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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

shows that the Megazyme© method seems to be the most reliable method due to a hydrolysis step (with HCl or H_2SO_4) combined with enzymatic incubation (McCleary & Draga, 2016). This work also showed that sulfuric acid instead of hydrochloric acid can be used for dissolving mushroom probes for optimal measurement of total glucan content. HCl and H_2SO_4 behave similarly, but for some species (*Gandoderma lucidum* and *Poria cocos* – not investigated in this study) H_2SO_4 seems to result in higher beta-glucan values due to a better dissolving of the mushroom samples.

3. Results and discussion

The screening of 39 culinary and wild mushrooms showed remarkable results, especially the variability of the beta-glucan contents in different species and the high levels of beta-glucans in species such as *Boletus edulis* (Bull. ex Fr., stipe part) or *Piptoporus betulinus* (Bull. ex Fr.) Karst. with more than 50 g/100 g dm. These result are in good accordance and support the work of McCleary and Draga (2016), who screened several mushroom species with the Megazyme© assay.

Earlier studies about the total carbohydrate content of *Boletus* spp. showed very high contents as well (Ouzouni & Riganakos, 2007; Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004, modified by Kalac, 2009). Complete carbohydrate values should be considered to include chitin or sugars beside beta-glucans, but values between 61.7% (Manzi et al., 2004) and 65.4% (Ouzouni & Riganakos, 2007) also indicate possible high beta-glucan contents.

The beta-glucan contents of nine culinary mushrooms, including *Lentinula edodes* (*Berk.*) (Shiitake) and five different *Pleurotus* species, show beta-glucan contents of 15–22 g/100 g dm (dry mass) (Table 1). In particular, *Lentinula edodes* and *Pleurotus ostreatus* and *Pleurotus eryngii* are well known for their medicinal potential, and many studies could show the positive effects on the immune system due to their beta-glucans Lentinan (*Lentinula edodes*) and Pleuran (*Pleurotus* spp.) (Chang & Buswell, 2003; Mizono, Minato, & Tsuchida, 1996; Synytsya, Mickova, Jablonsky, Slukova, & Copikova, 2008). *Pleurotus citrinopileatus, Pleurotus Pulmonarius* and *Pleurotus djamor* show quite similar contents with 15.32–21.70 g/100 g dm. Other species, such as *Agaricus bisporus* (white and brown varieties), show low contents (8.6–12.30 g/100 g dm) with more in the stipe than the cap. The popular culinary mushroom *Cantharellus cibarius* (Chanterelle) contains higher values of beta-glucans (23.59 g/100 g dm in cap, 26.93 g/100 g dm in the stipe). These values can be compared to a study from Barros and co-workers (Barros, Venturini, Baptista, Estevinho, & Ferreira, 2008; modified by Kalac, 2009): all carbohydrates were 31.9% (with consideration of other carbohydrates such as chitin or sugars and oligosaccharides).

Tables 2 and 3 show the results for the beta-glucan contents of thirty wild growing mushrooms from the Niederrhein area in North-Rhine Westphalia, Germany. The results vary widely.

Some species such as *Cortinarius violaceus* (L. ex Fr.) Gray, *Leucocybe connata* (Schumach.) Vizzini or *Laccaria amethystina* (Cooke) show very similar or even higher beta-glucan values than well-known species with bioactive properties (*Lentinula edodes*, *Pleurotus* spp.) (Table 1). Other (edible) species such as *Macrolepiota fuliginosa* (Barla) Bon. (13.95 g/100 g dm in cap and 13.12 g/100 g dm in stipe) or *Helvella crispa* (Bull.) (13.94 g/100 dm in cap, 17.26 g/100 g dm in stipe) present much lower contents (approximately 13–17 g/100 g dm) but still have more beta-glucans than *Agaricus bisporus* which is the most famous edible mushroom but does not present a very high beta-glucan content (8–12 g/100 g dm). This low beta-glucan content in *Agaricus bisporus* is also comparable to the result of McCleary and Draga (2016).

Some mushrooms seem to be composed of unusually high amounts of alpha-glucans; in particular, the cap probes seem to be formed of polysaccharides with alpha-bindings: The species *Boletus erythropus* Pers. showed unusually high amounts of alpha-glucans: 4.25 g/100 g dm in cap and 12.16 g/100 g dm in stipe. Thus, alpha-glucan accounts for approximately a quarter of all glucan in cap material and they account for almost 50% of the stipe. In addition, the cap probe of *Phallus impudicus* (L. ex Fr.) showed a very high alpha-glucan content in its cap, which equals up to about half of all glucans (30.84 g/100 g dm). In addition, the species *Coprinus comatus* (O.F. Müll.) Pers. shows a high alpha-glucan content in its cap of 4.52 g/100 g, which equals approximately 30% of the total glucan content. *Kuehneromyces*

Table 1
Commercially cultivated mushrooms, their dry matter and glucan content (all glucans: alpha-glucans and beta-glucans).

Mushroom	Dry matter in %	All glucans in g/100g dm	±sd	α-Glucans in g/100g dm	±sd	β-Glucans in g/100g dm	±sd	% α-Glucans/ all glucans	% β-Glucans/ all glucans
Agaricus bisporus (J. E. Lange) Imbach (white mushroom) cap	92.169	10.051	2.228	1.547	0.378	8.608	2.373	15.392	85.643
Agaricus bisporus (J. E. Lange) Imbach (white mushroom) stalk	92.203	14.963	4.979	2.667	1.224	12.296	4.077	17.824	82.176
Agaricus bisporus (J. E. Lange) Imbach (brown button mushroom) cap	90.833	12.348	4.514	3.511	2.383	8.837	3.046	28,434	71.566
Agaricus bisporus (J. E. Lange) Imbach (brown button mushroom) stalk	92.278	14.647	4.874	4.568	2.845	10.079	2.230	31,187	68.813
Lentinula edodes (Berk.) Pegl.(shiitake) cap	93.457	20.539	5.953	0.760	0.403	19.779	6.237	3.700	96.300
Lentinula edodes (Berk.) Pegl. (shiitake) stalk	93.330	26.749	3.953	1.440	0.855	25.309	4.384	5.383	94.617
Cantharellus cibarius (Fr.) (chanterelle) cap	91.491	25.307	1.667	1.721	1.004	23.586	1.662	6.800	93.200
Cantharellus cibarius (Fr.) (chanterelle) stalk	91.224	28.468	2.255	1.538	0.579	26.930	2.456	5.613	94.536
Pleurotus ostreatus (Jaqu. ex Fr.) P. Kumm. (oyster mushroom)	94.280	25.636	1.576	1.405	0.037	24.231	1.550	5.481	94.519
Pleurotus eryngii (DC ex Fr.) Gill. (king oyster mushroom)	85.420	19.237	1.576	3.916	0.335	15.321	1.741	20.357	79.643
Pleurotus citrinopileatus (Sing.) (golden oyster mushroom)	90.020	18.260	1.472	2.718	0.138	15.542	1.415	14.885	85.115
Pleurotus pulmonarius (Fr. ex Fr.) Quél. (lung oyster mushroom)	87.880	19.382	0.635	1.916	0.064	17.466	0.610	9.885	90.115
Pleurotus djamor (Rumph. ex Fr.) Boedijn (pink oyster mushroom)	90.230	23.579	1.083	1.876	0.208	21.703	0.821	7.956	92.044
Yeast beta-glucan control (Megazyme: 49%)	97.233	49.305	1.326	1.304	0.389	48.001	0.936	2.644	97.356
Internal mushroom powder control (23%)	94.056	26.946	0.251	3.130	0.065	23.816	0186	11.616	88.384

Table 2
Collected wild mushrooms (dividable in cap and stipe parts), their dry matter and glucan content (all glucans, alpha-glucans and beta-glucans).

Mushroom *	Dry matter in %	All glucans in g/100g dm	±sd	α-Glucans in g/100g dm	±sd	β-Glucans in g/100g dm	±sd	% α-Glucans/ all glucans	% β-Glucans/ all glucans
Macrolepiota fuliginosa (Barla) Bon (parasol mushroom) cap	91.25	15.826	1.388	1.874	0.316	13.952	1.491	11.841	88.159
Macrolepiota fuliginosa (Barla) Bon (parasol mushroom) stalk	91.966	14.488	2.396	1.371	0.095	13.118	2.304	9.463	90.549
Coprinus comatus (O.F. Müll.) Pers. (shaggy ink cap) cap	93.824	19.493	2.597	4.516	2.058	14.977	0.623	23.167	76.833
Coprinus comatus (O.F. Müll.) Pers. (shaggy ink cap) stalk	89.409	18.991	1.608	1.514	0.196	17.477	1.663	7.972	92.028
Paxillus involutus (Batsch) Fr. (roll-rim) cap	89.17	16.420	1.612	3.103	0.267	13.317	1.876	18.899	81.101
Paxillus involutus (Batsch) Fr. (roll-rim) stalk	91.932	20.722	1.215	3.63	0.257	17.092	0.973	17.518	82.482
Hypholoma fasciculare (Huds. ex Fr.) Kummer (sulphur tuft) cap	92.505	23.28	0.432	1.961	0.102	21.319	0.49	8.426	91.576
Hypholoma fasciculare (Huds. ex Fr.) Kummer (sulphur tuft) stalk	91.388	24.931	3.328	1.755	0.051	23.176	3.301	7.039	92.961
Xerocomellus chrysenteron (Bull.) Sutara (redcracking bolete) cap	91.559	9.373	0.217	1.57	0.563	7.803	0.623	16.750	83.250
Xerocomellus chrysenteron (Bull.) Sutara (redcracking bolete) stalk	88.252	14.33	1.093	1.231	0.212	13.099	0.945	8.590	91.410
Xerocomus badius (Fr.) E.-J. Gilbert (*Imleria badia*. bay bolete) cap	91.235	23.227	1.236	3.025	0.381	20.202	1.356	13.024	86.976
Xerocomus badius (Fr.) E.-J. Gilbert (*Imleria badia*. bay bolete) stalk	91.849	32.058	2.352	1.017	0.291	31.041	2.477	3.172	96.828
Armillaria mellea (Vahl ex Fr.) P. Kummer (honey fungus) cap	94.193	34.177	1.692	0.652	0.031	33.526	1.679	1.908	98.095
Armillaria mellea (Vahl ex Fr.) P. Kummer (honey fungus) stalk	92.434	39.307	5.136	0.538	0.08	38.769	5.099	1.369	98.631
Boletus erythropus Pers. (penny bun) cap	87.442	20.785	1.397	4.251	1.169	16.534	1.67	20.452	79.548
Boletus erythropus Pers. (penny bun) stalk	89.989	25.971	5.81	12.168	2.644	13.803	4.293	46.852	53.148
Cortinarius violaceus (L. ex Fr.) Gray (violet webcap) cap	92.835	32.561	2.781	3.819	0.849	28.742	2.982	11.729	88.271
Cortinarius violaceus (L. ex Fr.) Gray (violet webcap) stalk	90.372	40.748	1.929	4.559	0.529	36.189	1.924	11.188	88.812
Kuehneromyces mutabilis (Schaeff. ex Fr.) Sing & Smith (sheated woodtuft) cap	86.794	29.753	2.44	11.056	0.815	18.697	2.362	37.159	62.841
Kuehneromyces mutabilis (Schaeff. ex Fr.) Sing & Smith (sheated woodtuft) stalk	90.382	44.511	3.55	3.915	0.322	40.596	3.71	8.796	91.204
Lycoperdon perlatum (Pers.) (common puffball) cap	93.298	17.924	0.565	2.466	0.15	15.458	0.611	13.758	86.242
Lycoperdon perlatum (Pers.) (common puffball) stalk	91.166	27.032	1.722	7.283	0.971	19.749	2.14	26.942	73.058
Suillus grevillei (Klotzsch) Singer (larch bolete) cap	91.94	17.227	1.68	4.294	0.756	12.933	1.93	24.926	75.074
Suillus grevillei (Klotzsch) Singer (larch bolete) stalk	90.746	28.552	2.319	1.802	0.205	26.75	2.435	8.122	93.688
Helvella crispa (Bull) (common helvel) cap	91.578	16.171	0.737	2.234	0.322	13.937	0.905	13.815	86.185
Helvella crispa (Bull) (common helvel) stalk	89.403	18.703	0.768	1.428	0.25	17.275	0.743	7.635	92.365
Laccaria amethystina (Cooke). (amethyst deceiver) cap	83.513	28.368	3.293	2.122	0.329	26.246	3.47	7.507	92.520
Laccaria amethystina (Cooke) (amethyst deceiver) stalk	84.436	40.202	3.047	2.819	0.491	37.383	2.922	7.012	92.988
Leccinum palustre (Korhonen) cap	94.49	17.422	3.095	2.602	0.326	14.82	3.061	14.935	85.065
Leccinum palustre (*Korhonen*) stalk	94.522	36.185	1.352	4.271	0.278	31.914	1.391	11.803	88.197
Boletus edulis (Bull. ex Fr.) (porcini) cap	81.915	19.993	2.987	3.105	1.04	16.888	3.026	15.530	84.470
Boletus edulis (Bull. ex Fr.) (porcini) stalk	77.992	63.386	9.876	5.484	0.299	57.902	9.909	8.652	91.348
Clitocybe nebularis (Batsch ex Fr.) Kummer (cloud funnel) cap	88.581	23.497	2.18	2.728	0.287	20.769	1.937	11.610	88.390
Clitocybe nebularis (Batsch ex Fr.) Kummer (cloud funnel) stalk	89.121	29.402	2.277	3.872	0.465	25.53	2.325	13.169	86.831
Scleroderma citrinum (Pers.) (common earthball) cap	87.793	33.518	2.917	1.589	0.327	31.929	2.682	4.741	95.259
Scleroderma citrinum (Pers.) (common earthball) stalk	86.143	24.578	2.003	0.568	0.085	24.101	2.009	2.311	98.059
Lepista nuda (Bull ex Fr.) Cooke (wood blewit) cap	93.695	15.761	3.205	1.118	0.147	14.643	3.295	7.093	92.907
Lepista nuda (Bull ex Fr.) Cooke (wood blewit) stalk	68.519	31.882	1.649	4.6	1.048	26.84	0.421	14.428	82.805
Leucocybe connata (Schumach.) Vizzini (white domcap) cap	94.085	21.849	2.836	1.691	0.236	20.158	2.8	7.739	92.261
Leucocybe connate (Schumach.) Vizzini (white domcap) stalk	93.098	43.564	2.697	3.84	0.888	39.724	2.995	8.8146	91.184
Russula ochroleuca (Fr.) (ochre brittlegill) cap	92.999	18.608	0.993	1.173	0.115	17.435	0.911	6.304	93.696
Russula ochroleuca (Fr.) (ochre brittlegill) stalk	85.564	17.987	2.435	0.451	0.041	17.536	2.475	2.507	97.493

Table 2 (*continued*)

Mushroom *	Dry matter in %	All glucans in g/100g dm	±sd	α-Glucans in g/100g dm	±sd	β-Glucans in g/100g dm	±sd	% α-Glucans/ all glucans	% β-Glucans/ all glucans
Russula amara (*Russula caerulea*) (Kucera) (humpback brittlegill) cap	92.893	22.094	2.226	1.351	0.073	20.742	2.294	6.115	93.885
Russula amara (*Russula caerulea*) (Kucera) (humpback brittlegill) stalk	93.084	21.359	2.25	0.841	0.181	20.518	2.131	3.935	96.065
Phallus impudicus (L. ex Fr.) (common stinkhorn) cap	87.797	30.842	0.658	14.926	1.392	15.916	0.735	48.395	51.605
Phallus impudicus (L. ex Fr.) (common stinkhorn) stalk	87.627	20.604	1.394	3.334	0.3	17.27	1.552	16.181	83.819
Yeast beta-glucan control (*Megazyme: 49%*)	97.233	49.305	1.326	1.304	0.389	48.001	0.936	2.644	97.356
Internal mushroom powder control (23%)	94.056	26.946	0.251	3.130	0.065	23.816	0186	11.616	88.384

* Mushrooms were identified morphologically and by sequencing parts of the ITS region.

Table 3
Collected wild mushrooms (NOT dividable in cap and stipe parts) their dry matter and glucan content (all glucans, alpha-glucans and beta-glucans).

Mushroom	Dry matter in %	All glucans in g/100g dm	±sd	α-Glucans in g/100g dm	±sd	β-Glucans in g/100g dm	±sd	% α-Glucans/ all glucans	% β-Glucans/ all glucans
Trametes versicolor (L.) Lloyd. (turkey tail)	87.892	61.194	11.611	0.406	0.232	60.788	11.795	0.663	99.337
Piptoporus betulinus (Bull. ex Fr.) Karst. (birch bracket mushroom)	90.825	54.152	3.856	2.351	0.189	51.801	4.024	4.341	95.659
Laetiporus sulphureus (Bull. ex Fr.) Murr. (chicken of the woods)	89.627	52.612	6.691	5.606	0.587	47.006	6.517	10.655	89.345
Phlebia tremellosa Merulius tremellosus (Schrad) (jelly rot)	96.547	54.331	2.551	0.776	0.202	53.555	2.452	1.428	98.572
Grifola frondosa (Dicks. ex Fr.) Gray (Maitake)	92.783	31.03	4.021	5.039	0.793	25.991	3.643	16.239	83.761
Fomes fomentarius (L. ex Fr.) (hoof fungus)	86.122	24.943	1.832	2.448	0.521	22.495	2.329	9.814	90.186
Auricularia auricula (L.) Underwood (Jew's ear)	91.276	42.136	4.669	0.381	0.054	41.755	4.644	0.904	99.096
Yeast beta-glucan control (Megazyme: 49%)	97.233	49.305	1.326	1.304	0.389	48.001	0.936	2.644	97.356
Internal mushroom powder control (23%)	94.056	26.946	0.251	3.130	0.065	23.816	0186	11.616	88.384

* Mushrooms were identified morphologically and by sequencing parts of the ITS region.

mutabilis (Schaeff. ex Fr.) Sing&Smith also showed a very high alpha-glucan content with 11.06 g/100 g dm (all glucans: 29.75 g/100 g dm.

Alpha-glucans (phytoglycogen) and starch are usually low in. commonly cultivated mushrooms, less than approximately 10% (McCleary & Draga, 2016; Synytsya et al., 2008). The previously discussed mushroom species have clearly higher alpha-glucan contents. Usually, the bioactive beta-glucan components are the polysaccharides with immune-modulating and antitumor effects. While their effects are studied extensively, little is known about the bioactivity, if any, of alpha-glucans in mushroom cell walls (Volman, Mensink, Van Griensven, & Plat, 2010). Most of the mushroom species that we could divide into caps and stipes showed higher beta-glucan contents in their stipes than in their caps (Table 2); only a few species show similar contents in their stipes and caps (*Macrolepiota fuliginosa*, *Russula ochroleuca* (Fr.) and *Russula amara* (Kucera) or higher beta-glucan contents in the cap *(Boletus erythropus* and *Scleroderma citrinum* (Pers.). All other species in our study present higher beta-glucan contents in their stipes. This was also shown in a previous study on *Lentinula edodes* (Bak et al., 2014) and *Pleurotus ostreatus* and *P. eryngii* (Synytsya et al., 2008). For commercially cultivated mushrooms, the stipe parts of cultured mushrooms are cut off after harvest and before sale.

These observations would indicate that there are commercial opportunities for the currently discarded mushroom stipes as a good source of beta-glucan for nutriceutical products.

For example, stipe-cuts and rests from *Lentinula edodes* have high beta-glucan contents (25.31 g/100 g dm). Among the wild grown mushrooms, *Boletus edulis* (which is also known as a common culinary mushroom) shows extreme differences in beta-glucan contents in its stipes (57.9 g/100 g dm) and caps (16.89 g/100 g dm) (Table 2). This species is also well cultivated, and cut-offs from stipe parts could be used as a good source of beta-glucans.

Bracket fungi (and shelf fungi, Table 3) contain very high beta-glucan contents. For morphological reasons, these species were not divided into stipes and caps. This group includes species from different families including *Meruliaceae* (*Phlebia tremellosa* resp. *Merulius tremellosus* (Schrad), *Poriaceae* (*Piptoporus betulinus, Grifola frondosa* (Dicks. ex Fr.) Gray, *Laetiporus sulphureus* (Bull. ex Fr.) Murr., *Trametes versicolor* (L.) Lloyd., *Fomes fomentarius* (L. ex Fr.) and *Auriculariaceae* (*Auricularia auricula* (L.)). These species are generally not cultivated for consumption and exist as saprophytes or parasites in all of Europe and North America (Gerhardt, 2001). *Grifola frondosa* is perhaps the best known species for high beta-glucan content and is well known for being rich in beta-glucans and having pharmacological effectiveness (Inoue, Kodama, & Nanba, 2002; Zhuang & Wasser, 2004). In addition, *Trametes versicolor* (turkey tail) has been recently reported to have high beta-glucan content (Cui & Chisti, 2003; McCleary & Draga, 2016). Interestingly, our results show that other species of Bracket fungi also show very high beta-glucan contents: *Trametes versicolor* shows an extremely high beta-glucan content with 60.79 g/100 g

beta-glucan in its dry mass; *Laetiporus sulphureus* (chicken of the woods, 47.01 g/100 g dm), *Phlebia tremellosa (Merulius tremellosus*, jelly rot, 53.55 g/100 g dm) and *Piptoporus betulinus* (birch Bracket mushroom, 51.80 g/100 g dm) also have high beta-glucan contents. Only a few, isolated studies exist addressing beta-glucans from these fungi, and even less is known about their bioactivities (e.g., Alquini, Carbonero, Rosado, Cosentigo, & Iacomini, 2004; Olennikov, Agafonova, Rokhin, & Brovskii, 2011). This fact could lead to new approaches for biotechnological applications with mycelia cultures to find new and economical advantageous sources for beta-glucans. The submerged cultivation of higher basidiomycetes is a faster and easier way to produce beta-glucans in comparison to the production and extraction process of mushroom fruiting bodies (Komura et al., 2010). The extraction methods and the productions of these kinds of medical products are key features for the development of profitable biotechnological methods to obtain highly effective metabolites (Valverde, Hernándes-Pérez, & Paredes-López, 2015).

Many studies from recent decades prove the bioactive effects of beta-glucans in well-known species such as *Lentinula edodes, Grifola frondosa* or *Pleurotus* spp. The non-edible mushrooms may also have great potential for medical purposes using beta glucan extracts, however, the toxic properties of some of these species needs to be remembered.

In conclusion, this comprehensive study shows an overview of the bioactive beta-glucan contents (in contrast to a huge amount of studies focussing only on the total (!) carbohydrates) and confirms the latest result about beta-glucan research (McCleary & Draga, 2016) for a wide variety of wild and cultivated mushrooms. Our study points out that the Bracket fungi *Trametes versicolor, Piptoporus betulinus* or *Phlebia tremellosa* contain more the 50% beta-glucans by dry mass. In most analysed wild mushrooms, the beta-glucan contents were significantly higher in stipes than in caps.

Conflict of interest

There is no conflict of interest.

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