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Production parameters and nutritional value of *Pleurotus ostreatus* mushroom cultivated on agricultural wastes

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Abstract The influence of two alternative agro-residues (barley and oat straw-BO and beech wood residues-BW) used as main cultivation substrates in respect to wheat straw-WS was examined on the production and quality characteristics of *Pleurotus ostreatus*, strain AMRL 144. Evaluation included fungal bio-transformation efficiency with respect to substrate colonization and carposome production time, biological efficiency-BE%, as well as mushroom number, size, colour and firmness. The effect of these wastes was also evaluated on moisture content, crude protein, total polysaccharides, lipid and phenolic content of whole sporophores. First mushrooms appeared 19-42 days after inoculation, with WS and BOS substrates promoting earlier sporophore initiation than BW did. As for BE%, the highest values were recorded at BOS and BW wastes (75.30% and 64.26% respectively) compared to the conventional WS. Mushroom protein content was significantly increased when BW was used (28.25% versus 11.02 in WS) and so did colour lightness in BOS (L*=78.16). Firmer mushrooms were also produced in BOS and BW substrates (7.25 N) than in WS (6.56 N), while polysaccharides were synthesised in high amount, mostly in mushrooms cultivated in BW (35.47%) and consisted mainly of glucose (in higher than 70.5% w/w). Phenolic synthesis was promoted in BO and BW (640 mg/l GAE) versus WS (400 mg/l GAE) substrates. Mushroom lipid content, on the contrary, was not affected by the substrate used.

Keywords: Mushroom cultivation, agro-residues fermentation, protein, carbohydrates, lipids

1. Introduction

The growing number of wastes produced in modem agriculture has a huge impact on the environment. The burning and improper disposal of this vast number of wastes produced annually from agricultural-based industries pose a potential harm to the health of humans and wildlife and have created major-global environmental problems (Anwar et al., 2014). Mushroom cultivation is a biotechnological process that converts various agricultural lignocellulosic organic residues into high value-added products (carposomes, polysaccharides, enzymes etc), aiming also at "zero economy". The use of substrates containg mixtures of supplements is more beneficial than the use of homogeneous substrates, as due to the enriched nutritional synthesis it increases the nutritional content, speeds-up growth and increases mushroom yield. The evaluation of wastes, however, requires the determination of parameters such as the mineral content, the amount of cellulose, hemicellulose, lignin and total carbon to total nitrogen ratio, as they affect considerably growth and quality quaracteristics of mushrooms (Philipoussis, 2009; Ritota and Manzi, 2019). For exemple, growth rates of *Pleurotus*, spp., *Agrocybe aegerita* and *Volvariella volvacea* mushrooms as well as crop yield were found to be negatively correlated to lignin content and to a lesser degree to nitrogen content, whereas carbon and nitrogen content of the substrates affected the time of fructification and productivity (Philippoussis et al. 2003; 2007). The fungal strain used also influences considerably the colonization rates and therefore the incubation and crop cycle duration (Philippoussis et al., 2003; Diamantopoulou et al., 2019).

Pleurotus ostreatus mushrooms are fleshy edible fungi that due to their nutritional and medicinal properties are immensely popular in Greece. *Pleurotus* spp. the second most cultivated edible mushroom worldwide after *Agaricus bisporus* not only has medicinal properties but also economic and ecological value, while it requires a shorter growth time in comparison to other edible mushrooms (Sánchez, 2010; (Rathore et al., 2017; Roncero-Ramos and Delgado-Andrade, 2017). They are cultivated and consumed not only for their unique aroma and texture but also for nutritional and medicinal advantages (Kalač, 2013). Nutritionally they are registered as a complete healthy food providing all those nutrients in adequate proportions including all the essential amino acids that cannot be synthesized by human body along with the recuperative compounds (β-glucans, tocopherol, terpenoids and polysaccharides etc.). They are also known for their anti-tumour and anti-cancer activities, while they contain low number of calories, fat and, fact that constitutes them a great food option for people with cardiovascular problems sodium (Rathore et al., 2017; Roncero-Ramos and Delgado-Andrade, 2017). However, the type and chemical composition of substrates used affect the nutritional characteristics (proximate composition) of mushrooms produced even if species of

the same genus are cultivated, usually the protein and carbohydrate content (Zhang and Fadel, 2002; Ritota and Manzi, 2019). As the choice of the most suitable waste material as growth substrate is very important for maximizing yield and nutritional value of mushrooms, the present study aims at the evaluation of two alternative agro-wastes (barley and oat straw and beech wood residues) for *P. ostreatus* cultivation.

2. Materials and Methods

Edible fungus: *P. ostreatus* AMRL 144 strain maintained on the fungal culture collection of the Laboratory of Edible Fungi (LEF)/ITAP was used in the present study.

The lignocellulosic residues: wheat straw, barley and oat straw and beech wood residues derived from different Greek farms and industries were cut mechanically, weighted and soaked in water for 12h and after 2h drainage for the adjustment of moisture content (65-70%) were supplemented with additives (wheat bran, soybean and calcium carbonate) in various proportions to achieve a final C/N=20-30 and pH=6.4-7.0 (Philippoussis et al., 2001). Five replicates of polypropylene-autoclavable bags per substrate were used. Specifically, bags were filled with 1 kg substrate, autoclaved at 121 ± 1 °C for 2 h (1.1 atm) and inoculated with mushroom grain spawn along the central vertical axis of the bag. Substrate colonization took place in growth chambers at 25±1.0 °C, RH%=85% in the dark. At the end of complete colonization, bags were transferred to the fruiting room for fructification (16±0.5 °C, RH%=90%, 700 lux/12h/day) and productivity evaluation. Mature carposomes were harvested daily, counted and weighed, while pileus diameter and stipes' length were measured. BE% [BE=weight of fresh mushrooms (g)/weight of dry substrate (g) × 100] and earliness, which is the days elapsed between the day of inoculation and the day of the first harvest were also determined. After harvesting, mushrooms were freeze-dried and milled to produce mushroom powder.

Analytical Methods: Total-intracellular polysaccharide (IPS) determination was conducted according to Diamantopoulou et al. (2014) using D-glucose as standard

The total phenolic content (TPC) in the mushroom samples was estimated by using the Folin-Ciocalteau assay measuring the absorbance at 765 nm (Singleton and Rossi, 1965). The total lipid and the crude protein content of dried mushrooms were determined via modified Folch and Bradford assays (Folch et al., 1957; Bradford, 1976 respectively). Color recording [factors L*, whiteness, a* and b* shade-intensity of green (-a*), red (+a*), blue (-b*) and yellow (+b*)] was carried out by a MINOLTA Chromameter (CR-300) and the value ΔE (total color) was calculated by $\Delta E = [(L^* - 97)^2 + (a^* - (-2))^2 + b^2)]^{1/2}$ equation. Firmness was tested using TA.HDplus Texture Analyse, expressed in N.

Data analysis: Variance analysis was performed using the Least Significant Difference (LSD) test at 5 % level of probability to compare mean values of parameters tested.

2. Results – Discussion

WS substrate supported the fastest earliness (19 days) followed by BOS (30 days) and BWS (42 days). However, the highest values of BE% were recorded for BOS and BW; 75.30% and 64.26% respectively. Although *P. ostreatus* gave in BS higher BE values comparing to BW substrate, the number of produced mushrooms in WS proved to be fewer. This is since heavier fruiting bodies were produced in BW residues, while mushrooms with the lowest average weight appeared in BOS **(Table 1)**.

Table 1. Biological efficiency (BE%), earliness, number and average weight (g) of mushrooms using WS, BOS and BW as substrates for *P. ostreatus* cultivation

Substrates	BE%	Number	Average weight (g)	Earliness (days)
WS*	55.14 a**	66a	10.72b	19a
BOS	75.30 b	108b	8.60a	30b
BW	64.26a,b	60a	12.65c	42c

*WS wheat straw, BOS barley and oats straw, BW beech wood
**Values (means of five replicates) in the same strain not sharing common letters are significantly different at P=0.05

Various substrates appeared to affect the carposomes' nutritional composition, as *P. ostreatus* strain yielded IPS between 30.46 to 42.45 g/100g dw, with WS and BW substrates showing higher values (42.45 and 35.47 g/100g dw respectively) than the ones of mushrooms produced on BOS substrate (30.46 g/100g of dry biomass) **(Figure 1)**. Afterwards, the results from carbohydrate composition of total IPS produced by *P. ostreatus* showed that glucose was the main constituent presented in all examined carposomes (higher than 70.5% w/w), followed by fructose and mannitol (17.4% and 10.50% w/w, respectively in BOS substrate). Crude protein content seemed to be enhanced on mushrooms deriving from BW substrate (28.25 g/100 g dw) compared to WS and BOS (11.02 and 13.82 g/100g of dry biomass respectively), while lipid content, on the contrary, was not affected by the substrate used with values ranging from 2.17% in BWS and 2.39% w/w in WS and BOS. The mushroom moister content was similar too (88-90%). Extracts derived from mushrooms cultivated on BOS and BW substrates revealed high values of TPC content (640 mg/l GAE), while phenolic synthesis was less in carposomes of WS. Finally, concerning the mechanical behavior, firmer mushrooms were produced in BOS and BW substrates (7.0-7.25N) comparing to WS (6.56 N), while colour lightness was significantly increased in pilei of mushrooms produced at BOS residues (L*=78.16), followed by WS and BW as well as total color (ΔE=14.36) **(Table 2)**.

In view of the conclusion, this study supports the potential utilization of BOS (barley and oats straw) and BWS (beech wood residues) for mushroom cultivation. Not only they appeared to be adequately nutritive substrates for substitution of wheat straw but also, they enhanced nutritional and antioxidant characteristics of the produced mushrooms, affecting in this way their quality Many other

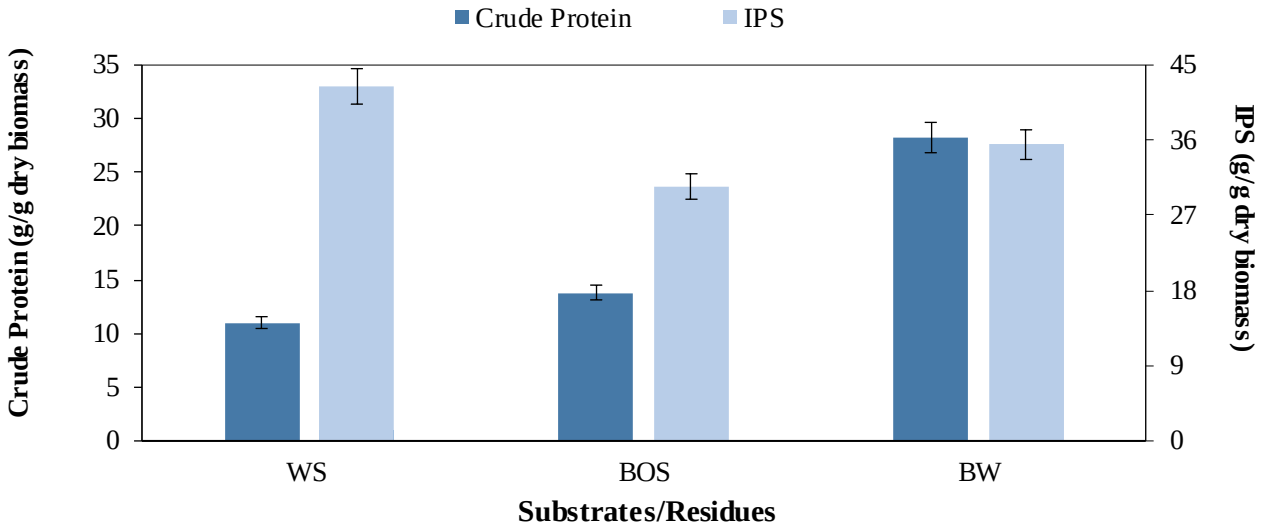

Figure 1. Total intracellular polysaccharide (IPS) content and total protein concentration in dry *Pleurotus ostreatus* mushroom AMRL 144 cultivated on three different substates (wheat straw-WS, barley and oat straw-BOS,beech wood-BW). Each point is the mean value of at least three independent measurements, SD < 5%.

Table 2. Firmness (N), colour and brightness analysis of *P. ostreatus* AMRL 144 mushrooms cultivated in WS, BOS and BW as substrates

	P. ostreatus AMRL 144		
Parameters/ Substrates	**Lightness (L*)**	**Colour (ΔE)**	**Firmness (N)**
WS*	72.30a**	12.35a	6.56a
BOS	78.16b	14.36b	7.00b
BW	70.29a	12.69a	7.25b,c

*WS wheat straw, BOS barley and oats straw, BW beech wood
**Values (means of five replicates) in the same strain not sharing common letters are significantly different at P=0.05

lignocellulosic residues have been also studied before; cotton waste promoted earliness in fructification and high sporophore yields of *P. ostreatus* and *P. pulmonarius* strains when ten selected wild and commercial strains of *P. ostreatus*, *P. eryngii*, *P. pulmonarius*, *Agrocybe aegerita* and *Volvariella volvacea* were cultivated on wheat straw, cotton waste and peanut shells (Philippoussis et al., 2001). Similarly, high sporophore yields were obtained by *P. ostreatus* and *P. pulmonarius* on both cotton waste and peanut shells. Additionally, mixtures of wheat straw, corn-cobs and oak-wood sawdust were also used as alternative substrates to the traditional cultivation of *Lentinula edodes* on hardwood, achieving high BE values, while promoted early fructification and mushroom quality (Philippoussis et al., 2003; Philippoussis et al., 2007). Yildiz et al. (2002) have also reported that the chemical composition of the substrates used significantly affected fructification, mushroom number and weight, nutritional and bioactive compounds' production of *P. ostreatus*. This study is further supported by the findings of Strapáč et al. (2017), which reported enhanced total phenolic content of *P. ostreatus* and *P. pulmonarius* carposomes produced in beech and linden substrates. Hence, the findings of the present study suggest that the use of lignocellulosic residues in mushroom cultivation could be a successful way of recycling agricultural wastes with positive impact to both economy and environment.

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