Chitin-Glucan Complex from *Agaricus blazei*, a Potential Raw Material for Production of Food Additives

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Abstract: *Agaricus blazei*, a mushroom native to Brazil, is a perspective source for food industry. This mushroom has been widely used in folk medicine due to its possible medicinal value. The most important components of fruiting bodies of *A. blazei* are specific β-glucans with β-(1→3), β-(1→4) andβ-(1→6) glycosidic linkages. These polysaccharides are supposed to be responsible for some healthy properties of mushrooms (anticarcinogenic and antimutagenic ones). β-Glucans are associated with chitin forming water-insoluble chitin-glucan complex. This complex was isolated from fresh and dried mushrooms (separately from caps and stems) by alkali treatment using NaOH solution at 9°C for 2 h. The structure of chitin-glucan complex was analysed by diffuse reflectance FT-IR spectroscopy. β-Glucans were also analysed by Megazyme enzymatic method based on exo-1,3-β-glucanase and β-glucosidase catalysed hydrolysis and photometric determination of the released glucose.

Keywords: chitin-glucan complex; *Agaricus blazei*

INTRODUCTION

Edible mushrooms are a potential source of biologically active dietary fibres. Fungal cell walls contain besides chitin, hemicelluloses, mannans, and the most interesting functional components, β-glucans. Mushroom β-glucans have been established as immunomodulators for over 40 years. These polysaccharides have also significant effects as a prophylactic treatment to reduce the mortality of anthrax infection in mice in synergism with antibiotics [1]. The antitumour activity was mainly due to indirect host mediated immunotherapeutic effect [2–4]. Moreover, glucans are active as antioxidants and is able to protect animals and man against radiation [5]. *Agaricus blazei*, a mushroom native to Brazil, is a perspective source for food industry. This mushroom has been widely used in folk medicine due to its possible medicinal value, while it a relatively new addition to the family of medicinal mushrooms with research beginning in the 1980s. Earlier research has shown that *A. blazei* contains more β-glucans than any other medicinal mushrooms tested so far. It has been reported [6] that β-glucans from *A. blazei* is highly branched mixed-linkage polysaccharide. Sugar analysis confirmed that the carbohydrate moiety was composed predominantly of Glc and small amounts of Rha, Xyl, Man and Gal. According to results of these authors, three types of repeating units are possible: a (1→6)-linked backbone, a (1→3)-linked backbone, or an alternately (1→3), (1→6)-linked backbone. For all this purpose, the knowledge of structural properties of β-glucans is an obvious need because of the linkage between structure and functionality. The structural features of β-glucans from mushrooms *A. blazei* are important determinants of their physical properties and functionality. FT-IR spectroscopy has been shown to be a useful tool in structural and quantitative characterisation of microbial and fungal chitin-glucan complexes [7]. In this work the study of insoluble chitin-glucan complexes obtained from fresh and dried fruity bodies of *A. blazei* (separately caps and stems) by alkali deproteinisation is reported.

EXPERIMENTAL

Fresh and dried fruity bodies of *A. blazei* (separately caps and stems) were used for isolation of

Table 1. Specification of the *A. blazei* fruit body samples

Sample	Delivery date	Specification	Dry matter (% m/m)
1	6. 2. 2004	fresh caps	11.22
2	6. 2. 2004	fresh stems	11.22
3	9. 1. 2004	dried caps	90.67
4	9. 1. 2004	dried stems	93.36
5	25. 5. 2004	dried caps	92.32
6	25. 5. 2004	dried stems	92.28

protein free chitin-glucan complexes by alkali treatment using NaOH solution at 90°C for 2 h. The specification of the samples of *A. blazei* fruit bodies is shown in Table 1. The structure of obtained chitin-glucan complexes was analysed by FT-IR spectroscopy. Diffusion reflectance FT-IR spectra of solid samples were measured on Nicolet 740 (Nicolet Analytical Instruments, USA) spectrometer with DCT 680, 256 scans were accumulated with a spectral resolution of 4.0 cm^{-1}. Neutral sugar composition of the complex was studied by total acid hydrolysis (4M HCl, 120°C, 6 h) and HPAEC-PAD. β-Glucans were also analysed by Megazyme enzymatic set (Megazyme, Ireland) based on exo-1,3-β-glucanase and β-glucosidase catalysed hydrolysis and photometric determination of the released glucose.

RESULTS AND DISCUSSION

The contents of α- and β-glucans in the *A. blazei* fruit body samples obtained by enzymatic method are shown in Table 2. The total glucan contents were similar for fresh and dried fruit bodies, but showed marked topological difference: ~ 5–6% m/m for caps and ~ 8–12% m/m for stems. Fresh fruit bodies contain significantly less amount of α-glucans than dried fruit bodies, and fresh stems contain more α- and β-glucans than fresh caps. In contrast, in the case of dried fruit bodies, caps contain less amounts of α-glucans than stems, while the amounts of β-glucans were comparable (~ 4.4–6.0% m/m). Diffuse reflectance FT-IR spectra of the *A. blazei* fruit body samples are shown in Figure 1. The band assignment (Table 3) was made

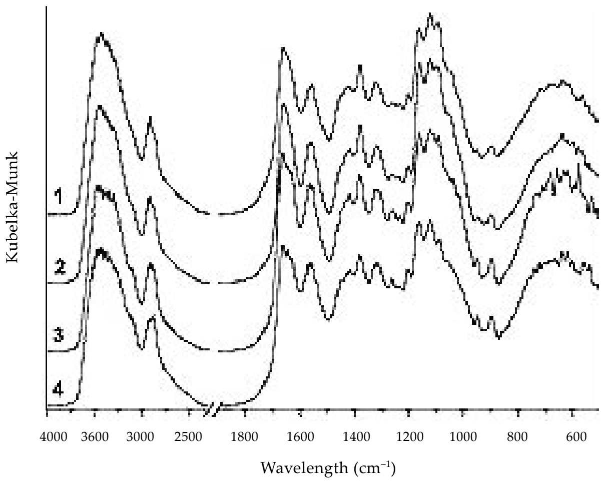

Figure 1. Diffuse reflectance FT-IR spectra of samples 1–4

Table 2. Contents of α- and β-glucans in the *A. blazei* fruit body samples

Sample	Total glucans (% m/m)		α-Glucans (% m/m)		β-Glucans (% m/m)	
	in wet matter	in dry matter	in wet matter	in dry matter	in wet matter	in dry matter
1	0.63	5.61	0.01	0.09	0.62	5.57
2	1.36	12.12	0.07	0.62	1.29	11.50
3	4.52	4.99	0.51	0.56	4.01	4.42
4	9.45	10.12	3.82	4.09	5.64	6.04
5	5.41	5.86	0.25	0.27	5.16	5.59
6	7.35	7.96	3.19	3.46	4.16	4.51

Table 3. FT-IR band positions (in cm^{-1}) and assignments for the *A. blazei* fruit body samples

Samples				Assignment [7–10]
fresh caps 1	fresh stems 2	dried caps 3, 5	dried stems 4, 6	
3427	3448	3448	3446	OH stretching (water, sugars)
3224	3236	3230	3232	NH stretching (chitin)
3107	3109sh	3111	3116	NH stretching (chitin)
	3087sh	3097	3099	CH stretching (aromatics)
2962sh	2956sh	2962sh	2960sh	CH stretching (pyrane ring)
2935sh	2935sh	2943sh	2937sh	CH_3 asym. stretching (chitin)
2920	2924	2924	2920	CH_2 stretching (sugars)
2894	2887	2891	2889	CH_3 sym. stretching (chitin)
2856	2854sh	2850sh	2860sh	CH_2 stretching (sugars)
1662	1660	1664	1664	Amide I (chitin)
1644	1647sh	1649	1645	HOH bending (bound water)
1632	1631sh	1631	1631	Amide I (chitin), CC stretching (aromatics)
1566	1568	1568	1568	Amide II (chitin)
1555	1556	1554	1554	Amide II (chitin)
	1536sh	1531sh	1541	CC stretching (aromatics)
1452	1448	1446	1450	CH_2 bending (sugars)
1429	1429	1431	1427	CH_3 asym. bending (chitin)
1417	1417	1417	1415	CCO, CCH, COH bending (pyrane ring)
1379	1379	1381	1381	CH_3 sym. bending (chitin)
1323	1315	1325	1315	Amide III (chitin)
1259	1261	1261	1261	CCO, CCH, COH bending (pyrane ring)
1234	1240	1232	1236	CCO, CCH, COH bending (pyrane ring)
1203	1203	1203	1203	CCO, CCH, COH bending (pyrane ring)
1161	1161	1161	1161	COC, CC stretching (β-glycosidic bond)
1122	1122	1124	1122	CC,CO stretching, CH def (pyrane ring)
1095	1103	1093	1090	CC,CO stretching, CH def (pyrane ring)
1045	1043sh	1039	1039sh	CC,CO stretching (β-anomer)
951	949	953	951	CH_3 rocking (chitin)
899	899	899	899	CH deformation (β-anomer)
		760	760	CH deformation (α-anomer)

according to thereferences [7–10]. The FT-IR spectra of all the samples exhibit several intense bands of α-chitin at ca. 950, 1320, 1555, 1568, 1662, 2890, 2935, 3110 and 3230 cm^{-1} indicating the presence of this polysaccharide. No specific marker bands of β-chitin [10] were found in the FT-IR spectra. Therefore, the chitin component of chitin-glucan complexes, which were isolated from *A. blazei* fruit bodies, is mainly in α-form. The presence of intense characteristic bands of amide groups confirms that this chitin component is highly acetylated. The bands at ca. 1160, 1040 and 899 cm^{-1} are characteristic for β-linked polysaccharides (chitin and β-glucan). The bands of α-glucans are less pronounced for all the samples. Dried fruit bodies had more intense vibration bands of aromatics at ca. 550–700, 1535, 1630 and 3090 cm^{-1} and less intense features of CH_2 groups of sugars at ca. 1450, 2855 and 2920 cm^{-1}. This fact may be explained by partial degradation of mushroom polysaccharides in dried fruit bodies.

CONCLUSIONS

Obtained results confirmed that both enzymatic analysis and FT-IR spectroscopy are useful for identification of different glucan types in various parts of intact fruiting bodies of *A. blazei*. On the other hand, the FT-IR spectra of cup and stalk of the same mushroom show marked differences, indicating variety in the chemical composition of different parts of the same fruity body.

Acknowledgements: This work was supported by the Internal Grant of the Institute of Chemical Technology, Prague (No. 321080015).

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