Reuse of degraded *Pleurotus ostreatus* substrate through supplementation with wheat bran and Calprozime® quantitative parameters

Reutilización del sustrato degradado de *Pleurotus ostreatus* mediante la suplementación con salvado de trigo y el suplemento comercial Calprozime® parámetros cuantitativos

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ABSTRACT

In this study, the agronomic viability of *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. was studied by reusing spent substrates that were previously used for crops of this mushroom. After the physical and chemical characterization of the substrates, we evaluated the quantitative production parameters for one growing season. The experiment used wheat straw (WS) and spent *Pleurotus* substrate (SPS) as a base material to generate prepared substrates that could be reutilized for mushroom production. These base substrates were supplemented with wheat bran (WB) (two doses, 300 and 600 g/6 kg) and the commercial supplement Calprozime® (120 g/6 kg). We obtained a biological efficiency (BE) between 50 and 63%, a high quantity of mushrooms (between 26 and 39 mushrooms/bag) and an excellent unit weight of the fruiting bodies (between 24.34 and 39.54 g) with the substrates supplemented with a 120 g/6 kg dose of Calprozime®.

Key words: edible fungi, waste utilization, yield components, earliness, agronomic characteristics.

RESUMEN

En el presente trabajo se estudia la viabilidad agronómica del cultivo de *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm., mediante la reutilización de sustratos previamente empleados en cultivos del mismo hongo. Tras la caracterización física y química de los sustratos, se han evaluado los parámetros de producción cuantitativos en un ciclo de cultivo. Como material de base, se parte de la paja de trigo y el sustrato degradado por *P. ostreatus* (SAP), para generar sustratos que permitan su reutilización en la producción de setas. Estos sustratos base, se suplementaron, con salvado de trigo (dos dosis, 300 y 600 g/6 kg) y el suplemento comercial Calprozime® (120 g/6 kg). En los sustratos donde se aplicó 120 g/6 kg de Calprozime®, se obtuvo una eficiencia biológica comprendida entre 50 y 63%, un buen número de setas total (entre 26 y 39 setas/bolsa) y buenos pesos unitarios de los carpóforos (entre 24,34 y 39,54 g).

Palabras clave: setas comestibles, aprovechamiento de desechos, caracteres de rendimiento, precocidad, características agronómicas.

Introduction

The commercial production of mushrooms of the *Pleurotus* genus is currently, along with other species of edible mushrooms (*Agaricus bisporus* [Lange] Imbach, and *Lentinula edodes* [Berkeley] Pegler), a modern and unique economic activity within the field of agronomy, with a remarkable presence both in Spain and around the world (Sánchez and Mata, 2012). Approximately 13,500 t of this fungus is produced in Castilla - La Mancha (67% of the national total) (Pardo *et al*., 2009). The mushroom growing sector in Spain generates about $5{\cdot}10^5$ t of spent compost, while the EU, as a whole, produces more than $3.5{\cdot}10^6$ t (Pardo *et al*., 2009; Picornell *et al*., 2009). This lignocellulosic material, called spent mushroom substrate, can be used in various fields of agriculture (Tajbakhsh *et al*., 2008, Faraco *et al*., 2009, Pathak *et al*., 2009), but these uses are not enough to take advantage of the high volume generated annually, which accumulates in collection centers located in the production areas of Spain. These spent substrates are potential contaminants, not to mention, a waste of energy.

This study aimed to carry out a quantitative agronomic evaluation of spent *Pleurotus* substrate (SPS) and its mixture with wheat straw (WS) at different proportions used as lignocellulosic sources in new growing cycles of *P. ostreatus*, unsupplemented and supplemented with different doses of wheat bran (WB) or Calprozime® (CPZ®), a commercial supplement. CPZ® optimizes the energy/protein nutrition means and provides proteins because mycelium needs certain amino acids (Fig. 1) The

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use of spent mushroom substrates that remain after the cultivation of *P. ostreatus* in new production cycles would be an agronomically viable alternative to using WS partially, which is currently used as a base material virtually exclusively (even more so if you consider the economic problems associated with the use of this cereal farmer's by-product and the high market price of WS, especially in drought years). If SPS could be easily available at a low cost, it could be integrated into new formulations and methodologies, diminish the environmental impact of the waste produced during mushroom cultivation, limit grower dependence on straw, and decrease the environmental impact of its overgrowing accumulation.

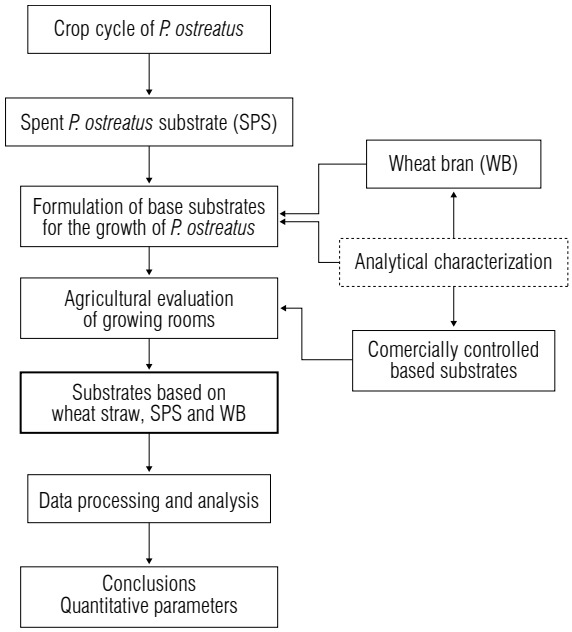

FIGURE 1. Schematic experiment approach in reuse of degraded *Pleurotus ostreatus* substrate.

Materials and methods

Analytical methodology for the characterization of the materials

For the characterization of the raw materials and processed substrates, we determined the following parameters: moisture (MAPA, 1994), pH (Ansorena, 1994), total nitrogen (Tecator, 1987; MAPA, 1994), ash (MAPA, 1994), organic matter (Ansorena, 1994), C:N ratio, crude fiber (ANKOM, 2008), crude fat (ANKOM, 2009), nitrogen free extractives (NFE) (Alvira *et al*., 1987), cellulose and neutral detergent-soluble fiber (NDS) (ANKOM, 2005; 2006a, b). Furthermore, an exploration of mites (Krantz, 1986) and nematodes (Nombela and Bello, 1983) was performed.

Preparation of the substrates and experimental design

The corresponding experimental design of this trial was a balanced plan factorial design, 3 x 4 with six replicates (randomized block factorial with two factors) (Tab. 1). Factor 1 corresponded to the type of base substrate: WS (6 kg/bag); WS (3 kg/bag) + SPS (3 kg/bag); and SPS (6 kg/bag), and factor 2 corresponded to four combinations of WB (300 and 600 g per 6 kg) + CPZ® (120 g/6 kg), resulting in 12 different treatments that had two commercial substrates added (6 kg bag and sack in a commercial form). Gypsum was added to all the treatments at 50 g kg^{-1} of the base material (except the commercial substrate). While the treatments with the WS base substrate was dosed with $CaCO_3$ at 10 g kg^{-1} of the base material, the combinations of WS+ SPS were dosed with $CaCO_3$ at 15 g kg^{-1} of the base material and the treatments based on the SPS substrate were dosed with $CaCO_3$ at 20 g kg^{-1} of the base material; the commercial substrates were not dosed with $CaCO_3$. For an appropriate statistical analysis, the commercial substrate T3 was not included since no mushrooms spawned from the mycelium (possibly due to anaerobic and/or antibiosis contamination problems) (Tab. 1).

TABLE 1. Treatments tested (quantities per bag) in reuse of degraded *Pleurotus ostreatus* substrate.

Treatment	WS	SPS	WB	CPZ®	Gypsum	$CaCO_3$
	(kg)				(g)	
T1	6	0	0	0	300	60
T2	6	0	300	0	300	60
T3	6	0	600	0	300	60
T4	6	0	0	120	300	60
T5	3	3	0	0	300	90
T6	3	3	300	0	300	90
T7	3	3	600	0	300	90
T8	3	3	0	120	300	90
T9	0	6	0	0	300	120
T10	0	6	300	0	300	120
T11	0	6	600	0	300	120
T12	0	6	0	120	300	120
T13	Commercially controlled based substrates (6 kg/bag)					
T14	Commercially controlled based substrates (sac commercial format)					

WS, wheat straw; SPS, spent *P. ostreatus* substrate; WB, wheat bran; CPZ→, Calprozime®; T1, WS 6 Kg + $CaCO_3$ 60 g; T2, WS 6 kg + WB 300 g + $CaCO_3$ 60 g; T3, WS 6 kg + WB 600 g + $CaCO_3$ 60 g; T4, WS 6 kg + CPZ→ 120 g + $CaCO_3$ 60 g; T5, WS 3 kg + SPS 3 kg + $CaCO_3$ 90 g; T6, WS 3 kg + SPS 3 kg + WB 300 g + $CaCO_3$ 90 g; T7, WS 3 kg + SPS 3 kg + WB 600 g + $CaCO_3$ 90 g; T8, WS 3 kg + SPS 3 kg + CPZ→ 120 g + $CaCO_3$ 90 g; T9, SPS 6 kg + $CaCO_3$ 120 g; T10, SPS 6 kg + WB 300 g + $CaCO_3$ 120 g; T11, SPS 6 kg + WB 600 g + $CaCO_3$ 120 g; T12, SPS 6 kg + CPZ→ 120 g + $CaCO_3$ 120 g; T13, commercially controlled based substrates (6 kg/bag); T14, commercially controlled based substrates (sac commercial format).

The first step in the preparation of the tested substrates consisted in chopping and pre-soaking the WB and subsequently mixing them with the substrates to adjust their moisture content. Then, we proceeded to a pasteurizing

heat treatment (60 - 65°C, 8 h) and progressive decrease for at least 15 h to a "seeding" temperature (25°C). Finally, the supplementation and "seeding" were carried out (dose of 30 g kg^{-1} of a variety of selected mycelium Mispajmycelium S-100) before manual bagging in the CIES pilot plant (CIES, 2007).

All of the substrates were packed into transparent polythene bags that were 29 cm in diameter and had a height ranging from 25 to 35 cm, depending on the type of substrate, holding approximately 6.5 kg of weight. These bags are made with four uniformly drilled holes of 2.2 cm in diameter over the side surface of each one.

Driving and monitoring of the crop cycle

The research was conducted over an 85 d cycle. The development of the crop cycle was in an experimental greenhouse located at the Center for Research, Experimentation and Mushroom Services (CIES), located in the town of Quintanar del Rey (Cuenca, Spain) under controlled conditions (room temperature, substrate temperature, relative humidity, and carbon dioxide concentration) within the recommended ranges for a variety of selected mycelium and in each stage of cultivation (CIES, 2007). Incubation of the substrates lasted approximately 17 d (excluding treatments with a low germination rate), without external ventilation or lighting. During the incubation period, the relative humidity inside the greenhouse ranged between 83 and 93%, while the substrate temperature ranged between 21 and 29°C and the room temperature ranged between 19 and 24°C. After this, we proceeded to induce fruiting with ventilation (to keep CO_2 levels regulated between 0.14 to 0.09%), a reduction of the room temperature (24 to 19°C) and substrate temperature (28 to 23°C), and a reduction of the humidity (89.5 to 89.0%) and lighting for 12 h.

Evaluation of the quantitative parameters

Depending on the level of spawn run time of the substrate by the mycelium and tested contaminations, we established a parameter designated as the germination index (GI) with a scale from 0 (no invasion) to 5 (full invasion). The mushrooms were harvested daily at their optimal commercial development. The quantity of "cones" and mushrooms harvested were determined by counting throughout the entire mushroom growth cycle; it was defined as a group of fruit bodies that simultaneously fruited from the same drilled hole in the substrate bag. To calculate the yield of mushrooms produced daily, each bag was weighed to the nearest gram. The estimated net yield was performed by weighing the fruit bodies after cutting the unmarketable stipe and calculating the percentage of shrinkage resulting from this operation. Once fruiting occurred, the BE was calculated and expressed as a percentage of the fresh weight of the harvest over the dry weight of the substrate that was used. The BE was established from the yield provided by each packet, taking into consideration the charge density of the substrate in the bags and the moisture content. The weight unit of the mushrooms (gross with unmarketable stipe and net without stipe), expressed in grams, was determined from the yields obtained and the quantity of sporophores harvested.

The earliness was established as the time in days from the "seeding" of the substrate to the first flush harvested (weighing the daily relative production of the substrate). A flush corresponds to each production cycle that is repeated rhythmically during a harvest. Similarly, we performed a second estimate of the earliness considering the total harvest.

The fruiting degree was defined as the ratio between the quantity of cones produced and the quantity of holes made in the bags.

Statistical analysis

To carry out the statistical analysis, two software packages were used: Statgraphics® Plus version 5.1 and SPSS®. Descriptive statistical techniques, a principal component analysis, a variance analysis and correlation and regression methods were used to evaluate the data. The differences were considered significant for $P \leq 0.05$.

Results and discussion

Germination index

The treatments with the commercial substrate (T14), the unsupplemented WS (T1), and supplemented WS with 300 g of WB (T2) and with 600 g of WB (T3) provided the worst substrate coverage per the vegetative mycelial growth. Most likely, this was due to the particle size of the WS in these treatments, which were the last to be manually bagged with a small size residual WS accompanied by dust, covering the whole surface (≤2 cm). In preparing the mixture of the processed substrates, the formation of agglomerates was observed, which may have contributed to a worse distribution of the "seed" on the media at the time of inoculation and generated a low oxygen diffusion through the compaction of the bag. These conditions favored the increase of CO_2 concentrations (presumably produced by the fungus) and the decrease of O_2 levels inside the bag, therefore causing an inhibitory effect on the growth and development of the fungus. This explanation is

shared with Zhang *et al*. (2002) and López-Rodríguez *et al*. (2008), among other researchers. According to Salmones *et al*. (2005) and Okano *et al*. (2007), the fungus in the mycelial growth phase (incubation period) preferably consumes soluble carbohydrates and hemicellulose with respect to the cellulose and lignin. Based on this, another reason could be that the aforementioned prepared substrates did not allow, or decreased, the availability of soluble carbohydrates or other compounds more easily assimilated by the fungus in the mycelial stage of growth.

In the rest of the other substrates that were tested, the expansion of the oyster mushroom mycelium was considered acceptable, with GI values ranging between 4.42 and 5.00 (Tab. 2). This suggests that colonization was supplied with soluble carbohydrates, easily assimilating compounds, and substrates high in fiber.

TABLE 2. ANOVA of the substrate germination index in reuse of degraded *Pleurotus ostreatus* substrate.

Substrate	Germination index
T1	1.71±0.48 b
T2	2.71±0.33 b
T3	2.63±0.27 b
T4	4.67 ± 0.12 a
T5	4.42 ± 0.11 a
T6	4.54±0.32 a
T7	4.79±0.14 a
T8	4.83±0.12 a
T9	4.92±0.08 a
T10	5.00±0.00 a
T11	4.96±0.04 a
T12	5.00±0.00 a
T14	0.29±0.14c
Average	3.88
Fisher F	50.90
Significance level F Fisher	0.00***

*** Significance with $P \leq 0.001$. Means with different letters in each column indicate significant differences according to the Tukey-HSD test ($P \leq 0.05$). See abbreviations in Tab. 1.

Quantitative production parameters

The quantitative production parameter aspects are presented in Tab. 3. The days from inoculation to the first primordia induction in the treatments with low germination index ranging from 11.25 d (commercial substrate) to 19.83 d (T2); the other treatments were less early, with a duration that ranged between 28.18 d (T10) and 43.12 d (T11). This trend manifested by the second earliness (the days from inoculation to total induction). For anomalous treatments, including the commercially controlled based substrates, a high earliness was achieved, ranging between 15.17 d (T3) and 22.93 d (T2). The remaining tested treatments took longer to reach this same stage of the growth and development of the oyster mushroom (from 34.98 d (T12) to 50.05 d (T11)).

There are other scientific studies in which a shorter duration of this phase of the growth cycle of *P. ostreatus* was reflected: with just 10 d using a substrate mixture of rice bran (40%), rice straw (35%) and *Juncus effuses* (25%) (Fonseca *et al*., 2009) and 14 d when adding supplements prepared from denatured soy flour and other organic protein sources (Gea *et al*., 2009), but there have also been higher values: from 15 to 32 d with different species of the genus *Pleurotus* and other edible fungi grown on wheat straw, cotton waste and peanut shells (Philippoussis *et al*., 2001), 30 d with coffee pulp (Rodríguez and Gómez, 2001), from 26 to 42 d using a substrate mixture of sawdust, cornstalk, coffee pulp and sugar cane bagasse (Garzón and Cuervo, 2008), and from 25 to 30 d with wheat straw, a mixture of wheat straw and eucalyptus chips, eucalyptus chips and aspen chips (Varnero *et al*., 2010).

The total gross and net yields were the results of two flushes in most of the substrates; the substrate mixed with WS 6 kg+ 600 g WB and the substrate mixed with WS 3 kg + SPS 3 kg + 600 g WB did not produce mushrooms in the second flush. For the treatment with SPS supplemented with 300 g of WB and a commercial substrate the turnout was 54.47 and 57.32%, respectively. Omitting these latter treatments, the participation of the first flush in the total harvest ranged from 63.17 to 100.00%. The production of *Pleurotus* sp. after the first flush was drastically reduced and the blossoming was delayed from 10 to 20 d (depending on the species of the *Pleurotus* genus used, the type of strain grown and the nature of the produced substrate (Upadhyay *et al*., 2002). This yield loss in the blossoming was accompanied by an increase in quantity could have been due to a decrease of nutrients or to an accumulation of unfavorable toxic substances for the fruiting according to Upadhyay *et al*. (2002). The wheat straw used for the cultivation of oyster mushroom was a poor source of nitrogen (0.5 to 0.8%). Gregori *et al*. (2008) and Kurt and Buyukalaca (2010), in their enzymatic studies, found an apparent reduction of laccase, manganese-dependent peroxidase (MnP), manganese-independent peroxidase (MiP), and lignin peroxidase (Lip) enzymes as the fruiting and blossoming quantity increased. Kurt and Buyukalaca (2010) showed that the highest protein contents of *P. ostreatus* were determined at the end of the first harvest. The sawdust bran produced the highest protein content (9.75 mg mL^{-1}) at the end of the first harvest while sesame straw produced the lowest protein content (2.38 mg mL^{-1}). The maximum laccase activity of *P. pulmonarius* was obtained using a C/N ratio

of 30:1 on corncob with a solid state fermentation. The C/N ratios higher than 30:1 had a negative effect on the laccase production. In general, there was a clear decreasing trend in the net and gross yields accompanied by an increase in the WB dose, while the opposite occurred with the 120 g CPZ® supplementation (Tab. 3).

Again, the treatments with low germination indexes were those that showed the worst fructification index (quantity of cones/hole), which together with the commercially controlled based substrates and the mixture of WS + SPS supplemented with 600 g of WB offered values ranging between 0.08 cones/hole (T3) and 0.20 cones/hole (T14). Applying 120 g of CPZ® provided the higher values of this index, which ranged between 0.67 cones/hole (T4) and 0.96 cones/hole (T12); as the WB dose increased in each of the substrates, the quantity of cones/hole was reduced.

This fructification index data coincided with the results obtained in the quantity of cones/bag and quantity of mushrooms/hole (Tab. 3).

The higher values of the gross weight unit of the mushrooms corresponded with the substrates of: SPS without supplementation (T9, 39.91 g), WS with 120 g of CPZ® (T4, 39.54 g), and mixture of WS + SPS without supplementation (T5, 35.72 g) and supplemented with 120 g of CPZ® (T8, 35.53 g). The average weight unit of the mushrooms researched by Pardo *et al*. (2005a) had values ranging between 20.50 and 31.50 g, using various substrates made with different combinations of WS, barley straw, kenaf, vine and grape seed flour; but was between 12.00 and 52.00 g in another study by Pardo *et al*. (2005b), who tested scrape, straw, kenaf, vine shoot and "alperujo"; and between 14.60 and 25.90 g when using pasteurization and thermophilic conditioned treatments, benomylmoisturization and pasteurization, and semianaerobia fermentation with the same substrates, as obtained by Pardo *et al*. (2007).

The treatments based on unsupplemented WS (T1) and supplemented with 300 g (T2) and 600 g (T3) of WB had the lower BEs, ranging from 0.87% (T3) to 11.33% (T1); these treatments also offered the worst germination index.

This group of substrates, with a low BE, should be mixed with treatments based on SPS + WS and supplemented with 300 g (T6, 18.15%) and 600 g (T7, 2.77%) of WB, with a good germination index and with little or no production in the second flush. This might suggest that the WB in the added doses did not provide sufficient nutrients for the production of fruiting bodies and even generated pollution (anaerobic and antibiosis) because the reduction

TABLE 3. ANOVA of the quantitative parameters of reuse of degraded *Pleurotus ostreatus* substrate.

Substrate	Earliness (days)		Gross yield (g/bag)	Index of the fructification (number cones/hole)	Number mushrooms/bag	UW	BE
	1st flush "seeding"	Total "seeding"					
T1	11.38±7.20 c	16.10±10.21 d	185.00±128.74 e	0.17±0.12 de	6.50±4.35 c	9.22±5.88 d	11.33±7.99 de
T2	19.83±9.22 b	22.93±10.29 cd	85.50±40.66 f	0.17±0.08 de	4.00±2.14 c	11.96±5.67 cd	4.67±2.16 e
T3	15.17±9.63 b	15.17±9.63 d	20.50±13.15 f	0.08±0.05 e	1.50±1.02 c	5.36±3.87 d	0.87±0.55 e
T4	34.25±2.20 b	40.58±3.15 ab	880.00±103.77 b	0.67±0.11 ab	25.50±5.05 b	39.54±5.93 a	49.60±4.89 abc
T5	31.68±1.09 b	42.45±1.36 ab	673.83±74.36 c	0.63±0.06 b	20.50±3.37 b	35.72±4.58 a	37.42±4.62 abcd
T6	35.03±2.28 b	46.00±4.09 a	330.50±91.33 d	0.42±0.05 c	18.50±4.48 b	18.14±2.16 c	18.15±5.12 de
T7	36.83±9.84 b	48.83±9.84 a	58.50±27.44 f	0.13±0.06 de	2.83±1.72 c	19.90±13.48 c	2.77±1.29 e
T8	32.83±1.79 b	43.00±2.47 ab	1139.67±152.65 a	0.79±0.10 a	36.33±6.81 a	35.53±4.94 a	62.48±8.34 a
T9	32.02±2.31 b	47.88±1.90 a	804.67±69.47 b	0.63±0.06 b	20.50±1.12 b	39.91±4.50 a	41.83±3.60 abcd
T10	28.18±2.78 b	47.20±3.26 a	574.33±106.04 c	0.63±0.14 b	23.67±7.94 b	33.35±7.58 a	29.35±4.95 bcd
T11	43.12±6.91 a	50.05±4.87 a	459.17±87.13 cd	0.54±0.10 bc	15.83±3.40 b	29.53±2.09 b	23.15±4.87 cd
T12	28.78±5.80 b	34.98±7.21 b	1081.33±223.81 a	0.96±0.20 a	38.67±8.61 a	24.34±5.57 b	58.48±12.67 ab
T14	11.25±7.14 c	19.53±12.36 cd	535.83±347.26 c	0.20±0.15 d	33.00 ± 20.95a	5.37 ± 3.43d	14.92±9.74 de
Average	26.57	34.44	525.29	0.46	19.03	23.68	27.31
Fisher F	2.61	3.39	7.21	7.14	2.86	4.55	10.97
S_L	0.007**	0.001***	0.00***	0.00***	0.003**	0.00***	0.00***

UW, weight unit of uncut mushrooms (g); BE, biological efficiency (kg/100 kg of dry substrate); S_L, F Fisher level significance.

*** Significance with $P \leq 0.001$, ** with $P \leq 0.05$. Means with different letters in each column indicate significant differences according to the Tukey-HSD test ($P \leq 0.05$). See abbreviations in Tab. 1.

of BEs with WB occurred in all of these base substrates. Kurt and Buyukalaca (2010), growing *P. ostreatus* and *P. sajor-caju* in WS supplemented with WB (2:1), obtained BEs of 112.70 and 100.20%, respectively, which were much higher than those achieved with WS (59.60 and 48.20%), and they concluded that WB contains soluble carbohydrates of a low molecular weight that are rapidly taken up by the mycelium of the fungus.

Similar results were also achieved in other studies (Permana *et al*., 2000; Wang *et al*., 2001). Permana *et al*. (2000) studied sugarcane bagasse supplemented with soybean meal or wheat bran and observed it to be a valuable substrate for mushroom production with *P. sajor-caju*, *P. eryngii* and *A. aegerita*. Comparable lignin degradation, fruiting body yield and increased *in vitro* digestibility, as obtained with other traditional substrates, were achieved by Wang *et al*. (2001), who considered un-pretreated spent beer grains as a basic substrate material for the cultivation of *P. ostreatus*. The effects of spent grain types, additives, substrate moisture content, and substrate packing density on the yield and nutrition of fruit bodies were investigated. The cultivation results showed that few fruit bodies were formed on the spent grain alone; however, a significantly high BE (19.1%) was obtained with the addition of wheat bran (45%). It was also found that the cultivation of *P. ostreatus* increased the crude protein content, while it decreased the ratio of lignin to cellulose in the spent grain substrate.

In this Experiment, the remaining differentiated treatments showed BE values ranging from 23.15% (T11) to 62.48% (T8); both of the WS in the mixture of WS+SPS and SPS offered the higher BE values: T4, 49.60%; T8, 62.48%; and T12, 58.48% (the latter treatments had CPZ®). There have been other studies with *P. ostreatus* that have provided BE values similar to those obtained here: Vogel and Salmones (2000), supplementing wheat straw with soybean meal and calcium sulfate obtained BE values of 64.50%, similar to those obtained by Upadhyay and Vijay (1991) working with rice bran (61%). Yildiz *et al*. (2002) tested *Fagus orientalis* sawdust, alone and mixed with rice straw, grass, waste paper and hazel leaf, and reached BEs of 8.60, 64.30, 43.70, 40.60 and 102.00%, respectively; the mixture of sawdust and hazel leaf provided the best results. Working with the oyster mushroom, Obodai *et al*. (2003) obtained BEs of 61.04 and 50.64% when tested on *Triplochiton scleroxylon* sawdust and rice straw, respectively. Shan *et al*. (2004), in research based on *P. ostreatus* and using oak sawdust as a substrate, obtained a BE of 64.7%. In the breeding study carried out by Marino *et al*. (2006), with an axenic culture of strains of *P. ostreatus* that are heat resistant in *Eucalyptus* sp. sawdust, obtained BEs between 35.80 and 43.10%; this kind of sawdust was supplemented with wheat bran and rice. Fanadzo *et al*. (2010) evaluated the BE with various substrates (WS, corn stover and *Hyparrhenia filipendula*) and supplements (corn bran and cottonseed) in *P. sajor-caju* and *P. ostreatus*, finding that WS obtained a higher BE (71%) than corn stover (40%) and *H. filipendula* (35.4%) for the species *P. sajor-caju*. However, corn stover (97%) was more suitable for *P. ostreatus* than WS (45.6%) although the cotton seed supplementation (25%) improved the BE in the cultivation of *P. ostreatus* using WS (70.4%). This experiment also showed that supplemented corn bran is not recommended for an increased BE. Sales-Campos *et al*. (2010), working with *P. ostreatus* cultivated in the dusts of various Amazon woods and sugar cane bagasse supplemented with rice bran, wheat and corn, found BEs from 58.59 to 128.66%.

With these studies in mind, one can conclude that the best results were obtained when the base substrate was supplemented with organic compounds high in proteins, oils, vitamins, and trace elements, etc. For instance, Gea *et al*. (2009) improved BE values from 58.04 to 77.10% by supplementing and Kurt and Buyukalaca (2010) did so by adding WB (2:1).

Correlation matrix and "step by step" regression models

According to the results, Tab. 4 presents the correlation matrix between the GI, earliness, quantitative production parameters, and physicochemical characteristics of the substrates. There were significant correlations with negative correlation coefficients between the GI and parameters: the pH of the substrates and the crude fiber contents and cellulose thereof, and with a positive correlation coefficient for the ash contents and NDS values. The average weight unit of the uncut mushrooms significantly and negatively correlated with the crude fat content, and positively with the ash contents, as well as the BE.

It is noteworthy that all of the correlations were significant (except for between the BE and the days from inoculation to the first appearance of primordia) with a positive correlation coefficient (Tab. 5).

Table 6 shows the "step by step" regression analysis for the physicochemical properties of the substrates, GI, earliness, and quantitative production parameters of the current experiment. The corresponding "stepwise" regression analysis was the only significant model to explain the variability of the GI (R^2 = 62.10%) with the independent variable of lignin content for the tested substrates (negative coefficient).

TABLE 4. Correlation matrix between the germination index, earliness, and quantitative parameters of production and physicochemical characteristics in reuse of degraded *Pleurotus ostreatus* substrate.

	Germination index	1st flush "seeding"	Total "seeding"	Total quantity of mushrooms	UW	BE
pH	-0.761** (0.004)	-0.557 (0.060)	-0.601* (0.039)	-0.354 (0.259)	-0.404 (0.192)	-0.233 (0.466)
$Nitrogen_T$[1]	0.197 (0.539)	0.061 (0.851)	-0.039 (0.905)	-0.429 (0.164)	-0.254 (0.425)	-0.547 (0.066)
Ash	0.762** (0.004)	0.539 (0.070)	0.684** (0.014)	0.711** (0.010)	0.803** (0.002)	0.732** (0.007)
C/N ratio	-0.328 (0.298)	-0.155 (0.631)	-0.113 (0.726)	0.239 (0.454)	0.153 (0.634)	0.380 (0.223)
Crude fiber[1]	-0.724** (0.008)	-0.495 (0.102)	-0.533 (0.075)	-0.333 (0.291)	-0.389 (0.211)	-0.234 (0.463)
Crude fat[1]	-0.551 (0.064)	-0.426 (0.168)	-0.631* (0.028)	-0.666* (0.018)	-0.635* (0.026)	-0.646* (0.023)
NFE[1]	0.205 (0.523)	0.164 (0.611)	0.092 (0.777)	-0.133 (0.679)	-0.292 (0.357)	-0.313 (0.322)
Cellulose[1]	-0.698** (0.012)	-0.432 (0.161)	-0.421 (0.173)	-0.215 (0.502)	-0.282 (0.375)	-0.111 (0.731)
NDS[1]	0.577* (0.049)	0.388 (0.213)	0.336 (0.285)	0.134 (0.679)	0.073 (0.821)	-0.021 (0.948)

UW, weight unit of uncut mushrooms (g); BE, biological efficiency (kg/100 kg of dry substrate); $nitrogen_T$ total nitrogen; NFE, nitrogen free extractives; NDS, neutral detergent-soluble fiber; [1] g/kg dry matter.

* significant at $0.01<P\leq0.05$; ** significant at $0.001<P\leq0.01$.

TABLE 5. Correlation matrix between the germination index, earliness, yield components, and biological efficiency in reuse of degraded *Pleurotus ostreatus* substrate.

	Germination index	1st Flush "Seeding"	Total "Seeding"	Total quantity of mushrooms	UW	BE
Germination index	1.000					
1st Flush "Seeding"	0.822*** (0.001)	1.000				
Total "Seeding"	0.804** (0.002)	0.922*** (0.000)	1.000			
Total quantity of mushrooms	0.661* (0.019)	0.604* (0.038)	0.669* (0.017)	1.000		
UW	0.787** (0.002)	0.749** (0.005)	0.825*** (0.001)	0.693** (0.012)	1.000	
BE	0.609* (0.035)	0.572 (0.052)	0.623* (0.030)	0.959*** (0.000)	0.775** (0.003)	1.000

UW, weight unit of uncut mushrooms (g); BE, biological efficiency (kg/100 kg of dry substrate).
* significant at $0.01<P\leq0.05$; ** significant at $0.001<P\leq0.01$.

Germination index, earliness and quantitative production parameters (QPP): germination index (GI), days from inoculation to the formation of the first primordia (P2), days from inoculation to the onset of harvest (P4), No. of mushrooms (quantity of mushrooms), average weight unit of uncut mushrooms (UW, g), and biological efficiency (BE, kg/100 kg of dry substrate).

In another study, *P. ostreatus* was mixed with: maize straw, maize cob, palm kernel cake, saw dust, spent grain, rice bran, lime and water. The highest coefficient of determination R^2 (0.990) was obtained from the regression line between the BE and pileus width. The highest scattered points were obtained from the correlation between the BE and stipe girth. The coefficient of determination R^2 showed that the regression line approximately, perfectly fit the data point with respect to the BE and pileus width (Chukwurah *et al*., 2013). In another study, the yield performance of *P. pulmonarius* (Fries.) Quelet was monitored in four agro-industrial wastes (coir fiber, oil palm waste, *Gmelina arborea* sawdust and rice straw) (Jonathan *et al*., 2013). The rice straw produced the highest dry weight mean which was in accordance with Obodai *et al*. (2003), who reported that rice straw was the best substrate for *P. ostreatus* cultivation when compared with banana leaves, maize stover, corn husk, rice husk and elephant grass. The highest BE could have been due to efficient and effective utilization of substrates by *P. pulmonarius*. The regression

TABLE 6. Models obtained with "step by step" regression in reuse of degraded *Pleurotus ostreatus* substrate.

Explained variable	Independent variable	Equation	R^2 corrected	SE
GI	PCC	GI = 14.663*** – 0.177*** · lignin	62.10***	0.70396
P4	PCC + GI + P2	P4 = 13.881* + 1.143*** · P2 – 1.093* · CFa	90.30***	4.07770
No. mushrooms	PCC + QPP (– BE)	Nº mushrooms = 108.224*** – 0.406*** · hemicellulose	64.40***	7.41122
UW	PCC + QPP (– BE)	UW = 89.429* + 0.800*** · P4 – 0.223* · NFE	77.70***	5.74542
		UW = 205.595*** + 0.583***·P4 – 0.333*** · NFE – 1.063** · lignin	90.50***	3.75189
BE	PCC	BE = 182.140*** – 0.692** · hemicellulose	60.80**	13.59471
		BE = 163.518*** – 0.701*** · hemicellulose + 0.210* · C/N ratio	76.10***	10.61367
	PCC + QPP	BE = 267.445*** – 1.465*** · hemicellulose + 0.310*** · C/N ratio + 6.111*** · CFa	93.60***	5.48390
		BE = 83.244* + 1.630*** · Nº mushrooms – 0.202* ·NFE	94.40***	5.14613

Physical-chemical characteristics of substrate (PCC): pH (aq. 1:5, w/w), total nitrogen (g kg^{-1}, odm), ash (g kg^{-1}, odm), C/N ratio, crude fiber (CFi; g kg^{-1}, odm), crude fat (CFa; g kg^{-1}, odm), nitrogen free extractives (NFE; g kg^{-1}, odm), hemicellulose (g kg^{-1}, odm), cellulose (g kg^{-1}, odm), lignin (g kg^{-1}, odm), neutral-detergent soluble (NDS; g kg^{-1}, odm), odm, and dry matter.

Germination index, earliness and quantitative production parameters (QPP): germination index (GI), days from inoculation to the formation of the first primordia (P2), days from inoculation to the onset of harvest (P4), No. of mushrooms (quantity of mushrooms), average weight unit of uncut mushrooms (UW, g), and biological efficiency (BE, kg/100 kg of dry substrate).

R^2, determination coefficient (%); SE, standard error of the estimate. * significant at $0.01<P\leq0.05$; ** significant at $0.001<P\leq0.01$. Regressions include only those coefficients accompanying the independent variables are significant, provided that the significance of the model is significant.

equation showing the relationship between the BE and spent substrate was an indication that *P. pulmonarius* made good use of the substrate since the R^2 was found to be high (~0.60).

Conclusions

Finally, it should also be noted that the SPS substrates (6 kg), unsupplemented and supplemented with 120 g of CPZ®, and the mixture of WS (3 kg) +SPS (3 kg), unsupplemented and supplemented with 120 g of CPZ®; SPS and WS + SPS supplemented with 120 g of CPZ®, BE are achieved above 59%, and less than 63%. These substrates had a higher average weight unit of harvested fruit bodies throughout the experiment and a high total quantity of mushrooms. Consequently, these gradient based composts for *P. ostreatus* mixtures could be a low cost substrate with selective and balanced nutrients for the growth and development of oyster mushrooms.

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