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Reuse of spent mushroom substrate by modification and its qualitative parameters

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Abstract. In this study the agronomic viability of *Pleurotus ostreatus* (Jacq.) P. Kumm. is studied by reusing the spent substrates previously used in crops of the same mushrooms. After the physical and chemical characterization of the substrates, we have evaluated qualitative production parameters in one growing season. As base material, the experiment was arranged with wheat straw (WS) and spent *Pleurotus* substrate (SPS) to generate prepared substrates with the participation of the same, alone and mixed in different proportions with wheat bran (WB). Unsupplemented SPS, supplemented SPS with 600 g of WB, mixture of WS + unsupplemented SPS, and mixture of WS + supplemented SPS with 600 g of WB, are prepared substrates that have achieved acceptable crude protein content in fruit bodies at the expense losing texture, but not firmness. Also these substrates promote brightness, and yellow-blue (b*) and red-green (a*) chromaticity of the harvested mushrooms.

Key words: Agricultural wastes; Edible mushrooms; *Pleurotus ostreatus* (Jacq.) P. Kumm.; Breaking strength; Growing media.

INTRODUCTION

The potential production of edible fungi is promising in the world market, as the current production does not reach to cover its demand. Most producing countries are importers too, since the average consumption in these countries is very high. Nutritionally, these species have moderate amounts of high quality protein, contain all essential amino acids, are rich in lysine and leucine, vitamin C, B vitamins, minerals, and trace elements. Furthermore, lipid levels are low, the ratio of saturated to unsaturated fatty acids is low, contain relatively high amounts of carbohydrates, and most species have high amounts of nutritionally valuable fibers (Chang & Miles, 1997). Complementary to its nutritional properties, there are various health benefits known in the fields of Medicine and Therapeutics, such as antitumor, antibiotic, antifungal, and anti-inflammatory effects, they are hypocholesterolemic, and they promote a healthy immune system. Additionally, they have been widely used to treat cancer and HIV (Brizuela et al., 1998). Approximately, there are around 300 species of cultivated mushrooms, but only 30 have been domesticated and just 10 are commercially grown. The most important cultivated mushroom worldwide is *Agaricus bisporus* (Lange)

Imbach, followed by *Pleurotus ostreatus* (Jacq.) P. Kumm. and other species of the genus *Pleurotus*; *Lentinula edodes* (Berkeley) Pegler is third and other edible fungi are making headways into the market.

The reason for this growth is because of the characteristics of the genus *Pleurotus* species (Sanchez, 2010): they have excellent organoleptic quality. They are easy to grow on a wide variety of substrates within a wide temperature range, and they have a great potential in bioremediation processes. Moreover, little initial capital is required to establish warehouses for growing. The preparation of the substrate does not require a lengthy complex composting, nor an application of a casing at the end of mycelial growth (such as white mushrooms), or a water immersion or dipping phase (such as shiitake). Unlike white mushrooms (*Agaricus bisporus* (Lange) Imbach), these do not require a substrate with chemical selectivity because they can grow in nutrient media with a C/N ratio between 30 and 300 (Rodriguez Barreal, 1987; Garcia Rollán, 2007). However, they need to grow in a specific biological environment with accompanying flora to protect and promote growth (Muez & Pardo, 2002). Approximately, 13,500 t of this fungus are produced in Castilla - La Mancha (67% of the national total) (Pardo et al., 2009). The Mushroom Growing Sector in Spain generates about 5 10^5 Mg of spent compost, while the EU, as a whole, produces more than 3.5 10^6 Mg (Pardo et al., 2009; Picornell et al., 2010). This lignocellulosic material called mushroom spent substrate, can be used in various fields of Agriculture (animal feed (Zadrazil, 1980), amendments (Tajbakhsh et al., 2008), substrates of nurseries, nurseries, (Medina et al., 2009), Bioremediation (Faraco et al., 2009), Aquaculture, Vermiculture and Biofuel (Pathak et al., 2009). But these uses are not enough to output the high volumes generated year after year that are accumulated in collection centers located in production areas of Spain, which can be potential contaminants, not to mention, a waste of energy. *P. ostreatus* has specific enzymes capable of degrading cellulose, lignin, phenols, and polyphenols to 60% of the original content of the spent substrates. Currently, cereal straw (wheat in particular), with increasing constraints in availability and price, is almost the only material used at an industrial scale for the production of *P. ostreatus* in Spain. The feasibility of using alternative materials of high availability and low price is a line of research of great technological interest to keep up with improve productivity and reduce processing costs (Muez & Pardo, 2002; Pardo et al., 2007, 2009; Picornell et al., 2010).

According to various studies, the most commonly profitable and readily available spent substrate which generates high quality fruit bodies for *P. ostreatus* mushroom growing (although this fungus can be grown on virtually any lignocellulosic substrate (sawdust, cereal straw, etc.)) is the trunk of *Quercus humboldtii* Bonpland specie (oak) (Garcia Rollán, 2007; among many others). Commercially in most industrial exploits, 2–4 cm long (Sanchez, 2010) pieces of winter cereal straw (wheat, barley and rye) (Savalgi & Savalgi, 1994) is used in the substrate container for the production of *Pleurotus* genus and others, such as *Pleurotus eryngii* (DC.: Fr.) Quel., *Pleurotus sajor-caju* (Fr.) Singer, *Pleurotus pulmonarius* substrate (Fr.) Quel., etc. Khanna & Garcha (1982) refer to rice straw as the best substrate for the cultivation of *Pleurotus sajor-caju* (Fr.) Singer, while wheat straw (which is similar to rice straw) is the best substrate for the cultivation of *Pleurotus* spp. (Bonatti et al., 2004). Biodegradation of these cellulosic residues by *Pleurotus* spp. growing depends on the production of hemicellulases, cellulases, and ligninases enzymes (Kurt & Buyukalaca, 2010). These enzymes, and others, turn and degrade long and insoluble components of lignocellulosic materials into

soluble components and of low molecular weight that are taken by intracellular enzymes from fungi for their nutrition. Additional, enzymes play an important role in the growth and development of fungi (Kuforiji & Fasidi, 2008). However, the lignocellulosic materials are generally low in protein content with insufficient values of nitrogen, phosphorus, and potassium (Vijay et al., 2007) mushroom cultivation. Organic supplements commonly used in the preparation of growing substrates are organic in nature (Wang et al., 2001). Among these substrates, wheat and rice bran are the most used (Wang et al., 2001; Peksen & Yakupoğlu, 2009; Kurt & Buyukalaca, 2010).

The aim of this work is the qualitative agronomic evaluation of the spent *Pleurotus* substrate (SPS), and its mixture with WS in different proportions, as lignocellulosic source in new growing cycles of *P. ostreatus*, unsupplemented and supplemented with different doses of WB.

MATERIALS AND METHODS

Analytical methodology for the characterization of materials

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

Preparation of substrates and experimental design

Source materials used in the preparation of the substrates were spent *Pleurotus* substrates remaining after the growth of *P. ostreatus* (SPS), and the mixture of WS with unsupplemented and supplemented SPS with a dose of 600 g, 1,200 g, and 1,800 g of WB. For reference, two commercial substrates from different sources were used to WS, unsupplemented and supplemented with the same doses of WB. According to the corresponding experimental design, twelve different treatments were generated, beside the two substrates corresponding to the commercial reference. In all treatments calcium sulfate was added at 50 g kg^{-1} of base material. $CaCO_3$ was not added to the 4 base substrates consisting of WS solely, whereas the remaining treatments received various amounts of $CaCO_3$ depending on the amount of SPS used (20 g kg^{-1} of SPS). $CaCO_3$ or gypsum was not added to the commercial substrates (Table 1). The first step in the preparation of the tested substrates consisted of the chopping and pre-soaking of the WS and subsequently mixing them with the substrates to adjust their moisture content. Once ready, the substrates proceeded to a pasteurizing heat treatment (60–65 °C, 8 h), and a progressive decrease of at least 15 h to a 'seeding' temperature (25 °C). Finally supplementation and 'seeding' were carried out (dose, 30 g kg^{-1} of Gurelan mycelium H-107) before manual bagging in CIES pilot plant.

Table 1. Treatments tested (g bag^{-1}) in the Experiment

TREATMENT	WS	SPS	WB	GYPSUM	$CaCO_3$
T1	6,000	0	0	300	0
T2	5,400	0	600	300	0
T3	4,800	0	1,200	300	0
T4	4,200	0	1,800	300	0
T5	3,000	3,000	0	300	60
T6	2,700	2,700	600	300	54
T7	2,400	2,400	1,200	300	48
T8	2,100	2,100	1,800	300	42
T9	0	6,000	0	300	120
T10	0	5,400	600	300	108
T11	0	4,800	1,200	300	96
T12	0	4,200	1,800	300	84
T13	Commercially controlled based substrates (A) (6.5 kg bag^{-1})				
T14	Commercially controlled based substrates (B) (6.5 kg bag^{-1})				

WS, wheat straw; **SPS**, spent *Pleurotus ostreatus* (Jacq.) P. Kumm. substrate; **WB**, wheat bran; **T**, treatment; **T1**, WS 6,000 g; **T2**, WS 5,400 g + WB 600 g; **T3**, WS 4,800 g + WB 1,200 g; **T4**, WS 4,200 g + WB 1,800 g; **T5**, WS 3,000 g + SPS 3,000 g + $CaCO_3$ 60 g; **T6**, WS 2,700 g + SPS 2,700 g + WB 600 g + $CaCO_3$ 54 g; **T7**, WS 2,400 g + SPS 2,400 g + WB 1,200 g + $CaCO_3$ 48 g; **T8**, WS 2,100 g + SPS 2,100 g + WB 1,800 g + $CaCO_3$ 42 g; **T9**, SPS 6,000 g + $CaCO_3$ 120 g; **T10**, SPS 5,400 g + WB 600 g + $CaCO_3$ 108 g; **T11**, SPS 4,800 g + WB 1,200 g + $CaCO_3$ 96 g; **T12**, SPS 4,200 g + WB 1,800 g + $CaCO_3$ 84 g; **T13**, commercially controlled based substrates (Quintanar del Rey); **T14**, commercially controlled based substrates (Villamalea).

All substrates were packed into transparent polyethylene bags of 29 cm in diameter and a height ranging from 25 to 35 cm, depending on the type of substrate, sheltering 6.5 kg approximate of weight. Four holes 2.2 cm in diameter were uniformly drilled over the side surface of each of them.

Driving and monitoring of the crop 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 range for a variety of selected mycelium and in each stage of cultivation (CIES, 2007). This tunnel-shaped greenhouse is also equipped with lights of different wave lengths (among these are 12 fluorescent Sylvania lights), and various insect traps (20 cm x 14 cm) placed throughout the greenhouse to ensure a controlled environment. Incubation of the substrates lasted approximately 17 days with no outside ventilation or lighting. During the incubation period, the relative humidity inside the greenhouse ranged between 81% and 96%, while the substrate temperature ranged between 24 °C and 32 °C, and room temperature ranged between 21 °C and 28 °C. After this, we proceeded to the induction of fruiting by ventilation (to keep CO_2 levels regulated between 0.14% and 0.10%), reduction of room temperature (23 °C to 13 °C) and substrate temperature (25 °C to 16 ºC), and the reduction of humidity (96% to 93%) and **2*** lighting. Throughout each cycle, temperature, relative humidity and CO_2 concentration were automatically recorded by various instruments within the

greenhouse. These values are close to the microclimatic conditions recommended by other researchers (Pardo et al., 2005b; Garcia Rollán, 2007; Pardo et al., 2007; Gregori et al., 2008; López-Rodríguez et al., 2008; Gea et al., 2009; Kurt & Buyukalaca, 2010).

Evaluation of qualitative parameters

For the evaluation of quality parameters mushrooms of uniform size maturity were used and selected on the most optimal day of the harvest period. The color of the surface of fruit bodies was measured by reflection using a Minolta brand colorimeter, model CR-300, previously calibrated with a calibration plate CR-A43 (L* = 96.12, a* = -0.11, b* = +2.66) and illuminant D65. To evaluate the mechanical properties of mushrooms, in terms of firmness, an analyzer (TA-XT Plus of Stable Micro Systems) was used. To take this measure the fruit bodies were cut into small pieces (4 cm^2, approximately) and were introduced into the 5-bladed Kramer Shear Cell (KS5), arranged in two adjacent uniform layers and performed the test at a constant speed of 2 mm s^{-1}; thus breaking strength (Bs) was obtained, defined as the maximum force required to tear the fruit bodies (expressed in N). Protein content in the carpophores was calculated by multiplying the total nitrogen content by a conversion factor of 4.38 (Delmas, 1987). Total nitrogen content was determined by the Kjeldahl method (Tecator, 1989; MAPA, 1994). To determine the ash content of the fruiting bodies, we proceeded to direct calcination of the samples at 540 °C (MAPA, 1994).

Mushrooms were harvested daily at their optimal commercial development. The quantity of 'cones' and mushrooms harvested were determined by counting throughout the whole 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. The estimated net yield was performed by weighing the fruit bodies after removing the stipe and calculating the percentage of shrinkage resulting from this operation. Once fruiting occurred, the biological efficiency (BE) was calculated and expressed as a percentage of the fresh weight of the harvest over the dry weight of the substrate 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 their moisture content. The unit weight of mushrooms (gross and net) was determined from the yields obtained and the quantity of sporophores harvested.

4* Statistical analysis

The corresponding experimental design of this trial was a Balanced Plan Factorial Design 3 x 4 with 6 replicates (randomized block factorial with two factors).

To carry out the statistical analysis, two software packages were used: Statgraphics® Plus version 5.1 (Statistical Graphics Corp., 2001) and SPSS® (SPSS, 2004). Descriptive statistical techniques, principal component analysis, variance analysis and correlation and regression methods were used to evaluate the data.

Differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Analytical characterization of base material used and substrates made

The research was conducted over an 80 day cycle, similar to Gea et al. (2009). Additionally, this cycle was longer than the cycle used in Pardo et al. (2007) with 69 days, but shorter than the total cultivation time of *P. ostreatus* in Sales-Campos et al. (2010) with 100 days. In each treatment in this experiment, the third flush did not show results of agronomic interest. The total gross yield was concentrated in the first two flushes.

Gregori et al. (2008), during the cultivation of *P. ostreatus* maintained CO_2 concentration at approximately 1,300 ppm and light cycles of 10 h (both very similar to those used in this experiment). Bermúdez et al. (2002) demonstrated that the BE and yield of *P. ostreatus* cultivated with cocoa waste are reduced between 68% and 63% respectively when the mushroom is exposed to light for less than 12 hours during its' fructification phase.

The chemical characteristics results of the different source materials, substrates made and commercially controlled based substrates tested are shown in Table 2. The processed substrates show great variability in most of analytical parameters tested (Table 2). Within the same group of processed the substrates (WS, WS + SPS and SPS) with increasing the dose of WB, the analytical parameters increase as well; however, these increases stop when the dose of WB reaches to 1,800 g in the physicochemical characteristics of substrates made.

Substrates prepared from WS and WB at doses of 1,200 g and 1,800 g (T3 and T4) have higher total nitrogen, protein and ash contents than commercial substrates (T13 and T14). The substrate made by T4 has the highest NDS content (23.04%), due to its high hemicellulose content (25.63%). The substrate made by T3 has the second highest NDS content (18.37%) because of its high lignin (6.94%), and ash content (24.11%). Compared with commercial substrates, these substrates (T3 and T4) reach higher hemicellulose values, lower lignin values, and similar cellulose and NDS values. As for the developed substrates formed with WS + SPS in the same proportions, but decreasing, and with WB and $CaCO_3$ as supplements in increasing amounts (T5 to T8), these substrates reach higher values of pH and a similar moisture level to commercial substrates (T13 and T14) (Table 2); ash content is higher than commercial substrates (T6, 28.25%; T14, 9.51%) with a lower content of organic matter (T7, 76.94%; T13, 92.94%). The third group of substrates made, is formed by a mixture of SPS (in decreasing amounts) + WB (in increasing amounts) + $CaCO_3$ (in decreasing amounts) (T9 to T12). This group exhibits a pH and moisture content very close to those corresponding to the commercial substrates. However, total nitrogen and protein content of the treatments with higher doses of WB (T11, 10.30%, 64.60%, respectively; T12, 12.70%, 79.20%, respectively) expressed higher values than the reference commercial substrates. This superiority is manifested also in ash content, in all different treatments of this group.

Crude fiber, cellulose and lignin content of all tested substrates were inferior in all cases showed by the commercial control (a situation related to the lower content of organic matter).

Table 2. Elaborate physicochemical characterization of source materials and substrates used

		pH (aq. 1:5, p v^{-1})	Moisture (g kg^{-1})	Total nitrogen (g kg^{-1}, d.m.)	Protein (g kg^{-1}, d.m.)	Ash (g kg^{-1}, d.m.)	Organic Matter (g kg^{-1}, d.m.)	C/N ratio	Crude fiber (g kg^{-1}, d.m.)	Crude fat (g kg^{-1}, d.m.)	NFE (g kg^{-1}, d.m.)	Cellulose (g kg^{-1}, d.m.)	NDS (g kg^{-1}, d.m.)
BASE MATERIALS	**WS**	5.85	685	3.5	21.9	76.0	924.0	153.1	391.4	6.9	503.8	407.8	156.8
	SPS	5.50	689	4.8	30.0	89.0	911.0	110.1	190.2	6.6	684.2	453.1	166.0
	WB	6.64	112	23.9	149.4	61.8	938.2	22.8	137.3	30.0	621.5	134.1	353.8
SUBSTRATES MADE	**T1**	7.70	713	3.6	22.5	199.3	800.8	129.0	355.8	5.8	416.6	324.5	156.3
	T2	8.09	733	7.3	45.3	226.3	773.8	61.9	334.2	10.4	383.8	295.0	165.6
	T3	8.36	743	11.6	72.6	241.1	758.9	37.9	306.6	13.8	365.9	262.8	183.7
	T4	7.11	714	16.4	102.4	218.9	781.1	27.7	261.4	16.5	400.9	225.4	230.4
	T5	7.37	711	4.7	29.6	247.3	752.7	92.2	322.0	5.5	395.6	299.1	186.1
	T6	8.16	725	8.0	49.7	282.5	717.5	52.3	301.4	10.2	356.3	289.4	166.1
	T7	8.28	739	11.2	70.2	230.6	769.4	39.7	287.3	13.6	398.3	270.1	200.1
	T8	8.03	713	13.7	85.7	268.7	731.4	30.9	264.9	16.2	364.6	227.6	210.5
	T9	7.19	704	4.0	25.0	281.6	718.4	104.2	320.0	5.3	368.2	309.0	147.8
	T10	7.81	718	7.8	48.6	234.5	765.6	57.0	322.6	9.9	384.4	317.9	167.9
	T11	8.26	709	10.3	64.6	267.6	732.4	41.1	294.5	13.3	359.9	268.5	179.3
	T12	8.17	715	12.7	79.2	252.1	747.9	34.2	276.7	16.0	376.1	248.8	209.1
	T13	8.08	735	8.2	51.1	70.6	929.4	65.9	448.4	14.1	415.7	389.1	181.3
	T14	7.94	711	8.0	49.9	95.1	904.9	65.7	404.7	12.9	437.4	383.1	169.7
	Average	7.90	720.21	9.1	56.9	222.6	777.4	59.9	321.5	11.7	387.4	293.6	182.4
	CV (%)	5.17	1.73	41.2	41.3	28.7	8.2	50.0	16.3	33.7	6.3	16.9	12.7

WS, wheat straw; **SPS**, spent *Pleurotus ostreatus* (Jacq.) P. Kumm. substrate; **WB**, wheat bran; **T**, treatment; **T1**, WS 6,000 g; **T2**, WS 5,400 g + WB 600 g; **T3**, WS 4,800 g + WB 1,200 g; **T4**, WS 4,200 g + WB 1,800 g; **T5**, WS 3,000 g + SPS 3,000 g + $CaCO_3$ 60 g; **T6**, WS 2,700 g + SPS 2,700 g + WB 600 g + $CaCO_3$ 54 g; **T7**, WS 2,400 g + SPS 2,400 g + WB 1,200 g + $CaCO_3$ 48 g; **T8**, WS 2,100 g + SPS 2,100 g + WB 1,800 g + $CaCO_3$ 42 g; **T9**, SPS 6,000 g + $CaCO_3$ 120 g; **T10**, SPS 5,400 g + WB 600 g + $CaCO_3$ 108 g; **T11**, SPS 4,800 g + WB 1,200 g + $CaCO_3$ 96 g; **T12**, SPS 4,200 g + WB 1,800 g + $CaCO_3$ 84 g; **T13**, commercially controlled based substrates (Quintanar del Rey); **T14**, commercially controlled based substrates (Villamalea). **CV**, coefficient of variation; **NFE**, nitrogen free extractives; **NDS**, neutral detergent-soluble. Results expressed in **g kg^{-1} dry matter**, except pH, moisture (fresh matter) and C/N ratio.

Production qualitative parameters. Descriptive statistics and ANOVA

In Table 3 descriptive statistics of crude protein and ash contents, as well as brightness values (L*), yellow-blue (b*) and red-green (a*) chromaticity color, Bs, and C_E in the harvested mushrooms are shown. Of the fourteen different treatments that have been generated with different combinations, including commercially controlled based substrates, in T8 (WS 2,100 g + SPS 2,100 g + WB 1,800 g + $CaCO_3$ 42 g) did not develop the mycelium due to difficulties in germination resulting in no production of mushrooms. Most likely, this accident was caused by an improper manual aggregation of high doses of WB, which led to a reduction in the pore spaces in the prepared substrate. This reduction caused a rise in temperature to the point of inoculation and agglomeration which reduced gas exchange with increased levels of CO_2 and the inhibition of mycelial growth. Due to these reasons, the substrate of T8 was not considered in the statistical analysis of this Experiment. When the substrates were supplemented with doses of 600 g of WB (T2, T6 and T10), a higher crude protein content was obtained in mushrooms than in the respective unsupplemented substrates (T1, T5 and T9) and in the commercial substrates (T13 and T14). Only the dose of 1,800 g of WB has shown the lowest contents of crude protein. Wheat straw + SPS and SPS do not improve the crude protein content relative to WS (there are not significant differences). Organic supplements such as soybean meal, alfalfa, flour, and cotton seed powder not only increase yields, but also, the protein content of mushrooms (Zadrazil, 1980).

Some researchers give values of crude protein content of fruit bodies of *P. ostreatus* varying between 17.80% and 34.10% (Benavides & Herrera, 2009; Rodríguez Barreal, 1987; Wang et al., 2001). Protein content in the carpophores of *P. ostreatus* with substrates based on alder tree sawdust supplemented with leaves of two different species of *Ginkgo biloba* L. according to Siwulski et al. (2009), depended on the number of sheets added of *G. biloba* L. to the growing substrate but not on the botanical species (between 17.30% and 21.10%). Higher values in *P. ostreatus* were obtained by Pardo et al. (2005a) in terms of protein content depending on the substrate type, treatment, and mycelium used: between 14.04% (straw, benomyl dip and pasteurization, Gurelan mycelium) and 17.75% (straw + vine shoot 1: 1 (v/v)), pasteurization and thermophilic conditions, Gurelan mycelium); substrate used: between 15.14% (straw) and 17.30% (straw + kenaf); treatment used: between 16.54% (pasteurization and thermophilic conditions) and 16.52% (benomyl dip and pasteurization); and mycelium used: between 16.74% (Amycel mycelium) and 16.33% (Gurelan mycelium). Lower values than those presented in this Experiment were obtained by Pardo et al. (2005b). In another further investigation they reached crude protein content in mushrooms of 22.40% (grape stalk + 'alperujo') from 11.80% (grape stalk + straw). When analyzing the studied substrates treatments, pasteurization and thermophilic conditions improved significantly (20.80%) compared to benomyl dip and pasteurization (19.20%). Although statistically insignificant, there were different values depending on the format of packaging: 20.60% in bags of 15 kg and 19.20% in bags of 5 kg. Pardo et al. (2007) obtained higher values of *P. ostreatus* depending on the different types of substrate and treatment used: between 19.07% (straw, pasteurization and thermophilic conditions) and 23.98% (straw + kenaf 1: 1 (v/v), benomyl dip and pasteurization); type of substrate used: between 20.61% (straw + 'alperujo' 1: 1 (v/v)) and 22.90% (straw + kenaf 1: 1 (v/v)); type of treatment used: between 20.30% (pasteurization and thermophilic conditions) and 21.70%

(benomyl dip and pasteurization). Higher values than those obtained in this Experiment were obtained by Bermúdez et al. (2007) on *Pleurotus* spp. in blends of coffee pulp + cedar chips with strains of CCEBI 3021 and CCEBI 3027: 27% and 34%, respectively; substrates on coffee pulp: 30% and 38%, respectively; and substrates based on cedar chips: 21% and 22%, respectively. Fonseca et al. (2009) also achieved slightly higher values in protein content than in this Experiment using a substrates mixture of rice bran (40%), rice straw (35%) and *Juncus effusus* L. (25%) in the growing of *P. ostreatus* (30.52%). These authors claim that the determined digestibility for *P. ostreatus* is comparable to that of *P. sajor-caju* (Fr.) Singer. Varnero et al. (2010), growing *P. ostreatus* on different lignocellulosic substrates, reached values of total protein content in mushrooms that ranged from 22.90% (aspen shavings) to 25.60% (WS); although these differences were not statistically significant. In *P. sajor-caju* (Fr.) Singer specie (Oyetayo & Akindahunsi, 2004), working with shredded ears, reached values of mushroom protein content of 17.49% vs. 14.94% in substrate without supplement. In *P. eryngii* (DC.: Fr.) Quel., Hassan et al.(2010) give results with significant differences for sawdust (22.17%), soybean straw (24.08%), sugarcane bagasse (21.33%) and rice straw (22.75%). Manzi et al. (2004) indicate default values for the fruiting bodies of *P. eryngii* (DC.: Fr.) Quel. of: dry matter (13.40%), fat (0.80%), crude protein (2.20%) and ash (1.20%); all with respect to fresh weight of fruit body. It is important to note how protein content during the growth of *P. ostreatus* on different substrates increases the most after the first harvest or post-harvest, according to the prepared mixtures (Kurt & Buyukalaca, 2010). These researchers obtained the maximum postharvest with sawdust and bran (2:1) with a figure of 5.23 mg mL^{-1}; while lower values were reached with the mixture of grape vine + WB (2:1), where they obtained only contents of 2.38 mg mL^{-1} (sesame straw) and 9.75 mg mL^{-1} (sawdust + WB (2:1)). In *P. sajor-caju* (Fr.) Singer, postharvest, mushrooms protein content ranged from 3.29 mg mL^{-1} (rice straw) to 8.13 mg mL^{-1} (WS), while after the first harvest, with lower figures oscillation was between 2.46 mg mL^{-1} (grapevines) and 5.92 mg mL^{-1} (sawdust and rice bran (2:1)). In other edible fungi, for instance, *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Peksen & Yakupoğlu, 2009), higher crude protein contents were reached when combined with supplements: sawdust (75%) + waste tea (20%) obtained values of 20.17% and 12.52% depending on the strain studied. When tea waste content was reduced to 10%, crude protein contents of 13.24% and 13.94% were obtained. When WB participated in 18% in the mix with sawdust, depending on mycelium, values of 12.91% and 16.93% were obtained.

The highest values of ash content in harvested mushrooms in this current Experiment are presented in substrates composed of WS + SPS (T5 to T7): from 6.76 g $(100\ g)^{-1}$ (T7) to 8.11 g $(100\ g)^{-1}$ (T6). Substrates supplemented with WB (dose of 600 g and 1,200 g) have a greater ash content in their mushrooms than commercial substrates (T13 and T14), although T7 substrate (6.76 g 100 g^{-1}) has a smaller ash content than the T13 commercial substrate (7.17 g $(100\ g)^{-1}$). As with crude protein content of the harvested mushrooms, only the dose of 1,800 g of WB in this study, has the lowest ash content. Standard means values given by Benavides & Herrera (2009) and Rodriguez Barreal (1987) are included in the ranges given above. This interval is extended by others working with *P. ostreatus* (Manzi et al., 1999; Wang et al., 2001; Shashirekha et al., 2002); these researchers reached values of ash content in mushrooms between 6.70% and 15.40%. Baena (2005), growing oyster mushroom in green bagasse

manguey obtained ash content values in fruit bodies from 3.77% to 8.73% and Siwulski et al. (2009), in substrates based on sawdust alder supplemented with leaves of two different species of *Ginkgo biloba* L., concluded that the addition of the leaves of this plant does not affect the ash content: green leaves, between 6.90% (no leaves and a 10% of leaves) and 7% (a 1% of leaves); and yellow leaves, from 6.80% (no leaves) and 7.20% (with a 10% of leaves).

Table 3. ANOVA of the qualitative parameters of the Experiment. **5***

Sub-strate	Crude protein (g 100 g^{-1})	Ash contents (g 100 g^{-1})	Color			B_S (N)	C_E (mJ)
			L*	a*	b*		
T1	19.69abc	8.02a	62.99a	3.00b	12.59ab	250.93abc	1,022.77abcd
T2	23.05a	7.46a	63.22a	1.64d	10.25ab	142.27bcde	575.65bcde
T3	14.18abc	4.05abc	39.27ab	0.54e	5.67bc	96.25de	435.70de
T4	4.50c	1.38c	13.09b	0.18e	1.89c	32.08e	145.23e
T5	22.44a	7.01a	64.31a	2.92b	12.73ab	277.75a	1,241.95a
T6	24.49a	8.11a	73.76a	2.31bcd	12.90a	266.90ab	1,099.58abc
T7	21.03ab	6.76a	64.54a	1.66d	12.55ab	126.65cde	546.07cde
T9	19.30abc	7.88a	65.68a	2.78bc	12.40ab	305.12a	1,204.23a
T10	23.19a	7.67a	69.50a	2.28bcd	12.01ab	286.70a	1,217.95a
T11	26.30a	7.36a	78.60a	1.81cd	13.31a	219.28abcd	951.33abcd
T12	5.29bc	1.45bc	13.09b	0.18e	1.89c	32.08e	145.23e
T13	18.72abc	7.17a	62.02a	3.33ab	12.99a	262.95ab	1,376.38a
T14	17.39abc	6.19ab	60.29a	4.21a	13.76a	274.05a	1,170.43ab
Average	18.43	6.19	56.18	2.07	10.38	197.92	856.35
Fisher F	4.09	5.75	5.88	30.35	8.66	14.77	12.15
S_L	0.00***	0.00***	0.00***	0.00***	0.00***	0.00***	0.00***

WS, wheat straw; **SPS**, spent *Pleurotus ostreatus* (Jacq.) P. Kumm. substrate; **WB**, wheat bran; **T**, treatment; **T1**, WS 6,000 g; **T2**, WS 5,400 g + WB 600 g; **T3**, WS 4,800 g + WB 1,200 g; **T4**, WS 4,200 g + WB 1,800 g; **T5**, WS 3,000 g + SPS 3,000 g + $CaCO_3$ 60 g; **T6**, WS 2,700 g + SPS 2,700 g + WB 600 g + $CaCO_3$ 54 g; **T7**, WS 2,400 g + SPS 2,400 g + WB 1,200 g + $CaCO_3$ 48 g; **T8**, WS 2,100 g + SPS 2,100 g + WB 1,800 g + $CaCO_3$ 42 g; **T9**, SPS 6,000 g + $CaCO_3$ 120 g; **T10**, SPS 5,400 g + WB 600 g + $CaCO_3$ 108 g; **T11**, SPS 4,800 g + WB 1,200 g + $CaCO_3$ 96 g; **T12**, SPS 4,200 g + WB 1,800 g + $CaCO_3$ 84 g; **T13**, commercially controlled based substrates (Quintanar del Rey); **T14**, commercially controlled based substrates (Villamalea); **L*,** brightness; **a***, red-green color components; **b***, yellow-blue color components; **B_S**, breaking strength; **C_E**, compression energy, **S_L**, F significance level Fisher.
*** P-value < 0,001. For each column, values followed by different letters are significantly different from each other (p = 0.05, Tukey-HSD).

Pardo et al. (2005a), also in *P. ostreatus*, got different values in ash content of the fruit bodies, depending on the substrate type, treatment thereof, and mycelium used: cereal straw subjected to benomyl dip and pasteurization, between 6.83% (Gurelan mycelium) and 7.70% (Amycel mycelium); substrate used: between 7.16% (straw) and 7.44% (straw + kenaf 1:1 (v/v)); treatment used: between 7.33% (pasteurization and thermophilic conditions) and 7.24% (benomyl dip and pasteurization); and mycelium used: between 7.38% (Amycel mycelium) and 7.19% (Gurelan mycelium). In a subsequent experiment, Pardo et al. (2005b) present ash content values in mushrooms ranging from 6% (grape stalk + 'alperujo', pasteurization and thermophilic conditions and packaging in bags of 15 kg) and 7.50% (grape stalk + vine shoot, pasteurization and thermophilic conditions and packaging of 15 kg); when, as a main factor of ANOVA,

only the substrate type is considered, consisting of grape stalk + 'alperujo' provides mushrooms with an ash content of 6.40% and, on the other hand, if the substrate was grape stalk + vine shoot the mixture from which the highest ash content is obtained (7.30%); treating substrates by pasteurization and thermophilic conditions favors this qualitative parameter production (7%) significantly against the treatments of immersion, benomyl dip, and pasteurization (6.60%); format packaging substrate (sacks of 5 kg and 15 kg) produced no significant difference: 6.70% and 6.90% respectively. Pardo et al. (2007), also in *P. ostreatus*, achieve different ash values in the carpophores, depending on the substrate type and treatment used: between 6% (straw, semi-anaerobic fermentation) and 7.14% (benomyl dip and pasteurization in substrate based on straw + vine shoot 1:1 (v/v) and in substrate based on straw + alperujo 1:1 (v/v)); substrate type used: between 6.54% (straw) and 6.88% (straw + vine shoot 1:1 (v/v)); treatment type used, between 6.47% (pasteurization and thermophilic conditions) and 7% (benomyl dip and pasteurization). Forero et al. (2008), also with *P. ostreatus*, but on waste chili, give a range of values between 8.81% and 9.84%, justifying these figures by the high ash content of supplied based substrates. Fonseca et al. (2009), using a mixture of substrates (rice straw, 35%; *Juncus effusus* L., 25% and rice bran, 40%), only reached an ash content of 6.38% in *P. ostreatus* mushrooms.

In other species of the genus *Pleurotus* as *P. eryngii* (DC.: Fr.) Quel., Manzi et al. (1999), in WS + sugar beets (15%) obtained ash content between 6.90% and 10.50%; Manzi et al. (2004) in the same fungus species, obtained ash content values of the carpophores of 1.20% based on a fresh product with 86.60% moisture. Also in *P. eryngii* (DC.: Fr.) Quel., Hassan et al. (2010) presented similar values to those achieved in the present Experiment: sawdust (6.94%), soybean straw (7.66%), sugarcane bagasse (6.54%) and rice straw (8.02%). In another of species of *Pleurotus*, specifically *P. sajor-caju* (Fr.) Singer, Oyetayo & Akindahunsi (2004) reached ash content values of 10.51% in mushrooms when the growing was carried out in substrates of grated ears, which decays to 7.41% if there is no such supplementation. In the consulted literature, there were significantly lower ash values when other species of edible mushrooms and substrates were used; for instance, Peksen & Yakupoğlu (2009), growing *Ganoderma lucidum* (Curt.: Fr.) P. Karst., reached values comprised between 2.09% (sawdust, 80%, and tea residue, 20%) and 4.67% (sawdust, 80% WB, 18%); these researchers found that the mycelium types have a great influence on the ash content of the fruiting bodies too.

There are significant differences in the brightness of the harvested mushrooms. Of all the tested treatments (between 60.29 and 78.60), the worst values were exhibited by a supplementation of 1,800 g of WB (T4, L* = 13.09). Rodriguez Estrada et al. (2009) investigated in mushrooms of *P. eryngii* var. *erynngii* grown on substrates made of straw; these researchers achieved brightness values in mushrooms ranging from 54.70 (substrate covered by a shell) to 74.10 (substrate not covered). Also, supplementation with WB reduced the value of red-green color components of mushrooms (a*) as WS as in the mixture of WS + SPS and SPS, and the reduction was greater as the dose is increased. This trend is also reflected in the value of yellow-blue color components of mushrooms (b*) (Table 3).

Mushrooms supplemented with 1,800 g of WB the worst texture and firmness significantly showed. There is a tendency, although not statistically significant, to decrease both parameters with each increasing dose of bran. Texture (hardness, cohesiveness, springiness and chewiness) was controlled in *Pleurotus* spp. grown on rice

straw (Kotwaliwale et al., 2007); according to these authors, an increase of hardness, chewiness, and a decrease in cohesion and elasticity of the mushrooms can be attributed to a migration loss of moisture inside of them. Prepared unsupplemented substrates and commercial substrates, although statistically insignificant, obtained the highest values of Bs and compression energy (C_E).

Correlation matrix and 'step by step' regression models

In Table 4 is presented the correlation matrix between qualitative parameters of production and physicochemical properties of the substrates prepared in Experiment. All quality parameters of harvested mushrooms that have been analyzed are significantly and negatively correlated with total nitrogen contents, crude fat and NDS values of the substrates tested, but positively correlated with crude fiber and cellulose contents.

Table 4. Correlation matrix between production of quantitative parameters and physicochemical characteristics of the substrates

	Crude protein (g 100 g^{-1})	**Ash contents (g 100 g^{-1})**	**B_s (N)**	**C_E (mJ)**	**L***	**a***	**b***
pH	0.246 (0.466)	0.063 (0.853)	-0.243 (0.471)	-0.229 (0.498)	0.169 (0.620)	-0.265 (0.431)	0.062 (0.856)
Nitrogen$_T$[1]	-0.656* (0.028)	-0.815** (0.002)	-0.853*** (0.001)	-0.836*** (0.001)	-0.697* (0.017)	-0.922*** (0.000)	-0.756** (0.007)
C/N ratio	0.362 (0.274)	0.596 (0.053)	0.689* (0.019)	0.663* (0.026)	0.435 (0.181)	0.819** (0.002)	0.546 (0.083)
Crude fiber[1]	0.588 (0.057)	0.731** (0.011)	0.665* (0.025)	0.650* (0.030)	0.596 (0.053)	0.756** (0.007)	0.617* (0.043)
Crude fat[1]	-0.578 (0.062)	-0.757** (0.007)	-0.861*** (0.001)	-0.847*** (0.001)	-0.631* (0.037)	-0.927*** (0.000)	-0.711** (0.014)
NFE[1]	-0.211 (0.534)	-0.075 (0.825)	-0.118 (0.729)	-0.111 (0.744)	-0.190 (0.576)	0.139 (0.683)	-0.050 (0.885)
Cellulose[1]	0.700* (0.016)	0.853*** (0.001)	0.861*** (0.001)	0.843*** (0.001)	0.737** (0.010)	0.896*** (0.000)	0.773** (0.005)
NDS[1]	-0.713** (0.014)	-0.847*** (0.001)	-0.809** (0.003)	-0.772** (0.005)	-0.755** (0.007)	-0.778** (0.005)	-0.732** (0.010)

Bs, breaking strength; **C_E**, compression energy; **L*,** brightness; **a***, red-green color components; **b***, yellow-blue color components; **Nitrogen$_T$**, total nitrogen; **NFE**, nitrogen free extractives; **NDS**, neutral detergent-soluble; **[1], g kg^{-1} dry matter.**

Results in parentheses indicate statistical significance. No significant ($p > 0.05$) (non *); significant at 95% ($0.01 < p \leq 0.05$) (*); significant at 99% ($0.001 < p \leq 0.01$) (**); 99.9% significant ($p \leq 0.001$) (***).

Table 5 presents the correlation matrix between production and qualitative parameters germination index, the earliness and quantitative production parameters. All correlations obtained between germination index, earliness, number of mushrooms and biological efficiency and the quality parameters of harvested mushrooms are significant, although with varying degrees of significance, but with positive coefficients. The same is true when considering the correlation matrix between qualitative production parameters (Table 6), it is to say, there is a statistical significance between them with positive coefficients.

Table 5. Correlation matrix between production of qualitative parameters and the rate of germination, earliness, and production of quantitative parameters

	Crude Protein (g 100 g^{-1})	**Ash contents (g 100 g^{-1})**	**B_s (N)**	**C_E (mJ)**	**L***	**a***	**b***
Germination Index	0.920*** (0.000)	0.943*** (0.000)	0.905*** (0.000)	0.905*** (0.000)	0.929*** (0.000)	0.882*** (0.000)	0.922*** (0.000)
1^{rs} Flush 'Seeding'	0.948*** (0.000)	0.926*** (0.000)	0.722** (0.012)	0.722** (0.012)	0.958*** (0.000)	0.730** (0.011)	0.926*** (0.000)
Total 'Seeding'	0.974*** (0.000)	0.985*** (0.000)	0.841*** (0.001)	0.837*** (0.001)	0.991*** (0.000)	0.848*** (0.000)	0.974*** (0.000)
Total quantity of mushrooms	0.731** (0.011)	0.841*** (0.001)	0.963*** (0.000)	0.958*** (0.000)	0.779** (0.005)	0.920*** (0.000)	0.812** (0.002)
BE	0.631* (0.037)	0.741** (0.009)	0.929*** (0.000)	0.937*** (0.000)	0.671* (0.024)	0.908*** (0.000)	0.734** (0.010)

B_S, breaking strength; **C_E**, compression energy; **L*,** brightness; **a***, red-green color components; **b***, yellow-blue color components; **BE**, biological efficiency (kg 100 kg^{-1} of dry substrate).
Results in parentheses indicate statistical significance. Significant at 95% ($0.01 < p \leq 0.05$) (*); significant at 99% ($0.001 < p \leq 0.01$) (**); 99.9% significant ($p \leq 0.001$) (***).

Table 6. Correlation matrix between production of qualitative parameters

	Crude protein (g 100 g^{-1})	**Ash contents (g 100 g^{-1})**	**B_s (N)**	**C_E (mJ)**	**L***	**a***	**b***
Crude protein (g 100 g^{-1})	1.000						
Ash contents (g 100 g^{-1})	0.945*** (0.000)	1.000					
B_s (N)	0.783** (0.004)	0.875*** (0.000)	1.000				
C_E (mJ)	0.793** (0.004)	0.863*** (0.001)	0.996*** (0.000)	1.000			
L*	0.987*** (0.000)	0.972*** (0.000)	0.837*** (0.001)	0.839*** (0.001)	1.000		
a*	0.775** (0.005)	0.901*** (0.000)	0.941*** (0.000)	0.935*** (0.000)	0.829** (0.002)	1.000	
b*	0.949*** (0.000)	0.972*** (0.000)	0.865*** (0.001)	0.867*** (0.001)	0.979*** (0.000)	0.901*** (0.000)	1.000

B_S, breaking strength; **C_E**, compression energy; **L*,** brightness; **a***, red-green color components; **b***, yellow-blue color components; **BE**, biological efficiency (kg 100 kg^{-1} of dry substrate).
Results in parentheses indicate statistical significance. Significant at 99% ($0.001 < p \leq 0.01$) (**); significant at 99.9% ($p \leq 0.001$) (***).

Table 7 presents the 'step by step' regression models for qualitative production parameters depending on the physicochemical properties of the substrates made, germination index, earliness and production characteristics of quantitative parameters. It is observed that the values of NDS made substrates define models that explain the variability of Bs and yellow-blue color components (b*) of harvested mushrooms. Also, as an independent variable, with a positive coefficient, the cellulose content of the prepared substrates defines the variability of the ash content of the harvested fruit bodies.

Table 7. Models obtained by regressing 'step by step'

Explained variable	Independent variable	Equation	R^2 corrected	SE
Ash contents	PCC + QPP + CPP	Ash contents = -5.153*** + 0.137*** P4 + 0.020*** · cellulose	99.10***	0.24145
B_s	PCC + QPP + CPP	B_S = 86.865* + 0.223*** · C_E – 0.417* · NDS	99.40***	7.63723
C_E	PCC + QPP + CPP (– B_S)	C_E = 238.289** + 25.933*** · Nº mushrooms	90.80***	128.71255
L	PCC + QPP + CPP	L* = -5.723** + 0.985*** · P4 + 2.982** · DM	99.30***	1.96105
a*	PCC + QPP + CPP	a* = -5.42**1 + 0.010*** · B_S + 0.014** · NFE	93.70***	0.26359
b*	PCC+ QPP + CPP	b* = -6.005* + 0.154*** · L* + 1.502*** · a* + 0.026* · NDS	98.80***	0.49742

R^2, determination coefficient (%); **SE**, standard error of the estimate.
PHYSICO-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), cellulose (g kg^{-1}, odm), neutral-detergent soluble (NDS; g kg^{-1}, odm), odm, on dry matter.
INDEX GERMINATION, 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 mushrooms (quantity of mushrooms), biological efficiency (BE, kg 100 kg^{-1} of dry substrate).
QUALITATIVE production parameters (CPP): average unit weight of uncut mushrooms (g), dry matter (DM, g 100 g^{-1}), crude protein (g 100 g^{-1}), ash contents (g 100 g^{-1}), **B_S**, breaking strength; **C_E**, compression energy; **L*,** brightness; **a***, red-green color components; **b***, yellow-blue color components.
Significativo al 95% ($0,01 < p \leq 0,05$) (*); significativo al 99% ($0001 < p \leq 0,01$) (**); significativo al 99,9% ($p \leq 0,001$) (***). Regressions include only those whose coefficients accompanying the independent variables are significant, provided that the significance of the model is significant.

CONCLUSIONS

SPS unsupplemented, SPS supplemented with 600 g of WB, mixture WS + SPS unsupplemented and mixed WS + SPS supplemented with 600 g of WB, are produced substrates which have achieved acceptable crude protein contents in fruiting bodies, although with a loss in texture but not firmness. These substrates also favor brightness, and red-green (a*) and yellow blue (b*) chromacity color of the harvested mushrooms. Consequently these compost formulations based on degraded *Pleurotus ostreatus* could be a low cost substrate, with selective and balanced nutrients for the growth and development of oyster mushrooms.

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