BIOACTIVE COMPOUNDS AND HEALTH BENEFITS OF COMMON EDIBLE MUSHROOMS

A Project Paper

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Food Science and Technology

by

Lingxi Zhou

December 2018

ABSTRACT

Edible mushrooms have been a common dietary source with not only unique taste and nutritional values, but also their additional health benefits. Research focus on the health benefits of mushrooms has shifted from medicinal mushrooms to edible mushrooms in recent years. Bioactive compounds, including polysaccharides, phenolic compounds, amino acids, functional proteins and peptides, indole compounds, and terpenoids, have been studied extensively. Some common health benefits of mushrooms discussed in different researches include immunomodulatory effect, antioxidant activity, anticancer effect, antibacterial activity, and anticholesterol activity. This report provides a comprehensive review of current research on edible mushrooms regarding their major nutrients, bioactive compounds and health benefits. Three groups of bioactive compounds: beta-glucans, phenolic compounds and L-ergothioneine, are reviewed in detail. Anti-cancer activity, immunomodulatory effect and prebiotic potential are covered for beta-glucans. Antioxidant activity and antimicrobial activity are covered for phenolic compounds. Last but not least, antioxidant activity, cyto-protective effect and immunomodulatory effect are covered for L-ergothioneine.

BIOGRAPHICAL SKETCH

Lingxi Zhou is an MPS student in Food Science and Technology in Cornell whose study focus is bioactive compounds in natural products. She is working with Dr. Rui Hai Liu for her MPS program. Lingxi had her undergraduate study in the University of Hong Kong. She obtained two Bachelor degrees: Food and Nutritional Science, and Science Education. Apart from her passion in science and education, she is also very interested in Chinese musical instruments. She was the General Manager of Chinese Orchestra in the University of Hong Kong.

ACKNOWLEDGMENTS

I am very grateful to all the people who have supported my academic work and life during the past one and half years. Firstly, I want to thank Dr. Rui Hai Liu for being my academic advisor and guiding my MPS project. Secondly, I want to give my special thanks to Dr. Patrick Gibney for all the extra academic support he provided to me. Next I really want to thank all my friends. Here I especially want to thank Anqi Chen, who has been always supportive to my academic work, physical and mental health. Also I would like to mention all my good friends from the Department of Food Science: Junyi Chen, Pei Zhu, Xianjia Zeng, Yifan Yang, and Zhixin Wang, whom I had a lot of great time with. In addition, I also want to thank Dong Miao, my best friend in Hong Kong, who gave me a lot of life suggestions.

Most importantly I want to thank my parents who have always been strongly supportive to my academic work since I was young. I would like to thank them for all the love they have been giving to me.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Mushrooms have been an important dietary source in human foods for more than thousands of years. The special flavors and texture make mushrooms a popular culinary ingredient in many cuisines (Chatterjee et al., 2017; Kalač, 2016a; Valverde, Hernández-Pérez, & Paredes-López, 2015). According to the data from Food and Agriculture Organization Corporation Statistics Database (FAOSTAT), the world production of mushrooms and truffles in 2014 reached more than 10.3 million metric tons (Kalač, 2016a) . The top 5 leading countries of producing and consuming mushrooms include China, Italy, the USA, the Netherland and Poland, with China producing far more amount than the rest (Feeney et al., 2014; Kalač, 2016a; Valverde et al., 2015).

Mushrooms are not only consumed as a delicacy due to their great taste and rich nutrients but also used as 'medicine' to treat and prevent some diseases because of their medicinal properties (Chatterjee et al., 2017; Han, Ling, & Chen, 2015; Jayachandran, Xiao, & Xu, 2017; S. P. Wasser, 2014). The earliest use of mushrooms as medicine can be dated back to ancient China and ancient Egypt (Jayachandran et al., 2017). In a famous Chinese pharmacopeia *Compendium of Materia Medica* (*Bencao Gangmu*), there were records of medicinal values of mushrooms including both medicinal mushrooms and common edible mushrooms. Examples of medicinal mushrooms are *Ganoderma lucidum* (*Lingzhi*), *Trametes versicolor* (*Yunzhi*), and *Cordyceps sinensis (Dongchong Xiacao)* (Guggenheim, Wright, & Zwickey, 2014). Examples of edible mushrooms are *Agaricus bisporus, Lentinula edodes, Pleurotus spp., Flammulina velutipes*, *Hericium erinaceus, Auricularia* and so on (Kalač, 2016b, 2016c; Royse Daniel,

Baars, & Tan, 2017; Valverde et al., 2015). Early researches focused on purely medicinal mushrooms that are not commonly consumed in diet. But since many edible mushrooms also carry medicinal values, the number of scientific researches on edible mushrooms regarding their health benefits have increased significantly. In fact, it is not easy to separate medicinal mushrooms and edible mushrooms because many mushrooms are used both as food and medicine. Consuming mushrooms as functional food is becoming a popular trend. The health benefits of mushrooms include immunomodulatory, anti-allergic, antioxidant, anticancer, antidiabetic, antibacterial, cholesterol-lowering effect, and source of prebiotics for gut health (Chatterjee et al., 2017; Feeney et al., 2014; Jayachandran et al., 2017; Valverde et al., 2015; S. P. Wasser, 2014; J.-J. Zhang et al., 2016). The major contributors of these health effects are the bioactive compounds inside mushrooms including polysaccharides, phenolic compounds, free amino acid, indole compounds, free fatty acids, functional proteins, terpenoids and others (Chatterjee et al., 2017; Feeney et al., 2014; Kalač, 2016c; Valverde et al., 2015; S. P. Wasser, 2014; J.-J. Zhang et al., 2016).

The purpose of this review is to provide comprehensive information of three major bioactive compounds in mushrooms which are beta-glucan, phenolic compounds and L-ergothioneine and their health benefits. This article is based on a few good reviews done by previous scientists (Feeney et al., 2014; Kalač, 2013; Rathore, Prasad, & Sharma, 2017; Valverde et al., 2015) and plenty of contemporary literatures on the health benefits of edible mushrooms. It is hoped that this article could allow both scholars and the general public have a quick understanding of the potential of edible mushrooms as a functional food.

CHAPTER 2

COMMON EDIBLE MUSHROOM SPECIES

Mushrooms belong to Kingdom Fungi and are consist of thousands of species, among which some are edible, some have medicinal uses but some are poisonous. There are more than 2000 mushroom species reported to be edible but only about 35 species are commercially cultivated with around 20 are produced in industrial scale (Kalač, 2013; Sánchez, 2004, 2010; Valverde et al., 2015). According to the most current review of world mushroom production , *Lentinus edodes* is the most cultivated one, which accounted for around 22% of world mushroom cultivation in 2013, followed by *Pleurotus spp*., *Auricularia spp*., *Agaricus bisporus* and *Flammulina velutipes* (Royse Daniel et al., 2017). Table 1 shows the commons names, applications, and distribution of the most common edible mushrooms in the world.

Table 1 Common Edible Mushrooms in the World

Scientific names	Common names	Distribution	Reference
Agaricus bisporus	Button mushroom (white/brown mushroom)	Most cultivated species worldwide	Hall, Buchanan, Cole, Yun, and Stephenson (2003); Morin et al. (2012); F. S. Reis, L. Barros, A. Martins, and I. C. Ferreira (2012); Valverde et al. (2015)
Agaricus blazei	Sun mushroom	Native to Brazil; extensively cultivated in Japan	Firenzuoli, Gori, and Lombardo (2008); Hall et al. (2003); Valverde et al. (2015)
Lentinus edodes	Shiitake mushroom	Native to East Asia; extensively cultivated in Japan, China	Bisen, Baghel, Sanodiya, Thakur, and Prasad (2010); Hall et al. (2003); Mizuno (1995);

			Filipa S Reis et al. (2012); Royse Daniel et al. (2017); Sánchez (2010)
Pleurotus ostreatus	Oyster mushroom	cultivated worldwide	Hall et al. (2003); F. S. Reis, L. Barros, A. Martins, and I. C. F. R. Ferreira (2012); Sánchez (2010); Valverde et al. (2015)
Pleurotus eryngii	King oyster mushroom		
Pleurotus citrinopileatus	Golden oyster mushroom	Native to China, Japan, Korea and East Russia	Hall et al. (2003); Jicheng Liu et al. (2012); J. Zhang et al. (1994)
Volvariella volvacea	Straw mushroom, paddy straw mushroom, Chinese mushroom	Cultivated in East especially in China and Southeast Asia	Bao et al. (2013); B. Chen et al. (2013); Hall et al. (2003); Sánchez (2010)
Flammulina velutipes	Enokitake, golden needle mushroom, winter mushroom	Cultivated in East Asia; Wildly native to many countries	Hall et al. (2003); Filipa S Reis et al. (2012); Sánchez (2010)

Nutritional compositions and bioactive compounds

Major macro- and micro-nutrients

Mushrooms are high in water content and the dry matter normally range from 80 to140 g/kg fresh matter (Kalač, 2013). Despite their low dry matter, they are good sources of many nutrients including proteins, fatty acids, vitamins and minerals, but low in calories due to low fat content. Many mushrooms are rich in proteins compared with most of the vegetables; they usually contain proteins around 200-250g/kg dry matter and some contain complete profile of amino acids with leucine, valine, glutamine, glutamic acid and aspartic acid as the most abundant ones (Valverde et al., 2015). Mushrooms also contain different types of monosaccharides and

their derivatives, oligosaccharides and polysaccharides (Kalač, 2013). Mannitol (28.9 g/kg dry matter) and trehalose (39.2 g/kg dry matter) are the most abundant free sugar alcohol and sugar in mushrooms respectively (Kalač, 2013). Polysaccharides are very important components in mushrooms due to their structural role in cell walls. β-glucans are probably the most well-studied one due to their abundant health benefits, which will be discussed in details in the next section (Kalač, 2013). Most of the saccharides in mushrooms are not for calorie purpose because of their low digestibility but many of them attribute to great health benefits. Table 2 shows the approximate compositions and energy of some most commonly cultivated mushrooms.

Apart from the macronutrients, mushrooms also contain different vitamins including riboflavin, niacin, folates, thiamin, vitamin B6, vitamin E and vitamin D (Valverde et al., 2015). They are the only natural non-animal source of vitamin D; wild mushrooms usually have more vitamin D than cultivated ones because they are exposed to UV-light more often (Kalač, 2013; Filipa S Reis et al., 2012; Valverde et al., 2015). Previous reports reported that mushrooms contained 3000-7000 mg/kg dry matter of ergosterol, which is the provitamin of ergocalciferol (Vitamin D2) (Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002; Phillips et al., 2011). The vitamin C content reported in some mushrooms is around 100 - 400 mg/100g dry matter. (Kalač, 2013). Tocopherol content is usually low in mushrooms however relatively high tocopherol value was reported in *Boletus reticulatus* (2.5 mg/100 g dry weight), *Lycoperdon umbrinum* (1.67 mg/100g dry weight) and *Suillus variegatus* (1.45 mg/100g dry weight) (Heleno et al., 2011; Pereira, Barros, Martins, & Ferreira, 2012; Vaz et al., 2011). Different minerals are also found in mushrooms; some major mineral include sodium, potassium, calcium, magnesium, phosphorus, sulfur and chlorine (Kalač, 2013, 2016b; Valverde et al., 2015). Among these, potassium is the most abundant one, ranging from 2000 – 4000 mg/100g dry matter (Kalač,

2016b). Table 3 and 4 show the major minerals and vitamins in some most commonly cultivated mushrooms.

Table 2 Proximate Compositions (g/100g FW) and Energy (kcal/100g fresh matter) of Common Edible Mushrooms

Scientific names	Common names	Water content	Protein	Total lipids	Total Carbohydrates	Ash	Energy	Reference
Agaricus bisporus (white)	Button mushroom	92.45	3.09	0.34	3.26	0.85	22	(USDA, 2018f)
Agaricus bisporus (brown)	Button mushroom	92.12	2.50	0.10	4.30	0.98	22	(USDA, 2018g)
Agaricus blazei	Sun mushroom	NA	3.13	0.18	5.94	0.75	38	(Carneiro et al., 2013)
Lentinus edodes	Shiitake	89.74	2.24	0.49	6.79	0.73	34	(USDA, 2018b)
Pleurotus ostreatus	Oyster mushroom	89.18	3.31	0.41	6.09	1.01	33	(USDA, 2018e)
Pleurotus eryngii	King oyster mushroom	89	1.1	1.45	8.14	0.618	42	(Filipa S Reis et al., 2012)
Volvariella volvacea	Straw mushroom	89.88	3.83	0.68	4.64	0.97	32	(USDA, 2018c)
Flammulina velutipes	Golden needle mushroom	88.34	2.66	0.29	7.81	0.91	37	(USDA, 2018d)
Auricularia polytricha	Jaw's ear	92.56	0.48	0.04	6.75	0.15	25	(USDA, 2018a)

NA: not available

Table 3 Minerals (mg/100g FW) in Common Edible Mushrooms

Scientific names	Common names	Ca	Fe	Mg	P	K	Na	Zn	Reference
Agaricus bisporus (white)	Button mushroom	3	0.5	9	86	318	5	0.52	(USDA, 2018f)
Agaricus bisporus (brown)	Button mushroom	18	0.40	9	120	448	6	1.10	(USDA, 2018g)
Lentinus edodes	Shiitake	2	0.41	20	112	304	9	1.03	(USDA, 2018b)
Pleurotus ostreatus	Oyster mushroom	3	1.33	18	120	420	18	0.77	(USDA, 2018e)
Pleurotus eryngii	King oyster mushroom	3	NA	14	NA	309	5	NA	(Filipa S Reis et al., 2012)
Flammulina velutipes	Straw mushroom	0	1.15	16	105	359	3	0.65	(USDA, 2018d)
Volvariella volvacea	Golden needle mushroom	10	1.43	7	61	78	384	0.67	(USDA, 2018c)
Auricularia polytricha	Jaw's ear	16	0.56	25	14	43	9	0.66	(USDA, 2018a)

NA: not available

Table 4 Vitamins in Common Edible Mushrooms

Scientific names	Common names	Thiamin (mg/100g , FW)	Riboflavin (mg/100g FW)	Niacin (mg/100g FW)	Vitamin B6 (mg/100g FW)	Folate (μg/100g FW)	Vitamin D (D2+D3) (μg/100g FW)	Ergosterol (mg/100g FW)	Vitamin A (μg/100g FW)	Vitamin E (mg/100g FW)	Reference
Agaricus bisporus (white)	Button mushroom	0.081	0.402	3.607	0.104	17	0.2	56	0	0.01	(USDA, 2018f)
Agaricus bisporus (brown)	Button mushroom	0.095	0.490	3.800	0.110	25	0.1	62	0	0.01	(USDA, 2018g)
Lentinus edodes	Shiitake	0.015	0.217	3.877	0.293	13	0.4	85	NA	NA	(USDA, 2018b)
Pleurotus ostreatus	Oyster mushroom	0.125	0.349	4.956	0.110	38	0.7	64	2	0	(USDA, 2018e)
Flammulina velutipes	Golden needle mushroom	0.225	0.200	7.032	0.100	48	0.1	36	0	0.01	(USDA, 2018d)
Volvariella volvacea	Straw mushroom	0.013	0.070	0.224	0.014	38	NA	NA	0	NA	(USDA, 2018c)
Auricularia polytricha	Jaw's ear	0.081	0.204	0.070	0.088	19	0	NA	0	NA	(USDA, 2018a)

NA: not available

Major bioactive compounds

Apart from providing various nutrients, mushrooms also contain many bioactive compounds that are highly related to many health benefits. Undigestible polysaccharides are probably the most well-known bioactive compound in mushrooms due to their antitumor and immunomodulatory activity (Valverde et al., 2015). Some mushroom polysaccharides such as β-glucans have gone through clinical trials and have been used as cancer adjuvant therapy in Asian countries such as China for a long time (Valverde et al., 2015). Table 5 shows the β-glucan content in some commonly cultivated mushrooms. Phenolic compounds, another group of bioactive compounds in mushrooms, exhibited potential health benefits such as anti-allergic, anti-tumor, anti-inflammatory effects; most of these benefits are attributed to their excellent antioxidant activity (Cheung, Cheung, & Ooi, 2003; Kalač, 2016c; Valverde et al., 2015). Table 6 shows the content of phenolic compounds in several most commonly cultivated edible mushrooms.

Mushrooms also contain some unique proteins that possess a few health functions including anti-tumor, anti-bacterial, anti-fungal, and immunomodulatory activity (Valverde et al., 2015). These proteins include lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial proteins and laccases (X. Xu, Yan, Chen, & Zhang, 2011). A very special amino acid in mushrooms: L-ergothioneine has caught researchers' attention for a long time due to its great antioxidant activity (Cheah & Halliwell, 2012; Feeney et al., 2014). L-ergothioneine is a sulfur-containing amino acid that can be only synthesized in fungi, some cyanobacteria and mycobacteria (Genghof, 1970; Genghof & Van Damme, 1964; Pfeiffer, Bauer, Surek, Schömig, & Gründemann, 2011). Mushrooms as fungi are a good source of L-ergothioneine; some species that contain high value of ergothioneine are king oyster mushroom,

maitake, and shiitake (N Joy Dubost, Beelman, Peterson, & Royse, 2006; Ey, Schömig, & Taubert, 2007). Its various roles include free radical scavanger, metal chelator, cytoprotective agent and immunomodulatory factor. Table 7 shows the ergothioneine content in some common edible mushrooms.

Apart from the bioactive compounds listed above, there are also many others which catch scientists' attention as well. These compounds include terpenoids, alkoloids such as eritadenine, lactones, nucleotide analogs, and polyketide such as lovastatin (Feeney et al., 2014; Kalač, 2016c).

In the following section, the health benefits of three representative bioactive compounds: beta-glucans, phenolic compounds and L-ergothioneine are going to be discussed in detail.

CHAPTER 3

BETA-GLUCANS

Structures and contents

Polysaccharides in mushrooms are considered as a group of important bioactive compounds that possess many health benefits including anti-tumor, immune-modulatory, anti-inflammation, anti-diabetic, anti-cholesterol effects, serving as prebiotics and so on (Feeney et al., 2014; Han et al., 2015; Jayachandran et al., 2017; Valverde et al., 2015). Although there are different polysaccharides in mushrooms including chitin, hemicellulose, mannans, alpha- and beta-glucans, galactans, and xylans, beta-glucans are the major polysaccharides in most mushrooms which consist half of the fungal cell wall mass and have been the most well studied for their immunomodulatory and anti-tumor activity (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Valverde et al., 2015; S. Wasser, 2002).

The structures of beta-glucans from different sources such as cereals, yeast, bacteria and mushrooms are different. Beta-glucans found in cereals is a mixture of $\beta(1,3)$ and $\beta(1,4)$ glycosidic bonds (Figure 1) (Laroche & Michaud, 2007). However, beta-glucans found in some fungi and yeasts are composed of a β (1,3) linked glucose backbone with $\beta(1,6)$ linked glucose side chains (Figure 2) (Laroche & Michaud, 2007). Many mushroom species can produce $\beta(1,3)$-D-glucans branched with $\beta(1,6)$ linked side chains. The exact chemical structure such as branching degrees and molecular weight depend on the species of mushrooms, which could affect their predominant health benefits (Vannucci et al., 2013; F. Zhu, Du, Bian, & Xu, 2015). It has been found that beta-glucans derived from oat and barley are mainly responsible for lowering

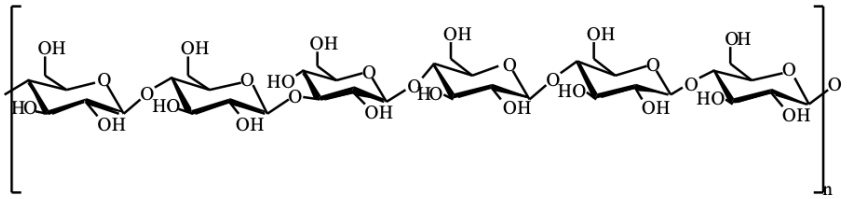

Figure 1. $\beta-(1,4)$ -glucopyranosyl units with one $\beta-(1,3)$ $glucopyranosyl\ unit$. Copyright 2007 Recent Patents on Biotechnology. (Laroche & Michaud, 2007)

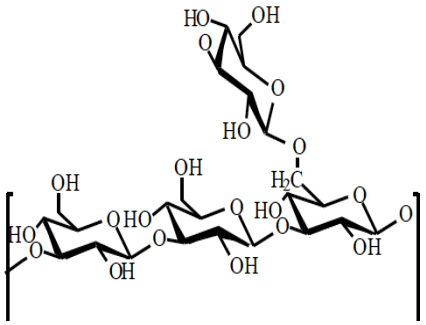

Figure 2. $\beta-(1,3)$-D-glucans with $\beta-(1,6)$ linked side chains. Copyright 2007 Recent Patents on Biotechnology. (Laroche & Michaud, 2007)

blood cholesterol and sugar but mushroom derived beta-glucans are more responsible for anti-tumor and immunomodulatory effect due to their particular structures (S. Wasser, 2002; F. Zhu et al., 2015).

The amount of beta-glucans varies in different mushroom species and cultivars. Sari, Prange, Lelley, and Hambitzer (2017) analyzed the beta-glucan content in nine culinary and thirty wild mushrooms in Germany. Among nine culinary mushrooms, *Cantharellus cibarius* (Chanterelle) contained more beta-glucans than others, with the mushroom cap having 23.59g/100g dry matter (dm) and stipe having 26.93g/100g dm (Sari et al., 2017). However, *Lentinula edodes* and *Pleurotus spp*. also contained significant amount of beta-glucans, ranging from 15.32g/100g dm to 25.31g/100g dm. Comparatively, *Agaricus bisporus* has lower beta-glucan content, which has around 9g/100g dm in the cap and around 10-12g/100g dm in the stipe

(Sari et al., 2017). The thirty wild mushrooms were found to have variable amount of beta-glucans but most of them, especially the bracket mushrooms, had higher amount of beta-glucans than *Agaricus bisporus*; *Trametes versicolor* (Turkey tail) had the highest beta-glucan content up to 60.79g/100g dm (Sari et al., 2017). The analytic method they applied is currently the most reliable one developed by Megazyme © International Ireland Ltd, which is an enzyme-based assay kit that can measure the total glucans and alpha glucans; beta-glucans are then calculated by difference (Sari et al., 2017). This method is much more accurate compared with the phenol-sulphuric-acid method, which is a commonly-used method for determining polysaccharides but cannot distinguish between alpha-glucans and beta-glucans (Sari et al., 2017). Bak, Park, Park, and Ka (2014) used the same assay kit to assess the beta-glucan content in fruiting bodies and mycelia in ten *Lentinula edodes* (Shiitake) cultivars in Korea. Their results showed that beta-glucans ranged from 20.06 ± 1.76% to 44.21 ± 0.13% of mushroom dry weight in the pileus sections of the fruiting bodies, from 29.74 ± 1.40% to 56.47 ± 4.72% in the stipe sections and from 15.59 ± 5.96% to 27.09 ± 2.11% in mycelia (Bak et al., 2014). Many studies have found that those mushrooms that can be divided into cap (pileus) and stipe part usually have higher amount of beta-glucans in the stipes than caps (Bak et al., 2014; Sari et al., 2017). However, commercial mushrooms always have their stipes cut before sale, which could lead to a big loss of beta-glucans.

Table 5 β-glucan Content in Commonly Edible Mushrooms

Scientific names	Common names	β-glucan (g/100g dry weight)		Reference
Agaricus bisporus (not specified)	Button mushroom	2.60		(Nitschke et al., 2011)
Agaricus bisporus (white)	Button mushroom	Cap: 8.608	Stalk: 12.296	(Sari et al., 2017)

Agaricus bisporus (brown)	Button mushroom	Cap: 8.837	Stalk: 10.079	(Sari et al., 2017)
Lentinus edodes	Shiitake	9.57		(Nitschke et al., 2011)
		Cap: 19.779	Stalk: 25.309	(Sari et al., 2017)
Pleurotus ostreatus	Oyster mushroom	9.05		(Nitschke et al., 2011)
		24.231		(Sari et al., 2017)
Pleurotus eryngii	King oyster mushroom	13.45		Nitschke et al. (2011)
		15.321		(Sari et al., 2017)
Pleurotus citrinopileatus	Golden oyster mushroom	15.542		(Sari et al., 2017)
Pleurotus pulmonarius	Phoenix mushroom	17.466		(Sari et al., 2017)
Pleurotus djamor	Pink oyster mushroom	21.703		(Sari et al., 2017)
Flammulina velutipes	Golden needle mushroom	8.98		Nitschke et al. (2011)

Table 6 Structural Features of Beta-glucans from Different Mushroom Species

Scientific names	Common names	Name of beta-glucans	Structural features	References
Agaricus brasiliensis	Himematsutake	Beta-glucan	β(1,3)-linked glucose backbone with a greater proportion of β(1,6)-linked branching chains	Camelini et al. (2005)
Schizophyllum commune	Common pelit gill	Schizophyllan	β(1,3)-linked glucose backbone with one β (1,6)-linked glucose residue every three residues	F. Zhu et al. (2015)
Lentinus edodes	Shiitake	Lentinan	β(1,3)-linked glucose backbone with two β (1,6)-linked glucose side chains every five residues	Schmid et al. (2001)

			β(1,3)-linked glucose backbone with two branch points every five residues; sides chains consist of both β(1,6)-linked and β(1,3)-linked glucose linkages	(Sasaki & Takasuka, 1976)
Pleurotus ostreatus	Oyster mushroom	Pleuran	β(1,3)-linked glucose backbone with one β-glucose residue linked by β (1,6) linkages every four residues	Karácsonyi and Kuniak (1994)
Pleurotus eryngii	King oyster mushroom	β-glucan	β(1,3)-linked glucose backbone with substitution of single unit of glucose by β(1,6) linkage on average to every three residues	(Carbonero et al., 2006)
Grifola fondosa	Maitake	Grifolan	β(1,3)-linked glucose backbone with single glucose unit linked by β (1,6) linkages every two residues	Fang et al. (2012)
Auricularia auricula	Jaw's ear	β-glucan	A main chain of β(1,4) linked glucose residues with glucopyranosyl residue linked at O-6 (70% ethanol/water solution extraction); A β(1,3)-linked backbone with two β-glucose linked by β(1,6) linkages every three residues (0.15M	Ma, Wang, and Zhang (2008); S. Xu, Xu, and Zhang (2012)

			aqueous NaCl extraction)	

Anti-tumor and immunomodulatory activity

The interest of fungal β-glucans can be traced back to early 1990s. The first isolated beta-glucan containing product was zymosan from yeast cell walls, which could regulate the innate immune functions (Fizpatrick & DiCarlo, 1964; Pillemer & Ecker, 1941). Since that discovery, fungal beta-glucans have attracted scientists' attention. The early research on anti-tumor and immunomodulatory effect of mushroom beta-glucans focused on lentinan (from *Lentinus edodes*), schizophyllan (from *Schizophyllum commune*), PSK, Krestin (from *Coriolus versicolor*) and grifolan (from *Grifola frondosa*) (Borchers et al., 1999; Brown & Gordon, 2003; C.Ooi & Liu, 2000). Lentinan is probably one of the earliest well-studied edible mushroom β-glucans since the other three are usually characterized as medicinal mushroom β-glucans. Chihara, Maeda, Hamuro, Sasaki, and Fukuoka (1969) isolated four polysaccharides from *Lentinus edodes* which included LC-33 (later known as lentinan), LC-1, EC-11 and EC-14 and tested their antitumor effect on mouse sarcoma 180. LC-11 and LC-33 showed great antitumor effect and the inhibitory ratio ranged from 73-100% (Chihara et al., 1969). Later more researches investigated the antitumor effect of lentinan on different tumor models such as MC-induced DBA/2.MC.CS-1 fibrosarcoma, gastric cancer, human colon carcinoma, oral squamous cell carcinoma, urothelial bladder cancer and so on (Harada, Itashiki, Takenawa, & Ueyama, 2010; Nakano et al., 1999; Ng & Yap, 2002; Suga, Shiio, Maeda, & Chihara, 1984; M. Sun, Zhao, Xie, Zhan, & Wu, 2015; L. Zhao, Xiao, & Xiao, 2013). There have been some clinical trials that tested the anti-tumor effect of lentinan in some patients but usually combined with chemotherapy

together (Oba, Kobayashi, Matsui, Kodera, & Sakamoto, 2009; Taguchi, 1987; P. Yang, Liang, Zhang, & Shen, 2008; S. Yoshino et al., 2000; Shigefumi Yoshino et al., 2010). The very first clinical trial was a four-year follow-up survey and randomized control trial on patients with advanced and recurrent stomach cancer to investigate the clinical efficacy when chemotherapeutic agent tegafur was combined with lentinan (Taguchi, 1987). The result showed significant increase of life span of the patients when lentinan was used together with the chemotherapy (Taguchi, 1987).

Since the first study of lentinan, the research on mushroom β-glucans has been expanded to different mushrooms species such as *Ganoderma lucidum* (reishi), *Tremella fuciformis* (snow fungus), *Griflola frondosa* (maitake), *Hericium erinaceus* (lion's mane), *Agaricus blazei* Murrill, *Flammulina velutipes* (golden needle mushroom), *Coriolus versicolor* (turkey tail), *Inonotus obliquus* (chaga mushroom), and *Pleurotus ostreatus* (Oyster mushroom) (Meng, Liang, & Luo, 2016). Among all these, *Ganoderma lucidum* is probably the most well-known medicinal mushroom for cancer treatment.

Some recent anti-cancer studies of beta-glucans based on animal cancer models are available. For example, Hetland et al. (2016) studied the anti-tumor effect of mushroom extract Andosan ™ (82% mushroom extracts from *Agaricus blazei* Murill in which beta-glucans are the major compounds) in A/J Min/+ Mice, which is a mouse model for colorectal cancer. Their results suggested that the mice in the treatment group had significant lower number of adenocarcinomas in the intestine and 60% reduction in the tumor load; in addition, there was reduction of expression of legumain, which is a tumor-associated proteolytic enzymes that is linked with tumor invasion and metastasis and increased production of Th1 type cytokines (Hetland et al., 2016). They also found that Andosan™ had a significant cytotoxic effect on

human colon cancer cell line Caco-2 (Hetland et al., 2016). Another study conducted by Roldan-Deamicis, Alonso, Brie, Braico, and Balogh (2016) investigated the anti-cancer effect of *Grifola frondosa* (Maitake) extracts in BALBc mice that were implanted with murine tumor cells LM3 to induce breast cancer. Mice were divided into control group and two treatment groups; one treatment group fed with Maitake extract ProX4 (commercially Maitake extract containing 30% of active proteo beta-glucan) and the other treatment group was fed with Maitake Standard (crude extract) (Roldan-Deamicis et al., 2016). The treatments were done before the implantation of tumor cells to assess the preventive effect of Maitake extracts on oncogenesis. The results indicated that Maitake ProX4 could block more than 60% breast cancer development via blocking of tumor invasiveness and reduction of angiogenesis but Maitake Standard could only block 26% of cancer development (Roldan-Deamicis et al., 2016).

There are also a few recent studies based on human subjects. For example, a randomized clinical trial designed by Wang, Bi, Zou, and Gu (2012) studied the efficacy of a combination treatment with lentinan and chemotherapy to treat individuals with oesophageal carcinoma in which general condition, symptoms and signs, quality of life and clinical efficacy were assessed. Their results showed that patients receiving the combination therapy had significantly greater improvement in general condition, symptoms and signs and quality of life compared with the control after just one course of treatment; in addition, the clinical efficacy was significantly greater than the control after two courses of treatment (Wang et al., 2012). Moreover, the immune function was assessed through analysis of pro-inflammatory and anti-inflammatory cytokines. The results indicated increase of pro-inflammatory cytokines in serum including IL-2, IK-6 and IL-12 but decrease of anti-inflammatory cytokines including IL-4, IL5 and IL-10 in both groups, which indicated up-regulation of immune function; the changes in treatment group

were greater than those in the control group (Wang et al., 2012). Their study suggests that the combination of mushroom beta-glucans and traditional chemotherapy may enhance the treatment efficacy of cancer through improving patients' immunity.

Although there are extensive studies of the antitumor effect of β-glucans from different mushrooms, the use of mushroom β-glucans to treat cancers is still under research and development. In fact mushroom polysaccharides are commonly used as adjuvant cancer therapy in Asian countries such as China and Japan (C.Ooi & Liu, 2000; Meng et al., 2016). A few important mushroom antitumor polysaccharides that are commonly used in oriental medicine include lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, grifolan from *Grifola frondosa*, PSK from *Coriolus versicolor* and polysaccharides from *Ganoderma lucidum* (C.Ooi & Liu, 2000; Meng et al., 2016). Nevertheless, they are still not widely used in Western medicine since the dose has not been standardized when used as cancer adjuvant therapy or as preventive functional food. In fact it is quite difficult to decide the suitable dose since different mushroom β-glucans have different anti-tumor effects, depending on their molecular weights, branching points, and confirmation (Borchers et al., 1999; Meng et al., 2016; F. Zhu et al., 2015). Therefore, more studies especially clinical trials are needed before mushroom β-glucans could be standardized as cancer therapy.

Right after the discovery of the anti-tumor activity of β-glucans, scientists started to study the mechanisms of it, which lead to the discovery of another important health function of β-glucans: immunomodulatory effect. The major role of β-glucans to inhibit the tumors is not a direct cytotoxic effect but to stimulate both the innate and adaptive immune systems and lead to a series of immune responses (Bohn & BeMiller, 1995). Innate immune system refers to the non-specific immunity which includes a lot of leukocytes including neutrophils, macrophages, natural

killer cells, and dendritic cells. They usually act by recognition of pathogen associated patterns and then initiate a series of immune responses including phagocytosis, free radical production, cytokine production and presentation of antigens to lymphocytes (Volman, Ramakers, & Plat, 2008). Adaptive immune system is more specific which involves production of antibodies by B lymphocytes to specific pathogens and attack of pathogen cells by cytotoxic and helper T cells (Volman et al., 2008). The antigen pieces presented by leukocytes and the cytokines produced by leukocytes are the activators of adaptive immunity (Volman et al., 2008).

Since beta-glucans are the major components of cell walls in fungi, plants and some bacteria which are not present in humans, they are recognized by human immune system as pathogen-associated molecule patterns therefore can stimulate immune responses (Brown & Gordon, 2003; Janeway, 1992). It has been found that β-glucans activate immune cells such as macrophages, neutrophils, eosinophils, monocytes, dendritic cells and natural killer cells via immune cell receptors including CR3, lactosylceramide, scavenger receptors, Dectin-1 and TLR-2/6 (Toll Like Receptors) (Brown & Gordon, 2003; Chan, Chan, & Sze, 2009). Thornton, Větvicka, Pitman, Goldman, and Ross (1996) studied sugar specificity for CR3 (CD11b/CD18) and found that CR3 could bind to pure beta-glucans from yeast, mushroom, seaweed or barley but also other molecules like N-acetyl-D-glucosamine (NADG), alpha- or beta-methylmannoside, and alpha- or beta-methyl-glucoside, which indicated that this receptor has a wide range of activators. However at least this study showed that CR3 (CD11b/CD18) is a leukocyte beta-glucan receptor which locates at the C-terminal to the I-domain of CD11b (Thornton et al., 1996). Later Dectin-1 was identified as a β-glucan receptor from screening of cDNA expression of macrophages with zymosan (yeast β-glucans) (Brown & Gordon, 2001). Dectin-1 is found to be expressed in populations of myeloid cells including monocytes,

macrophages, and neutrophil lineages; it is also expressed in dendritic cells and T cells (Taylor et al., 2002) . It has been shown that Dectin-1 activation collaborates with the induction of TLR2/6 which together lead to the production of signaling molecules such as NF-κB, signaling adaptor protein CARD9 and nuclear factor of activated T cells (NFAT) which further lead to the release of cytokines such as IL-12, IL-6, IL-10 and TNF-α (Brown & Gordon, 2003; Brown et al., 2003; Chan et al., 2009; Goodridge, Simmons, & Underhill, 2007; Gross et al., 2006; Rogers et al., 2005). Some of these cytokines and singling pathways are very important immune responses to cancers. Because those studies which studied the collaborative response of dectin-1 and TLR used zymosan which is an extract from yeast cell wall and is not pure β-glucans, it was unclear the way that pure β-glucans interact with TLR (Chan et al., 2009). Moreover, because zymosan is not pure beta-glucans and even the beta-glucans contained inside is structurally very different from mushroom beta-glucans, the interactions happened between zymosan and Dectin-1 and TLR may not necessarily be the same between mushroom beta-glucans and these receptors. A few studies and reviews emphasized that the immunomodulatory effect of β-glucans is highly associated with their molecular weights, solubility, chemical modifications, branching points, polymer lengths and confirmations (Borchers, Keen, & Gershwin, 2004; Brown & Gordon, 2003; Volman et al., 2008). Unfortunately there have not been too many studies that investigated the interaction between immune cell receptors and mushroom beta-glucans. However there are studies that measure changes of immune parameters when mushroom beta-glucans were used. Fruehauf, Bonnard, and Herberman (1982)'s research on lentinan is one of the earliest studies. They investigated the effect of lentinan on human monocytes and their results showed that lentinan increased production of IL-1 by K-562 cell line with only a very low concentration of 0.1 μg/ml (Fruehauf et al., 1982). Another early study was conducted by Herlyn, Kaneko, Powe,

Aoki, and Koprowski (1985), which is an *in vivo* study that studied the macrophage cytotoxicity affected by lentinan with the presence of antigen-specific monoclonal antibodies (MAbs). Their results showed that the murine peritoneal murine macrophages harvested after injection of 2.5 mg/ per kg of body weight of lentinan possessed the best cytotoxic effect on human tumor cells including melanoma, colorectal carcinoma and pancreatic carcinoma with the presence of IgG1, IgG2a and IgG3 MAbs (Herlyn et al., 1985). Some early human clinical studies were also available. For example, Matsuoka, Seo, Wakasugi, Saito, and Tomoda (1997) conducted immunological analysis on patients with various advanced cancers with lentinan administration together with chemotherapy. They found that patients who responded to lentinan showed a great increase of killer T cell/suppressor T cell ratio [CD11(-) CD8(+)/CD11(+) CD8(+)] and decrease of production in IL-6, G-CSF and PGE2 which indicated an increase in immune potential (Matsuoka et al., 1997).

The immunomodulatory effect of mushroom beta-glucans is still a popular research topic currently. Scientists are working on different mushroom species and the mechanisms of how mushroom beta-glucans mediate immune responses. Following are some recent studies within the latest 8 years.

A study conducted by Yan et al. (2018) assessed the immunomodulatory effect of a novel polysaccharide AAMP-A70 which is a branched beta-glucan from *Amillariella mellea* and they found that this novel beta-glucan activated the mouse macrophage cells RAW264.7 via NF-κB/MAPK signalling pathway and TLR2 receptor and increased the secretion of NO, ROS, and TNF-α, IL-6 and IL-1β phagocytosis (Yan et al., 2018). Similarly, Castro-Alves, Gomes, Menolli, Sforça, and Nascimento (2017) investigated the immunomodulatory effects of polysaccharide extracts from a commonly consumed mushroom *Pleurotus albidus* on murine

RAW 264.7 macrophages (Castro-Alves et al., 2017). They found that beta-glucans from this mushroom could stimulate the production of TNF-α and NO●, which are important marker for macrophage functions and signalling molecule involved in immune response respectively (Castro-Alves et al., 2017).

Besides *in vitro* cell studies, animal model-based studies have been also conducted to show immunomodulatory effect of beta-glucans. For example, Masuda et al. (2013) assigned oral administration of beta-glucans from Grifola frondosa (Maitake) to colon- and mammary-tumor-bearing mice to investigate if beta-glucans could initiate anti-cancer immunity. The results showed that beta-glucans from Maitake induced the maturation of dendritic cells via a C-type lectin receptor, which further induced the systemic tumor-antigen specific T cells; in addition, infiltration of the activated T cells into tumors was increased and the number of tumor-linked immunosuppressive elements was decreased (Masuda et al., 2013).

Although most studies of beta-glucans are still based on *in vitro* cell cultures or *in vivo* animal models, there is still some evidence from human studies. Jesenak et al. (2013) designed a double-blind, placebo-controlled, randomized clinical trial to test immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections. The study found that the treatment group had significantly decreased number of flu- and flu-like disease and number of respiratory tract infections through the immunomodulation of both innate and adaptive immunity; the effect of pleuran to stimulate the humoral immune system was indicated by the increased production of three immunoglobulin isotypes, increased number of NK cells and slower decline of the number of T-cytotoxic lymphocytes (Jesenak et al., 2013). Another human clinical trial conducted by Bobovčák, Kuniaková, Gabriž, and Majtán (2010) studied the immunomodulatory effect of pleuran on cellular immune response in athletes after 1

hour of intensive exercise since excessive high-intensity exercise could suppress the immune system. The study found that the control group had 28% reduction of natural killer cell activity (NKCA) compared with the baseline value but the treatment group did not have significant reduction in NKCA, which suggested that pleuran can prevent immune suppression due to intensive exercise (Bobovčák et al., 2010).

On the one hand, mushroom beta-glucans possess their immunomodulatory effects via immune-stimulation but on the other hand, they also present anti-inflammatory effect. Inflammation is the first reaction of human immune system to antigens, however, chronic inflammation is the major pathogenesis of autoimmune diseases and many chronic diseases such as diabetes, cancers, cardiovascular disease, neurological degeneration, inflammatory bowel disease and so on (Franceschi & Campisi, 2014). Chronic inflammation is characterized by persistent production of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α and dysregulation of cell signaling pathways (Du, Lin, Bian, & Xu, 2015; Franceschi & Campisi, 2014).

Wu *et al.* (2017) determined the *in vitro* anti-inflammatory activity of polysaccharide extracts from mycelia of *Grifola frondosa* in lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophage model. Their results showed that the high-pressure assisted polysaccharide extracts significantly inhibited the activation of NF-κB and reduced the secretion of TNF-α, IL-6 and IL-1β in LPS-stimulated macrophages (S. J. Wu et al., 2017). Another study conducted by Nishitani et al. (2013) investigated the anti-inflammatory effect of lentinan both *in vitro* and *in vivo*. Their results showed that lentinan significantly reduced IL-8 mRNA expression and NF-α activation in intestinal epithelial Caco-2 cells; in addition, lentinan also inhibited the expression of TNF receptor 1 (TNFR1) in Caco-cells in both protein and mRNA level (Nishitani

et al., 2013). In dextran sulfate sodium (DSS)-induced colitis mice, oral administration of lentinan to the mice showed the significant effect of amelioration on body weight loss, shorting of colon lengths, histological score and inflammatory mRNA expression in inflamed tissues (Nishitani et al., 2013). Moreover, lentinan inhibited the expression of TNFR1 in intestinal epithelial cells (Nishitani et al., 2013).

Compared with the research on immune-stimulation, the number of researches on anti-inflammation of mushroom beta-glucans is limited. More investigations are still necessary to understand the exact mechanisms of how beta-glucans could exhibit a balanced function between immune-stimulation and anti-inflammation.

The anti-tumor and immunomodulatory of mushroom beta-glucans makes mushrooms a potential source to act as a cancer adjuvant therapy and immune booster. However there is still a long way to go to finalize the dose and potential risk of using mushroom beta-glucans as a cancer treatment or as a functional food since the difference in beta-glucans from different mushrooms heavily affect their biological effects and makes the interactions between beta-glucans and immune system even more complicated.

Prebiotics

A prebiotic is defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). A similar term 'probiotics' on the other hand refers to the "preparations of or products containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health

effects in this host" (Schrezenmeir & de Vrese, 2001). In simple words, prebiotics are the 'food' of probiotics. A positive effect of prebiotics is to change the gut microbiota composition in the way that support the growth of probiotics such as bifidobacterial and lactobacilli which inhibit the growth of undesired bacteria, lower the incidence of infection, improve colonic integrity and enhance host immunity (Douglas & Sanders, 2008; G. T. Macfarlane, Steed, & Macfarlane, 2008; Palframan, Gibson, & Rastall, 2003). Gut microbiota is not only important for gut health but is also associated with several metabolic dysregulations, which could lead to inflammatory diseases such as diabetes, obesity and cancer (Jayachandran et al., 2017). It has been show that gut microbiota is essential to regulate immune homeostasis in the host, which affect both the innate and adaptive immunity (H.-J. Wu & Wu, 2012). Alternations of gut microbiota could lead to autoimmune diseases due to the dysregulation of the immune system (H.-J. Wu & Wu, 2012). Some well-studied and commercialized prebiotics include inulin, fructo-oligosaccharides, galacto-oligosaccharides, soya-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, lactulose, and polydextrose (Alander et al., 2001; Crittenden & Playne, 2002; Hayakawa et al., 1990; Kaneko, Yokoyama, & Suzuki, 1995; Losada & Olleros, 2002; S. Macfarlane, Macfarlane, & Cummings, 2006; Roberfroid, 2007).

Apart from the non-digestible oligosaccharides, non-digestible long polysaccharides have been attracting scientists' interests as a potential new source of prebiotics (Lam & Chi-Keung Cheung, 2013). Beta-glucans, as one type of non-digestible polysaccharides which are the major cell components in plants and fungi have been suggested as a good source of prebiotics (Lam & Chi-Keung Cheung, 2013). Starting from later 1990s and early 2000s, research on beta-glucans as prebiotics to promote growth of bifidobacterial and lactobacilli has been continuously conducted but early research is on cereal beta-glucans such as oat and barley (Dongowski, Huth,

Gebhardt, & Flamme, 2002; Drzikova, Dongowski, & Gebhardt, 2005; Jaskari et al., 1998; Kontula, von Wright, & Mattila-Sandholm, 1998). However besides cereals, mushrooms are also considered as a good source of beta-glucans and have a great potential to serve as prebiotics since their world production is huge and still keeps increasing (Kalač, 2016a). Until 2009, there was first research that studied the potential prebiotic activity of extracts from *Pleurotus ostreaus* and *Pleurotus eryngii* which contain both alpha-glucans and beta-glucans using nine strains of *Lactobacillus*, *Bifidobacterium* and *Enterococcus* (Synytsya et al., 2009). Their results showed that the extracts from both *Pleurotus* mushrooms supported the growth of *Lactobacillus* strains but *Pleurotus eryngii* favored the growth more than *Pleurotus ostreaus*; strain Bifi A and B utilized the mushroom extracts very differently, with Bifi A using *Pleurotus ostreaus* better than *Pleurotus eryngii* but with Bifi B only using *Pleurotus eryngii*; *Enteroccocus* strains could utilize both mushroom extracts but their growth rate was slower than *Lactobacillus* (Synytsya et al., 2009). These results suggested that different probiotic bacteria are selective about mushroom glucan sources with certain strains preferring certain chemical structures (Synytsya et al., 2009). This is an important implication when selecting mushroom beta-glucans as good prebiotic sources.

The number of researches about mushroom beta-glucans as a potential prebiotic source is still increasing. J. Zhao and Cheung (2011) showed that beta-glucans from different sources such as seaweed, barley and mushrooms can promote the growth of *Bifidobacterium infantis, Bifidobacterium longum, and Bifidobacterium*; the populations of three bacteria all increased in a large number. Among the three bacteria, *B. infantis* produced almost double the amount of short-chain fatty acids compared to the other two (J. Zhao & Cheung, 2011). Chou, Sheih, and Fang Tony (2013) not only studied the effect of mushroom polysaccharides on

bacterial growth in petri dish but also in simulated digestive environment. They found that low concentration of polysaccharides (0.1% - 0.5%) derived from the stipes of three mushroom species: *Lentinula edodes*, *Pleurotus eryngii*, and *Flammulina velutipes* can enhance the viability of *Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium longum subsp. longum* during cold storage; in addition, significant protective effects of polysaccharides from these three mushrooms on probiotics were found in a simulated environment of gastric juice and bile acid. Similarly, another study conducted by Nowak, Nowacka-Jechalke, Juda, and Malm (2017) also supports the finding that fungal polysaccharides can stimulate growth of prebiotics. They investigated the effect of polysaccharides derived from various wild mushroom species in Poland on the growth of *Lactobacillus acidophilus* and two stains of *Lactobacillus rhamnosus*; moreover, they also studied the bioavailability of the polysaccharides in artificial human gastric juice by calculating the degree of hydrolysis (Nowak et al., 2017). Their results indicated that mushroom polysaccharides stimulated the growth of *Lactobacillus acidophilus* and two stains of *Lactobacillus rhamnosus*, with some mushroom species significantly increasing the metabolism of glucose in the bacteria (Nowak et al., 2017). The bioavailability study showed that polysaccharides in mushrooms were highly resistant to acidity, which means that they could reach the colon where the probiotic bacteria are abundant (Nowak et al., 2017).

Besides bacteria culture study, there are some research carried out in animal models to study the beneficial effect of mushroom polysaccharides on gut health. Varshney et al. (2013) conducted an animal study by feeding the mice with either white-button mushroom diet or control diet for six weeks and checked the gut microbiota, urinary metabolome and resistance to gastrointestinal pathogens. What they found were that white-button mushroom diet changed the composition of the gut microbiota by increasing Bacteroidetes phylum and decreasing Firmicutes

phylum; in addition, mice that were introduced to *Citrobacter rodentium* infection had fewer inflammatory cells and less severe colitis in the GI mucosa when they were fed with white-button mushroom diet (Varshney et al., 2013). Another recent esearch conducted by Ren et al. (2018) investigated the potential of polysaccharides extract from *Hericium erinaceus* (Lion's Mane) to treat and prevent inflammatory bowel disease by testing its anti-inflammatory effect in C57BL/6 mice with dextran sulfate sodium (DSS) -induced colitis. *Hericium ernaceus* is a very valuable edible and medicinal mushroom commonly consumed in China. The results showed that polysaccharides from *Hericium erinaceus* can down regulate many key markers of oxidative stress including nitric oxide (NO), malondialdehyde (MDA), total superoxide dismutase (T-SOD), and myeloperoxidase (MPO) (Ren et al., 2018). In addition, it also suppressed the secretion of inflammatory molecules such as interleukin (IL)-6, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (Ren et al., 2018). The examination of the gut microbiota showed that the DSS-induced gut dysbiosis was reversed by the polysaccharides (Ren et al., 2018). This research demonstrates that polysaccharides from *Hericium erinaceus* exhibit great potential to ameliorate the inflammatory bowel disease by regulating the gut microbiota and reducing the clinical symptoms caused by inflammation.

So far the studies that only investigate the effect of pure mushroom beta-glucans as a prebiotic are still limited since most studies that looked at the mixture of polysaccharides. Moreover, more human studies are needed since the utilization of beta-glucans by probiotics could be very different *in vivo* since human gut environment is very complicated. The bioavailability of mushroom beta-glucans in human gut and the exact mechanism of how mushroom beta-glucans could benefit the gut heath are two possible future research directions.

CHAPTER 4

PHENOLIC COMPOUNDS

Types and contents

Phenolic compounds from different sources such as grains, vegetables, fruits, wine, and mushrooms have been extensively studied by scientists due to their strong antioxidant activity (Adom & Liu, 2002; Frankel, Waterhouse, & Teissedre, 1995; Kähkönen et al., 1999; Rice-Evans, Miller, & Paganga, 1997; J. Sun, Chu, Wu, & Liu, 2002). Phenolic compounds in edible mushrooms have been studied since 2003. Cheung et al. (2003) investigated the antioxidant capacity of phenolic compounds in water extracts and methanol extracts from *Lentinus edodes* and *Volvariella volvacea*. Later more mushroom species including cultivated mushrooms such as *Agaricus bisporus, Pleurotus ostreatus, Flammulina velutipes* and *Auricularia auricula-judae* and a large number of wild edible mushrooms had their phenolic contents and antioxidant activity determined (N. Joy Dubost, Ou, & Beelman, 2007; Palacios et al., 2011; Reis, Martins, Barros, & Ferreira, 2012; Wong & Chye, 2009).

The major phenolic compounds found in mushrooms are phenolic acids which can be divided into two groups, hydroxybenzoic acid and hydroxycinnamic acid (Ferreira, Barros, & Abreu, 2009). Hydroxybenzoic acids found in mushrooms include *p*-hydroxybenzoic, protocatechuic, gallic, gentisic, homogentisic, vannilic, 5-sulphosalicylic, syringic, veratric and vanillin (Ferreira et al., 2009). Hydroxycinnamic acids commonly reported in mushrooms include *p*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, 3-*o*-caffeoylquinic, 4-*o*-caffeoylquinic,

and 5-*o*-caffeoylquinic (Ferreira et al., 2009). Phenolic acids are usually present in bound forms through glycosidic bonds with simple sugars and polysaccharides in cell wall structure or through ester bonds with organic acids (Kozarski et al., 2015). Flavonoids are the second most abundant phenolic compounds in mushrooms; myricetin, chrysin, catechin, hesperetin, quercetin, rutin, and kaempferol have been found in mushrooms (Ferreira et al., 2009).

A few research studies the phenolic compounds in edible mushrooms from different countries. Islam, Yu, and Xu (2016) assessed the total phenolic content (TPC), total flavonoid content (TFC) and free phenolic acid content in 43 commonly consumed mushroom in China. The TPC ranged from 0.19 to 26.21 mg GAE/g dry weight (DW) with big variations between different species; among all mushroom species, stone ear (26.21mg GAE/g DW), porcino nero (11.94 mg GAE/g DW) and yellow bolete (10.62 mg GAE/g DW) had the highest TPC (Islam et al., 2016). The TFC ranged from 0.05 to 5.90 mg CAE/g, with pine spike (5.90 mg CAE/g DW), coral mushroom (3.65 mg CAE/g DW), nameko (3.00 mg CAE/g DW), jersey cow mushroom (2.18 mg CAE/g DW), yellow bolete (2.15 mg CAE/g DW), wild porcino nero (2.13 mg CAE/g DW) and stone ear (2.09 mg CAE/g DW) having relatively high TFC content (Islam et al., 2016). Free phenolic acids were assessed using HPLC UV-DAD and the results showed that gallic acid was the most abundant phenolic acid that were detected in most mushroom species, with princess matsutake having the highest content (1376.79 μg/g DW) (Islam et al., 2016). Other free phenolic acids found in some of the 43 mushroom species included gentisic acid, *p*-hydroxybenzoic acid, 2,3,4-hydroxubenzoic acid, 3,4-dihydroxybenzaldehyde, vanillic acid, caffeic acid, and *p*-comaric acid (Islam et al., 2016). Kaewnarin, Suwannarach, Kumla, and Lumyong (2016) determined the phenolic content, flavonoid content and phenolic profiles of four wild edible mushroom species in Thailand by using four different extraction solvents. What

they found was that water was particularly efficient for extraction of phenolics of these four mushroom species (Kaewnarin et al., 2016). The total phenolic compounds ranged from 2.13 to 20.2 mg GAE/g DW with *Russula emetica* having the highest total phenolic content in water extract (20.2 mg GAE/g DW) (Kaewnarin et al., 2016). The total flavonoid content ranged from 0.03 to 0.98 mg QE/g DW (mg quercetin equivalent/g DW), with *Rugiboletus extremiorientalis* having the highest total flavonoid content in methanolic extract (0.98 mg QE/g DW) (Kaewnarin et al., 2016). The HPLC analysis of phenolic compounds found 8 phenolic acids (gallic acid, protocatechuic acid, vanillic acid, syringic acid, sinapic acid, ferulic acid, m-courmaric acid, rosmarinic acid) and 4 flavonoid compounds (catechin, rutin, quercetin, apigenin) with some only appearing in one mushroom species (Kaewnarin et al., 2016). Gallic acid and protocatechuic acid were detected in all mushrooms; highest content of gallic acid and protocatechuic acid were found in the water extract of *Rugiboletus extremiorientalis* (36.545 μg/g DW and 31.017 μg/g DW respectively) (Kaewnarin et al., 2016). Another study conducted by Yahia, Gutiérrez-Orozco, and Moreno-Pérez (2017) identified the phenolic compounds in seventeen wild mushrooms including nine edible mushroom species in Central Mexico. The total soluble phenols in nine edible mushrooms ranged from 36.44 to 120.98 mg GAE/100g fresh weight (FW) with *Boletus luridus* having the highest amount (Yahia et al., 2017). The total flavonoid content ranged from 7.09 to 34.57 mg CAE/100mg FW with *Ramaria flava* having the highest content (Yahia et al., 2017). HPLC analysis of phenolic compounds detected nine phenolic acids in edible mushrooms including *p*-hydroxybenzoic acid, *o*-courmaric acid, chlorogenic acid, protocatechuic acid, caffeic acid, cinnamic acid, sinapic acid, ferulic acid, and vanillic acid; *R. flava* had highest content of sinapic acid (36.706 mg/100g DW) and *A.*

pantherina had the highest content of p-hydroxybenzoic acid (36.403 mg/100g DW) (Yahia et al., 2017).

However these studies may underestimate the total phenolic content and total phenolic acids in mushrooms since many phenolic acids exist in insoluble bound forms but the extraction methods in these studies could only extract the soluble phenolic compounds. Furthermore, the extraction solvents can affect the extraction efficiency but solvents used in these studies were all different, which makes the comparison of different mushroom species difficult. It is very important to develop consistent extraction methods that extract both soluble free and insoluble bound phenolic compounds in the future study, which could make the data more reliable and easier to be compared.

Table 7 Contents of Phenolic Compounds in Some Commonly Cultivated Mushrooms

Scientific names	Common names	Phenolic compounds (mg/GAE/g DW)	Sample origin	Reference
Agaricus bisporus	Button mushroom	3.748	China	(Guo et al., 2012)
		8	U.S.	(N. Joy Dubost et al., 2007)
Agaricus blazei	Sun mushroom	8.348	China	(Guo et al., 2012)
Lentinus edodes	Shiitake	4.294	China	(Guo et al., 2012)
		6.27	Taiwan, China	(J.-H. Yang, Lin, & Mau, 2002)
		4.32	U.S.	(N. Joy Dubost et al., 2007)
		36.19	Denmark	(Boonsong, Klaypradit, & Wilaipun, 2016)
		24.25		
		10.46		
Pleurotus eryngii	King oyster mushroom	3.171	China	(Guo et al., 2012)
		1.42	Turkey	(Oke & Aslim, 2011)
		0.93		

Pleurotus ostreatus	Oyster mushroom	4.002	China	(Guo et al., 2012)
		15.7	Taiwan, China	(J.-H. Yang et al., 2002)
		4.27	U.S.	(N. Joy Dubost et al., 2007)
Flammulina velutipes	Golden needle mushroom	3.459	China	(Guo et al., 2012)
		White: 8.38 Yellow: 9.26	Taiwan, China	(J.-H. Yang et al., 2002)
Volvariella volvacea		13.914	China	(Guo et al., 2012)
	Straw mushroom	22.97	Denmark	(Boonsong et al., 2016)
		27.89		
		1.99		
Auricularia auricula	Jaw's ear	4.000	China	(Guo et al., 2012)
		13.61	Turkey	(Oke & Aslim, 2011)
		10.54		
		2.90	Denmark	(Boonsong et al., 2016)
		2.75		
		2.17		
Auricularia polytricha	Wood ear	3.105	China	(Guo et al., 2012)

Table 8 Major Phenolic Compounds (μg/g DW) in Some Commonly Cultivated Mushrooms

Scientific names	Common names	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Reference
Agaricus bisporus	Button mushroom	15.5	0.5	63.7	10.4	16.4	94.9	15.4	3444.3	22.4	16.2	n.d,	258.7	NA	NA	NA	Palacios et al. (2011)
		n.d.	n.d.	n.d.	n.d.	n.d.	16	n.d.	n.d.	19	32	n.d.	464	13	NA	NA	(Kim et al., 2008)
Pleurotus ostreatus	Oyster mushroom	n.d	n.d.	n.d.	11.5	20.16	290.34	4.69	629.86	21.99	19.32	292.62	n.d.	NA	NA	NA	(Palacios et al., 2011)
		n.d.	n.d.	19	n.d.	n.d.	7	n.d.	16	21	18	n.d.	n.d.	9	NA	NA	(Kim et al., 2008)
Lentinus edodes	Shiitake	n.d.	3	n.d.	n.d.	n.d.	21	n.d.	n.d.	n.d.	16	n.d.	n.d.	n.d.	NA	NA	(Kim et al., 2008)
Pleurotus eryngii	King oyster mushroom	n.d.	n.d.	n.d.	n.d.	n.d.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8	NA	NA	(Kim et al., 2008)
		n.d.	32	n.d.	n.d.	6	n.d.	138	NA	NA	NA	NA	NA	NA	10	n.d.	(Oke & Aslim, 2011)
		10	n.d.	n.d.	4	n.d.	450	420	NA	NA	NA	NA	NA	NA	12	n.d.	(Oke & Aslim, 2011)
Flammulina velutipes	Golden needle mushroom	17	n.d.	26	n.d.	9	21	n.d.	15	n.d.	21	n.d.	15	n.d.	NA	NA	(Kim et al., 2008)
Auricularia auricula	Jaw's ear	76	NA	314	12	n.d.	636	488	NA	NA	NA	NA	NA	NA	104	254	(Oke & Aslim, 2011)

		200	NA	360	n.d.	n.d.	360	700	NA	NA	NA	NA	NA	NA	140	100	(Oke & Aslim, 2011)

1 Caffeic acid; 2 Catechin; 3 Catechin; 4 p-courmaric acid; 5 Ferulic acid; 6 Ferulic acid; 7 Ferulic acid; 8 Homogentisic acid; 9 Myricetin; 10 Protocatechuic acid; 11 Gentisic acid; 12 Pyrogallol; 13 Naringin; 14 Syringic acid; 15 Sinapinic acid

n.d.: not detected; NA: not available

Antioxidant activity

Free radicals are generated in many metabolic processes especially in cell respiration and immune response (Kozarski et al., 2015). Apart from endogenous generation of free radicals, there are also many external sources of free radicals including UV light, environmental toxins, ionizing radiation and so on (Kozarski et al., 2015). High amount of free radicals increase the oxidative stress in human bodies, which damages the tissues and contributes to many chronic diseases including cancer, cardiovascular disease, diabetes, neurodegenerative diseases (Santilli, D'Ardes, & Davì, 2015; Valverde et al., 2015). Although humans have endogenous antioxidant defense mechanism, imbalanced diet and unhealthy life style could lead to elevated oxidative stress which disrupt the antioxidant hemostasis in the body. Mushrooms have caught many researchers' attention due to their great nutritional values and high content of antioxidant compounds especially phenolic compounds (Kozarski et al., 2015). Phenolic compounds regulate the oxidative stress either through direct actions such as scavenging the free radicals, quenching singlet oxygen, chelating metals, and regenerating other antioxidants or through indirect regulations of the endogenous antioxidant defense mechanism via interactions with enzymes and cell receptors in different signaling pathways (Kozarski et al., 2015).

There are many studies of phenolic compounds and their antioxidant activity in different species of mushrooms. Islam et al. (2016) assessed the antioxidant activity of the phenolic compounds in 43 commonly consumed mushrooms in China. Different antioxidant assays showed varied antioxidant activities in different mushrooms, with highest DPPH value in porcino nero, highest FRAP value in mulberry yellow, highest ABTS value in stone ear and highest MCA value in Maitake (Islam et al., 2016). Another study conducted by Palacios et al. (2011) determined the phenolic contents and their antioxidant activities in eight cultivated and wild edible mushrooms commonly consumed in Spain. The antioxidant activity was assessed by monitoring inhibition of ABAP-induced lipid peroxidation (Palacios et al., 2011). The assay found that all mushrooms exhibited inhibitory effect but the inhibition did not correlate with total phenolic content nor flavonoid content (Palacios et al., 2011). Furthermore, it also did not correlate with the amount of homogenetisic acid which was the most abundant phenolic acid in these mushrooms (Palacios et al., 2011). They concluded that each phenolic compound or each group of compounds may exhibit different antioxidant activities, with some having much stronger activity than others (Palacios et al., 2011). Similarly, Kaewnarin et al. (2016) assessed the antioxidant activities of the phenolic compounds in four popular wild edible mushrooms in Thailand using DPPH assay, ABTS assay and FRAP assay. They found that antioxidant activities of phenolic compounds largely depended on mushrooms species, extraction methods and assay methods (Kaewnarin et al., 2016). Significant correlation was found between phenolic content and ABTS assay. There are more researches regarding the phenolic compounds and their antioxidant activities in edible mushrooms all around the world including Poland, Turkey, Ethiopia, Mexico, Bangladesh and so on (Chowdhury, Kubra, & Ahmed, 2015; Orhan & Üstün,

2011; Özyürek, Bener, Güçlü, & Apak, 2014; Radzki, Slawinska, Jablonska-Rys, & Gustaw, 2014; Woldegiorgis, Abate, Haki, & Ziegler, 2014).

While a lot of studies assessed the antioxidant activity of phenolic compounds in mushrooms and provided strong evidence to support the antioxidant activity, their results were not very reliable due to their chemical assessing methods. DPPH, ABTS and FRAP methods were applied to determine the antioxidant activity but these methods have disadvantages and cannot provide accurate results (Amorati & Valgimigli, 2015). DPPH• is a highly persistent radical which can be reduced to DPPHH by a reductant; due to the similar electronic configuration between DPPH• and peroxyl radicals, the rate constant between DPPH• and phenolic antioxidants and the rate constant between ROO• and phenolic antioxidants have the same ranking (Amorati & Valgimigli, 2015). However the linear relationship between rate constants of DPPH• and ROO• can be only maintained in aprotic solvents while most studies used polar protic solvents such as ethanol and methanol; these solvents can make the reaction between DPPH• and phenols much faster (Amorati & Valgimigli, 2015). $ABTS^{+}$• is a radical cation used in TEAC test (Trolox-Equivalent Antioxidant Capacity). The limitation of this test is that the reaction between antioxidants and $ABTS^{+}$• uses the electron transfer mechanism but the reaction between antioxidants and peroxyl radicals uses the H-atom transfer (Amorati & Valgimigli, 2015). Both DPPH• and $ABTS^{+}$• are used as radical probes in the antioxidant test but they are chemically different from the radicals responsible for the autooxidation in the real systems; these radical may be reduced by any reductant that may have no antioxidant activity therefore these methods can only indicate the reducing power but not the antioxidant activity (Amorati & Valgimigli, 2015). FRAP also has its limitation that the unpaired electrons in d-f orbitals and the reactivity of the inorganic Fe^{3+} cannot represent the characteristics of organic

peroxyl radicals (Amorati & Valgimigli, 2015). While TOSC assay is a good method to assess the antioxidant activity which can avoid the interference of the colored matrices, no research above used this assay (Amorati & Valgimigli, 2015). In conclusion, the above chemical assays cannot provide unbiased and accurate results of the antioxidant activity of the phenolic compounds.

Although there is a lack of reliable results from good chemical assays of the antioxidant activity of mushrooms, there are still a few *in vivo* animal studies that provide some evidence. Jun Liu, Jia, Kan, and Jin (2013) studied on the antioxidant activity of the phenolic compounds in white button mushroom in mice. They measured the concentration of antioxidant enzymes including SOD, GSH-Px and CAT in serums, livers and hearts in 30 mice that were divided into five different groups (Jun Liu et al., 2013). The results indicated that ethanolic extract at the dose of 600 and 1200 mg/kg body weight treatment had the antioxidant enzymes significantly increase in serums, liver and hearts compared with the normal control group (Jun Liu et al., 2013). The possible mechanism of the increased antioxidant enzymes is due to the enhanced mRNA expression of these enzymes by phenolic compounds (Jun Liu et al., 2013). Another study conducted by Khalili, Ebrahimzadeh, and Kosaryan (2015) assessed the iron-chelating activity of the phenolic compounds from the Angel's Wings mushroom (*Pleurotus porrigens*) in iron-overloaded mice. The results indicated a significant decrease of plasma iron concentration and a remarkable reduction in the extent of necrotic hepatocytes, fibrous tissues and pseudo-lobules in the livers of mice in the treatment group (Khalili et al., 2015).

The antioxidant activity of phenolic compounds is well proved in fruits, grains and vegetables, there is also a great potential for phenols in mushrooms to exhibit antioxidant activity (Adom & Liu, 2002; Chu, Sun, Wu, & Liu, 2002; J. Sun et al., 2002). However it is very

necessary for researchers to choose suitable chemical assays such as TOSC that could provide unbiased results. Also more in vivo studies are necessary to evaluate the antioxidant activity of mushrooms in complex living systems and find out the mechanisms behind.

Antimicrobial activity

Phenolic compounds have been investigated for their preventive effects against chronic diseases over the past 20 years (Daglia, 2012). In recent years, the antimicrobial activity of phenolic compounds has attracted scientists' attention because of their potential to become new antimicrobial agents since antibiotic resistance of microbials has been a big problem (Daglia, 2012). Mushrooms are a good sources of phenolic compounds especially phenolic acids and haven shown antimicrobial activity against several bacteria (Alves et al., 2013; Chowdhury et al., 2015; Nowacka et al., 2014, 2015; Smolskaitė, Venskutonis, & Talou, 2015).

Nowacka et al. (2014) studied the antibacterial activity of the phenolic extracts from 19 wild edible mushrooms in Poland. They assessed the minimal inhibitory concentration (MIC) of the phenolic extracts to inhibit the growth of four gram-positive (*Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*) and four gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*). What they found is that the phenolic extracts from 19 mushrooms exhibit moderate antimicrobial activity against both gram-positive and gram-negative bacteria; *Micrococcus luteus, Bacillus subtilis* and *Pseudomonas aeruginosa* were relatively susceptible to the phenolic extracts from mushrooms while *Proteus mirabilis, Klebsiella pneumoniae* and *Staphylococcus aureus* were less sensitive to the extracts (Nowacka et al., 2014). Another study which was also conducted by Nowacka et al. (2015) further studied the antimicrobial activity of phenolic extracts in 31 wild

mushroom species in Poland. The bacteria strains used were the same as the above study. The antibacterial assay showed that the phenolic extracts in the 31 mushroom species can inhibit both gram-positive and gram-negative bacteria with the inhibitory activity against gram-positive bacteria slightly higher than gram-negative bacteria (Nowacka et al., 2015). The bacteria strains that were more susceptible to the phenolic extracts were the same as the previous study which included *Micrococcus luteus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Nowacka et al., 2015). *Proteus mirabilis* were found to be the least susceptible to the mushroom phenolic extracts (Nowacka et al., 2015). Although these two studies assessed the antibacterial activity of a large number of mushroom species, they have not investigated the specific antimicrobial activity of different phenolic compounds from mushrooms separately. Alves et al. (2013) did take a further step to investigate the antimicrobial activity of 14 phenolic acids commonly identified in wild mushrooms against six gram-positive bacteria (methicillin-susceptible *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Listeria monocytogenes*) and five gram-negative bacteria (*Escherichia coli, Proteus mirabilis and Morganella morganii, Pasteurella multocida and Neisseria gonorrhoeae*). Their results indicated that 2,4-dihydroxybenzoic acid, protocatechuic acid, vanillic and *p*-coumaric acid showed greater antimicrobial activity against both gram-positive and gram-negative bacteria (Alves et al., 2013). They also found that cinammic acid derivatives had better antimicrobial activity against cocci bacteria (Alves et al., 2013). The structure-activity relationship analysis further showed that the presence of carboxylic acid, two hydroxyl groups in *para* and *ortho* positions of the benzene ring and a methoxyl group in the *meta* position is important for the antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (Alves et al., 2013).

The most antimicrobial studies were conducted *in vitro* and there is a lack of evidence *in vivo*. Therefore it is very necessary to conduct both animal study and human clinical trials in the future.

CHAPTER 5

L-ERGOTHIONEINE

Background

L-ergothioneine is a naturally amino acid derivative from histidine with methionine providing the methyl group and cysteine providing sulfur group (Fig. 3) (Donald B Melville, Eich, & Ludwig, 1957). It was first discovered by Charles Tanret when he was investigating the ergot fungus *Claviceps purpurea* (Tanret, 1909). Ergothioneine can be only synthesized by non-yeast fungi, some cyanobacteria and mycobacteria (Genghof, 1970; Genghof & Van Damme, 1964; Donald B. Melville, 1959; Pfeiffer, Bauer, Surek, Schömig, & Gründemann, 2011). Although animals are not able to synthesize ergothioneine, it is widely distributed in various cells and tissues, especially those that are usually exposed to high oxidative stress and inflammation such as erythrocytes, bone marrow, liver, kidney, semen and eyes (Cheah & Halliwell, 2012; Kalaras, Richie, Calcagnotto, & Beelman, 2017; Leone & Mann, 1951; Shires, Brummel, Pulido, & Stegink, 1997). Ergothioneine cannot cross the cell membrane directly, it needs to be transported through a transmembrane transporter protein OCTN1 encoded by gene SLC22A4 (Gründemann et al., 2005). Studies showed high expression of OCTN1 mRNA in kidney, ileum, bone marrow, cerebellum, spinal cord, coronary artery endothelium, lung, trachea, peripheral blood monocytes, macrophages, CD14+ cells, and synovia (Gründemann et al., 2005; Taubert, Jung, Goeser, & Schömig, 2009; X. Wu et al., 2000). High accumulation of ergothioneine and high expression of OCTN1 in many cells and tissues indicate the possible important physiological functions of this free amino acid.

Structure and content

Mushrooms are one of the great sources of ergothioneine (Cheah, Feng, Tang, Lim, & Halliwell, 2016; Cheah & Halliwell, 2012; N Joy Dubost et al., 2006; N. Joy Dubost et al., 2007; Kalaras et al., 2017). N. Joy Dubost et al. (2007) analyzed the ergothioneine concentration in six mushrooms including *Agricus bisoporus* (white, brown, and portabella)*, Lentinus edodes*, *Grifola frondosa,* and *Pleurotus osteratus*. The ergothioneine concentration ranged from 0.21 to 2.6 mg/g dry weight; the highest value was detected in *Pleurotus osteratus* and the lowerst value was detected in white button mushroom (N. Joy Dubost et al., 2007). S.-Y. Chen, Ho, Hsieh, Wang, and Mau (2012) also analyzed the ergothioneine content in the fruiting bodies of tweleve mushroom species and mycelia of seventeen mushrooms species. Ergothioneine was detected in all mushroom species; the species with very high ergothioneine content in fruiting bodies included *Pleurotus citrinopileatus*, *P. ostreatus* (Korea), *P. ostreatus* (Taiwan, China) and *Pleurotus salmoneostramineus* (2.85, 1.83, 1.46 and 1.24 mg/g dry weight, respectively), while the mushroom species with highest ergothioneine content in mycelia was *Pleurotus eryngii* (1514.6 mg/kg) (S.-Y. Chen et al., 2012). Overall the *Pleurotus spp* contain more ergothioneine than other species. Another study conducted by Kalaras et al. (2017) analyzed ergothioneine content in twelve mushroom species. The range of the ergothioneine content was 0.15-7.27mg/g dw with *Boletus edulis* having the highest content (Kalaras et al., 2017). Pileus were found to contain more ergothioneine than stipe tissues in those mushrooms that were assessed for both parts. A few mushroom species in Kalaras et al. (2017)'s study were also analyzed in S.-Y. Chen et al. (2012)'s study. However, ergothioneine content in S.-Y. Chen et al. (2012)'s study was higher than that in Kalaras et al. (2017)'s study. This is probably due to the sources of mushrooms which are affected by the growing environment, harvesting method and harvesting

time. Table 7 lists the ergothioneine content in several common edible mushrooms from different sources and studies.

Table 9 L-ergothioneine Content in Commonly Cultivated Mushrooms

Scientific names	Common names	L-ergothioneine content (mg/g DW)	Sample origin	Reference
Agaricus bisporus (white)	Button mushroom	0.21	U.S.	N. Joy Dubost et al. (2007)
Agaricus bisporus (not specified)	Button mushroom	0.93	Taiwan, China	S.-Y. Chen et al. (2012)
Agaricus bisporus (brown)	Button mushroom	0.40	U.S.	N. Joy Dubost et al. (2007)
Lentinus edodes	Shiitake	1.98	U.S.	N. Joy Dubost et al. (2007)
		0.41	Taiwan, China	S.-Y. Chen et al. (2012)
		0.40	Japan	Ito et al. (2011)
Pleurotus ostreatus	Oyster mushroom	2.59	U.S.	N. Joy Dubost et al. (2007)
		0.94	Japan	S.-Y. Chen et al. (2012)
		1.83	Korea	S.-Y. Chen et al. (2012)
		1.46	Taiwan, China	S.-Y. Chen et al. (2012)
		1.98	Japan	Ito et al. (2011)
Pleurotus eryngii	King oyster mushroom	0.62	Taiwan, China	S.-Y. Chen et al. (2012)
		1.41	Japan	Ito et al. (2011)
Pleutotus citrinopileatus	Golden oyster mushroom	3.94	U.S.	Kalaras et al. (2017)
Flammulina velutipes	Golden needle mushroom	0.45	Taiwan, China	S.-Y. Chen et al. (2012)

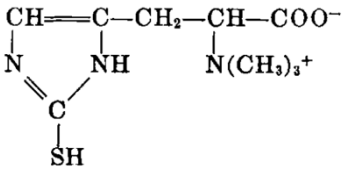

Figure 3. Structure of ergothioneine
(Melville, 1959)

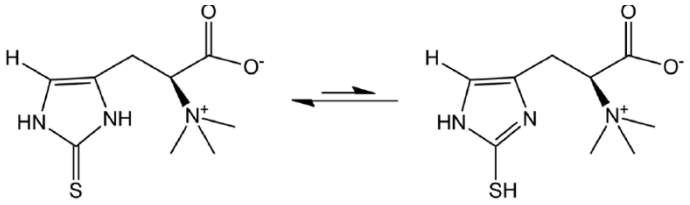

Figure 4. Tautomers of ergothioneine; ergothioneine predominately exists as thione form at physiological pH (Tang, Cheah, Yew, & Halliwell, 2018)

Health benefits of L- ergothioneine

Ergothioneine is well known of its potential health functions including scavenging of free radicals, chelation of metals, cytoprotection, gene regulation, and immunomodulation (Akanmu, Cecchini, Aruoma, & Halliwell, 1991; Aruoma, Spencer, & Mahmood, 1999; Cheah & Halliwell, 2012; MITSUYAMA, 1999). Paul and Snyder (2009) studied the cytoprotective effect of ergothioneine using RNA interference technique. They found cells that lacked ergothioneine transporters were more susceptible to the oxidative stress, lipid and protein oxidation; in addition, ergothioniene transporters is accumulated in mitochondria which indicates its protective function against the superoxide produced by mitochondria (Paul & Snyder, 2009). They also suggested that ergothioneine may be a potential candidate of vitamin due to its special physiological function (Paul & Snyder, 2009). Another study conducted by B.-Z. Zhu et al.

(2011) investigated the cytoprotective effect of ergothioneine on copper-induced oxidative damage on DNA and protein *in vitro*. They found that ergothioneine exhibited strong dose-dependent protection by forming the ergothioneine-copper complex (B.-Z. Zhu et al., 2011).

Hseu et al. (2015) reported the protective effect of ergothioneine on UVA-irradiated human keratinocytes (skin cells). Their study demonstrated that nanomolar concentrations of ergothioneine (125-500 nM) was able to protect human keratinocytes from damages caused by UVA effectively by inhibiting generation of ROS, DNA strand breaks, DNA fragmentation, mitochondrial dysfunction, and cell apoptosis (Hseu et al., 2015). They found that ergothioneine prevented cell apoptosis by downregulating caspase-3/-9 activation and dysregulating Bcl-2/Bax expression (caspase-3/-9 and Bcl-2/Bax are key apoptotic regulatory proteins) (Hseu et al., 2015). In addition, their results showed that ergothioneine upregulated the antioxidant genes HO-1, NQO-1, and γ-GCLC by increasing the expression of Nfr2 and inhibiting the degradation of synthesized Nfr2, which is a nuclear factor that regulates the antioxidant genes (Hseu et al., 2015). Furthermore, they found that induction of antioxidant genes and restore of glutathione in keratinocytes was due to the activation of the transcriptional signaling pathway Nrf2/ARE, which was further mediated by AKT and PKC pathways and ergothioneine was able to increase phosphorylation of AKT and PKC pathways. (Hseu et al., 2015).

Other studies report the immunomodulatory effect of ergothioneine (Cheah & Halliwell, 2012; Martin, 2010; Yoshida et al., 2017). Martin (2010) investigated whether ergothioneine could inhibit the pro-inflammatory induction of adhesion molecules that are expressed in atherosclerosis. The results showed that ergothioneine at 0.1-0.3mM significantly inhibited the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (ELAM-1 or E-selectin) (Martin,

2010). This study suggests that ergothioneine-rich food sources such as mushrooms may effectively prevent CVD. Yoshida et al. (2017) found that ergothioneine has immune-enhancing properties which increased the TLR signaling of mouse bone marrow-derived macrophages. TLR-signal-mediated cytokine production including IL-6 and IL-12p40 was increased; furthermore, ergothioneine enhanced Th17 differentiation in CD4+ T cells (Yoshida et al., 2017).

Apart from *in vitro* studies, there are also some epidemiological and *in vivo* studies that have demonstrated the physiological functions of ergothioneine either in animals or humans. Neuroprotective effect of ergothioneine has been widely studied. Cheah, Feng, et al. (2016) conducted an epidemiological study that investigated the association between ergothioneine and age and the association between ergothioneine and neurodegenerative disease. What they found is that the blood ergothioneine level was significantly lower in people older than 60 (Cheah, Feng, et al., 2016). They also found that people with mild cognitive impairment had significantly reduced ergothioneine level in blood compared with age-matched normal subjects (Cheah, Feng, et al., 2016). Their study suggests that ergothioneine may be closely associated with age-related neurodegenerative diseases. Song, Chen, Liao, Ou, and Tsai (2010) investigated the protective effect of ergothioneine on neuronal injury induced by cisplatin both *in vitro* and *in vivo*. Their results found that ergothioneine could significantly prevent neurotoxicity of pheochromocytoma (PC12) cells and primary cortical neuron (PCN) cells induced by cisplatin *in vitro* (Song et al., 2010). Their *in vivo* study which used CBA mice as study models also showed neuroprotection by ergothioneine including reduced lipid peroxidation, restoring of actetylcholinesterase (AchE) and maintaining the balance between glutathione/glutathione disulfide ratio in brain tissues (Song et al., 2010). Ergothioneine showed significant restoration of learning and memory deficits in mice with neurotoxicity (Song et al., 2010). Another study conducted by N.-C. Yang et al.

(2012) investigated the protective effect of ergothioneine on neuronal injury induced by beta-amyloid in mice. Accumulation of beta-amyloid is highly associated with Alzheimer's disease. Their results showed that ergothioneine significantly prevented the accumulation of beta-amyloid in hippocampus and reduced lipid peroxidation at the same time(N.-C. Yang et al., 2012). In addition, it restored the acetylcholinesterase activity and maintained the glutathione/glutathione disulfide ratio and superoxide dismutase activity in brain tissues (N.-C. Yang et al., 2012). The behavioral tests of mice showed improvement of memory and learning abilities in mice treated with ergothioneine (N.-C. Yang et al., 2012). Their research shows the potential of ergothioneine to improve the symptoms of Alzheimer's disease.

Regarding to the antioxidant and anti-inflammation of ergothioneine, Repine and Elkins (2012) reported reduced lung injury and lung inflammation in cytokine insufflated rats that were fed with ergothioneine either before or after insufflation. They suggested that ergothioneine which exhibits high antioxidant activity could be a potential treatment for acute respiratory distress (ARDs) because lung inflammation and lung oxidative stress are the major factors of ARDs (Repine & Elkins, 2012). Cheah, Tang, Yew, Lim, and Halliwell (2016) conducted a placebo-controlled, double-blind human clinical study which investigated the pharmocokenetics of ergothioneine in healthy human subjects and its antioxidant activity by examining selective biomarkers of oxidative damage and inflammation. Their results showed that plasma and whole blood ergothioneine concentrations significantly increased and the excretion of ergothioneine in urine was relatively low, which indicated that ergothioneine was well absorbed and retained in the body (Cheah, Tang, et al., 2016). The examination of biomarkers of oxidative damage and inflammation showed that there was some decreasing in these biomarkers but was not significant (Cheah, Tang, et al., 2016). They concluded that absorption and retention of ergothioneine in

human bodies suggests that it may play important physiological roles in the body however its antioxidant activity may be more potent under oxidative stress but not in healthy condition (Cheah, Tang, et al., 2016).

Although the physiological functions and health benefits of ergothioneine have been extensively studied by many researchers, especially its antioxidant activity and cytoprotective effect both *in vitro* and *in vivo*, the exact mechanisms of how ergothioneine ameliorates the symptoms of many diseases are still under investigation. Nevertheless, ergothioneine from dietary sources, especially from mushrooms which contain high amount of ergothioneine, has large potential to provide different health benefits.

CHAPTER 6

SAFETY OF MUSHROOM CONSUMPTION

Although mushrooms are a good source of different nutrients and bioactive compounds which possess many health benefits, safety issues related to mushroom consumption should be taken seriously since inadequate consumption could lead to health problems. Apart from the accidents of consuming poisonous mushrooms which are usually due to wrong identification of wild mushroom species, another important safety issue is the presence of excessive heavy metals in those edible mushrooms especially wild edible mushrooms. There have been a few studies that assessed the heavy metal content in wild edible mushrooms in different countries. X.-H. Chen, Zhou, and Qiu (2009) analyzed the heavy metal content in 11 mushroom species collected from three regions in southern part of China, in which 5 were from the urban area and 6 were from the rural areas. Their results indicated that the five wild mushroom species collected from the urban area had high contamination of Pb, Cd, and Hg; the highest concentration of Pb, Cd and Hg reached 10.8 mg/kg in *Calvatia craniiformis*, 91.8 mg/kg in *Macrolepiota crustosa* and 3.92 mg/kg in *Agaricus subrufescens* respectively. Some of them had heavy metal content far exceed the Chinese limit value (Pb limit: 1.0 mg/kg; Cd limit: 0.2 mg/kg; Hg limit: 0.1 mg/kg) (USDA Foreign Agricultural Service, 2018) . They concluded that consumption of these mushrooms from the urban area could possess health risks to humans and the source of contamination was mainly the automobile pollution (X.-H. Chen et al., 2009). Similarly, B. Liu et al. (2015) evaluated the heavy metal contamination of 8 wild edible mushroom species from 16 locations in Yunnan Province, which is a big supplier of wild edible mushrooms in China. They found that the highest value of As was 11.86 mg/kg in *Tuber indicum Cooke et Massee*, the highest value of

Cd was 2.88 mg/kg in *Tricholoma matsutake* and the highest value of Pb was 10.18 mg/kg in *Russula vinosa Lindbl* (B. Liu et al., 2015). Their results indicated that the concentrations of As, Pb and Cd in all 8 species were higher than the national standard value (Pb limit: 1.0 mg/kg; Cd limit: 0.2 mg/kg; As limit: 0.5mg/kg) (USDA Foreign Agricultural Service, 2018). The consumption of these contaminated mushrooms could lead to serious health problems and the source of contamination was very likely due to the rapid industrialization in Yunnan Province (B. Liu et al., 2015). Another study conducted by Schlecht and Säumel (2015) collected large amount of samples of 18 wild edible mushroom species from different habitats and 16 commercial cultivars of *Agricus bisporus* that were exposed to high traffic in the city of Berlin, Germany. Their purpose was to assess the health risks of consumption of mushrooms from the urban area that may have excessive accumulation of heavy metals due to pollutions (Schlecht & Säumel, 2015). Their results showed that the highest value of Pb reached 51.6 mg/kg in *M. procera* and the highest value of Cd reached 67.6 mg/kg in *B. recticulatus*. They suggested that the high traffic burden is the major cause of the accumulation of the heavy metals in the mushrooms and consumption of these mushrooms should be limited due to the potential health risks (Schlecht & Säumel, 2015).

Heavy metal contamination due to pollutions from industries and increased number of vehicles has been a growing safety problem for consumption of wild edible mushrooms. The consumption of wild edible mushrooms especially those in the urban areas should be limited to avoid health risks from the excessive heavy metals in these mushrooms.

Apart from heave metal contamination, natural toxins in mushrooms is another safety concern. Misidentification of poisonous wild mushrooms as edible ones could lead to severe poisoning effects such as renal failure. Leathem, Purssell, Chan, and Kroeger (1997) reported

four cases of mushroom poisoning which lead to renal failure; it was highly possible because of misidentification of *Amanita smithiana* as pine mushrooms and *Amanita smithiana* is a poisonous mushroom which contains nephrotoxic compounds (Leathem et al., 1997). Other Amanita spp. such as Amanita phalloides (Death cap) and Amanita muscarius (Fly agaric) are both poisonous mushrooms that contain phallotoxins, virotoxins and amatoxins (Garcia et al., 2015; Sadler, 2003). These toxins target several organs but mainly liver and kidney and the intoxication symptoms include gastrointestinal disorders, jaundice, seizures and coma (Garcia et al., 2015; Sadler, 2003). There is a high possibility that ingestion of these poisonous mushrooms can finally lead to death.

Last but not least, some edible mushroom species can be poisonous when ingested together with other food or beverage. One well-known example is *Coprinus atramentarius* (Ink caps), which is a safe edible mushroom when cooked and eaten alone but can cause poisoning when combined with alcohol (Reynolds & Lowe, 1965). Symptoms of intoxication include flushing, palpitation, dyspnea, hyperventilation, achycardia, and nausea (Reynolds & Lowe, 1965).

CHAPTER 7

CONCLUSION

Extensive research on potential health benefits of different bioactive compounds sheds a light on mushrooms which has a great potential to be included as part of a healthy diet. Recent years the research focus of mushrooms has shifted from medicinal to edible ones since many bioactive compounds such as non-digestible long polysaccharides, phenolic compounds, terpenoids, and ergothioneine can be commonly found in edible mushrooms. Compared with medicinal mushrooms, edible mushrooms, especially those commercially cultivated ones, are affordable and easily accessible for most people. This review first discusses about the basic nutritional values and major bioactive compounds in some common edible mushrooms. Then it evaluates the structures, contents and major health benefits of three groups of important bioactive compounds in mushrooms including beta-glucans, phenolic compounds and L-ergothioneine. Lastly, the safety of mushroom consumption is also briefly reviewed. The collective information about edible mushrooms in this article could help understand the potential benefits of daily consumed mushrooms.

REFERENCES

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*(21), 6182-6187.

Akanmu, D., Cecchini, R., Aruoma, O. I., & Halliwell, B. (1991). The antioxidant action of ergothioneine. *Archives of biochemistry and biophysics, 288*(1), 10-16.

Alander, M., Mättö, J., Kneifel, W., Johansson, M., Kögler, B., Crittenden, R., . . . Saarela, M. (2001). Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of Bifidobacterium lactis Bb-12 in the gastrointestinal tract. *International Dairy Journal, 11*(10), 817-825.

Alves, M. J., Ferreira, I. C. F. R., Froufe, H. J. C., Abreu, R. M. V., Martins, A., & Pintado, M. (2013). Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *Journal of Applied Microbiology, 115*(2), 346-357. doi:10.1111/jam.12196

Amorati, R., & Valgimigli, L. (2015). Advantages and limitations of common testing methods for antioxidants. *Free radical research, 49*(5), 633-649.

Aruoma, O., Spencer, J., & Mahmood, N. (1999). Protection against oxidative damage and cell death by the natural antioxidant ergothioneine. *Food and Chemical Toxicology, 37*(11), 1043-1053.

Bak, W. C., Park, J. H., Park, Y. A., & Ka, K. H. (2014). Determination of Glucan Contents in the Fruiting Bodies and Mycelia of Lentinula edodes Cultivars. *Mycobiology, 42*(3), 301-304. doi:10.5941/MYCO.2014.42.3.301

Bao, D., Gong, M., Zheng, H., Chen, M., Zhang, L., Wang, H., . . . Tan, Q. (2013). Sequencing and Comparative Analysis of the Straw Mushroom (Volvariella volvacea) Genome. *PLoS One, 8*(3), e58294. doi:10.1371/journal.pone.0058294

Bisen, P., Baghel, R. K., Sanodiya, B. S., Thakur, G. S., & Prasad, G. (2010). Lentinus edodes: a macrofungus with pharmacological activities. *Current Medicinal Chemistry, 17*(22), 2419-2430.

Bobovčák, M., Kuniaková, R., Gabrižˇ, J., & Majtán, J. (2010). Effect of Pleuran (β-glucan from Pleurotus ostreatus) supplementation on cellular immune response after intensive exercise in elite athletes. *Applied Physiology, Nutrition, and Metabolism, 35*(6), 755-762. doi:10.1139/H10-070

Bohn, J. A., & BeMiller, J. N. (1995). (1→3)-β-d-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers, 28*(1), 3-14. doi:https://doi.org/10.1016/0144-8617(95)00076-3

Boonsong, S., Klaypradit, W., & Wilaipun, P. (2016). Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agriculture and Natural Resources, 50*(2), 89-97. doi:https://doi.org/10.1016/j.anres.2015.07.002

Borchers, A. T., Keen, C. L., & Gershwin, M. E. (2004). Mushrooms, tumors, and immunity: an update. *Experimental biology and medicine, 229*(5), 393-406.

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Gershwin, M. E. (1999). Mushrooms, tumors, and immunity. *Proceedings of the Society for Experimental Biology and Medicine, 221*(4), 281-293.

Brown, G. D., & Gordon, S. (2001). A new receptor for β-glucans. *Nature, 413*, 36. doi:10.1038/35092620

Brown, G. D., & Gordon, S. (2003). Fungal β-Glucans and Mammalian Immunity. *Immunity, 19*(3), 311-315. doi:https://doi.org/10.1016/S1074-7613(03)00233-4

Brown, G. D., Herre, J., Williams, D. L., Willment, J. A., Marshall, A. S. J., & Gordon, S. (2003). Dectin-1 Mediates the Biological Effects of β-Glucans. *The Journal of Experimental Medicine, 197*(9), 1119-1124. doi:10.1084/jem.20021890

C.Ooi, V. E., & Liu, F. (2000). Immunomodulation and Anti-Cancer Activity of Polysaccharide-Protein Complexes. *Current Medicinal Chemistry, 7*(7), 715-729. doi:10.2174/0929867003374705

Camelini, C. M., Maraschin, M., de Mendonça, M. M., Zucco, C., Ferreira, A. G., & Tavares, L. A. (2005). Structural characterization of β-glucans of Agaricus brasiliensis in different stages of fruiting body maturity and their use in nutraceutical products. *Biotechnology Letters, 27*(17), 1295-1299.

Carbonero, E. R., Gracher, A. H. P., Smiderle, F. R., Rosado, F. R., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2006). A β-glucan from the fruit bodies of edible mushrooms Pleurotus eryngii and Pleurotus ostreatoroseus. *Carbohydrate Polymers, 66*(2), 252-257. doi:https://doi.org/10.1016/j.carbpol.2006.03.009

Carneiro, A. A. J., Ferreira, I. C. F. R., Dueñas, M., Barros, L., da Silva, R., Gomes, E., & Santos-Buelga, C. (2013). Chemical composition and antioxidant activity of dried powder formulations of Agaricus blazei and Lentinus edodes. *Food Chemistry, 138*(4), 2168-2173. doi:https://doi.org/10.1016/j.foodchem.2012.12.036

Castro-Alves, V. C., Gomes, D., Menolli, N., Sforça, M. L., & Nascimento, J. R. O. d. (2017). Characterization and immunomodulatory effects of glucans from Pleurotus albidus, a promising species of mushroom for farming and biomass production. *International Journal of Biological Macromolecules, 95*, 215-223. doi:https://doi.org/10.1016/j.ijbiomac.2016.11.059

Chan, G. C.-F., Chan, W. K., & Sze, D. M.-Y. (2009). The effects of β-glucan on human immune and cancer cells. *Journal of Hematology & Oncology, 2*(1), 25. doi:10.1186/1756-8722-2-25

Chatterjee, S., Sarma, M. K., Deb, U., Steinhauser, G., Walther, C., & Gupta, D. K. (2017). Mushrooms: from nutrition to mycoremediation. *Environmental Science and Pollution Research, 24*(24), 19480-19493. doi:10.1007/s11356-017-9826-3

Cheah, I. K., Feng, L., Tang, R. M. Y., Lim, K. H. C., & Halliwell, B. (2016). Ergothioneine levels in an elderly population decrease with age and incidence of cognitive decline; a risk factor for neurodegeneration? *Biochemical and Biophysical Research Communications, 478*(1), 162-167. doi:https://doi.org/10.1016/j.bbrc.2016.07.074

Cheah, I. K., & Halliwell, B. (2012). Ergothioneine; antioxidant potential, physiological function and role in disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1822*(5), 784-793. doi:https://doi.org/10.1016/j.bbadis.2011.09.017

Cheah, I. K., Tang, R. M. Y., Yew, T. S. Z., Lim, K. H. C., & Halliwell, B. (2016). Administration of Pure Ergothioneine to Healthy Human Subjects: Uptake, Metabolism, and Effects on Biomarkers of Oxidative Damage and Inflammation. *Antioxidants & Redox Signaling, 26*(5), 193-206. doi:10.1089/ars.2016.6778

Chen, B., Gui, F., Xie, B., Deng, Y., Sun, X., Lin, M., . . . Li, S. (2013). Composition and Expression of Genes Encoding Carbohydrate-Active Enzymes in the Straw-Degrading Mushroom Volvariella volvacea. *PLoS One, 8*(3), e58780. doi:10.1371/journal.pone.0058780

Chen, S.-Y., Ho, K.-J., Hsieh, Y.-J., Wang, L.-T., & Mau, J.-L. (2012). Contents of lovastatin, γ-aminobutyric acid and ergothioneine in mushroom fruiting bodies and mycelia. *LWT - Food Science and Technology, 47*(2), 274-278. doi:https://doi.org/10.1016/j.lwt.2012.01.019

Chen, X.-H., Zhou, H.-B., & Qiu, G.-Z. (2009). Analysis of several heavy metals in wild edible mushrooms from regions of China. *Bulletin of Environmental Contamination and Toxicology, 83*(2), 280.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry, 81*(2), 249-255. doi:https://doi.org/10.1016/S0308-8146(02)00419-3

Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T., & Fukuoka, F. (1969). Inhibition of Mouse Sarcoma 180 by Polysaccharides from Lentinus edodes (Berk.) Sing. *Nature, 222*, 687. doi:10.1038/222687a0

Chou, W. T., Sheih, I. C., & Fang Tony, J. (2013). The Applications of Polysaccharides from Various Mushroom Wastes as Prebiotics in Different Systems. *Journal of food science, 78*(7), M1041-M1048. doi:10.1111/1750-3841.12160

Chowdhury, M. M. H., Kubra, K., & Ahmed, S. R. (2015). Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. *Annals of clinical microbiology and antimicrobials, 14*(1), 8.

Chu, Y.-F., Sun, J., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry, 50*(23), 6910-6916.

Crittenden, R., & Playne, M. (2002). Purification of food-grade oligosaccharides using immobilised cells of Zymomonas mobilis. *Applied microbiology and biotechnology, 58*(3), 297-302.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174-181. doi:https://doi.org/10.1016/j.copbio.2011.08.007

Dongowski, G., Huth, M., Gebhardt, E., & Flamme, W. (2002). Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. *The Journal of nutrition, 132*(12), 3704-3714.

Douglas, L. C., & Sanders, M. E. (2008). Probiotics and prebiotics in dietetics practice. *Journal of the American Dietetic Association, 108*(3), 510-521.

Drzikova, B., Dongowski, G., & Gebhardt, E. (2005). Dietary fibre-rich oat-based products affect serum lipids, microbiota, formation of short-chain fatty acids and steroids in rats. *British Journal of Nutrition, 94*(6), 1012-1025.

Du, B., Lin, C., Bian, Z., & Xu, B. (2015). An insight into anti-inflammatory effects of fungal beta-glucans. *Trends in Food Science & Technology, 41*(1), 49-59. doi:https://doi.org/10.1016/j.tifs.2014.09.002

Dubost, N. J., Beelman, R. B., Peterson, D., & Royse, D. J. (2006). Identification and quantification of ergothioneine in cultivated mushrooms by liquid chromatography-mass spectroscopy. *International journal of medicinal mushrooms, 8*(3).

Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry, 105*(2), 727-735. doi:https://doi.org/10.1016/j.foodchem.2007.01.030

Ey, J., Schömig, E., & Taubert, D. (2007). Dietary sources and antioxidant effects of ergothioneine. *Journal of Agricultural and Food Chemistry, 55*(16), 6466-6474.

Fang, J., Wang, Y., Lv, X., Shen, X., Ni, X., & Ding, K. (2012). Structure of a β-glucan from Grifola frondosa and its antitumor effect by activating Dectin-1/Syk/NF-κB signaling. *Glycoconjugate Journal, 29*(5), 365-377. doi:10.1007/s10719-012-9416-z

Feeney, M. J., Dwyer, J., Hasler-Lewis, C. M., Milner, J. A., Noakes, M., Rowe, S., . . . Wu, D. (2014). Mushrooms and Health Summit proceedings. *J Nutr, 144*(7), 1128S-1136S. doi:10.3945/jn.114.190728

Ferreira, I. C., Barros, L., & Abreu, R. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry, 16*(12), 1543-1560.

Firenzuoli, F., Gori, L., & Lombardo, G. (2008). *The Medicinal Mushroom Agaricus blazei Murrill: Review of Literature and Pharmaco-Toxicological Problems* (Vol. 5).

Fizpatrick, F. W., & DiCarlo, F. J. (1964). ZYMOSAN. *Annals of the New York Academy of Sciences, 118*(4), 235-261. doi:10.1111/j.1749-6632.1964.tb33982.x

Franceschi, C., & Campisi, J. (2014). Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. *The Journals of Gerontology: Series A, 69*(Suppl_1), S4-S9. doi:10.1093/gerona/glu057

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins. *Journal of Agricultural and Food Chemistry, 43*(4), 890-894. doi:10.1021/jf00052a008

Fruehauf, J. P., Bonnard, G. D., & Herberman, R. B. (1982). The effect of lentinan on production of interleukin-1 by human monocytes. *Immunopharmacology, 5*(1), 65-74. doi:https://doi.org/10.1016/0162-3109(82)90037-6

Garcia, J., Costa, V. M., Carvalho, A., Baptista, P., de Pinho, P. G., de Lourdes Bastos, M., & Carvalho, F. (2015). Amanita phalloides poisoning: Mechanisms of toxicity and treatment. *Food and Chemical Toxicology, 86*, 41-55. doi:https://doi.org/10.1016/j.fct.2015.09.008

Genghof, D. S. (1970). Biosynthesis of ergothioneine and hercynine by fungi and Actinomycetales. *Journal of bacteriology, 103*(2), 475-478.

Genghof, D. S., & Van Damme, O. (1964). Biosynthesis of ergothioneine and hercynine by mycobacteria. *Journal of bacteriology, 87*(4), 852-862.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition research reviews, 17*(2), 259-275.

Goodridge, H. S., Simmons, R. M., & Underhill, D. M. (2007). Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. *The Journal of immunology, 178*(5), 3107-3115.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., . . . Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature, 442*(7103), 651.

Gründemann, D., Harlfinger, S., Golz, S., Geerts, A., Lazar, A., Berkels, R., . . . Schömig, E. (2005). Discovery of the ergothioneine transporter. *Proceedings of the National Academy of Sciences of the United States of America, 102*(14), 5256-5261.

Guggenheim, A. G., Wright, K. M., & Zwickey, H. L. (2014). Immune Modulation From Five Major Mushrooms: Application to Integrative Oncology. *Integr Med (Encinitas), 13*(1), 32-44.

Guo, Y.-J., Deng, G.-F., Xu, X.-R., Wu, S., Li, S., Xia, E.-Q., . . . Li, H.-B. (2012). Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. *Food & function, 3*(11), 1195-1205. doi:10.1039/C2FO30110E

Hall, I. R., Buchanan, P. K., Cole, A. L., Yun, W., & Stephenson, S. (2003). *Edible and poisonous mushrooms of the world* (Vol. 103): Timber Press Portland.

Han, M., Ling, M.-T., & Chen, J. (2015). The Key Role of mitochondrial apoptotic pathway in the cytotoxic effect of mushroom extracts on cancer cells. *Critical Reviews™ in Eukaryotic Gene Expression, 25*(3).

Harada, K., Itashiki, Y., Takenawa, T., & Ueyama, Y. (2010). Effects of lentinan alone and in combination with fluoropyrimidine anticancer agent on growth of human oral squamous cell carcinoma in vitro and in vivo. *International journal of oncology, 37*(3), 623-631.

Hayakawa, K., Mizutani, J., Wada, K., Masai, T., Yoshihara, I., & Mitsuoka, T. (1990). Effects of soybean oligosaccharides on human faecal flora. *Microbial Ecology in Health and Disease, 3*(6), 293-303.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., Santos-Buelga, C., & Ferreira, I. C. (2011). Targeted metabolites analysis in wild Boletus species. *LWT-Food Science and Technology, 44*(6), 1343-1348.

Herlyn, D., Kaneko, Y., Powe, J., Aoki, T., & Koprowski, H. (1985). MONOCLONAL ANTIBODY-DEPENDENT MURINE MACROPHAGE-MEDIATED CYTOTOXICITY AGAINST HUMAN TUMORS IS STIMULATED BY LENTINAN. *Japanese Journal of Cancer Research GANN, 76*(1), 37-42. doi:10.20772/cancersci1985.76.1_37

Hetland, G., Eide, D. M., Tangen, J. M., Haugen, M. H., Mirlashari, M. R., & Paulsen, J. E. (2016). The Agaricus blazei-Based Mushroom Extract, Andosan(™), Protects against Intestinal Tumorigenesis in the A/J Min/+ Mouse. *PLoS One, 11*(12), e0167754. doi:10.1371/journal.pone.0167754

Hseu, Y.-C., Lo, H.-W., Korivi, M., Tsai, Y.-C., Tang, M.-J., & Yang, H.-L. (2015). Dermato-protective properties of ergothioneine through induction of Nrf2/ARE-mediated antioxidant genes in UVA-irradiated Human keratinocytes. *Free Radical Biology and Medicine, 86*, 102-117. doi:https://doi.org/10.1016/j.freeradbiomed.2015.05.026

Islam, T., Yu, X., & Xu, B. (2016). Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. *LWT - Food Science and Technology, 72*, 423-431. doi:https://doi.org/10.1016/j.lwt.2016.05.005

Ito, T., Kato, M., Tsuchida, H., Harada, E., Niwa, T., & Osawa, T. (2011). Ergothioneine as an Anti-Oxidative/Anti-Inflammatory Component in Several Edible Mushrooms. *Food Science and Technology Research, 17*(2), 103-110. doi:10.3136/fstr.17.103

Janeway, C. A. (1992). The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunology Today, 13*(1), 11-16. doi:https://doi.org/10.1016/0167-5699(92)90198-G

Jaskari, J., Kontula, P., Siitonen, A., Jousimies-Somer, H., Mattila-Sandholm, T., & Poutanen, K. (1998). Oat β-glucan and xylan hydrolysates as selective substrates for Bifidobacterium and Lactobacillus strains. *Applied microbiology and biotechnology, 49*(2), 175-181.

Jayachandran, M., Xiao, J., & Xu, B. (2017). A Critical Review on Health Promoting Benefits of Edible Mushrooms through Gut Microbiota. *Int J Mol Sci, 18*(9). doi:10.3390/ijms18091934

Jesenak, M., Majtan, J., Rennerova, Z., Kyselovic, J., Banovcin, P., & Hrubisko, M. (2013). Immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections. *International Immunopharmacology, 15*(2), 395-399. doi:https://doi.org/10.1016/j.intimp.2012.11.020

Kaewnarin, K., Suwannarach, N., Kumla, J., & Lumyong, S. (2016). Phenolic profile of various wild edible mushroom extracts from Thailand and their antioxidant properties, anti-tyrosinase and hyperglycaemic inhibitory activities. *Journal of Functional Foods, 27*, 352-364. doi:https://doi.org/10.1016/j.jff.2016.09.008

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry, 47*(10), 3954-3962. doi:10.1021/jf990146l

Kalač, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture, 93*(2), 209-218.

Kalač, P. (2016a). Chapter 1 - Introduction *Edible Mushrooms* (pp. 1-6): Academic Press.

Kalač, P. (2016b). Chapter 2 - Proximate Composition and Nutrients *Edible Mushrooms* (pp. 7-69): Academic Press.

Kalač, P. (2016c). Chapter 4 - Health-Stimulating Compounds and Effects *Edible Mushrooms* (pp. 137-153): Academic Press.

Kalaras, M. D., Richie, J. P., Calcagnotto, A., & Beelman, R. B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. *Food Chemistry, 233*, 429-433. doi:https://doi.org/10.1016/j.foodchem.2017.04.109

Kaneko, T., Yokoyama, A., & Suzuki, M. (1995). Digestibility characteristics of isomaltooligosaccharides in comparison with several saccharides using the rat jejunum loop method. *Bioscience, Biotechnology, and Biochemistry, 59*(7), 1190-1194.

Karácsonyi, Š., & Kuniak, Ľ. (1994). Polysaccharides of Pleurotus ostreatus: Isolation and structure of pleuran, an alkali-insoluble β-d-glucan. *Carbohydrate Polymers, 24*(2), 107-111. doi:https://doi.org/10.1016/0144-8617(94)90019-1

Khalili, M., Ebrahimzadeh, M. A., & Kosaryan, M. (2015). In Vivo Iron-Chelating Activity and Phenolic Profiles of the Angel's Wings Mushroom, Pleurotus porrigens (Higher Basidiomycetes). *17*(9), 847-856. doi:10.1615/IntJMedMushrooms.v17.i9.50

Kim, M.-Y., Seguin, P., Ahn, J.-K., Kim, J.-J., Chun, S.-C., Kim, E.-H., . . . Chung, I.-M. (2008). Phenolic Compound Concentration and Antioxidant Activities of Edible and Medicinal Mushrooms from Korea. *Journal of Agricultural and Food Chemistry, 56*(16), 7265-7270. doi:10.1021/jf8008553

Kontula, P., von Wright, A., & Mattila-Sandholm, T. (1998). Oat bran β-gluco-and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. *International journal of food microbiology, 45*(2), 163-169.

Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Petrović, P., . . . Van Griensven, L. (2015). Antioxidants of edible mushrooms. *Molecules, 20*(10), 19489-19525.

Lam, K.-L., & Chi-Keung Cheung, P. (2013). Non-digestible long chain beta-glucans as novel prebiotics. *Bioactive Carbohydrates and Dietary Fibre, 2*(1), 45-64. doi:https://doi.org/10.1016/j.bcdf.2013.09.001

Laroche, C., & Michaud, P. (2007). New developments and prospective applications for β (1, 3) glucans. *Recent patents on biotechnology, 1*(1), 59-73.
Leathem, A. M., Purssell, R. A., Chan, V. R., & Kroeger, P. D. (1997). Renal Failure Caused by Mushroom Poisoning. *Journal of Toxicology: Clinical Toxicology, 35*(1), 67-75. doi:10.3109/15563659709001168
Leone, E., & Mann, T. (1951). Ergothioneine in the seminal vesicle secretion. *Nature, 168*(4266), 205.
Liu, B., Huang, Q., Cai, H., Guo, X., Wang, T., & Gui, M. (2015). Study of heavy metal concentrations in wild edible mushrooms in Yunnan Province, China. *Food Chemistry, 188*, 294-300. doi:https://doi.org/10.1016/j.foodchem.2015.05.010
Liu, J., Jia, L., Kan, J., & Jin, C.-h. (2013). In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (Agaricus bisporus). *Food and Chemical Toxicology, 51*, 310-316. doi:https://doi.org/10.1016/j.fct.2012.10.014
Liu, J., Sun, Y., Yu, H., Zhang, C., Yue, L., Yang, X., . . . Liu, J. (2012). Purification and identification of one glucan from golden oyster mushroom (Pleurotus citrinopileatus (Fr.) Singer). *Carbohydrate Polymers, 87*(1), 348-352. doi:https://doi.org/10.1016/j.carbpol.2011.07.059
Losada, M., & Olleros, T. (2002). Towards a healthier diet for the colon: the influence of fructooligosaccharides and lactobacilli on intestinal health. *Nutrition research, 22*(1-2), 71-84.
Ma, Z., Wang, J., & Zhang, L. (2008). Structure and chain conformation of β-glucan isolated from Auricularia auricula-judae. *Biopolymers, 89*(7), 614-622.
Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology, 104*(2), 305-344.
Macfarlane, S., Macfarlane, G., & Cummings, J. t. (2006). Prebiotics in the gastrointestinal tract. *Alimentary pharmacology & therapeutics, 24*(5), 701-714.
Martin, K. R. (2010). The bioactive agent ergothioneine, a key component of dietary mushrooms, inhibits monocyte binding to endothelial cells characteristic of early cardiovascular disease. *Journal of Medicinal Food, 13*, 1340+.
Masuda, Y., Inoue, H., Ohta, H., Miyake, A., Konishi, M., & Nanba, H. (2013). Oral administration of soluble β-glucans extracted from Grifola frondosa induces systemic antitumor immune response and decreases immunosuppression in tumor-bearing mice. *International Journal of Cancer, 133*(1), 108-119. doi:10.1002/ijc.27999
Matsuoka, H., Seo, Y., Wakasugi, H., Saito, T., & Tomoda, H. (1997). Lentinan potentiates immunity and prolongs the survival time of some patients. *Anticancer research, 17*(4A), 2751-2755.
Mattila, P., Lampi, A.-M., Ronkainen, R., Toivo, J., & Piironen, V. (2002). Sterol and vitamin D2 contents in some wild and cultivated mushrooms. *Food Chemistry, 76*(3), 293-298.
Melville, D. B. (1959). Ergothioneine**Much of the unpublished work from the author's laboratory reviewed in this article was supported by grants from the National Science Foundation and by research grant E-1745 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service. In R. S. Harris, G. F. Marrian, & K. V. Thimann (Eds.), *Vitamins & Hormones* (Vol. 17, pp. 155-204): Academic Press.
Melville, D. B., Eich, S., & Ludwig, M. L. (1957). The biosynthesis of ergothioneine. *Journal of Biological Chemistry, 224*(2), 871-877.

Meng, X., Liang, H., & Luo, L. (2016). Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. *Carbohydrate Research, 424*, 30-41. doi:https://doi.org/10.1016/j.carres.2016.02.008

MITSUYAMA, H. (1999). Uptake and antioxidant effects of ergothioneine in human erythrocytes. *Clinical Science, 97*(4), 407-411.

Mizuno, T. (1995). Shiitake, Lentinus edodes: functional properties for medicinal and food purposes. *Food Reviews International, 11*(1), 109-128.

Morin, E., Kohler, A., Baker, A. R., Foulongne-Oriol, M., Lombard, V., Nagye, L. G., . . . Martin, F. (2012). Genome sequence of the button mushroom Agaricus bisporus reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proceedings of the National Academy of Sciences, 109*, 17501-17506.

Nakano, H., Namatame, K., Nemoto, H., Motohashi, H., Nishiyama, K., & Kumada, K. (1999). A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unresectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group. *Hepato-gastroenterology, 46*(28), 2662-2668.

Ng, M.-L., & Yap, A.-T. (2002). Inhibition of Human Colon Carcinoma Development by Lentinan from Shiitake Mushrooms (Lentinus edodes). *The Journal of Alternative and Complementary Medicine, 8*(5), 581-589. doi:10.1089/107555302320825093

Nishitani, Y., Zhang, L., Yoshida, M., Azuma, T., Kanazawa, K., Hashimoto, T., & Mizuno, M. (2013). Intestinal Anti-Inflammatory Activity of Lentinan: Influence on IL-8 and TNFR1 Expression in Intestinal Epithelial Cells. *PLoS One, 8*(4), e62441. doi:10.1371/journal.pone.0062441

Nitschke, J., Modick, H., Busch, E., von Rekowski, R. W., Altenbach, H.-J., & Mölleken, H. (2011). A new colorimetric method to quantify β-1,3-1,6-glucans in comparison with total β-1,3-glucans in edible mushrooms. *Food Chemistry, 127*(2), 791-796. doi:https://doi.org/10.1016/j.foodchem.2010.12.149

Nowacka, N., Nowak, R., Drozd, M., Olech, M., Los, R., & Malm, A. (2014). Analysis of phenolic constituents, antiradical and antimicrobial activity of edible mushrooms growing wild in Poland. *LWT - Food Science and Technology, 59*(2, Part 1), 689-694. doi:https://doi.org/10.1016/j.lwt.2014.05.041

Nowacka, N., Nowak, R., Drozd, M., Olech, M., Los, R., & Malm, A. (2015). Antibacterial, Antiradical Potential and Phenolic Compounds of Thirty-One Polish Mushrooms. *PLoS One, 10*(10), e0140355. doi:10.1371/journal.pone.0140355

Nowak, R., Nowacka-Jechalke, N., Juda, M., & Malm, A. (2017). The preliminary study of prebiotic potential of Polish wild mushroom polysaccharides: the stimulation effect on Lactobacillus strains growth. *European Journal of Nutrition*. doi:10.1007/s00394-017-1436-9

Oba, K., Kobayashi, M., Matsui, T., Kodera, Y., & Sakamoto, J. (2009). Individual patient based meta-analysis of lentinan for unresectable/recurrent gastric cancer. *Anticancer research, 29*(7), 2739-2745.

Oke, F., & Aslim, B. (2011). Protective effect of two edible mushrooms against oxidative cell damage and their phenolic composition. *Food Chemistry, 128*(3), 613-619. doi:https://doi.org/10.1016/j.foodchem.2011.03.036

Orhan, I., & Üstün, O. (2011). Determination of total phenol content, antioxidant activity and acetylcholinesterase inhibition in selected mushrooms from Turkey. *Journal of Food Composition and Analysis, 24*(3), 386-390. doi:https://doi.org/10.1016/j.jfca.2010.11.005

Özyürek, M., Bener, M., Güçlü, K., & Apak, R. (2014). Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms. *Food Chemistry, 157*, 323-331.

Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M. A., Martínez, J. A., . . . Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry, 128*(3), 674-678. doi:https://doi.org/10.1016/j.foodchem.2011.03.085

Palframan, R., Gibson, G., & Rastall, R. (2003). Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. *Letters in Applied Microbiology, 37*(4), 281-284.

Paul, B. D., & Snyder, S. H. (2009). The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death And Differentiation, 17*, 1134. doi:10.1038/cdd.2009.163

Pereira, E., Barros, L., Martins, A., & Ferreira, I. C. (2012). Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. *Food Chemistry, 130*(2), 394-403.

Pfeiffer, C., Bauer, T., Surek, B., Schömig, E., & Gründemann, D. (2011). Cyanobacteria produce high levels of ergothioneine. *Food Chemistry, 129*(4), 1766-1769.

Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J., . . . Haytowitz, D. B. (2011). Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *Journal of Agricultural and Food Chemistry, 59*(14), 7841-7853.

Pillemer, L., & Ecker, E. (1941). Anticomplementary factor in fresh yeast. *J Biol Chem, 137*(1), 139-142.

Radzki, W., Slawinska, A., Jablonska-Rys, E., & Gustaw, W. (2014). Antioxidant capacity and polyphenolic content of dried wild edible mushrooms from Poland. *International journal of medicinal mushrooms, 16*(1).

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition, 5*(2), 35-46. doi:https://doi.org/10.1016/j.phanu.2017.02.001

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. *Food and Chemical Toxicology, 50*(2), 191-197.

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology, 50*(2), 191-197. doi:https://doi.org/10.1016/j.fct.2011.10.056

Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. F. R. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology, 50*(5), 1201-1207. doi:https://doi.org/10.1016/j.fct.2012.02.013

Ren, Y., Geng, Y., Du, Y., Li, W., Lu, Z.-M., Xu, H.-Y., . . . Xu, Z.-H. (2018). Polysaccharide of Hericium erinaceus Attenuates Colitis in C57BL/6 Mice via Regulation of Oxidative Stress, Inflammation-related Signaling Pathways and Modulating the Composition of the

Gut Microbiota. *The Journal of Nutritional Biochemistry*. doi:https://doi.org/10.1016/j.jnutbio.2018.03.005
Repine, J. E., & Elkins, N. D. (2012). Effect of ergothioneine on acute lung injury and inflammation in cytokine insufflated rats. *Preventive Medicine, 54*, S79-S82. doi:https://doi.org/10.1016/j.ypmed.2011.12.006
Reynolds, W. A., & Lowe, F. H. (1965). Mushrooms and a Toxic Reaction to Alcohol. *New England Journal of Medicine, 272*(12), 630-631. doi:10.1056/NEJM196503252721209
Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152-159. doi:https://doi.org/10.1016/S1360-1385(97)01018-2
Roberfroid, M. B. (2007). Inulin-type fructans: functional food ingredients. *The Journal of nutrition, 137*(11), 2493S-2502S.
Rogers, N. C., Slack, E. C., Edwards, A. D., Nolte, M. A., Schulz, O., Schweighoffer, E., . . . Brown, G. D. (2005). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity, 22*(4), 507-517.
Roldan-Deamicis, A., Alonso, E., Brie, B., Braico, D. A., & Balogh, G. A. (2016). Maitake Pro4X has anti-cancer activity and prevents oncogenesis in BALBc mice. *Cancer Medicine, 5*(9), 2427-2441. doi:10.1002/cam4.744
Royse Daniel, J., Baars, J., & Tan, Q. (2017). Current Overview of Mushroom Production in the World. *Edible and Medicinal Mushrooms*. doi:doi:10.1002/9781119149446.ch2
10.1002/9781119149446.ch2
Sadler, M. (2003). Nutritional properties of edible fungi. *Nutrition Bulletin, 28*(3), 305-308. doi:10.1046/j.1467-3010.2003.00354.x
Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied microbiology and biotechnology, 64*(6), 756-762.
Sánchez, C. (2010). Cultivation of Pleurotus ostreatus and other edible mushrooms. *Applied microbiology and biotechnology, 85*(5), 1321-1337.
Santilli, F., D'Ardes, D., & Davì, G. (2015). Oxidative stress in chronic vascular disease: From prediction to prevention. *Vascular Pharmacology, 74*, 23-37. doi:https://doi.org/10.1016/j.vph.2015.09.003
Sari, M., Prange, A., Lelley, J. I., & Hambitzer, R. (2017). Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. *Food Chemistry, 216*, 45-51. doi:https://doi.org/10.1016/j.foodchem.2016.08.010
Sasaki, T., & Takasuka, N. (1976). Further study of the structure of lentinan, an anti-tumor polysaccharide from Lentinus edodes. *Carbohydrate Research, 47*(1), 99-104. doi:https://doi.org/10.1016/S0008-6215(00)83552-1
Schlecht, M. T., & Säumel, I. (2015). Wild growing mushrooms for the Edible City? Cadmium and lead content in edible mushrooms harvested within the urban agglomeration of Berlin, Germany. *Environmental Pollution, 204*, 298-305. doi:https://doi.org/10.1016/j.envpol.2015.05.018
Schmid, F., Stone, B. A., McDougall, B. M., Bacic, A., Martin, K. L., Brownlee, R. T., . . . Seviour, R. J. (2001). Structure of epiglucan, a highly side-chain/branched (1→ 3; 1→ 6)-β-glucan from the micro fungus Epicoccum nigrum Ehrenb. ex Schlecht. *Carbohydrate Research, 331*(2), 163-171.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics—approaching a definition. *The American journal of clinical nutrition, 73*(2), 361s-364s. doi:10.1093/ajcn/73.2.361s

Shires, T. K., Brummel, M. C., Pulido, J. S., & Stegink, L. D. (1997). Ergothioneine distribution in bovine and porcine ocular tissues. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 117*(1), 117-120.

Smolskaitė, L., Venskutonis, P. R., & Talou, T. (2015). Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species. *LWT - Food Science and Technology, 60*(1), 462-471. doi:https://doi.org/10.1016/j.lwt.2014.08.007

Song, T.-Y., Chen, C.-L., Liao, J.-W., Ou, H.-C., & Tsai, M.-S. (2010). Ergothioneine protects against neuronal injury induced by cisplatin both in vitro and in vivo. *Food and Chemical Toxicology, 48*(12), 3492-3499. doi:https://doi.org/10.1016/j.fct.2010.09.030

Suga, T., Shiio, T., Maeda, Y. Y., & Chihara, G. (1984). Antitumor Activity of Lentinan in Murine Syngeneic and Autochthonous Hosts and Its Suppressive Effect on 3-Methylcholanthrene-induced Carcinogenesis. *Cancer Research, 44*(11), 5132-5137.

Sun, J., Chu, Y.-F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry, 50*(25), 7449-7454.

Sun, M., Zhao, W., Xie, Q., Zhan, Y., & Wu, B. (2015). Lentinan reduces tumor progression by enhancing gemcitabine chemotherapy in urothelial bladder cancer. *Surgical Oncology, 24*(1), 28-34. doi:https://doi.org/10.1016/j.suronc.2014.11.002

Synytsya, A., Míčková, K., Synytsya, A., Jablonský, I., Spěváček, J., Erban, V., . . . Čopíková, J. (2009). Glucans from fruit bodies of cultivated mushrooms Pleurotus ostreatus and Pleurotus eryngii: Structure and potential prebiotic activity. *Carbohydrate Polymers, 76*(4), 548-556. doi:https://doi.org/10.1016/j.carbpol.2008.11.021

Taguchi, T. (1987). Clinical efficacy of lentinan on patients with stomach cancer: end point results of a four-year follow-up survey. *Cancer detection and prevention. Supplement : official publication of the International Society for Preventive Oncology, Inc, 1*, 333-349.

Tang, R. M. Y., Cheah, I. K.-M., Yew, T. S. K., & Halliwell, B. (2018). Distribution and accumulation of dietary ergothioneine and its metabolites in mouse tissues. *Scientific reports, 8*(1), 1601.

Tanret, C. (1909). Sur une base nouvelle retiree du seigle ergote, l'ergothioneine. *Rend. Acad. Sci., 149*, 222-224.

Taubert, D., Jung, N., Goeser, T., & Schömig, E. (2009). Increased ergothioneine tissue concentrations in carriers of the Crohn's disease risk-associated 503F variant of the organic cation transporter OCTN1. *Gut, 58*(2), 312-314.

Taylor, P. R., Brown, G. D., Reid, D. M., Willment, J. A., Martinez-Pomares, L., Gordon, S., & Wong, S. Y. (2002). The β-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *The Journal of immunology, 169*(7), 3876-3882.

Thornton, B. P., Větvicka, V., Pitman, M., Goldman, R. C., & Ross, G. D. (1996). Analysis of the sugar specificity and molecular location of the beta-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). *The Journal of immunology, 156*(3), 1235-1246.

USDA. (2018a). National Nutrient Database for Standard Reference Legacy Release - Full Report (All Nutrients): 11228, Jew's ear, (pepeao), raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11228?n1=%7BQv%3D1%7D&fgcd=&man=&

lfacet=&count=&max=25&sort=default&qlookup=Auricularia&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018b). National Nutrient Database for Standard Reference Legacy Release - Full Report (All Nutrients): 11238, Mushrooms, shiitake, raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11238?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=shiitake&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018c). National Nutrient Database for Standard Reference Legacy Release - Full Report (All Nutrients): 11238, Mushrooms, straw, canned, drained solids. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11989?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=Volvariella+volvacea&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018d). National Nutrient Database for Standard Reference Legacy Release - Full Report (All Nutrients): 11950, Mushrooms, enoki, raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11950?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=Flammulina+velutipes&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018e). National Nutrient Database for Standard Reference Legacy Release - Full Report (All Nutrients): 11987, Mushrooms, oyster, raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11987?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=Pleurotus+ostreatus&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018f). National Nutrient Database for Standard Reference Legacy Release - Full Report: 11260, Mushrooms, white, raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11266?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=Agaricus+bisporous+&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA. (2018g). National Nutrient Database for Standard Reference Legacy Release - Full Report: 11266, Mushrooms, brown, italian, or crimini, raw. Retrieved from https://ndb.nal.usda.gov/ndb/foods/show/11266?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=agaricus+bisporus&ds=&qt=&qp=&qa=&qn=&q=&ing=
USDA Foreign Agricultural Service. (2018). China: China Releases the Standard for Maximum Levels of Contaminants in Foods. Retrieved from https://gain.fas.usda.gov/Recent%20GAIN%20Publications/China%20Releases%20the%20Standard%20for%20Levels%20of%20Contaminants%20in%20Foods%20_Beijing_China%20-%20Peoples%20Republic%20of_5-9-2018.pdf
Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International journal of microbiology, 2015*.
Vannucci, L., Krizan, J., Sima, P., Stakheev, D., Caja, F., Rajsiglova, L., . . . Saieh, M. (2013). Immunostimulatory properties and antitumor activities of glucans. *International journal of oncology, 43*(2), 357-364.
Varshney, J., Ooi, J. H., Jayarao, B. M., Albert, I., Fisher, J., Smith, R. L., . . . Cantorna, M. T. (2013). White Button Mushrooms Increase Microbial Diversity and Accelerate the

Resolution of Citrobacter rodentium Infection in Mice. *The Journal of nutrition, 143*(4), 526-532. doi:10.3945/jn.112.171355

Vaz, J. A., Barros, L., Martins, A., Santos-Buelga, C., Vasconcelos, M. H., & Ferreira, I. C. (2011). Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Food Chemistry, 126*(2), 610-616.

Volman, J. J., Ramakers, J. D., & Plat, J. (2008). Dietary modulation of immune function by β-glucans. *Physiology & Behavior, 94*(2), 276-284. doi:https://doi.org/10.1016/j.physbeh.2007.11.045

Wang, J.-L., Bi, Z., Zou, J.-W., & Gu, X.-M. (2012). Combination therapy with lentinan improves outcomes in patients with esophageal carcinoma. *Molecular medicine reports, 5*(3), 745-748.

Wasser, S. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied microbiology and biotechnology, 60*(3), 258-274. doi:10.1007/s00253-002-1076-7

Wasser, S. P. (2014). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. *Biomed J, 37*(6), 345-356.

Woldegiorgis, A. Z., Abate, D., Haki, G. D., & Ziegler, G. R. (2014). Antioxidant property of edible mushrooms collected from Ethiopia. *Food Chemistry, 157*, 30-36.

Wong, J. Y., & Chye, F. Y. (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis, 22*(4), 269-277. doi:https://doi.org/10.1016/j.jfca.2008.11.021

Wu, H.-J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes, 3*(1), 4-14. doi:10.4161/gmic.19320

Wu, S. J., Chen, Y. W., Wang, C. Y., & Shyu, Y. T. (2017). Anti-inflammatory properties of high pressure-assisted extracts of Grifola frondosa in lipopolysaccharide-activated RAW 264.7 macrophages. *International Journal of Food Science & Technology, 52*(3), 671-678. doi:10.1111/ijfs.13320

Wu, X., George, R. L., Huang, W., Wang, H., Conway, S. J., Leibach, F. H., & Ganapathy, V. (2000). Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1466*(1), 315-327.

Xu, S., Xu, X., & Zhang, L. (2012). Branching structure and chain conformation of water-soluble glucan extracted from Auricularia auricula-judae. *Journal of Agricultural and Food Chemistry, 60*(13), 3498-3506.

Xu, X., Yan, H., Chen, J., & Zhang, X. (2011). Bioactive proteins from mushrooms. *Biotechnology advances, 29*(6), 667-674.

Yahia, E. M., Gutiérrez-Orozco, F., & Moreno-Pérez, M. A. (2017). Identification of phenolic compounds by liquid chromatography-mass spectrometry in seventeen species of wild mushrooms in Central Mexico and determination of their antioxidant activity and bioactive compounds. *Food Chemistry, 226*, 14-22. doi:https://doi.org/10.1016/j.foodchem.2017.01.044

Yan, J., Han, Z., Qu, Y., Yao, C., Shen, D., Tai, G., . . . Zhou, Y. (2018). Structure elucidation and immunomodulatory activity of a β-glucan derived from the fruiting bodies of Amillariella mellea. *Food Chemistry, 240*, 534-543. doi:https://doi.org/10.1016/j.foodchem.2017.07.154

Yang, J.-H., Lin, H.-C., & Mau, J.-L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry, 77*(2), 229-235. doi:https://doi.org/10.1016/S0308-8146(01)00342-9

Yang, N.-C., Lin, H.-C., Wu, J.-H., Ou, H.-C., Chai, Y.-C., Tseng, C.-Y., . . . Song, T.-Y. (2012). Ergothioneine protects against neuronal injury induced by β-amyloid in mice. *Food and Chemical Toxicology, 50*(11), 3902-3911. doi:https://doi.org/10.1016/j.fct.2012.08.021

Yang, P., Liang, M., Zhang, Y., & Shen, B. (2008). Clinical application of a combination therapy of lentinan, multi-electrode RFA and TACE in HCC. *Advances in Therapy, 25*(8), 787. doi:10.1007/s12325-008-0079-x

Yoshida, S., Shime, H., Funami, K., Takaki, H., Matsumoto, M., Kasahara, M., & Seya, T. (2017). The Anti-Oxidant Ergothioneine Augments the Immunomodulatory Function of TLR Agonists by Direct Action on Macrophages. *PLoS One, 12*(1), e0169360. doi:10.1371/journal.pone.0169360

Yoshino, S., Tabata, T., Hazama, S., Iizuka, N., Yamamoto, K., Hirayama, M., . . . Oka, M. (2000). Immunoregulatory effects of the antitumor polysaccharide lentinan on Th1/Th2 balance in patients with digestive cancers. *Anticancer research, 20*(6C), 4707-4711.

Yoshino, S., Watanabe, S., Imano, M., Suga, T., Nakazawa, S., Hazama, S., & Oka, M. (2010). Improvement of QOL and prognosis by treatment of superfine dispersed lentinan in patients with advanced gastric cancer. *Hepato-gastroenterology, 57*(97), 172-177.

Zhang, J., Wang, G., Li, H., Zhuang, C., Mizuno, T., Ito, H., . . . Li, J. (1994). Antitumor Polysaccharides from a Chinese Mushroom, "Yuhuangmo" the Fruiting Body of Pleurotus citrinopileatus. *Bioscience, Biotechnology, and Biochemistry, 58*(7), 1195-1201. doi:10.1271/bbb.58.1195

Zhang, J.-J., Li, Y., Zhou, T., Xu, D.-P., Zhang, P., Li, S., & Li, H.-B. (2016). Bioactivities and Health Benefits of Mushrooms Mainly from China. *Molecules, 21*(7), 938.

Zhao, J., & Cheung, P. C. K. (2011). Fermentation of β-Glucans Derived from Different Sources by Bifidobacteria: Evaluation of Their Bifidogenic Effect. *Journal of Agricultural and Food Chemistry, 59*(11), 5986-5992. doi:10.1021/jf200621y

Zhao, L., Xiao, Y., & Xiao, N. (2013). Effect of lentinan combined with docetaxel and cisplatin on the proliferation and apoptosis of BGC823 cells. *Tumor Biology, 34*(3), 1531-1536. doi:10.1007/s13277-013-0680-8

Zhu, B.-Z., Mao, L., Fan, R.-M., Zhu, J.-G., Zhang, Y.-N., Wang, J., . . . Frei, B. (2011). Ergothioneine Prevents Copper-Induced Oxidative Damage to DNA and Protein by Forming a Redox-Inactive Ergothioneine−Copper Complex. *Chemical Research in Toxicology, 24*(1), 30-34. doi:10.1021/tx100214t

Zhu, F., Du, B., Bian, Z., & Xu, B. (2015). Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis, 41*, 165-173. doi:https://doi.org/10.1016/j.jfca.2015.01.019