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Wheat bran biodegradation by edible *Pleurotus* fungi – A sustainable perspective for food and feed

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ABSTRACT

Wheat bran, a side product of the milling industry, can serve as valuable food component, feed ingredient or feedstock for biorefineries. However, all these applications bear drawbacks of sensory, physiological and technological challenges. The present study investigates an alternative utilization strategy of wheat bran as substrate for mushroom production and evaluates residual substrates for further sustainable application possibilities. Substrates containing 250 g/kg, 500 g/kg and 980 g/kg of wheat bran were inoculated with spores of *Pleurotus eryngii* and *Pleurotus ostreatus* followed by solid-state fermentation. Highest biomass yield, protein content and dry matter were obtained on 980 g/kg of bran when inoculated with *Pleurotus eryngii*. Beyond that, fermentation also markedly decreased the phytate content, the viscosity and the neutral detergent fiber level in this substrate. Furthermore, the substrate containing 980 g/kg bran displayed a remarkable decrease in neutral detergent fiber. *Pleurotus* fungi production on wheat bran leads to improved edible mushroom quality compared to commonly used substrates and also offers some innovative application possibilities of the fermented substrate in animal feeding.

1. Introduction

Due to its annual worldwide production volume of up to 150 million tons, wheat bran is an economically important by-product accumulating during the milling process (Prückler et al., 2014; Reisinger et al., 2013). It has already been used as a food component (Aravind, Sissons, Egan, & Fellows, 2012; Prückler et al., 2015; Wójtowicz & Mościcki, 2011), as a feed ingredient (Friedt & McKinnon, 2012; Kraler et al., 2014; Moradi, Zaghari, Shivazad, Osfoori, & Mardi, 2013) and as a raw material for a future biorefinery (Apprich et al., 2014; Reisinger, Tirpanalan, Huber, Kneifel, & Novalin, 2014; Tirpanalan et al., 2015). Despite its nutritional value and potential health benefits, only minor amounts of wheat bran are applied in food (Anil, 2012; Prückler et al., 2014). This limitation is mainly due to negative changes in the physical and sensory properties of the food system upon wheat bran incorporation.

Owing to its pronounced fiber content the incorporation of wheat bran in animal feeds bears several advantages like improved gut health and cost-efficiency (Donkoh & Zanu, 2010; Grizard & Barthomeuf, 1999). For this reason, certain proportions of wheat bran have been routinely used in poultry and pig feeding (Schedle, 2016). However, the relatively low energy level of wheat bran associated with high fiber content necessitates high feed intake values (Bach Knudsen, 2014). An increase in viscosity of the chymus caused by large non-starch polysaccharide (NSP) molecules reduces the rate of gastric emptying and the absorption of nutrients in the small intestine (Bach Knudsen, 2011). Furthermore, wheat bran contains significantly higher levels of phytate compared to whole wheat kernel (Dost & Tokul, 2006; Guo et al., 2015). The phytate complex impairs the digestion of protein and hampers the energy utilization of feedstuff (Collopy & Royse, 2004; Selle & Ravindran, 2007).

Herein, so-called white-rot fungi, a group of basidiomycetes, possess the ability to degrade agro-industrial lignocellulosic by-products by their oxidative ligninolytic systems involving key

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enzymes like laccases (Lac), lignin and manganese peroxidases (MnP) (Castoldi et al., 2014; Elisashvili et al., 2008). Hence, solid-state fermentation (SSF) of wheat bran is of interest, as it has the potential of disintegrating the lignocellulosic fraction via the intrinsic enzyme secretion of the fungi. Beside this biodegradation, SSF using white-rot cultures may also enable the conversion of the substrate to edible mushrooms creating interesting revenue streams towards wheat bran valorization to feed application in parallel. Last but not least, based on some potential phytase activities produced during SSF, a contribution towards improved digestibility of wheat bran could be anticipated (Collopy & Royse, 2004).

Following a cascade-like strategy, the sustainable utilization of side streams along the agri food chain has become a major issue within modern biorefinery concepts. The novelty inherent in the combined approach of this study is to focus not only on using bran as a substrate for mushroom production, but also assessing the perspective of secondary utilisation of side products from mushroom production as an interesting animal feed perspective. Hence, the study carried out under pilot-scale conditions was undertaken to investigate some innovative combination of wheat bran application in the food and feed area: (i) the feasibility of wheat bran for being utilized as a substrate for mushroom cultivation using *Pleurotus eryngii* and *Pleurotus ostreatus*, two edible mushrooms, and (ii) the degree of fungal biodegradation of wheat bran enabling further innovative end-use strategies as an animal feed compound.

2. Materials and methods

2.1. Substrate preparation

Wheat bran samples used in this study were obtained from GoodMills Austria (Schwechat, Austria). Three different substrate mixtures containing 150, 500 and 980 g/kg bran (denoted as 'Substrates A, B and C', respectively) were mixed with beech wood and chalk as given in Table 1 and set to a moisture content of approximately 650 g/kg using distilled water. Portions of about 1.3 kg of substrate were filled in polypropylene bags equipped with micro filters enabling controlled air exchange and autoclaved at 121 °C for 90 min. After cooling to room temperature in a laminar-flow workbench, the substrates were inoculated with 50 g/kg grain spawn of the fresh weight. Two different species of white-rot fungi were used for inoculation: *Pleurotus ostreatus* strain JS2 Aloa and *Pleurotus eryngii* strain Korea. Grain spawns, beech wood chips and sawdust as well as chalk were purchased from Mushroom Production Center (Innsbruck, Austria). All experiments were performed in duplicates.

2.2. Mushroom cultivation procedure

The inoculated polypropylene bags were incubated in a climate room at 23 °C with a relative humidity of 85% for 21 d without exposure to light. After the mycelium formation period, room temperature was dropped to 18 °C and the relative humidity rose to 95%. In order to trigger primordium formation, slashes were cut into polypropylene bags at a distance of around 10 cm and a light exposure interval of 8 h per d was applied. The harvest period for mushroom bodies lasted from d 35 to d 65.

The biological efficiency (BE) was calculated by the formula according to Wang et al. (2013): BE = (weight of fresh mushrooms (g)/weight of substrate (g)) × 100.

Table 1
Proportional composition of wheat bran-based substrates A, B and C [g/kg].

	Wheat bran	Beech wood chips	Beech wood sawdust, large	$CaCO_3$
A[a]	150	280	550	20
B[a]	500	160	320	20
C[a]	980	–	–	20

[a] Letters A, B and C represent different proportions of wheat bran in the substrates (150, 500, 980 g/kg, respectively).

2.3. Sample preparation

Fresh mushrooms were stored at −18 °C until the end of the harvest period. For the analyses each batch was defrosted, mixed and dried to constant weight for 18 h at 60 °C, before grinding to 1 mm sieve size using a rotary mill (Retsch GmbH, Haan, Germany).

2.4. Analyses of substrates and mushrooms

Initial substrates and residual substrates were dried at 60 °C for 18 h and subjected to moisture, ash, protein, fat, neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses according to the standard methods of VDLUFA (2012). Mushrooms were subjected to dry matter and protein analysis according to the standard method of VDLUFA (2012). Protein levels were calculated based on total nitrogen content using a conversion factor of 5.26 and of 4.38 for wheat bran and mushrooms, respectively (Rodrigues et al., 2015; Tkachuk, 1969). Starch contents were determined using a commercial test kit from Megazyme (Total Starch, K-TSTA). Unless stated otherwise, all analyses were carried out in duplicates.

2.5. Phytic acid content

Among the substrates examined only 'substrate C' was of feed-grade. For this reason, phytic acid levels were only analyzed in these test series using the method of Makkar, Siddhuraju, and Becker (2007). Previously dried and ground samples were extracted with 3.5 g/100 mL (w/v) HCl for one h. Solids were separated by centrifugation at 14.196×*g* for 5 min, and 1 mL of the supernatant was diluted to 25 mL using distilled water. Phytic acid was separated through an AG1 X8 chloride anion exchange (200–400 mesh) column. Elution of inorganic phosphorus and other interfering compounds was carried out with 0.1 mol/L NaCl, while the phytic acid was eluted with 0.7 mol/L NaCl. Samples were measured spectrophotometrically at an absorbance of 500 nm.

2.6. Viscosity

The viscosity of 'substrate C' was determined in order to indirectly monitor the enzymatic cleavage of non-starch polysaccharides (NSP) as an indication for the degradation of hemicelluloses. For that purpose a rotation viscometer RVA 4500 (Perten Instruments, Hamburg, Germany) equipped with a beaker (diameter: 3.5 cm; height: 7.0 cm) and a stirrer (diameter: 3.3 cm; height: 1.2 cm) was employed. Dried samples were diluted with distilled water to a solid to liquid ratio of 1:6.25. The viscometer was programmed according to the protocol of Kraler et al. (2014) with minor modifications. The starting temperature was set to 37 °C and the rotation frequency of the stirrer to 1.060 rpm. After 10 s, the frequency was decreased to 160 rpm, and after 5 min the temperature was raised to 95 °C for subsequent 3 min followed by cooling to the initial temperature for further 5 min. The program was stopped after 12 min. All measurements were performed in triplicates.

2.7. Determination of C/N ratio

The C/N ratio was determined by means of an element analyzer