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Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms

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ABSTRACT

Mushrooms have unique sensory properties and nutritional values as well as health benefits due to their bioactive compounds, especially beta-glucans. Well-known edible and medicinal mushroom species as well as uncommon or unknown species representing interesting sources of bioactive beta-glucans have been widely studied. Commercially cultivated and wild growing mushrooms were analysed for their beta-glucan contents. Enzymatic determinations of all glucans, alpha-glucans and beta-glucans in 39 mushrooms species were performed, leading to very remarkable results. Many wild growing species present high beta-glucan contents, especially Bracket fungi. The well-known cultivated species *Agaricus bisporus*, *Lentinula edodes* and *Cantharellus cibarius* as well as most screened wild growing species show higher glucan contents in their stipes than caps.

1. Introduction

Mushrooms have a long history in culinary and medicinal usage. More than 2000 species of mushrooms are known to exist in nature, but only a few are considered food. The most cultivated mushroom species are *Agaricus bisporus, Lentinula edodes* and *Pleurotus* spp., and their production is continuously increasing in China, which is the largest producer worldwide (Aida, Shuhaimi, Yazid, & Maaruf, 2009; Patel & Goyal, 2012). Due to their unique sensory properties, nutritional values and health benefits, mushrooms have become the focus of international medicinal research in recent years. The dry matter content is very low in mushrooms (approximately 10%). Mushrooms have higher protein contents than most vegetables and provide all essential amino acids for adult requirements (Flegg & Maw, 1997; Kalac, 2013). Furthermore, high insoluble fibre contents (chitin and other polysaccharides) present nutritional advantages, and low lipids and glycogen contents result in low energy values (Kalac, 2009, 2013). In addition to a wide variety of compounds that are beneficial to health, such as phenolic substances, sterols, alkaloids, lactones, terpenes and ceramides, bioactive polysaccharides and polysaccharide-protein complexes are the most studied group of functional compounds in medicinal mushrooms (De Silva et al., 2013; Hishida, Nanba, & Kuroda, 1988; Villares, Mateo-Vivaracho, & Guillamon, 2012). These glucans show bioactive properties such as immune-modulating, antitumor (Sari et al., 2016), antiviral (Zhang, Cui, Cheung, & Wang, 2007) and hepato-protective effects (Wasser, 2014).

The major structural feature of mushroom beta-glucans is a beta-1,3-D-glucan main chain with single D-glucosyl residues linked beta-1,3 along this main chain. Some of this glucan can be extracted from the fruiting body of the mushroom, and soluble beta-glucans are also produced by cultured mycelia (Chang & Wasser, 2012).

Because beta-glucans are not synthesized by the human body, they are recognized by the immune system and induce both adaptive and innate immune responses (Brown & Gordon, 2005). In this context, the use of mushroom extracts with soluble beta-glucans vs. the consumption of the whole fruiting body is discussed for digestibility and bioactivity (Kalac, 2013; Wasser, 2014). In addition, chitin and alpha-glucans are present in mushrooms; the total polysaccharide contents of mushrooms range between 50% and 90% (Wasser, 2002). Thus, the determination of exact beta-glucan content is still difficult due to finding an optimized method (e.g.

Abbreviations: dm, dry mass; GOPOD, glucose oxidase/peroxidase.

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McCleary & Draga, 2016; Synytsya & Novak, 2014). Often the phenolic sulphur method is described, which does not differentiate between different kinds of carbohydrates. In the recent five years alternative methods for beta-glucan (especially 1,3-1,6 beta glucans) are described. For example, the congo red coloration method for beta 1,3-glucans or the aniline blue coloration method for beta 1,3-1,6 beta-glucans (Nitschke et al., 2011) or measurement kits like Megazyme© assay or Glucatell©-kit were used (Gründemann et al., 2015). The most recent work by McCleary and Draga (2016) addresses the analytical problems, compared methods and reported beta-glucan values for a wide range of mushrooms and commercial mycelial products. Furthermore, mushrooms are a potential source of soluble and insoluble dietary fibre (Manzi & Pizzoferato, 2000). Many species of mushrooms are known for their high beta-glucan contents and are well studied: The anticarcinogenic properties of beta-glucans from *Lentinula edodes* (Lentinan) (e.g., Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970), *Grifola frondosa* (Grifolan) (e.g., Ohno et al., 1984), *Ganoderma lucidum* (Reishi) (e.g., Liu et al., 2004), *Trametes versicolor* (Krestin, proteo-glucan) (e.g., Mizuno, 1999) and many others are shown in a large number of studies. Mushrooms make up a considerable but largely untapped source of new powerful products with pharmaceutical properties, but many wild species (edible and non-edible) have not yet been investigated for their beta-glucan contents. These mushrooms can be valuable sources for nutritional and pharmacological compounds. The consumption of both the fruiting body and extracts (water or other solvents) from fruiting bodies or mycelia shows positive effects on health. Therefore, a large number of medicinal mushroom drugs and preparations with immunomodulating properties can be found on the market today (El Enshasy & Hatti-Kaul, 2013; Wasser, 2014). However, currently, very little is known about the glucan contents of wild mushrooms. Therefore, it was the goal of this study to analyze the glucan contents in a wide variety of wild grown mushroom, especially Bracket fungi, and compare them with cultivated mushrooms.

2. Materials and methods

2.1. Mushroom samples

The fruiting bodies of common culinary mushrooms were purchased in local supermarkets in Mönchengladbach, North-Rhine Westphalia, Germany (*Agaricus bisporus* (J.E. Lange) Imbach white and brown varieties, *Lentinula edodes* (Berk.) Pegl., and *Cantharellus cibarius* (Fr.) or were ordered from a local mushroom grower (*Pleurotus ostreatus* (Jaqu. ex Fr.) P. Kumm., *Pleurotus eryngii* (DC ex Fr.) Gill., *Pleurotus citrinopileatus* (Sing.), *Pleurotus pulmonarius* (Fr. ex Fr.) Quél., and *Pleurotus djamor* (Rumph. ex Fr.) Boedijn.

Thirty wild mushroom species were collected in a local area of 1 km^2 in the western part of North-Rhine Westphalia, Germany between 24.9.2015 and 08.11.2015. The temperature varied between −1.2 °C and 20 °C with rainfall between 0.05 and 42 L/m^2 per day. All mushroom samples were divided into caps and stipes, if possible, and finally dried at 60 °C for 16 h.

2.2. Dry matter analysis

For optimal comparability, the dry matter was determined for all hot-air-dried samples by mixing with sea sand and heating at 103 ± 2 °C until weight constancy. The dry matter was calculated by dividing the sample weight after and before heating. All values were calculated on the dry mass in gram per 100 g dry mass of the mushroom.

2.3. Identification of species

All species were determined morphologically according to the literature (Gminder, 2008; Ostry, Anderson, & O'Brien, 2011). DNA-analysis of the mushroom species was performed by Alvalab molecular analysis service, LA Rochela, Spain to ensure all determinations by using PCR and sequencing of parts of the ITS-region (Alvarado et al., 2015).

2.4. Detection of beta-glucan contents

The 1,3-1,6-beta-glucans were determined in quadruplicate using an assay kit (Megazyme© Ltd., Bray, Wicklow County, Ireland) according to the manufacturer's instructions. All enzymes used were purchased from Megazyme© Ltd. The dried mushroom samples (100 mg) were milled using an analytical mill (IKA GmbH, Staufen, Germany), sieved using a 0.5 mm screen and weighed into culture tubes, and 1.5 mL of concentrated HCl (37%) was added. After heating at 30 °C for 45 min, 10 mL of distilled water was added, and the samples were incubated in a boiling water bath for 2 h. After a neutralization step with 2 M KOH, the samples were adjusted to 100 mL with a sodium acetate buffer (pH 5.0). To measure the total glucan contents, 0.1 mL aliquots were mixed with *exo*-1,3-beta-glucanase (20 U/mL) and beta-glucosidase (4 U/mL) and incubated in a water bath at 40 °C for 60 min. Then, 3 mL of glucose-oxidase-peroxidase-reagent (GOPOD) was added and again incubated at 40 °C for 20 min.

For the determination of the alpha-glucan contents, dried extract samples (100 mg in quadruplicate) were stirred with 2 mL of KOH (2 M) in an ice water bath for 20 min. After adding 8 mL of sodium acetate buffer (pH 3.8) and 0.2 mL of amyloglucosidase (1630 U/mL), the samples were incubated in a water bath at 40 °C for 30 min. Next, 0.1 mL aliquots were mixed with 0.1 mL of sodium acetate buffer (pH 5.0) and 3 mL of GOPOD. They were incubated again at 40 °C for 20 min. The beta-glucan contents of a yeast standard and an internal mushroom powder control were determined. All samples were measured at 510 nm in a photometer (LKB Biochrom, Cambridge, England) against a reagent blank. The beta-glucan content was determined by subtracting the alpha-glucan content from the total glucan content. In both steps the total glucan/alpha-glucan contents as well as the D-glucose in oligosaccharides, sucrose and free D-glucose contents are measured. The enzymatic assay test for detecting 1,3-1,6-beta-glucans in mushrooms is a complete method for the quantitative determination of special-linked beta-glucans in yeast and fungi. All glucans are split into their glucose monomers and are measured photometrically. Standard errors of approximately <5% are achieved routinely (Megazyme© International Ireland Ltd, 2013).

Because there is currently no standard method for quantitatively determining the beta-glucan contents in mushroom extracts, the phenol-sulphuric-acid method is commonly used for polysaccharide determination (Masuko et al., 2005). However, this method does not specifically measure either beta-glucan or alpha-glucan content. The current method specifically measures alpha-glucan and beta-glucan (by difference). Further information on size and shape of the beta-glucans can be obtained, but are not the topic of this particular study.

In our experiment, we used an enzyme-based test kit by Megazyme© Ltd. (Bray, Wicklow County, Ireland). The previously described method is expected to deliver reliable results for beta-glucan content determination (Bak, Park, Park, & Ka, 2014; Chatterjee et al., 2013).

Very recently, McCleary and Draga (2016) published very valuable data about beta-glucans in different mushroom species. Different alternative methods (e.g. GEM assay) are compared to the Megazyme© assay kit with different optimization steps. The study