Mushrooms of Genus *Pleurotus* as a Source of Dietary Fibres and Glucans for Food Supplements

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Abstract

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Fruit bodies (separately pilei and stems) of mushrooms *Pleurotus ostreatus* (four strains) and *Pleurotus eryngii* were characterised as a source of polysaccharides. The contents of glucans and dietary fibres were determined with using the respective Megazyme enzymatic kits. Enzymatic analysis of the fruit bodies confirmed significant differences in the contents of these components among the species and strains. The stems contained more insoluble dietary fibres than the pilei in all the cases and more β-glucans in most cases. However, relatively high contents of β-glucan (20–50% of dry matter) could be a result of incomplete enzymatic hydrolysis of insoluble α-1,3-glucans. Nevertheless, low food quality stems of mushrooms *Pleurotus* sp. could be a valuable source of cell wall glucans for the preparation of food supplements.

Keywords: mushrooms *Pleurotus*; glucans; dietary fibres; food supplements

For millennia, humankind has been valued mushrooms as an important edible and medical resource (CHANG 1980; BREENE 1990; WASSER 2002). The dry matter of mushroom fruit bodies is about 5–15%, they have a very low fat content and contain 19–35% proteins. Mushroom fruit bodies are rich in vitamins, mainly B_1, B_2, C, and D_2 (MANZI *et al.* 1999; 2004; MATTILA *et al.* 2000), and contain some important elements such as K and P (VETTER 2007). The content of carbohydrates, which are mainly present as polysaccharides or glycoproteins, ranges 50–90%; the most abundant polysaccharides are chitin, α- and β-glucans and other hemicelluloses (e.g. mannans, xylans and galactans). Mushroom polysaccharides are present mostly as glucans with different types of glycosidic linkages, such as branched (1→3),(1→6)-β-glucans and linear (1→3)-α-glucans, but some are true heteroglycans (WASSER 2002).

Mushrooms are a potential source of dietary fibres due to the presence of non-starch polysaccharides. Total dietary fibre (TDF) in mushrooms is the sum of intrinsic non-digestible carbohydrates, mainly chitin (VETTER 2007). Mushroom glucans are also components of soluble (SDF) or insoluble (IDF) dietary fibres (PROSKY *et al.*

1988). Their solubility in water strongly depends on the molecular structure and conformation. Glucans bound to proteins or to chitin are usually insoluble in water.

Mushroom-derived substances with antitumour, immunomodulating, and antioxidative properties are currently used as dietary supplements. Mushroom polysaccharides with antitumour action differ greatly in their chemical compositions and configurations, as well as in their physical properties. Immunomodulatory and antitumour activities are exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers; the differences in their activities can be correlated with the solubility in water, molecular size, branching rate and form (Augustín 1998; Ooi & Lui 1999; Wasser 2002; Novak & Vetvicka 2008). Besides the well-known antitumour β-(1→3),(1→6)-glucans (Kuniak *et al.* 1992, 1998), a wide range of biologically active glucans of different structures have been described. Linear or branched polysaccharides chains occur with a backbone composed of α- or β-linked glucose units, and their various side chains can be attached in different ways. The main source of biologically active polysaccharides appears to be fungal cell walls consisting mainly of chitin-glucan complexes. However, fungal chitin has no antitumour activity (Mizuno *et al.* 1995).

Commercial importance of fungal polysaccharides has attracted much attention in the field of functional foods, especially, commonly cultivated mushrooms of the genus *Pleurotus* are interesting because of their β-glucans demonstrating significant immunomodulative properties. Pleuran, a specific glucan isolated from *Pleurotus* sp., has a suppressive effect on tumours (Kuniak *et al.* 1992; Karácsonyi & Kuniak 1994). Mushrooms of genus *Pleurotus* are cultivated in several countries because of their high adaptability. Annual production of these mushrooms is more than 900 000 tons. There are a lot of different species in the genus *Pleurotus* that have pharmacological properties, for example *P. florida, P. tuber-regium, P. sajor-caju, P. pulmonarius, P. ostreatus,* and *P. eryngii* (Ragunathan *et al.* 1996). It has been reported earlier that the fruit bodies and sclerotia of these species contain specific glucans (Karácsonyi & Kuniak 1994; Cheung & Lee 1998; Chenghua *et al.* 2000; Manzi & Pizzoferrato 2000; Rout *et al.* 2005). Biologically active glucans themselves or their complexes with proteins and other polysac-

charides isolated from the fruit bodies of these species are interesting for the preparation of novel food supplements.

The aim of this study was to characterise different parts of the fruit-bodies of the mushrooms *P. ostreatus* (four strains) and *P. eryngii* cultivated in the Czech Republic as a raw material for the preparation of food supplements and functional foods. The contents of glucans and dietary fibres in the pilei and stems were determined by enzymatic methods.

MATERIALS AND METHODS

Materials. The mushrooms *P. ostreatus* (strains 70, 77, L22, 137) and *P. eryngii* (non-specified strain) were cultivated under controlled conditions by the mushrooms grower Rudolf Ryzner in the region South Moravia, the Czech Republic. The fruit bodies (Figure 1) were subdivided into stems and pilei and homogenised with the laboratory dispenser DI 25 basic equipped with the dispersing element S25 N-25 G (IKA-Werke GmbH, Germany). The homogenised samples were kept at –20°C.

Analytical methods. The contents of total and α-glucans were determined in raw fruit bodies according to the Mushroom and Yeast β-glucan Assay Procedure K-YBGL 10/2005 (Megazyme, Ireland). Here, the estimation of non-starch glucans was based on the difference between glucose contents after total acidic hydrolysis of glucans and specific enzymatic hydrolysis of α-1,4-glucans. The samples were previously dried under a stream of nitrogen to ensure the correct acid concentration required for the hydrolysis. The glucan contents were calculated in dry matter. Total dietary fibres were determined as soluble and insoluble fractions according to the enzymatic method AOAC 991.43 combined with Total Dietary Fibre, K-TDFR 06/0 (Megazyme, Ireland). Relative standard deviations of the methods used were 6.1% (total and α-glucans) and 7.2% (total dietary fibres).

RESULTS AND DISCUSSION

Dietary fibres

Figure 2 shows the dietary fibre contents in dry matter (TDF – total dietary fibres, IDF – insoluble dietary fibres, SDF – soluble dietary fibres) in the fruit bodies (pilei and stems separately)
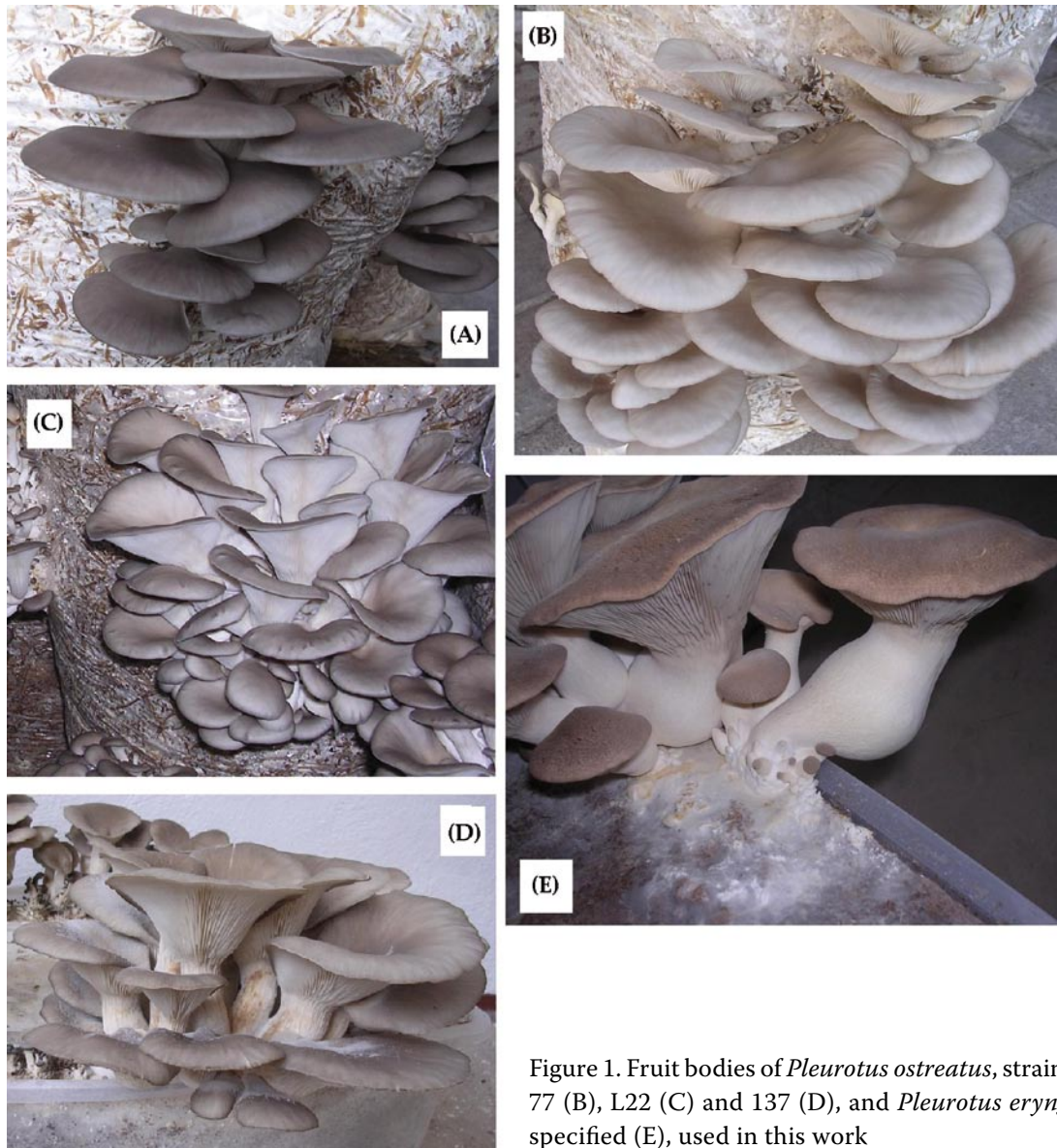
Figure 1. Fruit bodies of *Pleurotus ostreatus*, strains 70 (A), 77 (B), L22 (C) and 137 (D), and *Pleurotus eryngii*, non-specified (E), used in this work

of both *Pleurotus* species. The dry matter values of the fruit bodies (pilei and stems) ranged from 9.0% to 14.3%, confirming the high moisture content of these samples (Breene 1990; Manzi *et al.* 1999). The TDF contents were 34.5–63.1% in pilei and 38.9–64.8% in stems. The highest TDF level (64.8%) was observed in the stems of strain 77, the lowest TDF level (34.5 %) in the pilei of strain L22 *P. ostreatus*. The TDF values of the latter strain are close to the earlier reported TDF values of *P. sajor-caju*: 33.1% for pilei and 35.5% for stems (Cheung 1996). Fin all the samples, the IDF contents (29.2–61.4%) were significantly higher than those of SDF (2.0–4.9%). The stems contained higher amounts of IDF (36.7–61.4%)

than, the pilei (29.2–51.4%). The SDF contents in the pilei (2.0–4.7%) and stems (3.3–4.9%) were comparable but showed evident specificity as to the strains and species. As a rule, the stems contained more TDF and IDF than the pilei; en exception was strain 77 of *P. ostreatus* showing reciprocal relations. For comparison, Manzi *et al.* (2001, 2004) reported dietary fibre values in fresh fruit bodies of *P. ostreatus* (47.3% TDF, 42.4% IDF and 5.0% SDF) and *P. eryngii* (34.6% TDF, 30.7% IDF and 4.0% SDF) related to dry matter like in the present work. Thus, our results are comparable with the literature data, although strains 77 and 70 showed significantly higher values of TDF and IDF which could be explained by the strain specificity.
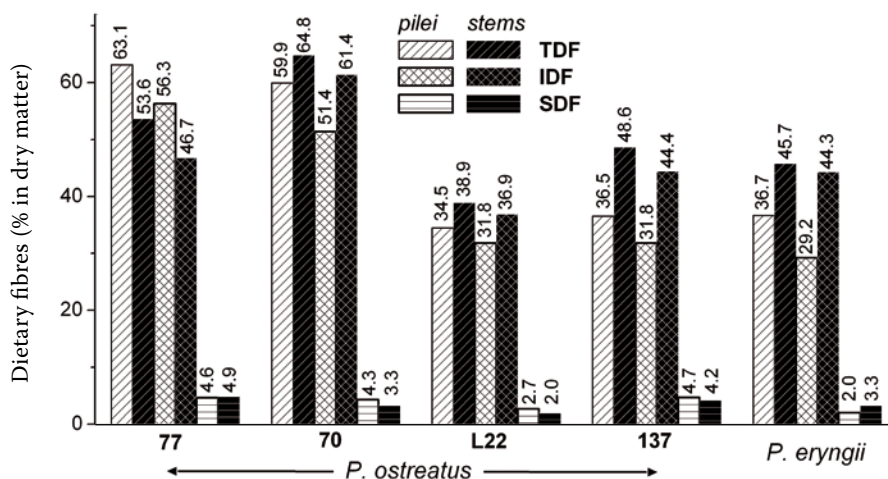
Figure 2. Contents of dietary fibres (TDF – total dietary fibres, SDF – soluble dietary fibres, IDF – insoluble dietary fibres) in pilei and stems of *P. ostreatus* (strains 77, 70, L22 and 137) and *P. eryngii* (non-specified)

Contents of glucans

The α- and β-glucan contents in the pilei and stems of *P. ostreatus* and *P. eryngii* are summarised in Figure 3. Total glucans were obtained by complete acidic hydrolysis of the samples, α-glucans by enzymatic hydrolysis with α-amylase and α-glucosidase. The fruit bodies contained small amounts of α-glucans: 3.4–7.9 in pilei and 3.0–7.6 in stems (*P. ostreatus*), and 3.6% in pilei and 4.3% in stems (*P. eryngii*). The contents of β-glucans, which were calculated as a difference between the total and α-glucans, were 27.4–39.2 in the pilei and 35.5–50.0 in the stems (*P. ostreatus*), and 20.4% in the pilei and 39.1% in the stems (*P. eryngii*). It is evident that the glucan contents showed topological specificity in the fruit bodies and significantly differed between the species of *Pleurotus* as well as between the individual strains of *P. ostreatus*. The fruit bodies of *P. eryngii* contained a lower amount of glucans than were those found in most

strains of *P. ostreatus*. In all the cases, the stems contained more β-glucans than the pilei. The highest level of β-glucans was found in the stems of strain 77 of *P. ostreatus* (50.0% in stems, 39.2% in pilei), while strain L22 showed the lowest level of β-glucans in the case of this species (35.5% in stems, 27.4% in pilei).

In contrast, according to enzymatic analysis based on direct hydrolysis by lichenase and β-glucanase, Manzi *et al*. (2000, 2001, 2004) reported about 1–2 order lower contents of β-glucan in fresh fruit bodies of *P. ostreatus* (0.24–0.38% and 1.6%) and *P. eryngii* (0.22–0.38% and 3.1%) related to dry matter. We suggest that out of the possible reasons for the low β-glucan content mentioned by the authors (Manzi & Pizzoferrato 2000), the most probable appears to be the presence of inert material in the fibre residue, maybe an insoluble chitin-glucan complex, that prevents the diffusion of enzymes during the β-glucan determination. This assumption is supported by the evident
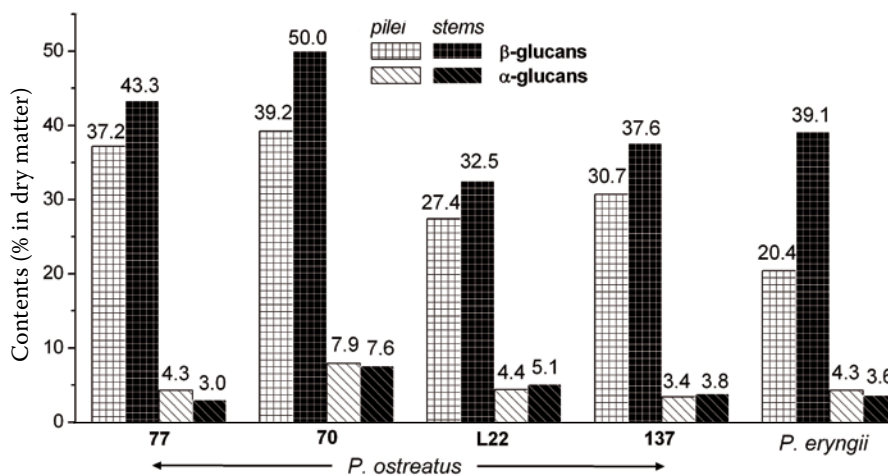

Figure 3. Contents of α- and β-glucans in pilei and stems of *P. ostreatus* (strains 77, 70, L22 and 137) and *P. eryngii* (non-specified)

increase of β-glucan content in the fruit bodies after cooking (partial degradation of insoluble chitin-glucan complex) or in the samples without previous washing with aqueous ethanol that may lead to removal of the low molecular fraction of β-glucan (Manzi & Pizzoferrato 2000; Manzi *et al.* 2001).

On the other hand, following the assumption mentioned, we cannot exclude that the relatively high contents of β-glucan obtained in this work (Figure 3) resulted from incomplete enzymatic hydrolysis of some cell wall α-glucans integrated into insoluble chitin-glucan complexes. Such α-glucans, which are not available for the enzymes commonly used in the set, can be structurally different from amylose or phytoglycogen. Linear α-1,3-glucan (pseudonigeran) as well as more complex polysaccharides including mixed α,β-glucans have been isolated from various mushrooms and microscopic fungi (Horisberger *et al.* 1972; Kiho *et al.* 1989; Chen *et al.* 1998; Zhang *et al.* 1999; Jin *et al.* 2004; Wang *et al.* 2007); and our preliminary investigation confirmed their presence in the fruit bodies of *Pleurotus*. The susceptibility of specific fungal α-glucans to enzymatic hydrolysis by α-amylase and α-glucosidase has not yet been studied, so we cannot be fully confident that the method of β-glucan assay used in this work is more effective than that used by Manzi *et al.* (2000, 2001, 2004). To clarify this problem, we will report on further investigation devoted to the spectroscopic characterisation and enzymatic analysis of polysaccharide fractions isolated from the fruit bodies of *P. ostreatus* and *P. eryngii* (Synytsya *et al.* 2009).

CONCLUSION

It can be concluded that the fruit bodies of *P. ostreatus* and *P. eryngii* contain significant amounts of β-glucans, which are components of both insoluble and soluble dietary fibres. The contents of these polysaccharides in the fruit bodies vary with the strains and species. The stems are a better source of IDF and glucans than are the gastronomically attractive pilei, and, therefore, the stems can be used for the preparation of biologically active polysaccharide complexes utilisable as food supplements. In relation to the significant difference between β-glucan contents obtained in this work and those reported earlier, further work is now in progress with the aim to evaluate the possible influence of non-starch α-glucans on the enzymatic analysis for β-glucan in the fruit bodies of *Pleurotus*.

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