Water-solubility of β-Glucans in Various Edible Mushrooms

- Research Note -

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Abstract

The amount of β -glucans in 12 edible mushroom species was determined and their water-solubility was assessed. A large variability in β -glucan content was observed in the mushroom species, ranging from 4.71 to 46.20% on a dry basis. Gyrophora esculenta, Lentinus edodes, Coriolus versicolor, Ganoderma lucidum, and Flammulina velutipes had high levels of β -glucan. Soluble β -glucan content, which plays a key role in the physiological effects of mushrooms, also varied greatly according to the mushroom species, ranging from 2.12 to 19.66%. Water-solubility of β -glucan in the edible mushrooms, as a percentage of total β -glucan content varied from 42.55 to 73.35%.

Key words: edible mushrooms, β -glucan, water-solubility

INTRODUCTION

Mushrooms have been consumed in oriental culture not only for their special flavor and texture, but also for their nutritional value. Mushrooms are considered to be healthy because they are low in fat and calories but contain appreciable amounts of dietary fiber (1). Mushrooms are also known as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer, mainly due to the presence of dietary fiber (2). Fungal cell walls contain chitin (Nacetyl-D-glucosamine-polymer), mannans, other hemicelluloses and, among the most interesting functional components, β-glucans (3). These compounds are believed to contribute to some of the healthy properties of mushrooms, such as enhancement of macrophage function, host resistance to many bacterial, viral, fungal and parasitic infections, stimulation of the immune system, and reduction of blood cholesterol levels and glycemic response in vivo (4).

Numerous mushrooms and their extracts have been studied *in vitro* and *in vivo* for their anti-tumor activities (5,6). Anti-tumor polysaccharides are, in most cases, (1 \rightarrow 3)- β -D-glucans, which primarily consist of (1 \rightarrow 3)- β -D-linked D-glucose as a backbone and some (1 \rightarrow 6)- β -D-linked D-glucose branch units (7). Many biologically active β -glucans having (1 \rightarrow 3)- and (1 \rightarrow 6)-linkages isolated from mushrooms are widely used as anti-tumor and immunomodulating agents (8-12). β -Glucans in mushrooms are distributed both in soluble and in insoluble

dietary fractions. However, scant information is available about the amounts of soluble and insoluble β -glucans in edible mushrooms.

The objective of this study was to determine the water-soluble, water-insoluble, and total β -glucan contents, and to estimate water-solubility of β -glucans in various edible mushrooms.

MATERIALS AND METHODS

Samples

Fruiting bodies of 12 different species of mushrooms were collected from local grocery stores. These included Ganoderma lucidum, Lentinus edodes, Coriolus versicolor, Agaricus bisporus, Flammulina velutipes, Agaricus blazei Murill, Pleurotus ostreatus, Phellinus linteus, Gyrophora esculenta, Auricularia auricula, Inonotus obliquus, and Pleurotus eryngii. Fresh mushrooms were cut into small pieces and dried at 40°C for 2 days using a dry oven. The dried mushroom samples were milled to pass through a 0.5 mm screen using a Cyclotec 1093 sample mill (Tacator, Hoganas, Sweden).

Determination of β-glucan content

β-D-Glucans in mushroom samples were determined using a mushroom β-glucan assay kit (Megazyme Pty, Ltd., Wicklow, Ireland). The milled mushroom sample (100 mg) and 8 mL of aqueous ethanol (80% v/v) were added to a tube and stirred vigorously on a vortex mixer. The tube was centrifuged (1,500 \times g, 10 min), and the supernatant was carefully decanted to remove low mo-

lecular weight sugars. Ice-cold sulfuric acid (60% v/v, 2 mL) was added to each tube while stirring on a magnetic stirrer for 1 hr. The tubes were mixed with 12 mL of distilled water and then incubated in a boiling water bath at 100°C for 2 hr. The contents of each tube were adjusted to 100 mL with distilled water and centrifuged (1,500×g, 10 min). Aliquots (0.2 mL) of the centrifuged extracts were transferred to glass test tubes and analyzed with glucose-peroxidase reagent (GOPOD) to measure total glucan content. For measurement of α -glucan, low molecular weight sugars were also removed as previously described. Two mL of 2 M KOH was added to each tube to suspend pellets and to dissolve phytoglycogen and starch by stirring for 20 min in an ice water bath. The suspension was added with 8 mL of 1.2 M sodium acetate buffer (pH 3.8), mixed with 0.1 mL of amyloglucosidase (3,300 U/mL), and incubated in a water bath at 40°C for 30 min. The tubes were centrifuged (1,500×g, 10 min), and aliquots (0.1 mL) of supernatants were analyzed with glucose-peroxidase reagent to measure α -glucan content. β -Glucan content was determined by substracting the α -glucan from the total glucan content.

Determination of soluble and insoluble β-glucan

The mushroom sample (100 mg) was mixed with 10 mL of distilled water, and then subjected to continuous shaking for 30 min at 38°C, followed by centrifugation (5,000×g, 10 min). β -Glucan content in the sediment was measured as insoluble β -glucan. Soluble β -glucan was determined by substracting the insoluble from the total β -glucan content. Solubility of β -glucan was calculated as a percentage of the total β -glucan content in the mushroom sample.

RESULTS AND DISCUSSION

β-Glucan contents of various edible mushrooms Table 1 shows glucan (total, α - and β -glucan) con-

tents of the 12 edible mushroom species. A large variability in glucan contents was observed among the mushroom species. Total glucan (α - and β -glucan) in the samples ranged from 5.23 to 46.49% on a dry basis. α -Glucan content ranged from 0.21 to 10.88%. *Agaricus blazei* Murill showed the highest α -glucan value of 10.88%. In most mushrooms, however, α -glucan was found at levels below 1%. The α -glucan content was estimated to be phytoglycogen and starch present in mushrooms (13,14).

β-Glucan content, determined by substracting α -glucan from total glucan content, ranged from 4.71 to 46.20%. Gyrophora esculenta appeared to have the highest concentration of β-glucan. Lentinus edodes, Coriorus versocolor, Ganoderma lucidum, and Flammulina velutipes showed higher levels of β -glucan than the other samples. Cereals contain essentially β -glucans with β - $(1\rightarrow 3)$, $(1\rightarrow$ 4) linkages, while mushrooms have large amounts of (1 \rightarrow 3)- β -D-glucans, sometimes referred to as $(1\rightarrow3)$, $(1\rightarrow$ 6)-β-D-glucan (15). They have a common structure, a main chain consisting of $(1\rightarrow 3)$ -linked β -D-glucopyranosyl units along which are randomly dispersed single β -D-glucopyranosyl units attached by $(1\rightarrow 6)$ -linkages, resulting in a comb-like structure (7). Only those polysaccharides having $(1\rightarrow 3)$, $(1\rightarrow 6)$ - β -D-linkages have been associated with immunomodulating activity and inhibition of tumor growth (13).

Water-soluble and insoluble β-glucan contents of various edible mushrooms

β-Glucans in mushrooms are distributed in both the soluble and insoluble fraction of mushroom dietary fiber. Table 2 shows α -, β - and total glucan contents in waterinsoluble fractions in the 12 edible mushrooms. The total glucan in the insoluble fractions of mushrooms varied from 3.10 to 26.79%, whereas α - and β -glucan contents ranged from 0.17 to 3.93% and from 2.59 to 26.54% on a dry basis, respectively.

	Glucan content (%)		
	Total	α	β
Pleurotus ostreatus	10.80 ± 0.51	0.72 ± 0.06	10.08 ± 0.58
Pleurotus eryngii	11.72 ± 1.46	2.22 ± 0.13	9.50 ± 1.58
Ganoderma lucidum	22.24 ± 1.32	0.63 ± 0.07	21.61 ± 1.39
Lentinus edodes	33.99 ± 0.65	5.91 ± 0.57	28.08 ± 0.08
Agaricus bisporus	7.19 ± 0.74	0.60 ± 0.07	6.59 ± 0.67
Phellinus linteus	12.82 ± 2.37	0.37 ± 0.12	12.45 ± 2.25
Auricularia auricula	8.86 ± 0.20	0.31 ± 0.10	8.55 ± 0.30
Gyrophora esculenta	46.49 ± 0.24	0.29 ± 0.03	46.20 ± 0.27
Coriolus versicolor	22.69 ± 2.15	0.45 ± 0.31	22.24 ± 1.84
Flammulina velutipes	18.55 ± 1.37	0.21 ± 0.15	18.34 ± 1.52
Inonotus obliquus	5.23 ± 0.45	0.52 ± 0.14	4.71 ± 0.59
Agaricus blazei Murill	18.41 ± 1.45	10.88 ± 1.52	7.53 ± 2.97

¹⁾Values are means of triplicate analyses ± standard deviations.

Table 2. Insoluble glucan contents¹⁾ of various edible mushrooms

	Insoluble glucan content (%)		
	Total	α	β
Pleurotus ostreatus	3.44 ± 0.27	0.52 ± 0.10	2.92 ± 0.37
Pleurotus eryngii	4.35 ± 0.47	0.76 ± 0.16	3.59 ± 0.64
Ganoderma lucidum	6.16 ± 0.08	0.40 ± 0.08	5.76 ± 0.16
Lentinus edodes	13.78 ± 0.16	2.98 ± 0.21	10.80 ± 0.04
Agaricus bisporus	3.37 ± 0.02	0.29 ± 0.07	3.08 ± 0.08
Phellinus linteus	5.69 ± 0.90	0.33 ± 0.05	5.36 ± 0.95
Auricularia auricula	3.51 ± 0.40	0.21 ± 0.02	3.30 ± 0.42
Gyrophora esculenta	26.79 ± 0.82	0.25 ± 0.01	26.54 ± 0.82
Coriolus versicolor	11.84 ± 1.19	0.22 ± 0.03	11.62 ± 1.22
Flammulina velutipes	7.03 ± 0.29	0.17 ± 0.01	6.86 ± 0.28
Inonotus obliquus	3.10 ± 0.02	0.51 ± 0.07	2.59 ± 0.09
Agaricus blazei Murill	7.11 ± 0.08	3.93 ± 0.32	3.18 ± 0.40

¹⁾Values are means of triplicate analyses ± standard deviations.

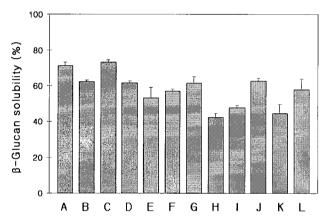


Fig. 1. Water-solubility of β-glucans from various edible mushrooms. A, Pleurotus ostreatus; B, Pleurotus eryngii; C, Ganoderma lucidum; D, Lentinus edodes; E, Agaricus bisporus; F, Phellinus linteus; G, Auricularia auricula; H, Gyrophora esculenta; I, Coriolus versicolor; J, Flammulina velutipes; K, Inonotus obliquus; L, Agaricus blazei Murill.

Water-soluble β -glucan was calculated as the difference between the total and insoluble β -glucan. Soluble β -glucan content varied greatly from 2.12 to 19.66%. This result was similar to the result which the distribution of β -glucan in the dietary fiber fractions was variable in different mushroom species (13). *Gyrophora esculenta*, *Lentinus edodes* and *Ganoderma lucidum* showed relatively high concentrations of soluble β -glucan, greater than 15%.

Water-solubility of β-glucan in the edible mushrooms varied from 42.55 to 73.35% (Fig. 1). Ganoderma lucidum showed the highest β-glucan solubility. Ganoderma lucidum, a medicinal mushroom, is popularly used as a dietary supplement, primarily because of its anti-tumor activity (16). Despite having the highest amount of soluble β-glucan, Gyrophora esculenta showed the lowest β-glucan solubility. The water-solubilities of β-glucan in Pleurotus ostreatus and Lentinus edodes were relatively high. The difference in water-solubility of β-glucan in

mushroom was probably due to different molecular structures or molecular weights. On the other hand, the insoluble dietary fiber fraction of mushrooms was much higher than soluble dietary fiber (17), since chitin, a structural polymer of fungal cell walls, is located in the insoluble fiber fraction. Soluble components of β -glucan in mushrooms probably play an important physiological role in human diet due to a cumulative action of different fiber components.

REFERENCES

- Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. 1999. Nutrients in edible mushrooms: an interspecies comparative study. Food Chem 65: 477-482.
- Bobek P, Galbavy S. 1999. Hypocholesterolemic and antiatherogenic effect of oyster mushroom in rabbit. Nahrung 43: 339-342.
- Manzi P, Marconi S, Altero A, Pizzoferrato L. 2004. Commercial mushrooms: nutritional quality and effect of cooking. Food Chem 84: 201-206.
- 4. Cheung PCK. Functional properties of edible mushrooms. 1998. *J Nutr* 128: 1512-1516.
- Kim JE, Lee WS, Chung HY, Jang SJ, Kim JS, Lee JB, Song CS, Park SY. 2004. The selective antitumor activity of water-soluble extracts of the fruiting bodies and the cultivated mycelia of *Agaricus blazei* Murill. *Food Sci Biotechnol* 13: 347-352.
- Chun HS, Choi EH, Kim HJ, Choi CW, Hwang SJ. 2001. In vitro and in vivo antitumor activities of water extracts from Agaricus blazei Murill. Food Sci Biotechnol 10: 335-340.
- Bohn JA, BeMiller JN. 1995. (1→3)-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohyd Polymers 28: 3-14.
- Chihara G, Hamuro H, Maeda Y, Arai Y, Fukuoka K. 1970. Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan from Lentinus edodes. Cancer Res 30: 2776-2781.
- Komatsu N, Okubo S, Kikumoto S, Kimura K, Saito G. 1969. Host-mediated antitumor action of schizophyllan, a glucan produced by Schizophyllum commume. Gann 60: 137-144.

- Mizuno T, Ohsawa K, Hagiwara N, Kuboyama R. 1986. Fractionation and characterization of antitumor polysaccharides from maitake, *Grifola frondosa*. *Agric Biol Chem* 50: 1679-1688.
- 11. Mizuno T, Hagiwara T, Nakamura T, Ito H, Shimura K, Sumiya T, Asakura A. 1990. Antitumor activity and some properties of water-soluble polysaccharides from "Himematsutake", the fruiting body of *Agaricus blazei* Murill. *Agric Biol Chem* 54: 2889-2896.
- 12. Tsukagoshi S, Ohashi F. 1974. Protein-bound polysaccharide preparation, PS-K, effective against mouse sarcoma 180 and rat ascites hepatoma AH-13 by oral use. *Gann* 65: 557-558.
- 13. Manzi P, Pizzoferrato L. 2000. Beta-glucans in edible mushrooms. *Food Chem* 68: 315-318.
- Hammond JBW. 1979. Changes in composition of harvested mushrooms (*Agaricus bisporus*). *Phytochemistry* 18: 415-418.
- Mullins JT. 1990. Regulatory mechanism of β-glucan synthetases in bacteria, fungi and plants. *Physiological Plantarum* 78: 309-314.
- 16. Sliva D. 2004. Cellular and physiological effects of *Ganoderma lucidum*. Med Chem 4: 873-879.
- 17. Manzi P, Aguzzi A, Pizzoferrato L. 2001. Nutritioanl value of mushrooms widely consumed in Italy. *Food Chem* 73: 321-325.

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