015) in cream cheese have been developed. In addition, innovative techniques, such as spray-drying, to process liquid by-products obtained from *Stropharia rugoso-annulata* mushroom have been reported (Chen, Lai, Shen, Li, & Zhou, 2014).

Microencapsulated *Suillus luteus* and *Coprinus atramentaria* extracts showed synergistic antioxidant effect when incorporated to cottage cheese, preserving nutritional properties and colour (Ribeiro, et al., 2015). Francisco et al. also used microencapsulation to functionalise yoghurts with *A.bisporus* extracts encapsulated in spray-dried maltodextrin crosslinked with citric acid, achieving noticeable improvement in the stability and hydrophilicity of the extracts (Francisco, et al., 2018). Despite all these recent studies in mushroom extracts incorporation to functional foods, more work is necessary to obtain information regarding the bioaccessibility and bioavailability of these type of compounds (Reis, Martins, Vasconcelos, Morales, & Ferreira, 2017).

Extracts from *A.bisporus* have a wealth of secondary metabolites, many of which possessing significant biological activities and providing the basis of novel drugs or nutraceuticals (Atila et al., 2017). A nutraceutical can be defined as a substance that may be considered a food constituent to provide prophylaxis to the onset of disease. Dietary fibres, proteins, peptides, amino acids, keto acids, minerals, and anti-oxidative vitamins are examples of nutraceuticals extracted from

mushroom species (Rathore, Prasad, & Sharma, 2017). High contents of these compounds are found within *Agaricus* spp (Stojkovic, et al., 2014) making them a potent source of potentially bioactive compounds. Nevertheless, long standing traditional use and intense research has provided evidence that several fungi contain important bioactive components that are beneficial in an array of diseases, including immune- and inflammatory disorders, cancer, hypertension, stroke, diabetes and hyperlipidemia (Rathore, Prasad, & Sharma, 2017).

Novel approaches to identify fungal nutraceuticals are being introduced in the identification of the fungal ingredients with nutraceutical interest. In general terms, *in vitro* bioassays are much more suitable for this purpose, but the efficiency of the screening activities greatly depends on the assay quality. For this purpose, a high throughput screening panel of Chemical Activated LUciferase gene eXpression (CALUX®) can be used in activity screens of chemicals and complex chemical mixtures as present in plant- and fungal extracts (van der Burg, et al., 2013). This panel contains assays measuring pathways that are relevant for obesity and metabolic syndromes, endocrine functioning pathways, (anti)inflammatory and immune modulatory pathways, cancer and antioxidants/chemoprevention.

Another application based on mushroom compounds was reported in the CN102177960A Patent, where the production of a film-free preservative from *A.bisporus* was detailed (Patent, 2011). The film-free preservative is an aqueous solution composed of chitosan (1.2-2.5 %), citric acid (0.4-0.6 %), sodium chloride (0.4-0.6 %) vitamin C (0.2-0.3 %) and 0.0001-0.0003 % of cysteine, in percentage by mass. Comparing with prior methods, the film-free preservative has advantages such as no film formation, no observable colour change, convenient operation while shelf-life is prolonged by 3-5 days at normal temperatures (25 ˚C) and by 15 to 20 at low temperatures (5 ºC). In addition, the preservative is edible and the process is environmentally-friendly with relative low cost. This method may reinforce the existing cell layers by acting as an additional chitosan cell membrane.

The production of valuable compounds from enzymes recovered from industrial by-products of *Lentinula edodes* mushrooms could be a promising alternative to obtain economic profitable enzyme preparations (Schimpf & Schulz, 2016). Other authors studied the production of chitosan through the deacetylation of chitin isolated from the *Ganoderma lucidum basidiomycetes* mushroom, as alternative to the crustaceous shell common source. They concluded that chitosan obtained from this mushroom, following a new protocol, showed significant similitudes with commercial products (Mesa Ospina et al, 2015).

6. Conclusions and future perspectives

Compounds that can be extracted from mushrooms fruiting bodies and/or their by-products show high potential by their specific functionalities and a rising number of studies have been reported

in the last years focusing on their nutritional value, health-promoting benefits, polysaccharide sources and antioxidant and antimicrobial properties of chemicals extracted from them.

Throughout this review, the high potential of *A.bisporus* as a source of valuable compounds has been proved and the possibilities for their application are continuously increasing since they can be applied in many different fields. In addition, the innovation by applying new techniques or procedures to extract these compounds with high yields and selectivities, in particular by using green extraction techniques, become a cornerstone to achieve success in this field of research. However, more and deeper studies are still necessary to get a solid introduction of these products into the market, due to the lack of information on safety issues and robust extraction procedures. In addition, restrictive legislations depending on the final application should be still implemented to ensure consumer's safety.

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