



BIOCONVERSION OF AGRICULTURAL WASTES USING PARENTAL, HYBRID AND RECONSTITUTED STRAINS OF *Pleurotus* AND *Lentinula*
BIOCONVERSIÓN DE RESIDUOS AGRÍCOLAS USANDO CEPAS PARENTALES, HÍBRIDAS Y RECONSTITUIDAS DE *Pleurotus* Y *Lentinula*

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Abstract

The production of parental, hybrid and reconstituted strains of *Pleurotus* and *Lentinula* was evaluated using two agricultural wastes such as wheat straw (WS) and a mixture of oak sawdust, wheat straw, millet seed, cotton seed hull and CaCO₃ (AP). The productivity, morphology parameters and chemical composition of the mushrooms, and also the chemical composition of the substrates before and after harvest were determined. The parental strain *Pleurotus djamor* (PD) produced on WS showed the highest productivity: biological efficiency (141.86%), production rate (2.27%) and yield (47.88%). The hybrid strain *Pleurotus djamor*x*Lentinula edodes* (PD₄xLC₃) cultivated on AP presented the highest productivity parameters: biological efficiency (120.01%), productivity rate (2.70%) and yield (41.10%). All the strains presented fruit bodies with pileus diameter corresponding to 5 - 9.9cm and length of stipe < 5 cm. The parental strain of *Pleurotus ostreatus* PO and the hybrids PO₅xLC₂ and PD₄xLC₃ cultivated on WS presented fruit bodies with highest protein content between 27.16 to 27.76% respectively, and the reconstituted strain of *Pleurotus djamor* PD₁xPD₄ produced on AP presented a protein content of 33.90%. The substrate WS used in the fructification of the parental strain of *Pleurotus djamor* (PD) exhibited the highest biodegradation value of lignin (28.50%), while the substrate AP used in the production of the reconstituted strain PO₁xPO₂ presented the highest biodegradation value of lignin (39.50%).

Keywords: Agricultural wastes, chemical composition, morphology, productivity.

Resumen

Se evaluó la producción de cepas parentales, híbridas y reconstituidas de *Pleurotus* y *Lentinula* utilizando dos residuos agrícolas, paja de trigo (WS) y mezcla de: aserrín de encino, paja de trigo, semilla de mijo, cáscara de semilla de algodón y CaCO₃ (AP). Se determinaron las productividades, morfología y la composición química de las cepas, así como de los sustratos antes y después de la cosecha. La cepa parental *Pleurotus djamor* (PD) fructificada en WS mostró la más alta productividad: eficiencia biológica (141.86%), tasa de producción (2.27%) y rendimiento (47.88%). La cepa híbrida *Pleurotus djamor*x*Lentinula edodes* (PD₄xLC₃) cultivada en AP presentó también alta productividad: eficiencia biológica (120.01%), tasa de productividad (2.70%) y rendimiento (41.10%). Todas las cepas presentaron carpóforos con diámetro de píleo entre 5 - 9.9 cm y tamaño del estípite < 5 cm. La cepa parental de *Pleurotus ostreatus* PO y los híbridos PO₅xLC₂ y PD₄xLC₃ cultivados en WS presentaron carpóforos con mayor contenido proteico entre 27.16 y 27.76% respectivamente, y la cepa reconstituida de *Pleurotus djamor* PD₁xPD₄ cultivada en AP presentó un contenido de proteína de 33.90%. El sustrato WS usado en la fructificación de la cepa parental de *Pleurotus djamor* (PD) exhibió mayor biodegradación de lignina (28.50%), mientras la cepa reconstituida PO₁xPO₂ presentó la mayor biodegradación de lignina (39.50%) en el sustrato AP.

Palabras clave: Residuos agroindustriales, composición química, morfología, productividad.

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1 Introduction

Fungi possess two types of extracellular enzymatic systems to degrade polysaccharides and lignocellulosic materials. Mushroom with the ability to degrade the lignin are known white-rot fungi, while the brown-rot fungi groups are able to attack cellulose and only modify lignin (Leonowicz *et al.*, 1999; Sánchez, 2009). Mushroom industry uses wastes from agro food industry decreasing the impact in the environment; these are usually rich in lignocellulosic compounds and are the result after processing of different kind of plants (Levanon *et al.*, 1993; Philippoussis *et al.*, 2001; Philippoussis *et al.*, 2007).

Chemical dedikaritozation is one of the successful method to produce new strains (Valenzuela-Cobos *et al.*, 2017), which consists in the recovery of the two monokaryotic components from a dikaryon using toxic substances such as sodium taurocholate, colic acid, peptone or glucose (Miles and Raper, 1956). The new fungi strains are produced pairing compatible monokaryotic components breaking the incompatibility barrier (Leal-Lara and Eger-Hummel, 1982). Worldwide the edible fungi with highest production in the world are: *Lentinula edodes* and *Pleurotus* spp. (Sugimoto *et al.*, 2001). These species need different conditions to grow and produce fruit body; *Pleurotus* spp. requires tropical climates (Fultz, 1988; Kashangura *et al.*, 2006), while *Lentinula edodes* needs long incubation times, specific substrates (Gaitán-Hernández *et al.*, 2006; Sharma *et al.*, 2015).

The advantages of the development of a hybrid and reconstituted strain are to improve the commercial attributes, decreasing incubation time and using different agricultural wastes for mushroom cultivation (Eichlerová and Homolka, 1999; Chakraborty and Sikdar, 2008; Guadarrama-Mendoza *et al.*, 2014).

The purpose of this research was to evaluate the productivity, morphology parameters and the chemical composition of the fruit bodies of parental, hybrid and reconstituted strains using two different substrates in the cultivation, and compare the chemical composition of the substrates before and after harvest.

2 Materials and methods

2.1 Biological material

In this experiment was used the following mushroom strains: two parental strains i.e. *Pleurotus ostreatus* (PO) and *Pleurotus djamor* (PD); one parental strain of *Lentinula edodes* (LC); three hybrid strains PO₂xLC₂, PO₅xLC₂, PD₄xLC₃ and three reconstituted strains PO₁xPO₂, PD₁xPD₄, LC₂xLC₂. The hybrid and reconstituted strains were obtained by pairing compatible neohaplonts recovered by chemical dedikaryotization and are maintained on MEA dishes. Stocks of all strains are deposited at the fungal collection of the Cellular Cultures Laboratory of the Unidad Profesional Interdisciplinaria de Biotecnología (UPIBI-IPN).

2.2 Substrates preparation

Mushrooms were cultivated in two different agricultural wastes for the formulation of the substrate: WS (100% wheat straw) and AP (mixture of 44% oak sawdust, 30% wheat straw, 16% millet seed, 5% cotton seed hull and 5% CaCO₃). The substrates were hydrated, reaching 80% and 65% moisture respectively. After that, the substrates were placed (0.5 kg wet weight) in plastic bags and sterilized for 2 h at 15 psi (121 °C). Subsequently, the bags were cooled down and the inoculated with 10% (w/w) of wheat grain and incubated in a dark room at temperature of 28±2 °C.

2.3 Induction to form fruit bodies

As soon as the mycelium of the strains had completely colonized the substrates, the bags with substrate were transferred to the fructification room with favorable conditions: relative humidity was maintained between 85 and 90%, temperature of 18±1 °C, air recirculation and period of illumination of 12 h.

2.4 Productivity parameters of the fruit bodies

The productivity of the fruit bodies was evaluated based on the biological efficiency (BE; fresh weight of harvested mushrooms/substrate dry weight x 100), yield (Y; fresh weight of harvested mushrooms / substrate fresh weight x 100) and production rate, daily average biological efficiency (PR; ratio of

BE/total number of production days starting from inoculation) (Royse, 1989; Salmones *et al.*, 1997).

2.5 Morphology of the fruit bodies

The size of the mushrooms was determined according to pileus diameter: group 1 (G1) < 5 cm, group 2 (G2) 5-9.9 cm and group 3 (G3) 10-14.9 cm (Salmones *et al.*, 1997). At the same time, the length of stipe was classified according to (Cruz, 2009) (G1) < 5 cm and group 2 (G2) 5-9.9 cm.

The color was measured using a Minolta Chroma Meter CR-300 (Minolta Camera Co., Ltd., Osaka, Japan), which determined the chromatic coordinates L^* (luminosity), b^* (yellow-blue component) and a^* (red-green component). The analyses of color were made by triplicate, the first measurement was realized in the centre of the mushroom and the other two between 1 and 2 cm from the first (Roy *et al.*, 1995; Pardo *et al.*, 2004). The whiteness index (WI), yellowness index (YI) and browning index (BI) (which represents the purity of brown colour and is considered an important parameter associated with browning) were calculated, see Eq. (1)-(4), (Rhim *et al.*, 1999; Maskan, 2001; Bozkurt and Bayram, 2006; Valencia del Toro *et al.*, 2018):

$$WI = \left[100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \right]^{1/2} \quad (1)$$

$$YI = \left(\frac{142.86b^*}{L^*} \right) \quad (2)$$

$$BI = 100 \left(\frac{x - 0.31}{0.17} \right) \quad (3)$$

where:

$$x = \frac{a^* + 1.75L^*}{5.645L^* + -3.012b^*} \quad (4)$$

2.6 Chemical composition of the fruit bodies

Fruit bodies were dried at 60 °C for 24 h and then milled to perform proximal analysis using standard methods. Moisture, ash, crude fiber and crude fat were determined according to the Association of Official Analytical Chemists methods (AOAC, 1997). Total nitrogen was evaluated with the micro-kjeldahl method, crude protein was calculated from total nitrogen content by employing the converting factor

4.38, total carbohydrates were calculated by the formula: $100 - (\%moisture + \%protein + \%fat + \%ash\ contents)$, and energy value was estimated according to the equation: $energy = 4 \times (\%protein + \%carbohydrate) + 9 \times (\%fat)$ (Manzi *et al.*, 2004; Valencia del Toro *et al.*, 2018). The energy value of mushrooms must be estimated based on the content of crude protein ($N \times 4.38$), fat and carbohydrate using specific modified factors 3.75, 8.37 and 4.2 kcal g^{-1} of each component, respectively (Lau, 1982).

2.7 Chemical composition of the agricultural wastes

To determine the chemical composition of the substrates a proximal analysis was realized: acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, cellulose, and lignin were determined using the methodology of (Gaitán-Hernández *et al.*, 2006).

2.8 Statistical analysis

In all experiments, a completely randomized design and the results were examined using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p < 0.05$ level, of productivity, morphology parameters and chemical composition of the fruit bodies and the chemical composition of the substrates, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. Additionally was determined the correlation coefficient between the productivity parameters and the chemical composition of the substrates. The analyses were carried out using statistical software (Statgraphic ver. 16).

3 Results and discussion

3.1 Productivity parameters

Table 1 shows the productivity parameters of the parental, hybrid and reconstituted strains were cultivated on two different substrates: wheat straw (WS) and mixture of oak sawdust, wheat straw, millet seed, cotton seed hull and $CaCO_3$ (AP), except for the parental strain of *Lentinula edodes* (LC) and its reconstituted LC₂×LC₂ only were cultivated on AP.

Table 1. Productivity parameters of parental, hybrid and reconstituted strains produced on WS and AP.

Strains	Type of strains	Substrates	Precocity (days)	Total cultivation time (days)	Biological efficiency (%)	Production rate (%)	Yield (%)
PO	Parental	WS	23.40±0.84 ^c	52.00±1.63 ^c	104.23±17.69 ^a	2.00±0.31 ^b	35.18±5.97 ^a
		AP	26.00±0.94 ^D	48.60±0.97 ^D	69.54±10.69 ^B	1.43±0.23 ^B	22.67±4.10 ^B
PD	Parental	WS	22.50±0.71 ^c	62.40±1.50 ^f	141.86±19.12 ^c	2.27±0.31 ^c	47.88±6.45 ^b
		AP	16.00±1.63 ^A	47.10±3.51 ^C	65.43±12.66 ^B	1.39±0.34 ^B	21.33±4.13 ^B
LC	Parental	WS	48.00±5.03 ^E	61.00±3.09 ^E	79.86±29.70 ^C	1.31±0.49 ^B	26.37±4.10 ^B
		AP	27.00±0.82 ^e	54.40±1.42 ^d	91.67±13.65 ^a	1.68±0.24 ^a	30.02±4.47 ^a
PO ₂ xLC ₂	Hybrid	WS	17.40±1.43 ^A	44.00±1.56 ^A	90.82±12.97 ^D	2.06±0.29 ^C	31.10±4.44 ^D
		AP	15.40±1.43 ^a	35.10±1.66 ^a	105.18±23.20 ^a	3.00±0.70 ^c	32.96±7.48 ^a
PO ₅ xLC ₂	Hybrid	WS	20.20±1.31 ^B	42.40±1.96 ^A	85.63±18.03 ^C	2.02±0.48 ^C	29.32±6.17 ^C
		AP	31.60±1.71 ^f	58.50±1.35 ^e	130.16±25.62 ^b	2.22±0.42 ^b	42.64±8.39 ^b
PD ₄ xLC ₃	Hybrid	WS	24.50±2.50 ^C	44.40±2.22 ^A	120.01±22.88 ^E	2.70±0.55 ^D	41.10±7.84 ^E
		AP	25.20±0.79 ^d	49.80±1.32 ^b	98.13±17.22 ^a	1.97±0.32 ^b	30.96±5.43 ^a
PO ₁ xPO ₂	Reconstituted	WS	22.60±2.37 ^C	48.20±2.30 ^D	74.51±9.77 ^C	1.55±0.25 ^B	24.64±3.23 ^B
		AP	19.20±0.92 ^b	50.20±1.87 ^b	106.69±20.68 ^a	2.12±0.42 ^b	33.66±6.52 ^a
PD ₁ xPD ₄	Reconstituted	WS	20.80±2.25 ^B	45.70±1.25 ^B	57.75±10.88 ^B	1.26±0.25 ^B	19.10±3.60 ^A
		AP	51.00±3.65 ^F	65.50±3.92 ^F	47.46±16.38 ^A	0.73±0.27 ^A	15.67±5.41 ^A

*All values are means ± standard deviation of ten replicates. Uppercase letters indicate difference between the productivity parameters of the mushrooms obtained on AP, while lowercase letters indicate difference between the productivity parameters of the mushrooms obtained on WS according to Duncan's test ($p < 0.05$), $n = 10$.

The parental strain of *Pleurotus djamor* (PD) cultivated on WS showed the highest productivity parameters: biological efficiency of 141.86%, productivity rate of 2.27% and yield of 47.88% in relation to the other strains cultivated in this substrate, while the hybrid PD₄xLC₃ cultivated on AP presented the highest productivity: biological efficiency of 120.01%, productivity rate of 2.70% and yield of 41.10% in accordance with the strains cultivated in this substrate. Philippoussis (2009) cultivated one strain of *P. ostreatus* and one strain of *P. pulmonarius* on wheat straw showing biological efficiencies between 85.40 to 90.90%, also produced these strains on softwood residues presenting biological efficiencies since 48.10 to 76.00%.

Parental strains cultivated on WS presented biological efficiencies between 104.23 to 141.86%, production rates ranged from 2.00 to 2.27% and yields since 35.18 to 47.88%, while the parental strains produced on AP showed biological efficiencies between 65.43 to 79.86%, production rates ranged from 1.31 to 1.43% and yields between 21.33 to 26.37%. Mandeel et al. (2005) cultivated one strain of *Pleurotus ostreatus* on four different substrates (paper, cardboard, fiber, sawdust) showed biological efficiencies between 59.6 to 117.5%. Gaitán-Hernández et al. (2006) reported productivity parameters for four strains of *Lentinula edodes* cultivated on three different substrates (vineyard pruning, barley straw and wheat straw): biological efficiencies between 37.02 to 93.25%, production

rates ranged from 0.39 to 1.17% and yields between 10.35 to 23.86%, while Zied et al., 2016 cultivated six strains of *Lentinula edodes* on three different mixtures of eucalyptus sawdust, wheat bran, cotton seed meal, corn flour, rice bran and CaCO₃ under semi-controlled conditions (14 °C at night and 22 °C in the day) showing yields between 19.30 to 22.70%; and under uncontrolled conditions (16 °C) presenting yields ranged from 13.80 to 20.60%. Hybrid strains cultivated on WS presented biological efficiencies between 91.67 to 130.16%, production rates since 1.68 to 3.00% and yields ranged from 30.02 to 42.64%, while the hybrids cultivated on AP present biological efficiencies since 85.63 to 120.01%, production rates between 2.02 to 2.70% and yields ranged from 29.32 to 41.10%. On the other hand, the reconstituted strains cultivated on WS showed biological efficiencies ranged from 98.13 to 106.69%, production rates since 1.97 to 2.12% and yields between 30.96 to 33.66%, whereas the reconstituted strains produced on AP showed biological efficiencies ranged from 47.46 to 74.51%, production rates between 0.73 to 1.55% and yields since 15.67 to 24.64%. Mallick and Sikdar (2014) reported productivity parameters of six hybrids *Pleurotus florida* x *Lentinus edodes* showing precocity between 25 to 34 days, biological efficiencies since 44.22 to 107.38%, while (Chakraborty and Sikdar, 2008) reported for 2 hybrids *Pleurotus florida* x *Volvariella volvacea* showing precocity ranged from 20 to 40 days and biological efficiencies ranged from 56 to 142%.

Table 2. Morphology parameters of fruit bodies of parental, hybrid and reconstituted strains, cultivated on WS and AP.

Strains	Type of strains	Substrates	Diameter of pileus (cm)	Length of stipe (cm)	Whiteness index (WI)	Yellowness index (YI)	Browning index (BI)	Color
PO	Parental	WS	6.71±2.13 ^b	2.70±0.64 ^c	64.95±5.15 ^b	40.81±3.99 ^b	38.81±5.22 ^b	Pale yellow
		AP	5.36±1.71 ^A	2.22±0.65 ^B	59.80±7.05 ^C	51.62±10.55 ^B	50.77±12.78 ^B	Pale yellow
PD	Parental	WS	6.82±1.66 ^b	1.59±0.38 ^a	66.15±2.60 ^b	32.97±4.80 ^a	34.05±3.10 ^a	Pink
		AP	6.15±1.49 ^B	1.65±0.64 ^A	77.21±1.98 ^E	24.10±1.93 ^A	24.19±2.41 ^A	Pink
LC	Parental	AP	6.80±2.25 ^C	3.86±1.53 ^C	32.98±7.89 ^A	56.71±8.01 ^B	71.37±12.65 ^D	Dark coffee
PO ₂ xLC ₂	Hybrid	WS	5.72±1.08 ^a	2.26±0.45 ^b	60.53±3.80 ^a	55.81±7.30 ^d	55.21±9.80 ^c	Pale yellow
		AP	6.47±1.34 ^B	2.34±0.57 ^B	51.98±4.56 ^B	63.73±7.66 ^C	68.77±10.91 ^C	Pale coffee yellow
PO ₅ xLC ₂	Hybrid	WS	5.66±1.48 ^a	2.42±0.50 ^b	61.90±5.21 ^a	53.39±7.56 ^c	51.95±11.66 ^d	Pale yellow
		AP	6.27±1.88 ^B	2.50±5.44 ^B	57.21±5.50 ^C	56.78±9.03 ^B	58.26±12.28 ^B	Pale yellow
PD ₄ xLC ₃	Hybrid	WS	8.09±1.96 ^c	2.58±0.53 ^c	71.37±1.11 ^c	29.24±0.98 ^a	32.21±1.60 ^a	Pink
		AP	6.39±2.09 ^B	2.40±0.50 ^B	62.64±1.74 ^D	54.03±3.19 ^B	51.79±4.17 ^B	Pale yellow
PO ₁ xPO ₂	Reconstituted	WS	5.97±1.23 ^a	2.30±0.63 ^b	61.42±4.31 ^a	51.60±6.69 ^c	50.69±9.12 ^c	Pale yellow
		AP	5.97±1.58 ^B	2.51±0.57 ^B	59.38±4.95 ^C	52.90±6.94 ^B	52.89±9.75 ^B	Pale yellow
PD ₁ xPD ₄	Reconstituted	WS	6.42±1.84 ^b	2.27±0.46 ^b	67.02±1.77 ^b	33.97±12.55 ^a	36.30±8.05 ^a	Pink
		AP	5.61±1.32 ^A	2.22±0.44 ^B	62.66±1.45 ^D	53.98±1.99 ^B	51.70±2.85 ^B	Purplish pale pink
LC ₂ xLC ₂	Reconstituted	AP	6.68±2.51 ^B	3.76±1.61 ^C	31.35±9.64 ^A	50.94±16.31 ^B	65.53±20.17 ^C	Dark coffee

*All values are means ± standard deviation of ten replicates. Uppercase letters indicate difference between the morphology parameters of the mushrooms obtained on AP, while lowercase letters indicate difference between the morphology parameters of the mushrooms obtained on WS according to Duncan's test ($p < 0.05$, $n = 10$).

The uses of different substrates improve the biological efficiency, production rate and yield (Royse *et al.*, 2004; Yang *et al.*, 2013). The variability of the productivity parameters is directly related to the strains and the supplementation in the substrates (Sánchez *et al.*, 2002; Gaitán-Hernández *et al.*, 2014). Nutrients in the composition of the substrate are one of the factors that limit colonization, which influences in the edible mushroom production (Philippoussis, 2009).

The hybrids of different genera presented advantages such as: reduction in precocity the parental strain (LC) cultivated on AP needed 48 days for the start of the primordia, while the hybrids *Pleurotus*x*Lentinula* produced on both substrates presented precocity between 15 to 31 days. Total cultivation time is another attribute was improved, the parental strain of *Pleurotus djamor* (PD) cultivated on WS required 62 days for the 3 harvests, while the hybrid PD₄xLC₃ produced on WS required 58 days. Productivity was improved; the hybrid PO₅xLC₂ cultivated on WS showed highest biological efficiency, production rate and similar yield in comparison to its parental strain of *Pleurotus ostreatus* (PO) and *Lentinula edodes* (LC).

3.2 Morphological and physical characteristics

Among the phenotypic characteristics determined from the fruit bodies of the parental, hybrid and reconstituted strains were: diameter of pileus, length

of the stipe and color of the mushrooms (Table 2). Table 2 shows that the fruit bodies of the strains cultivated on WS presented diameter of pileus being in a range from 5.66 to 8.09 cm corresponding to Group 2 (5 - 9.9cm), while the fruit bodies of the strains produced on AP showed diameter of pileus in a range from 5.36 to 6.80 cm, also corresponding to Group 2. Salmones *et al.* (2004) reported for three parental strains of *Pleurotus djamor* and four hybrid strains of *Pleurotus djamor* presented diameter of pileus with high presence to Group 1 and Group 2 and low presence to Group 3, while Valencia del Toro *et al.* (2003) reported size diameter of *Pleurotus* spp. corresponding to Group 2. Valencia del Toro *et al.* (2018) indicated that the size of the pileus can increase the market value.

The fruit bodies of the strains produced on WS showed length of stipe since 1.59 to 2.70 cm corresponding to Group 1 (< 5 cm); likewise the fruit bodies of the strains produced on AP exhibited length of stipe since 1.65 to 3.86 cm (Group 1). Valencia del Toro *et al.* (2018) presented result of fruit bodies of three strains of *Pleurotus* produced in five substrates with length of stipe corresponding to Group 1. The edible mushroom markets prefer mushrooms with large pileus and short stipe due to unpleasant flavor (Kamat *et al.*, 2010; Lechner and Albertó, 2011).

Whiteness index (WI) indicated that mushrooms of the hybrid PD₄xLC₃ cultivated on WS presented the highest value being of 71.37, while the fruit bodies of the parental of *Pleurotus djamor* (PD) produced on AP showed the highest value being of

77.21. The yellowness index (YI) indicated that the mushrooms of $PO_2 \times LC_2$ showed the highest values being of 55.81 cultivated on WS and 63.73 produced on AP. Browning index (BI) indicated that the hybrid $PO_5 \times LC_2$ produced on WS presented the highest value being of 51.95, while the parental LC cultivated on AP also showed highest value being of 71.37.

Browning index (BI) is influenced by the strain or by the substrate, however, it is moreover related to enzymatic and non-enzymatic processes in the fruit bodies that take place during maturation or in the post-harvest stages (Maskan, 2001, Mohapatra *et al.*, 2010). Salmenes *et al.* (2004) cultivated 3 parental strains of *Pleurotus djamor* and hybrids in different substrates presented color variations between fruiting bodies.

The color of the mushrooms of the reconstituted and hybrid strains was similar to the fruit bodies of some parental strain, with exception of the hybrids $PO_5 \times LC_2$ and $PD_4 \times LC_3$ cultivated on AP showing colorations of the fruit bodies different from the parental strains. Salmenes *et al.* (2004) pointed out the cultivation in different substrates present variations between the color of the fruit body.

3.3 Chemical characteristics of the fruit bodies

The chemical composition of the mushrooms of the parental, hybrid and reconstituted strains produced on WS and AP is presented in Table 3.

The fruit bodies of the reconstituted strain $LC_2 \times LC_2$ produced on AP showed the lowest moisture content being of 82.22%. The highest value of moisture was presented for the mushrooms of the parental strain of *Pleurotus ostreatus* (PO) and its reconstituted $PO_1 \times PO_2$ cultivated on WS being in a range between 94.13 to 95.52%. The fruit bodies of the parental strain of *Lentinula edodes* (LC) produced on AP showed the lowest fat content being of 0.83%, while the mushrooms of the hybrid strain $PO_2 \times LC_2$ cultivated on WS showed the highest fat content being of 4.99%. The moisture and fat content of the fruit bodies is influenced by the composition of the substrates used in the production of the mushroom (Liu *et al.*, 2005; Valencia del Toro *et al.*, 2018)

Mushrooms of the hybrid strain $PD_4 \times LC_3$ produced on WS presented the highest crude fiber content being of 21.46%, while the lowest fiber content was exhibited by the hybrid $PO_2 \times LC_2$ being between 6.01 to 6.88%. The high fiber content in edible fungi promotes intestinal regulation and helps the body digestion and elimination of undigested food

(Silva *et al.*, 2002).

The highest content of crude protein was showed by the fruit bodies of the reconstituted strain $PD_1 \times PD_4$ cultivated on AP being of 33.90%, while the fruit bodies of the parental strain of *Pleurotus ostreatus* (PO) cultivated on WS showing the highest protein content being of 27.76%. Crisan and Sands (1978) indicated that the protein content of the fungi depends on the composition of the substrates used in the cultivation, the size of the pileus, and the strain. Supplementation of substrates to raise the nutritional value available for the mushrooms tends to produce carpophores with less water and higher protein content (Pardo-Giménez *et al.*, 2016).

The fruit bodies of the hybrid $PD_4 \times LC_3$ cultivated on WS and AP presented the highest ash content being in a range between 12.64 to 12.69%, while the mushrooms of the hybrid $PO_5 \times LC_2$ and the reconstituted $PO_1 \times PO_2$ produced on AP showed the lowest ash content being in a range from 5.82 to 6.48%.

Mushrooms of the hybrid strain $PD_4 \times LC_3$ cultivated on WS and AP showed the lowest carbohydrate content, while the fruit bodies of the parental strain of *Lentinula edodes* (LC) and its reconstituted $LC_2 \times LC_2$ produced on AP showed the highest carbohydrate content being in a range from 74.04 to 77.59%. Carbohydrates were the most abundant macronutrients, followed by protein and ash.

Similar studies have been previously reported, Fernandes *et al.* (2015) presented fruit bodies of one *Pleurotus ostreatus* strain cultivated on 3 different substrates (paper remnants) showing moisture content between 84.30 to 91.00%, crude protein ranged from 9.29 to 14.70%, fat since 1.18 to 1.68%, ash between 5.69 to 15.90%, carbohydrates in a range between 73.20 to 78.60% generating a caloric energy from 342.00 to 385.00 kcal. On the other hand, Heleno *et al.* (2015) showed mushrooms for one strain of *Lentinula edodes* presenting crude protein content of 16.00%, fat content of 1.14%, ash of 6.24%, carbohydrates of 76.62% presenting a caloric energy of 380.74 kcal. Selvakumar *et al.* (2015) presented fruit bodies of one hybrid *P.djamor* \times *P.ostreatus* cultivated on rice straw showing the following nutritional composition: moisture content of 84.25%, protein content of 29.40%, fat of 2.02%, ash of 3.90%, crude fiber of 7.85%, carbohydrates of 50.10% exhibiting a caloric energy of 268.28 kcal. The nutritional composition of the fruit bodies is influenced by the substrate used in the cultivation of the mushrooms and the strain (Manzi *et al.*, 2001).

Table 3. Chemical composition of the mushrooms of the parental, hybrid and reconstituted strains cultivated on WS and AP.

Strains	Type of strains	Substrates	%Moisture	%Fat	%Crude Protein	%Ash	%Crude Fiber	% Carbohydrate	Energy value (kcal/100g dm)
PO	Parental	WS	95.52±0.66 ^c	1.66±0.18 ^a	27.76±0.19 ^d	9.20±1.01 ^b	8.50±0.34 ^b	61.38±1.24 ^b	344.45±4.06 ^b
		AP	91.98±0.83 ^E	1.26±0.06 ^B	22.66±0.44 ^B	10.07±0.56 ^D	8.55±0.29 ^B	66.01±0.44 ^D	347.18±2.15 ^C
PD	Parental	WS	91.95±0.59 ^b	2.59±0.27 ^d	23.62±7.88 ^b	7.65±0.11 ^a	14.70±0.60 ^c	66.15±8.01 ^c	361.37±11.06 ^d
		AP	89.03±0.69 ^D	1.83±0.21 ^D	23.82±3.71 ^C	8.96±0.22 ^C	11.67±0.80 ^C	65.38±4.03 ^C	352.34±5.53 ^C
LC	Parental	AP	84.28±0.33 ^B	0.83±0.07 ^A	16.26±1.68 ^A	8.86±0.94 ^C	8.48±0.30 ^B	74.04±2.60 ^E	360.57±6.46 ^C
		WS	91.04±0.87 ^b	4.99±0.05 ^c	19.86±0.50 ^a	7.62±0.24 ^a	6.01±1.08 ^a	67.53±0.68 ^c	377.41±1.44 ^e
PO ₂ xLC ₂	Hybrid	AP	86.21±1.41 ^C	3.90±0.09 ^F	23.27±0.60 ^B	8.32±0.55 ^C	6.88±0.60 ^A	64.51±1.13 ^C	364.58±2.65 ^C
		WS	91.50±0.54 ^b	1.92±0.04 ^b	27.16±0.45 ^d	9.44±1.19 ^b	17.98±1.46 ^d	61.48±0.84 ^b	345.42±4.20 ^b
PO ₅ xLC ₂	Hybrid	AP	86.67±0.83 ^C	2.89±0.13 ^E	25.66±0.09 ^D	6.48±0.16 ^A	8.66±1.18 ^B	64.97±0.11 ^C	364.29±0.97 ^C
		WS	87.97±0.14 ^a	2.34±0.28 ^d	27.20±0.46 ^d	12.69±0.82 ^c	21.46±0.86 ^c	36.31±2.05 ^a	243.33±8.28 ^a
PD ₄ xLC ₃	Hybrid	AP	85.94±0.85 ^C	1.58±0.44 ^C	22.24±1.27 ^B	12.64±0.71 ^E	13.30±0.97 ^D	50.24±1.43 ^A	282.49±2.74 ^A
		WS	94.13±0.39 ^c	1.44±0.10 ^a	24.72±0.36 ^b	8.48±2.27 ^b	9.35±0.95 ^b	65.36±2.04 ^b	351.35±8.57 ^c
PO ₁ xPO ₂	Reconstituted	AP	89.49±0.84 ^D	1.20±0.04 ^A	23.59±0.25 ^B	7.45±0.07 ^B	14.09±1.61 ^D	67.76±0.26 ^D	356.45±0.37 ^C
		WS	96.32±1.28 ^d	2.03±0.15 ^c	25.55±0.37 ^c	9.38±0.63 ^b	10.29±1.55 ^c	63.05±0.45 ^b	348.70±2.20 ^b
PD ₁ xPD ₄	Reconstituted	AP	92.94±1.58 ^E	1.30±0.15 ^B	33.90±0.76 ^E	7.69±0.63 ^B	6.80±0.57 ^A	57.10±0.27 ^B	339.56±0.96 ^B
		WS	82.22±0.87 ^A	0.96±0.08 ^A	15.57±1.02 ^A	5.89±0.60 ^A	7.94±0.78 ^B	77.59±1.60 ^E	374.66±4.16 ^D

*All values are means ± standard deviation of ten replicates. Uppercase letters indicate difference between chemical composition of the mushrooms obtained on AP, while lowercase letters indicate difference between chemical composition of the mushrooms obtained on WS according to Duncan's test ($p < 0.05$), $n = 10$.

Table 4. Chemical composition of WS and AP before and after harvest.

Strains	Type of strains	Substrates	Acid Detergent Fiber (%)	Neutra Detergent Fiber (%)	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Control	Without strain	WS	38.77±0.26 ^c	65.82±0.76 ^g	11.99±0.06 ^d (0%)	27.05±0.52 ^d (0%)	53.84±0.70 ^f (0%)
		AP	40.42±0.46 ^G	68.98±0.20 ^G	12.94±0.11 ^F (0%)	28.56±0.66 ^E (0%)	56.04±0.23 ^G (0%)
PO	Parental	WS	30.57±0.13 ^d	50.44±0.13 ^f	11.17±0.08 ^c (6.8%)	19.87±0.17 ^c (26.5%)	39.27±0.15 ^e (27.1%)
		AP	31.40±0.39 ^F	43.60±0.49 ^C	8.56±0.34 ^B (33.8%)	12.20±0.87 ^A (57.3%)	35.03±0.35 ^D (37.5%)
PD	Parental	WS	25.82±1.26 ^c	43.28±0.49 ^d	8.57±0.30 ^a (28.5%)	17.45±1.56 ^b (35.5%)	34.70±0.58 ^c (35.5%)
		AP	18.59±0.34 ^A	40.58±0.17 ^B	10.15±0.08 ^D (21.6%)	21.99±0.17 ^D (23.0%)	30.43±0.19 ^A (45.7%)
LC	Parental	AP	28.40±0.47 ^D	45.52±0.26 ^D	12.35±0.10 ^F (4.6%)	17.12±0.73 ^B (40.1%)	33.17±0.17 ^B (40.8%)
		WS	24.78±0.14 ^b	41.22±0.43 ^b	11.15±0.25 ^c (7.0%)	16.44±0.54 ^b (39.2%)	30.07±0.20 ^a (44.1%)
PO ₂ xLC ₂	Hybrid	AP	28.08±0.29 ^D	38.13±0.99 ^A	9.51±0.19 ^C (26.5%)	10.05±1.06 ^A (64.8%)	28.62±1.16 ^A (48.9%)
		WS	25.75±0.23 ^c	39.30±0.19 ^a	10.24±0.29 ^b (14.6%)	13.55±0.34 ^a (49.9%)	29.06±0.43 ^a (46.0%)
PO ₅ xLC ₂	Hybrid	AP	29.70±0.15 ^E	46.35±0.30 ^b	10.65±0.23 ^E (17.7%)	16.65±0.39 ^B (41.7%)	35.70±0.54 ^D (36.3%)
		WS	24.77±0.22 ^b	42.89±0.16 ^d	11.11±0.02 ^c (7.3%)	18.13±0.29 ^b (33.0%)	31.78±0.15 ^b (41.0%)
PD ₄ xLC ₃	Hybrid	AP	27.80±0.15 ^C	47.58±0.33 ^E	12.73±0.19 ^F (1.6%)	19.77±0.30 ^C (30.8%)	34.85±0.51 ^C (37.8%)
		WS	22.59±0.49 ^a	42.17±0.10 ^c	10.50±0.08 ^b (12.4%)	19.58±0.57 ^c (27.6%)	31.67±0.18 ^b (41.2%)
PO ₁ xPO ₂	Reconstituted	AP	30.89±0.88 ^F	51.13±0.54 ^F	7.83±0.20 ^A (39.5%)	20.24±0.65 ^C (29.1%)	43.30±0.73 ^F (22.7%)
		WS	30.62±0.36 ^d	48.24±0.07 ^e	10.54±0.53 ^b (12.1%)	17.62±0.37 ^b (34.9%)	37.70±0.46 ^d (30.0%)
PD ₁ xPD ₄	Reconstituted	AP	26.14±0.10 ^B	48.29±0.17 ^E	9.15±0.10 ^C (29.3%)	22.15±0.27 ^D (22.4%)	39.15±0.07 ^E (30.1%)
		WS	27.31±0.16 ^C	43.75±0.59 ^C	12.47±0.11 ^F (3.6%)	16.44±0.72 ^B (42.4%)	31.28±0.62 ^A (44.2%)

*All values are means ± standard deviation of triplicate measurements. Uppercase letters indicate difference between the chemical composition of the substrate AP, while lowercase letters indicate difference between chemical composition of the substrate WS according to Duncan's test ($p < 0.05$), $n = 10$.

*The biodegradation of the cellulose, hemicellulose and lignin was calculated according to the following equation: %Biodegradation = 100-(%Final composition of the substrate*100/% Initial composition of the substrate). Values in brackets represent the percentage of biodegradation of the substrates.

3.4 Chemical characteristics of the substrates

The values of acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, cellulose and lignin varied between the two substrates: WS and AP used in the cultivation of the parental, hybrid and reconstituted strains (Table 4). The lowest degradation of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose and cellulose when was used the substrate (AP) in the mushroom cultivation.

The values of acid detergent fiber, neutral detergent fiber, hemicellulose, cellulose and lignin decreased in the substrates used in the fructifications of all strains. The substrate WS used in the fructification of the hybrid PO₅xLC₂ presented the

highest degradation value of cellulose (46.00%), while the substrate AP used in the production of the hybrid PO₂xLC₂ presented the highest biodegradation of cellulose (48.90%) On the other hand, the substrate WS used in the fructification of the parental strain of *Pleurotus djamor* (PD) exhibited the highest biodegradation value of lignin (28.50%), while the substrate AP used in the fructification of the reconstituted strain PO₁xPO₂ presented the highest biodegradation value of lignin (39.50%). Gaitán-Hernández *et al.* (2006) indicated the contents of neutral detergent fiber (NDF), hemicellulose, cellulose and lignin decreased using barley straw such as substrate for cultivation of 3 strains of *Lentinula edodes*.

Similar results have been published, Gaitán-Hernández *et al.* (2006) cultivate three strains of *Lentinula edodes* in three different substrates (vineyard pruning, barley straw and wheat straw) showing the following chemical composition of the substrates after harvest: neutral detergent fiber in a range between 58.64 to 71.98%, hemicellulose ranged from 10.76 to 25.51%, cellulose since 29.34 to 48.39% and lignin in a range between 9.00 to 18.69%, while (Bae *et al.*, 2006) cultivated *Pleurotus ostreatus*, *Pleurotus eryngii*, *Flammulina velutipes* using as a substrate (mixture of sawdust, rice bran and corn cob) showing the following chemical composition of the mixture after harvest: neutral detergent fiber of 78.20%, acid detergent fiber of 60.40%, lignin of 20.00%, hemicellulose of 17.80%, cellulose of 40.40%. Kwak *et al.* (2008) cultivated *Pleurotus eryngii* using a mixture of sawdust, rice bran, corn cob supplemented with poultry litter showing the following composition of the substrate after harvest: hemicellulose content of 13.90%, cellulose of 32.20%, lignin of 17.40%. Blanchette (1991) indicated that the degradation of lignocellulosic residues is influenced by the characteristic of the substrate, the environmental factors and the genetic factors between the strains.

Conclusions

The hybrid strain PD₄xLC₃ cultivated on AP presented highest productivity in comparison with the parental strains. Also the hybrid strains PO₅xLC₂ and PD₄xLC₃ cultivated on WS presented fruit bodies with highest protein content in relation with the other strains used in this research. The use of different agricultural wastes used in the mushroom cultivation provided a directly relation with the productivity and the color of the fruit bodies.

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