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Bioconversion of agrowastes by *Lentinula edodes*: the high potential of viticulture residues

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Abstract The production of four strains of edible mushroom *Lentinula edodes* was evaluated through solid-state fermentation (SSF) of vineyard pruning (VP), barley straw (BS), and wheat straw (WS). Biological efficiency, proximal composition, and energy value of the fruiting bodies, as well as substrate chemical changes after harvest, were determined. The shortest primordium formation time (28 days), highest biological efficiency (93.25%), highest yield (37.46%), and shortest production cycle (6 days) were observed in VP. The fruiting bodies obtained from VP had high energy value (379.09 to 392.95 kcal) and contents of protein (12.37 to 17.19%), but low contents of fat (1.82 to 2.15%). After SSF, phenol concentration decreased on VP (1.2 mmol/L) and BS (0.31 mmol/L), but on WS remained practically the same. Hemicellulose decreased in all substrates; cellulose increased on WS and decreased in the rest of the treatments. Lignin decreased on WS and BS, but its concentration increased on VP. The variability observed in the degradation capacity of lignocellulosic components was influenced by the substrate's nature, environmental factors, and genetic factors among strains. VP has great potential for shiitake production due to its low cost, short production cycles, and high biological efficiency.

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Introduction

Fungi are primary causative agents of organic matter disintegration. Many fungi are important for their role in the decomposition of plant residues, releasing available nutrients and carbon dioxide for the plants. Basidiomycetes are key organisms in the degradation of the plant's primary cell wall and have been used biotechnologically to obtain protein-rich biomass for human and animal consumption as well for bioremediation processes (Abdullan and Iqbal-Zafar 1999). *Lentinula edodes* (Berk.) Pegler, known as shiitake, produces hydrolytic and oxidative enzymes that are responsible for the selective degradation of organic substrates. The production of enzymes is specifically related to and dependent on substrate composition and environmental factors such as temperature and moisture. The bioconversion of agricultural residues produces a strong environmental impact by avoiding waste accumulation.

Due to this biodegradative characteristic, *L. edodes* has been traditionally cultivated on hardwood logs, mainly oak, to obtain fruiting bodies for human consumption (Kozak and Krawczyk 1993; Sobata and Nall 1994). However, this cultivation system represents a limiting factor and potential danger to the environment due to the slow growth rate and the overuse of the oak, jeopardizing the population of this important forest element. Thus, efforts to develop a more efficient, faster, and more reliable production system have focused on the use of an enriched sawdust substrate (Przybylowicz and Donoghue 1990). Shiitake is the most important mushroom among the species industrially cultivated. In 1997, the production worldwide was more than 1,564,000 tons, with China, Japan, Taiwan, and Korea being the main producing countries (Chang and Miles 2004; Lin et al. 2000; Savoie et al. 2000). Currently, in addition to having a wide market for direct consumption, it contains bioactive compounds that

are important for the pharmacology, food, and cosmetology industries (Kües and Liu 2000).

Experimental cultivation of shiitake has been developed using shavings, e.g., *Carpinus*, *Bursera*, *Alnus*, and *Eliocarpus* (Mata et al. 1990; Morales and Martínez-Carrera 1991; Morales et al. 1991), and even coffee pulp and sugar cane bagasse (Mata and Gaitán-Hernández 1992, 1994; Salmones et al. 1999) in Mexico. Although the availability of some of these substrates is limited, there are other lignocellulosic residues such as straw of different cereals and vineyard pruning (VP). There are 26,000 ha cultivated with grapes, producing approximately 250,000 tons of pruned material per year in Mexico. On the other hand, barley and wheat crops cover 383,000 and 560,000 ha, generating approximately 0.7 and 2.7 million tons of straw residue, respectively (FAO 2004), which are widely used in the cultivation of *Agaricus bisporus* (Lange) Imbach and *Pleurotus* spp. In contrast, VP, which has great potential for mushroom production (Sánchez et al. 2002), is practically never used. Mexico is a leader in the production of *A. bisporus* and *Pleurotus* spp. mushrooms in Latin America, with a reported production of approximately 40,000 tons for the year 2002. In contrast, only 30 tons of *L. edodes* is generated per year (Lahman and Rinker 2004).

The aim of the present study is to evaluate the efficiency of the bioconversion of some abundant lignocellulosic by-products for shiitake cultivation.

Materials and methods

Strains

The four *L. edodes* strains evaluated in this study were as follows: CS.2 from Fungi Perfecti, USA, IBUG-17, donated by Ruth de Leon from "Planta Piloto de Hongos Comestibles y Medicinales de Guatemala", and strains V084 and S610 (commercial strains from Somycel, USA), donated by Jean Michel Savoie from Station de Recherches sur les Champignons (INRA) of France. The strains are deposited in the Fungi Strain Collection of the Institute Ecology (Xalapa, Mexico) and are registered as IE-105, IE-123, IE-245, and IE-247, respectively. The strains were maintained on malt extract agar (MEA) (BIOXON, USA) at 25°C.

Spawn

Millet seeds (*Panicum miliaceum* L.) (88.5%), adjusted to approximately 55% moisture, were mixed with coffee pulp powder (8.8%), $CaS0_4$ (1.3%), and peat moss (1.3%). Percentages are based on dry matter. The spawn reached a final moisture content of 70%. Two hundred fifty grams (fresh weight) of this mixture was placed in plastic bags and was sterilized for 1.5 h at 121°C. The sterile mixture was inoculated with 1 cm^2 MEA with mycelium of *L. edodes* of each of the strains and incubated in complete darkness for 15 days at 25±1°C. For additional spawn, new bags were filled with the sterile mixture and inoculated with the first spawn, developed previously for use in the substrate.

Substrate for fruiting and cultivation methods

Fungus was produced using VP (*Vitis vinifera* L.), barley straw (BS; *Hordeum vulgare* L.), and wheat straw (WS; *Triticum aestivum* L.). The substrates were chopped into small particles that ranged from 5 to 8 cm in length using an electric chopper and were hydrated separately in a container for 12 h. After that, they were drained, reaching 60, 73, and 75% moisture, respectively. The substrates were placed (1.2 kg wet weight) in 19.5×48 cm polypropylene bags with a micropore filter (Unicorn Import and Manufacturing, Commerce, TX) and sterilized for 1.5 h at 121°C. The bags were cooled down and then inoculated using 5% (w/w) of spawn and incubated in a dark room at a controlled temperature of 25±1°C.

When the mycelium of *L. edodes* had completely covered the substrates, the samples were transferred to a production room with favorable fruiting conditions, and the polypropylene bags were removed. Relative humidity was maintained between 85 and 90% with an air temperature of 18±1°C. Air recirculation was used for cooling to maintain air distribution and low CO_2 levels. A photoperiod of 12 h was provided with 350 lx illumination with light lamps during the day to favor fruiting and obtain fruiting bodies with normal morphology and pigmentation.

Fungi removal took place during the mature stage. Maturity was determined by exposing the gills with the cap margin completely extended. Production data were evaluated based on biological efficiency (BE; ratio of fresh mushroom / substrate dry weight, expressed in percentages), production rate (PR; ratio of BE / total number of production days, starting from inoculation), and yield (Y; fresh weight of harvested mushrooms / substrate fresh weight, expressed in percentages). Also considered was the production period (PP), number of crops, and size of the mushrooms produced according to pileus diameter: group 1 (G1) <5 cm, group 2 (G2) 5–9.9 cm, and group 3 (G3) 10–15 cm. Bioconversion was determined by substrate dry weight loss after mushroom harvest.

Chemical composition of shiitake fruiting bodies and substrates

To determine the chemical composition of mature *L. edodes* fruiting bodies that were obtained from the first crop, a proximal analysis was conducted: moisture (AOAC No. 7.003; AOAC 1990), total minerals (AOAC No. 7.0009; AOAC 1990), crude fat (AOAC No. 7.060; AOAC 1990), and crude protein (*N*×4.38), using the combustion method with Leco FP-528 equipment (AOAC No. 990.03; AOAC 1990). Available carbohydrates were calculated by difference. Total calories (energy value) were calculated based on dry matter according to Lau's (1982) equation:

kcal/100 g=2.62×(protein %)+4.2×(carbohydrates %)+8.37×(fat %). The proximal substrate analysis was conducted following the methodology previously mentioned for fruiting bodies (AOAC 1990). Neutral detergent fiber (NDF), hemicellulose, cellulose, and lignin were determined using Goering and Van Soest's (1970) technique. Total phenol content was assessed according to Box (1983), and total sugars using the phenol-H_2SO_4 colorimetric method (Dubois et al. 1956). The C:N ratio value was obtained according to Sánchez and Royse's description (2002), where C:N=%C / %N and C (%)=0.58× organic matter (%).

Experimental design and statistical analysis

A completely randomized design with factorial arrangement was applied to proximate analysis and substrate composition values. Samples were tested by triplicate in the fruiting bodies ash and fat contents; duplicates were used for proteins and moisture. A completely randomized design with factorial arrangement was also applied to production values; six samples were evaluated per treatment. An analysis of variance was conducted for all values and a comparison of means according to Duncan's test ($p<0.05$) using the SAS version 8.02 statistical package (SAS Institute 1998).

Results and discussion

Fruiting body production

The influence of substrates during the early stages of *L. edodes* primordium development as well as in the PP is shown in Table 1. Mean days to primordium emergence were 39, 48, and 50 for VP, BS and WS, respectively. With the exception of strain IE-245, PP was similar on VP, which emerged in a short time. PP was longer in cereals straw, but very similar among cereals, with an average of 40 days. IE-245 took less time to start fruiting, with an average of 31 days, while average time for the rest of the strains fluctuated between 43 (IE-123) and 57 days (IE-247) for the three substrates. VP was the best substrate for IE-245 due to early primordia formation (28 days) and the long PP (69 days). With this residue, IE-105, IE-123, and IE-247 displayed the shortest PP with 6, 11, and 14 days, respectively. Although production on BS and WS started considerably later than VP, it remained for a longer period.

Total fresh mushroom production varied from 369.9 (WS-IE-247) to 2691.4 g (VP-IE-245). The number of crops obtained for IE-245 and IE-247 in the tested substrates were three and two, respectively. IE-105 and IE-123 strains fluctuated from one to three and from two to three, respectively. Production distribution displayed a similar pattern in each substrate, with more that 70% of the total obtained in the first crop (>71% on VP, >76% on BS, >80% on WS) (Table 1).

Fungi came in three size groups with the exception of strain IE-247 on WS, which produced only size G3. The predominant production corresponded to G2. The best represented size group was G2 on VP and BS, on the average, while G3 was on WS as substrate. The largest presence of G3 on VP was with IE-247 (66.27%), on BS with IE-105 (40.19%), and on WS with IE-247 (100%). The sum of mean percentages of G2 and G3, obtained from all strains for each substrate, represented more than 86% of production on VP, while on BS and WS, it was 74 and 83%, respectively (Table 2).

Mean BE on VP varied between 44.61 (IE-247) and 93.25% (IE-245), with an average BE of 59.32% (Table 3) and no statistical difference among three of the four

Table 1 Production of fresh *Lentinula edodes* on VP, BS, and WS

Strain	Substrate	IP	PP	Flushes	Total weight (g)[a]	Production by flush (%)[b]		
						First	Second	Third
IE-105	VP	38	6	1	1,368.5±33.76$^{a–d}$	100.00		
	BS	57	28	2	961.2±127.69$^{a–d}$	89.97	10.03	
	WS	61	40	3	844.5±34.29$^{a–c}$	85.18	13.66	1.16
IE-123	VP	41	14	2	1,501.9±61.71^{c,d}	99.18	0.82	
	BS	47	43	3	1,150.7±65.48$^{a–d}$	76.45	15.85	7.70
	WS	41	30	2	676.9±42.42^{a,b}	97.72	2.28	
IE-245	VP	28	69	3	2,691.4±114.49^{e}	71.30	19.00	9.70
	BS	36	46	3	1,215.2±62.36$^{b–d}$	79.16	18.60	2.24
	WS	29	55	3	1,829.6±33.61^{d}	80.17	13.35	6.48
IE-247	VP	48	11	2	1,073.1±54.14$^{a–d}$	95.71	4.29	
	BS	53	34	2	1,329.1±147.89^{d}	89.91	10.09	
	WS	70	38	2	369.9±36.60^{a}	86.94	13.06	

Values are means ± standard deviation of six replicates. Values in a column with different superscripts are significantly different ($p<0.05$, Duncan)

IP incubation period (time necessary for primordium formation), *PP* production period (time from primordium formation to the last harvest)

[a]Fresh weight of mushrooms harvested from six replicates

[b]Distribution of total weight mushrooms obtained in each flush, estimated in percentage

Table 2 Mushroom size of *Lentinula edodes* harvested from VP, BS, and WS

Strain	Substrate	Production of each size group (%)[a]		
		G1	G2	G3
IE-105	VP	12.72	55.07	32.21
	BS	4.12	55.69	40.19
	WS	6.56	29.90	63.54
IE-125	VP	27.50	60.53	11.97
	BS	15.01	62.59	22.40
	WS	5.29	68.87	25.84
IE-245	VP	8.96	54.00	37.04
	BS	37.45	59.37	3.18
	WS	55.11	42.81	2.08
IE-247	VP	3.96	29.77	66.27
	BS	47.00	49.56	3.44
	WS			100.00

[a]Groups of pileus size according to its diameter: G1 <5 cm, G2 5–9.9 cm, G3 10–15 cm

evaluated strains. The highest BE was also accomplished with strain IE-247 (88.60%) on BS, with no difference from the rest of the strains ($p>0.05$). The best BE was obtained with IE-245 (78.48%) on WS, and no significant difference was observed for the other three strains. The highest mean BE per substrate was observed on BS (77.6%), significantly different from the BE obtained on VP and WS. Standard deviation data were high in some cases, because the mushroom production was variable among samples (Table 3).

Table 3 Productivity of *Lentinula edodes* in VP, BS, and WS

Substrate	Strain	BE (%)	PR (%)	Y (%)
VP	IE-105	$47.41\pm7.02^{c,d}$	$1.07\pm0.12^{a,b}$	$19.09\pm2.81^{b-e}$
	IE-123	$52.04\pm12.83^{b-d}$	$1.03\pm0.37^{a,b}$	$20.94\pm5.14^{b,c}$
	IE-245	93.25 ± 23.80^{a}	$0.95\pm0.23^{a-c}$	37.46 ± 9.54^{a}
	IE-247	44.61 ± 11.25^{d}	$0.77\pm0.09^{a-c}$	$17.96\pm4.51^{b-e}$
Means		59.32 ± 13.72^{b}	0.95 ± 0.20^{a}	23.86 ± 5.5^{a}
BS	IE-105	$64.08\pm4.56^{a-d}$	$0.76\pm0.36^{a-c}$	$16.10\pm10.64^{b-e}$
	IE-123	$76.71\pm21.82^{a-c}$	$0.87\pm0.12^{a-c}$	$19.26\pm5.45^{b-e}$
	IE-245	$81.01\pm20.78^{a,b}$	$0.97\pm0.30^{a-c}$	$20.33\pm5.19^{b-d}$
	IE-247	88.60 ± 49.29^{a}	1.17 ± 1.04^{a}	22.23 ± 12.32^{b}
Means		77.6 ± 24.11^{a}	0.94 ± 0.45^{a}	19.48 ± 8.4^{b}
WS	IE-105	42.26 ± 10.29^{d}	$0.48\pm0.22^{b,c}$	$11.81\pm2.85^{c-e}$
	IE-123	40.65 ± 12.74^{d}	$0.73\pm0.44^{a-c}$	$11.30\pm3.53^{d,e}$
	IE-245	$78.48\pm10.09^{a-c}$	$1.05\pm0.51^{a,b}$	21.86 ± 2.80^{b}
	IE-247	37.02 ± 10.99^{d}	0.39 ± 0.17^{c}	10.35 ± 3.05^{e}
Means		49.60 ± 10.80^{b}	0.66 ± 0.33^{b}	13.83 ± 3.05^{c}

Values are means ± standard deviation of six replicates. Means in a column with different superscripts of each strain in the three substrates and only among means are significantly different ($p<0.05$, Duncan)

BE biological efficiency, mushroom fresh weight/substrate dry weight; *PR* production rate, BE/days for obtaining each flush including incubation time; *Y* Yield, mushroom fresh weight/substrate fresh weight

Mean PR on VP varied from 0.77 (IE-247) to 1.07% (IE-105), with the highest mean value (0.95%) not significantly different from BS (0.94%) but significantly different from WS (0.66%). The four strains showed similar behavior on VP. The highest PR was observed with strain IE-247 (1.17%) on BS, similar to the rest of the strains ($p>0.05$). IE-245 showed a higher PR (1.05%), statistically the same as IE-105 and IE-123 (Table 3) on WS. The highest Y obtained was 37.46% (IE-245) on VP; it was 22.23% (IE-247) on BS and 21.86% (IE-245) on WS, with no significant difference between the two last strains. Mean Y on VP was higher than on BS and WS ($p>0.05$) (Table 3).

Mata et al. (1998) cultivated strain IE-247 on WS with peat moss, pasteurized at 65°C for 24 h, registering a BE of 38 to 44%. In this study, a BE of 37.02% was found; however, this strain on VP obtained a BE of 44.61% and on BS of 88.60%. Savoie et al. (2000) obtained a BE of approximately 60% with IE-247 and pasteurized WS, which is higher than that obtained in the current study. However, this value is smaller that the value reached with VP and BS. Recently, Gaitán-Hernández and Mata (2004) reported a BE of 46.9% for IE-245 and 50% with IE-247 on WS pasteurized through hot water soak.

The highest BE values on VP (93.25%), BS (88.60%), and WS (78.48 %) were located within the range cited in previous studies (18 to 130%) where supplemented sawdust and alternative substrates such as sugar cane and coffee residues were used (Leifa et al. 1999; Mata and Gaitán-Hernández 1994; Morales and Martínez-Carrera 1991; Morales et al. 1991; Morais et al. 2000; Pire et al. 2001; Royse 1985, 1996; Salmones et al. 1999). Morais et al. (2000) used supplemented and sterile chestnut wood sawdust (*Castanea* sp.), obtaining a BE of 18.9 to 59.5%. Philippoussis et al. (2002) observed mycelial growth and fruiting in WS similar to that observed in oak sawdust. Growth tended to be related to substrate nitrogen content and pH. Previous reports assess that nitrogen could be a growth-limiting factor in shiitake cultivation (Kalberer 2000).

Mean PR on VP (0.95%), BS (0.94%), and WS (0.66%) are considered acceptable when compared to those cited by Royse (1985) for shiitake on sterilized enriched sawdust substrates (0.29 to 0.79%) utilizing conventional systems. Delpech and Olivier (1991) obtained Y of 11.9 to 15.9% with strain IE-247 using supplemented WS pasteurized with steam at 60°C for 24 h. In our study, this strain yielded 17.96 (VP), 22.23 (BS), and 10.35% (WS). The highest Y was reached with IE-245 on VP (37.46%) and WS (21.86%), and IE-247 on BS (22.23%); these values are equal to or higher than those reported by Kilpatrick et al. (2000) for sterile WS (5 to 31%) supplemented with wheat bran and millet. In general, based on BE, PR, Y, and the number of crops obtained, the best substrates were VP and BS, and IE-245 and IE-247 the best strains (Tables 1 and 3).

The mature fruiting bodies produced in the three substrates showed normal development and morphology. Protein content was relevant, particularly in strain IE-105, exceeding that reported by Salmones et al. (1999), with this strain using sterilized substrates (Table 4). The

Table 4 Chemical composition of *Lentinula edodes* fruiting bodies harvested from VP, BS, and WS

Strain	Substrate	M	A	CF	CP	CH	EV
IE-105	VP	91.69±0.32^{a,b}	5.40±0.10^{c}	2.15±0.06^{c}	17.19±0.30^{a}	75.26±0.13^{f}	379.09±2.57^{d}
	BS	93.08±0.00^{a}	5.89±0.04^{b}	2.17±0.06^{b,c}	16.83±0.24^{a}	75.13±0.11^{f}	377.78±2.28^{d}
	WS	91.85±2.21^{a,b}	6.08±0.07^{a}	2.02±0.03^{d,e}	16.12±0.20^{b}	75.81±0.14^{f}	377.48±1.96^{d}
IE-123	VP	90.41±0.00^{a,b}	5.00±0.05^{e}	1.93±0.02^{e,f}	14.77±0.00^{d,e}	78.33±0.03^{c,d}	383.84±0.42$^{b–d}$
	BS	89.92±2.25^{b}	5.32±0.07^{c}	2.27±0.09^{b}	16.85±0.024^{a}	75.62±0.37^{f}	380.76±4.18^{c,d}
	WS	91.14±0.00^{a,b}	5.22±0.01^{d}	1.91±0.09^{e,f}	13.87±0.23^{f}	78.94±0.17^{b,c}	383.91±2.93$^{b–d}$
IE-245	VP	86.46±0.00^{c}	3.57±0.02^{g}	1.82±0.02^{f}	12.37±0.67^{g}	82.22±0.70^{a}	392.95±6.86^{a}
	BS	91.43±0.03^{a,b}	5.19±0.06^{d}	1.95±0.08^{e}	15.15±0.25^{c,d}	77.68±0.31^{d}	382.30±3.75$^{b–d}$
	WS	91.47±2.06^{a,b}	6.07±0.02^{a}	2.76±0.10^{a}	14.51±0.18^{e,f}	76.68±0.28^{e}	383.14±3.46$^{b–d}$
IE-247	VP	86.68±0.00^{c}	3.36±0.03^{h}	1.91±0.03^{e,f}	15.14±0.44^{c,d}	79.55±0.44^{b}	389.90±4.59^{a,b}
	BS	92.20±0.00^{a,b}	5.37±0.05^{c}	2.22±0.04^{c,b}	15.65±0.16^{b,c}	76.76±0.15^{e}	382.00±1.92$^{b–d}$
	WS	92.02±0.38^{a,b}	4.25±0.04^{f}	2.12±0.07^{c,d}	14.38±0.15^{e,f}	79.20±0.14^{b}	388.05±2.19$^{a–c}$

Values are means ± standard deviation of three replicates (ash, fat) and all the rest with two replicates. Means in a column with different superscripts are significantly different ($p<0.05$, Duncan). All values are on a dry matter basis, except moisture (percentage of fresh mushroom weight)

M moisture, *A* ash, *CF* crude fat, *CP* crude protein [*N*×4.38(kcal)], *CH* carbohydrates, *EV* energy value [kcal/100 g=2.62×(% of protein) +4.2×(% of carbohydrates)+8.37×(% of fat)]

values also coincide with those reported for shiitake (13.4 to 17.5%) (Crisan and Sands 1978).

Minerals, protein, and crude fat contents were similar to those cited by Morais et al. (2000) for mushrooms cultivated on sterile wood shavings. Fat varied from 1.82 to 2.76% on dry base. Carbohydrates ranged from 75.13 to 82.22%, exceeding values reported by Crisan and Sands (1978) for shiitake and *Agaricus* sp. (56.9%) and similar to *Pleurotus* spp. (74%) (Mendívil-Salmón et al. 2001). The energy value determined herein coincided with the 387 kcal value also reported by Crisan and Sands (1978). Shiitake fruiting bodies are an important source of secondary metabolites that are useful to man, with immunological, antimicrobial, and antifungal activities such as those of lentinan and lanthionine (Hatvani 2001). They also produce pharmaceutically useful protein inhibitors (Odumi et al. 1999). This bioconversion process offers a nutritious alternative, because fungi fruiting bodies contain high percentages of total carbohydrates and proteins, and their nutritional value is as high as those of beans (Brisko et al. 2002).

Biodegradation and chemical composition of initial and residual substrates

Average biodegradation was 33.08, 38.51, and 41.74% for VP, BS, and WS, respectively. Although cereals had the highest value of biodegradation, according to BE, PR, and Y, assimilation was more efficient on VP. The two most productive strains presented high biodegradation rates, IE-245 in VP (34.19%) and WS (63.84%) and IE-247 in BS (52.56%).

The proximal composition of residues varied significantly before and after solid-state fermentation (SSF) ($p<0.05$) (Table 5). The ash content increased by more than 300% in VP and BS, with a positive correlation between mineralization and BE in BS ($r^2=0.83$) and WS ($r^2=42$) and inversely proportional in VP ($r^2=-0.66$). Crude fat was differed among residues, the highest value corresponding to WS ($p<0.05$). After SSF, crude fat content decreased in WS, increased in BS ($p<0.05$), and tended to increase in VP. Total protein content was higher on WS, where minimum BE mean value was obtained (Table 3). After SSF, it decreased in all treatments with WS; while it increased ($p<0.05$) on BS and on VP, it did not change except with strain IE-123, where it increased.

The higher the initial C:N ratio, the higher the average BE (Tables 3 and 6). After SSF, the BE coefficient decreased on VP and BS, except in treatments with IE-245, where the best BE were obtained, while this coefficient increased ($p<0.05$) on WS (Table 5). With an evaluation of mycelial growth and produced biomass, it was proven that wood enriched with 25 to 30% rice bran stimulated *L. edodes* growth due to substrate C:N ratio (30:1) and because its structure favor gas exchange, which is indispensable for enzymatic degradation activity. However, it has been observed that supplements with high cellulose and hemicellulose contents, such as sugar cane molasses, do not increase mushroom growth (Rossi et al. 2003). This is due to the fact that when shiitake grows in lignocellulosic substrates, cellulose degradation is lower (14%) than with lignin (40 and 60%) (Morais et al. 2000). Song and Chow (1987) reported abundant shiitake growth at a 30:1 C:N ratio.

With the exception of the VP-IE-105 and WS-IE-105 treatments, total sugars content increased significantly ($p<0.05$) (Table 5). Preferable carbon sources for shiitake are glucose, trehalose, and fructose, as shown in studies conducted with synthetic medium (Song and Chow 1987).

Phenolic content decreased after SSF in all treatments except WS-IE-245 ($p<0.05$), although this occurred less in strains with higher BE (Tables 3 and 5). In general, phenol concentration decreased by 1.23 mmol/L on VP, by

Table 5 Composition of VP, BS, and WS before and after solid-state fermentation of *Lentinula edodes*

Stage	Strain	Substrate	Moisture	Ash	Fat	Protein	C/N ratio	Total sugar (mg/g)	Total phenol (mmol/L)
Control	–	VP	7.02±0.20$^{b–d}$	3.18±0.02^{k}	0.63±0.02^{f}	4.98±0.02^{c,d}	49.40±0.01^{f}	209.41±0.91^{e}	2.20±0.03^{a}
	–	BS	4.62±0.16^{l}	6.45±0.10^{j}	0.93±0.07^{d,e}	3.89±0.22^{e}	61.09±0.06^{c}	42.23±1.55^{l}	1.54±0.03^{b}
	–	WS	5.19±0.09^{h,i}	7.79±0.07^{i}	1.24±0.06^{b,c}	7.50±0.09^{a}	31.23±0.02^{n}	196.68±1.96^{g}	0.83±0.03^{f,g}
Final residues	IE-105	VP	6.73±0.56$^{c–e}$	13.83±0.50^{d}	0.91±0.05^{d,e}	4.85±0.03^{c,d}	45.16±0.26^{h}	203.06±2.41^{f}	0.63±0.12^{i}
		BS	6.69±0.64$^{c–e}$	10.60±0.12^{g}	1.47±0.03^{a}	5.26±0.52^{b,c}	43.18±0.06^{i}	157.79±1.10^{h}	1.18±0.02^{d,e}
		WS	5.30±0.15$^{g–i}$	12.52±0.18^{f}	0.78±0.10^{e,f}	4.16±0.03^{e}	53.44±0.11^{d}	200.45±3.30^{f,g}	0.77±0.13^{g,h}
	IE-123	VP	5.07±0.69^{h,i}	8.97±0.03^{h}	0.81±0.05^{e,f}	5.48±0.39^{b}	42.23±0.01^{j}	244.05±0.54^{b}	0.92±0.00^{f}
		BS	6.41±0.40$^{d–f}$	17.46±0.20^{b}	1.22±0.07^{c}	5.66±0.00^{b}	37.02±0.54^{l}	144.51±6.85^{j}	1.14±0.02^{e}
		WS	7.53±0.22^{a,b}	13.09±0.25^{e,f}	0.77±0.24^{e,f}	3.04±0.04^{f}	72.62±0.21^{b}	238.83±0.21^{c}	0.65±0.05^{h,i}
	IE-245	VP	7.38±0.21$^{a–c}$	6.23±0.06^{j}	0.77±0.09^{e,f}	4.68±0.10^{d}	50.96±0.03^{e}	241.32±3.97^{b,c}	1.47±0.00^{b}
		BS	6.01±0.49$^{e–g}$	16.05±0.32^{c}	1.42±0.02^{a,b}	4.96±0.19^{c,d}	73.03±0.16^{i}	152.83±0.75^{I}	1.33±0.08^{c}
		WS	5.80±0.58$^{f–h}$	13.45±0.08^{d,e}	0.81±0.06^{e,f}	2.46±0.05^{g}	89.56±0.08^{a}	224.71±3.59^{d}	1.31±0.07^{c}
	IE-247	VP	6.10±0.46^{e,f}	10.63±0.01^{g}	0.61±0.21^{f}	4.85±0.20^{c,d}	46.80±0.01^{g}	252.55±0.75^{a}	0.84±0.00^{f,g}
		BS	5.25±0.35^{h,i}	27.89±0.28^{a}	1.24±0.05^{b,c}	4.99±0.05^{c,d}	36.70±0.14^{m}	76.004±1.99^{k}	1.27±0.09^{c,d}
		WS	7.84±0.09^{a}	13.09±0.10^{e,f}	1.03±0.08^{d}	5.30±0.05^{b,c}	41.59±0.05^{k}	220.40±0.73^{d}	0.62±0.04^{i}

All values are means ± standard deviation of triplicate measurements. Means in a column with different superscripts are significantly different (p<0.05, Duncan)

0.31 mmol/L on BS, and remained practically the same on WS. One of the enzymes involved in the oxidation of phenolic compounds is Mn-dependent peroxidase, which is present in *L. edodes*, an enzyme that acts on the lignin degradation system (Kirk and Farrell 1987). VP after SSF showed the largest decrease in phenols, a condition possibly related to the higher concentration of lignin in the substrate.

NDF varied among the substrates (p<0.05), having a positive correlation with BE. With the exception of WS-IE-105, after SSF, NDF decreased in all treatments (p<0.05). Initial hemicellulose, cellulose, and lignin concentration varied among the substrates (p<0.05), with the highest levels of cellulose and lignin in VP and BS (Table 6). The initial amount of hemicellulose was similar on BS and WS and was significantly lower on VP (p<0.05). After SSF, hemicellulose decreased in all substrates, with a positive correlation on VP between its decrease and BE (r^2=0.87); while on BS and WS, no association was observed (Tables 3 and 6). Hemicellulose is considered to include linear and branched chains of monosaccharides other than glucose, polypeptide chains, and a fraction of low molecular weight cellulose chains, all of which are compounds that are relatively easy to break down, hence the supply of energy for lignin oxidation. This assumption supports our results, as substrates with higher percentages of hemicellulose (Table 6) showed higher biodegradation rates (BS and WS).

Table 6 Fiber fractions of VP, BS, and WS before and after solid-state fermentation by *Lentinula edodes* strains

Stage	Strain	Substrate	NDF	Hemicellulose	Cellulose	Lignin
Control	–	VP	71.98±0.71^{b}	15.82±0.16^{b}	41.46±2.05^{c,d}	14.69±2.92^{b,c}
	–	BS	87.12±0.78^{a}	25.51±0.73^{a}	48.39±0.16^{a}	13.22±1.35$^{b–d}$
	–	WS	69.11±0.89^{c}	27.28±2.02^{a}	29.34±1.39^{h}	12.50±2.53$^{b–e}$
Final Residues	IE-105	VP	63.47±0.11^{g,h}	10.76±0.80^{c,d}	33.93±0.67^{g}	18.78±1.36^{a}
		BS	62.37±1.18^{h,i}	9.94±0.25^{d}	40.88±2.19^{c,d}	11.56±0.76$^{c–f}$
		WS	67.55±0.47^{c,d}	9.69±0.03^{d}	48.87±1.74^{a}	9.00±1.24^{e,f}
	IE-123	VP	64.87±0.25$^{e–g}$	8.60±0.74^{d,e}	37.58±1.21^{e,f}	18.69±0.72^{a}
		BS	59.45±0.34^{j}	13.98±0.19^{b}	34.24±1.88^{g}	11.23±2.03$^{c–f}$
		WS	63.73±0.78$^{f–h}$	6.26±0.09^{e,f}	47.51±0.01^{a}	9.95±0.70$^{d–f}$
	IE-245	VP	60.48±1.17^{i,u}	2.95±0.77^{g}	42.21±0.03^{b,c}	15.33±0.42^{b}
		BS	65.03±1.94$^{e–g}$	9.94±2.46^{d}	40.71±1.79^{c,d}	14.38±2.31^{b,c}
		WS	65.87±0.91$^{d–f}$	6.40±2.43^{e,f}	50.33±1.20^{a}	9.20±0.24^{e,f}
	IE-247	VP	64.51±1.66$^{e–h}$	14.01±1.10^{b}	35.36±0.75^{f,g}	15.14±1.32^{b}
		BS	66.56±0.41^{d,e}	13.17±1.13^{b,c}	39.19±0.25^{d,e}	14.20±0.46^{b,c}
		WS	58.64±2.61^{j}	5.39±2.54^{g}	44.58±0.30^{b}	8.66±0.24^{f}

All values are means ± standard deviation of triplicate measurements. Means in a column with different superscripts are significantly different (p<0.05, Duncan)

During delignification, cellulose is broken down simultaneously; hemicellulose is an energy source for white rot fungi, since lignin alone apparently cannot serve as a growth substrate (Boominathan and Reddy 1992). The initial cellulose content differed among residues ($p<0.05$); after SSF, it increased on WS and decreased in the rest of the treatments, except for VP-IE-245, where it did not vary ($p>0.05$) (Table 6). Although *L. edodes* tends to cause white rot by decomposing lignin, the lignocellulose, cellulose, and hemicellulose depolymerization through extracellular enzymes depends on substrate chemical composition, which affects the culture's BE (Morais et al. 2000). It has also been reported that high levels of lignin limit cellulose availability (Kilpatrick et al. 2000).

Leatham (1985) observed that hemicellulose activity in glucuronic–xylan polymers was constant during the initial and intermediate periods of shiitake growth, while proteinase activity was detected during the intermediate growth stage, and polygalacturonase activity was high during the entire development. He also found that endoglucanase and β-glucosidase activity increased slightly at the time of fruiting, although in general, there was no significant decrease in cellulose. Other authors have mentioned that endoglucanase production is associated with other primordia and fruiting formation stages where there has been a significant increase in enzymatic activity, diminishing the crop (Mata et al. 1998).

Lignin was similar in all substrates, decreasing after SSF on WS ($p<0.05$), tending to decrease on BS, and increasing on VP. Average lignin degradation was higher on WS (3.3%). The low C:N ratio in this substrate (Table 5) could have favored biodegradation; Blanchette (1991) mentioned that the substrate's low nitrogen levels stimulate lignin degradation in several white rot fungi, while high concentrations of nitrogen promote polysaccharide degradation. Van et al. (2003) observed that during the first stages of shiitake growth, cellulose and xylans are the substrate's most abundant components, while lignin mostly decomposes. They correlated this with low cellulose and high ligninolytic activity. During the advanced stages of the culture, they determined a decrease of approximately 22% in ligninolysis and a greater degradation of polysaccharides such as hemicellulose and xylose. Belinky et al. (2003) found an important correlation between high extracellular concentration of reactive oxygen species and ligninolytic activity, suggesting that these compounds have an important function during the degradation process. The variability observed in the degradation capacity of lignocellulosic components is influenced by the substrate's nature, by environmental factors, and most importantly by genetic factors among species or even among strains of the same species (Blanchette 1991).

Vineyard pruning is an excellent substrate for the cultivation of lignin-decomposing basidiomycetes. Sánchez et al. (2002) showed that VP by itself or in combinations with a greater proportion of VP, grape pomace, favors mycelium growth, shortens fruiting time, and increases BE in *Pleurotus* spp. The authors related this behavior during growth with low nitrogen and fat content and a larger amount of hemicellulose.

In conclusion, VP showed higher fungal production as compared with BS and WS. This substrate favored early primordia initiation and short PPs. In addition, availability and low cost make viticulture residues a viable alternative for the cultivation of shiitake.

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