

Nitrogen Supplementation on the Productivity and the Chemical Composition of Oyster Mushroom

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Abstract

Mushrooms have been used for food and medicinal purposes since ancient period. The β -glucans found in mushrooms are currently considered to be responsible for some of the medicinal properties of mushrooms, thereby motivating studies on characterization of these compounds. In our study, we tested the effect of nitrogen supplementation on the productivity and nutritional composition of *Pleurotus ostreatus* mushrooms. The fungi were grown in various substrates supplemented with urea or rice bran, and the biological efficiency, mineral composition, protein and β -glucan content were evaluated. The growth of *P. ostreatus* in substrates with nitrogen supplementation increases the mushroom's productivity and nutritional value. Our results also indicate that nitrogen supplementation, a simple technique, efficiently increases the β -glucan content of mushrooms.

Keywords: Rice bran, Urea, Biological Efficiency, Bioactive polysaccharides

1. Introduction

Mushrooms are becoming increasingly important and common in human diets, due to their nutritional (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008; Bernaś, Jaworska & Lisiewska, 2006) and medicinal characteristics (Jedinak, Dudhgaonkar, Jiang, Sandusky & Sliva, 2010). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluhan & Ranogajec, 2011). As dietary food, mushrooms are comparable to vegetables. Also, mushrooms are a good source of protein, containing all of the essential amino acids, which allows mushrooms to serve as a meat substitute. Moreover, mushrooms have high vitamin B content and a low lipid content, which renders them nutritionally ideal for people who have heart problems (Ghorai, Banik, Verma, Chowdhury, Mukherjee & Khowala, 2009).

In the last decades, an increasing number of studies on mushrooms have been conducted, which have been demonstrated the powerful properties of compounds extracted from mushrooms. Among these compounds, β -glucan have received considerable attention because of their medicinal properties, such as their ability to enhance macrophage functions, activate nonspecific immune responses, reduce cancer occurrences and developments (Jedinak et al., 2010; Wasser, 2011).

To improve the productivity and nutritional value of mushrooms several techniques, substrates, cultivation conditions and strains have been tested. Some studies show that supplementation with nitrogen source increase the biomass and mushroom's productivity (Curvetto, Figlas, Devalis & Delmastro, 2002; Buswell, Cai & Chang, 1995; Shashirekha, Rajarathnam & Bano, 2005). However, in the literature no studies related to increase β -glucans content in mushrooms was found. In this study we compare the addition of organic and inorganic nitrogen source on the productivity, β -glucan content, protein concentration and mineral composition of *P. ostreatus* mushrooms.

2. Material and Method

2.1 Microorganisms and inoculum production (spawn)

Pleurotus ostreatus strains (PLO 6 and PLO 2) were obtained from the Federal University of Viçosa and were maintained in Petri dishes containing potato dextrose agar (PDA) medium at 22 °C. Wheat grains were used as a substrate for the spawn. The grains were cooked for 30 min in water at a 1:3 ratio of wheat grains:water (w/w).

After cooking, the grains were drained and supplemented with 0.35% CaCO₃ and 0.01% CaSO₄. These grains (70 g) were packed into small glass jars and were sterilized in an autoclave at 121 °C for 90 min. After cooling, each jar was inoculated with 4 agar discs, 5 mm diameter, containing mycelium, and the jars were incubated in the dark, at room temperature, for 15 d.

2.2 Substrates and environmental conditions for mushroom production

The following substrates were obtained from local farms and were used for this study: eucalypt sawdust, corncobs, eucalypt bark, coffee husks and sugarcane bagasse (Table 1). Except for the control substrate, which lacked supplementation, all of the substrates were supplemented with 20% rice bran (w/w) or 0.5% urea (w/w). All of the substrates except the coffee husks were crushed and passed through a 0.5-mm sieve. The coffee husks were boiled for 2 h and were centrifuged, 1800 rpm x 5 min (Silva et al., 2012). The substrates were humidified until a moisture content of 70% was reached. The substrates were packed into polyethylene bags and were sterilized twice in an autoclave at 121 °C for 90 min. After sterilization, each bag was inoculated using 70 g of spawn and was incubated in the dark at room temperature for 20 d.

2.3 Mushroom harvesting

After incubation period, the bags were transferred to a cultivation chamber at 25 ± 2 °C and 90% relative air humidity in the presence of light throughout the entire harvesting period. The mushrooms were harvested at maximum development but with the hat closed. The mushrooms were weighed to determine their biological efficiency (BE), which was calculated with the following equation: BE = (fresh mass of mushroom / dry mass of substrate) x 100.

2.4 Nutritional composition

For mineral content determination, the dried mushrooms were triturated and submitted to nitroperchloric digestion (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss; 1995). Phosphorus content was determined by a colorimetric method (Murphy & Riley, 1962). The mushrooms' calcium and magnesium contents were determined by atomic absorption spectrometry, and potassium content was measured by flame spectrometry (Thiers & Hviid, 1962). Total protein content was determined by the Kjeldahl method using a conversion factor of 4.38 (Guo, Lin & Lin, 2007). Soluble protein content was determined with a colorimetric method (Bradford, 1976) using bovine serum albumin as a standard. All analyses were performed in duplicate.

β-glucan content was performed in triplicate according to the methodology employed by Park, Ikegakim, Alencar, & Aguiar (2003). The concentration of β-glucan in the mushrooms was calculated with the following equation: β-glucan (g 100 g⁻¹) = glucose (100 g⁻¹) x 0.9. The correction factor of 0.9 takes into account the structural differences between free glucose and β-glucan.

2.5 Statistical analysis

The experiment was designed in completed and randomized blocks with five replicates. The data were subjected to analysis of variance and mean values were compared by Tukey's test (p < 0.05) using Saeg software (version 9.1, Federal University of Viçosa).

3. Results

Regardless of the substrate used for cultivation, supplementation with nitrogen increased the mushrooms' BEs (Figure 1). The highest BE was achieved with rice bran supplementation, especially when the mushrooms' substrates were based on sugarcane bagasse and eucalypt bark. When rice bran and urea supplementation were compared, the best results were yielded by rice bran supplementation, especially when sugarcane bagasse, corncobs and coffee husks were used as substrates (Figure 1).

The influence of nitrogen supplementation on the level of mineral absorption was directly related to the composition of the substrate (Table 2). The supplementation of substrates with rice bran affected positively the level of phosphorus absorption while urea supplementation affected negatively.

For most substrates, nitrogen supplementation did not affect the percentages of protein in the mushrooms (Table 3). However, urea supplementation decreases the protein content in both strains when they were cultivated in eucalypt sawdust. Furthermore, the protein content of PLO 2 strain grown in sugarcane bagasse supplemented with urea increased 33.60% compared to the control, and the protein content of the PLO 6 strain grown in sugarcane bagasse or eucalypt bark supplemented with rice bran decreased 32.77 and 19.05%, respectively, compared to the control (Table 3).

Urea supplementation increased the soluble protein content increased 8.72% compared to the control, and the β-glucan content increased 20.87% with rice bran and 17.65% with urea supplementations (Table 4).

4. Discussion

Nitrogen supplementation enhanced mushroom BE, especially when organic sources were used (Figure 1). The supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize wastewater, soya cake powder and rice, has increased the BEs of various species of basidiomycetes (Loss, Royer, Barreto-Rodrigues, & Barana, 2009; Moonmoon, Shelly, Khan, Uddin, Hossain, Tania, & Ahmed, 2011). Organic sources of nitrogen can be easily used by fungi because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelial growth and mushroom formation. The BE increase can be due to the high availability of water in substrate add with rice bran (Figure 1), since addition of rice bran decreases the granulometry of substrate, which improve the moisture retention (Özçelik & Peksen, 2007). For mushroom formation, the fungus requires a considerable amount of water, due to the high content of water in mushrooms (Tewari, 1986).

The BE values observed in our study (Figure 1) were similar to observed in other basidiomycetes, which ranges from 18.9 to 100% (Jafarpour, Zand, Dehdashtizadeh & Eghbalsaied, 2010; Loss, et al., 2009; Wang, Sakoda & Suzuki, 2001).

In general, supplementation of the substrates with rice bran did not affect the mineral content of the mushrooms (Table 2). However, the uptake of phosphorus increased for most substrates, which may be a result of the elevated levels of phosphorus in the rice bran (Özçelik & Peksen, 2007). Thus, the increase in the mushrooms' productivity did not impair the mineral composition of the mushrooms (Table 2 and Figure 1). Furthermore, addition of urea inhibits the assimilation of phosphorus, potassium and magnesium in some substrates (Table 2). Such downregulation has also been reported for the assimilation of Mg and K in plants (Khan, Watanabe, & Watanabe, 2000). Urea may act as a chelator, decreasing the availability of minerals. Further, nitrogen supplementation did not affect calcium concentrations, which is in agreement with reports that calcium does not bioaccumulated in mushrooms (Kalac, 2009).

The protein content of mushrooms depends on several factors, such as substrate chemical composition, pileus size, cultivation time and strain (Bernaś, et al., 2006). The mushroom protein contents that were found in this study (Table 3) are in agreement with the range of mushroom protein contents reported in the literature (Bernaś, et al., 2006; Papaspyridi, Katapodis, Gonou-Zagou, Kapsanaki-Gotsi & Christakopoulos, 2010; Tshinyangu & Hennebert, 1996) varying between 17 and 42.5%, dependent on the correction factor (4.38, 6.25 or 6.38). These values were influenced by chemical composition of substrate, which reinforces the necessity of selecting suitable substrates and, in some cases, suitable nitrogen supplementation (Table 3).

Similar to the protein content, soluble protein in fungi also depend on the chemical composition of the substrate and fungus strain, varying between 5 and 14 mg g⁻¹ (Membrillo, Sánchez, Meneses, Favela & Loera, 2011; Paul, Singh, Tyagi, Singh & Dubey, 2010). The protein contents found in our study were slightly greater than the previously published values (Table 4). In both strains, urea supplementation increased the soluble protein content. These results were in keeping with theoretical predictions stating that increase in the availability of nitrogen may enable increase protein contents of plants, animals and fungi (Chandel, Banerjee, See, Meena, Sharma & Verulkar, 2010; Ferrise, Triossi, Stratonovitch, Bindi & Martre, 2010; Janicki, Holter & Hayes, 1985; Membrillo, Sánchez, Meneses, Favela & Loera, 2008).

The percentages of β -glucans found in this study were less (Table 3) than reported in the literature (Carbonero, Gracher, Smiderle, Rosado, Sasaki, Gorin, et al., 2006; Manzi & Pizzoferrato, 2000; Papaspyridi, et al., 2010), which range around 38%, 39% and 53% , in *P. ostreatus*, *P. eryngii* and *P. pulmonarius*, respectively. However, values lower than 30% have been found in *P. ostreatus* (Papaspyridi, et al., 2010). The low value observed in our study can be attributed to the physiological characteristic of our isolates.

Functional foods represent one of the most interesting areas of research and innovation in the food industry (Arias-Aranda & Romerosa-Mártinez, 2010). According to these authors, functional foods may help to prevent disease, reduce the risk of developing disease, or enhance health. Mushrooms represent an unlimited source of polysaccharides, mainly β -glucans, with antitumor and immunostimulating properties (Wasser, 2002). Thus, studies about β -glucans content in mushrooms are important, since these compounds are beneficial to health. Our results indicate that nitrogen supplementation, a simple technique, efficiently increases the β -glucan content in the mushrooms. So, *P. ostreatus* mushroom cultivated in substrate supplemented with nitrogen is a good food source containing protein, minerals and bioactive compound, such as β -glucans.

5. Conclusions

This study shows that the cultivation of *P. ostreatus* in substrates supplemented with nitrogen is a simple technique to increase the productivity and β -glucans content in mushrooms. The use of rice bran increases the productivity of mushrooms more than urea supplementation does.

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Table 1. Materials used as substrates for the production of *Pleurotus ostreatus* mushrooms

Agroindustrial residues (substrates)	
Es	Eucalypt sawdust
EsRb	Es + Rice bran (20% w/w)
EsUr	Es + Urea (0.5 % w/w)
Co	Corn cob
CoRb	Co + Rice bran (20% w/w)
CoUr	Co + Urea (0.5 % w/w)
Eb	Eucalypt bark
EbRb	Eb + Rice bran (20% w/w)
EbUr	Eb + Urea (0.5 % w/w)
Ch	Coffee husk
ChRb	Ch + Rice bran (20% w/w)
ChUr	Ch + Urea (0.5% w/w)
Sb	Sugarcane bagasse
SbRb	Sb + Rice bran (20% w/w)
SbUr	Sb + Urea (0.5% w/w)

Table 2. Mineral composition (as a percentage of dry mass) of *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Substrates*	Percentage of minerals in mushrooms								
	Magnesium**			Potassium					
	PLO 2 and PLO 6			PLO 2			PLO 6		
	Control	Rice bran	Urea	Control	Rice bran	Urea	Control	Rice bran	Urea
Es	0.17 ^A	0.16 ^A	0.14 ^B	2.63 ^A	2.23 ^A	1.86 ^B	2.42 ^A	2.41 ^A	1.39 ^B
Co	0.13 ^A	0.13 ^A	0.13 ^A	2.10 ^A	1.92 ^B	1.95 ^A	2.02 ^A	1.82 ^A	1.81 ^A
Eb	0.16 ^A	0.14 ^B	0.13 ^B	2.13 ^A	2.48 ^A	1.63 ^B	2.00 _{AB}	2.07 ^A	1.75 ^B
Ch	0.13 ^B	0.15 ^A	0.11 ^B	2.27 _{AB}	2.34 ^A	2.14 ^B	2.23 ^A	2.42 ^A	2.15 ^A
Sb	0.17 ^A	0.16 ^A	0.16 ^A	2.48 ^A	2.24 ^A	2.43 ^A	2.70 ^A	2.14 ^B	2.36 ^B
	Phosphorus**			Calcium***					
Es	1.38 ^A	1.14 ^B	0.91 ^C	0.0159					
Co	0.82 ^B	1.50 ^A	0.58 ^C	0.0043					
Eb	1.00 ^B	1.25 ^A	0.65 ^C	0.0131					
Ch	0.64 ^B	1.50 ^A	0.57 ^B	0.0056					
Sb	1.64 ^A	1.61 ^A	1.32 ^B	0.0054					

Means followed by different letters within the same line differ at Tukey's test (P<0.05).

* See table 1, ** Not significantly different between the strains (P< 0.05), *** Not significantly different between the strains and nitrogen supplementation (P< 0.05). Control = without nitrogen supplementation.

Table 3. Total protein (as a percentage of dry weight) of *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Substrates*	Total protein**					
	PLO2			PLO6		
	Control	Rice bran	Urea	Control	Rice bran	Urea
Es	28.60 ^A	22.86 ^B	22.56 ^B	27.38 ^A	27.68 ^A	18.75 ^B
Co	20.28 ^A	20.45 ^A	17.48 ^A	20.02 ^A	21.81 ^A	20.63 ^A
Eb	19.40 ^A	20.98 ^A	19.80 ^A	21.16 ^A	17.13 ^B	21.64 ^A
Ch	23.35 ^A	21.90 ^A	25.32 ^A	23.83 ^A	21.81 ^A	25.40 ^A
Sb	22.29 ^B	20.15 ^B	29.78 ^A	27.16 ^A	18.26 ^B	26.50 ^A

Means followed by different letters within the same line differ at Tukey's test ($P < 0.05$).

Control = without nitrogen supplementation. * See table 1, ** Nitrogen correction factor used = 4.38.

Table 4. Soluble protein (mg g^{-1} dry weight) and β -glucan (percentage of dry weight) concentrations in *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Nitrogen supplementation	Soluble protein*	β -glucans*
Control	8.558 ^B	22.99 ^B
Rice Bran	8.419 ^B	27.79 ^A
Urea	9.305 ^A	27.05 ^A

Means followed by different letters within the same line differ at Tukey's test ($P < 0.05$).

* Not significantly different between the strains and substrates ($P < 0.05$).

Control= without supplementation.

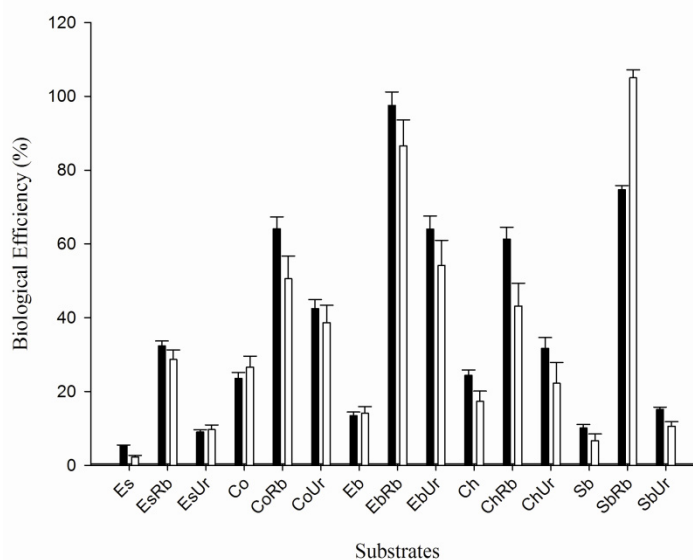


Figure 1. Biological efficiency of *P. ostreatus* (PLO 2 strain in black and PLO 6 strain in white) strains grown in different substrates supplemented with rice bran or urea. The following abbreviations are used: Es, eucalypt sawdust; Co, corncob; Eb, eucalypt bark; Ch, coffee husk; Sb, sugarcane bagasse; Rb, rice bran supplementation; and Ur, urea supplementation. These data represent the means \pm sd ($n = 5$)