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RESEARCH ARTICLE

Yield, and phenolic content of shiitake mushrooms cultivated on alternative substrates

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ABSTRACT

Background: The productivity of four strains of shiitake (*Lentinula edodes*) on diverse lignocellulosic by-products used as substrates were evaluated. The objective was to determine the correlation between the composition of the substrate with the productivity of carpophores and their polyphenol content. The fungi were produced on vineyard prunings (VP), sorghum stubble (SS), sugarcane bagasse (SB), and oak shavings (OS). The productivity was evaluated based on the biological efficiency (BE), production rate (PR), and yield (Y). To evaluate the effect on shiitake growth on the chemical composition of the substrates, the content of fiber [neutral detergent fiber (NDF), hemicellulose, cellulose, lignin] was determined before and after inoculation. Total phenolic compounds were quantified by spectrophotometry. **Results:** The highest BE was observed for the IE-256 strain on SS (145.11%), and the highest PR for the IE-245 strain on SS (1.69%). The highest Y was observed for the IE-256 strain on SS (41.96%). The fiber results were significantly affected by growth period, strain, substrate, and the selected combinations of strains and substrates. The phenolic compound content of the carpophores was also significantly affected by substrate and strain; the lowest and highest values were obtained for the IE-256 strain on OS (1.5983 mg GAE*g-1) and SS (2.7197 mg GAE*g-1), respectively. **Conclusions:** On average, the highest carpophores production per substrate and strain corresponded to SS and IE-245, respectively. The carpophores of the IE-256 strain cultivated on SS could potentially present greater antioxidant activity because of their high polyphenol content.

Keywords: Antioxidants; Fruiting substrate; Lignocellulosic wastes; Mushroom cultivation; Productivity; Shiitake

INTRODUCTION

The cultivation of fungi on agricultural by-products has been consolidated as a viable alternative for producing supplements for human diets as well as biofertilizers for agriculture in many countries, especially in tropical regions where millions of tons of waste are underutilized. The biotechnology to produce edible fungi has enabled a more efficient use of space, energy, and water resources in addition to the use of waste generated by agricultural activities, among other benefits (Philippoussis, 2009). However, it is important to select the most optimal wastes for the cultivation of specific fungi considering the location, quantity, and conditions of waste production and waste characteristics (Mata et al., 2013).

In recent years, the cultivation of edible mushrooms has greatly advanced in Latin American and especially in Mexico because of the adaptation of technologies and substrates appropriate to environment regional conditions. However, mushroom cultivation has mostly been restricted to species of *Agaricus* and *Pleurotus*. *Lentinula edodes* (Berk.) Pegler is one species that is produced in lower quantities but represent a great potential for large-scale cultivation in Mexico (Gaitán-Hernández et al., 2017). At the worldwide level, *L. edodes* occupies first place in terms of production volume among the cultivated edible mushrooms (Royse and Sánchez, 2017). It is mainly produced in China and Japan, and China represents both the largest exporter and consumption center in the world. In Ibero-America, ca. 1,300 tons of shiitake are produced. Brazil is the main producer (800 ton) followed by Spain (350 ton) and Mexico (30 ton) (Andrade Gallegos et al., 2012).

Traditionally, *L. edodes* has been cultivated on hardwood trunks, mainly oak (Stamets, 2000). However, this cultivation

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system represents a potential risk to the environment and is limited by the slow growth and excessive use of oak wood, an important forest species (Gaitán-Hernández et al., 2006). The traditional method of cultivation on trunks has been partially replaced by production systems using sawdust or wood shavings from different hardwood species. In these systems, it is necessary to sterilize the substrates and inoculate the fungi under controlled conditions (Savoie et al., 2000). Nevertheless, the low availability of these forest resources in some regions of Mexico and other countries has promoted the exploration of alternative substrates such as cereals straw (Gaitan-Hernandez and Mata, 2004; Philippoussis et al. 2002, 2007; Peralta and Frutis, 2010), viticulture residues (Gaitán-Hernández et al., 2006; 2011), coffee pulp (Mata et al., 2013) and sugarcane bagasse (Salmones et al., 1999; Rossi et al, 2003; Sobal et al., 2010), among others. In general, the supplementation of these materials with wheat husk/bran has been recommended. In addition, the presence of certain bioactive compounds as well as the chemical composition, flavor, and smell of harvested basidiomes has been found to significantly vary according to the characteristics of the utilized substrate (Gaitán-Hernández et al., 2017; Smith et al., 2002).

Lentinula edodes is a great source of nutrients and has also been used as a medicinal tonic for over two thousand years. Many of the properties attributed to shiitake by traditional Asian medicine have been scientifically proven in recent decades. Furthermore, *L. edodes* may have additional beneficial synergic and additive effects on human health because of its content of bioactive compounds. Therefore, because of their nutritional and medicinal value, shiitake mushrooms are recognized as a functional food (Zhu et al., 2015). In particular, these mushrooms have been shown to exhibit anticancer, antiviral, immunomodulatory, antioxidant, antibacterial, and antifungal activities (Kang et al., 2012). The bioactive compounds responsible for these properties include phenolic compounds (Yildiz et al., 2015), vitamin C, and polysaccharides (Jiang et al., 2010). In one study, Mattila et al. (2001) separated the phenolic compounds by high-resolution liquid chromatography and identified p-hydroxybenzoic acid, trans-cinnamic acid, and protocatechuic acid as the main phenols present in *Lentinula edodes*.

The influence of the substrates on the phenolic compound content of shiitake basidiomes has been little unknown (Gaitán-Hernández et al., 2011; 2017). Because phenolic compounds appear to be the main components responsible for the antioxidant activity of edible mushrooms (Elmastas et al., 2007), it is important to select the most ideal substrates for specific fungal strains that would increase the content of phenolic compounds in basidiomes, thereby obtaining mushrooms that represent a potential natural source of antioxidants. For this reason, in the present study, four shiitake strains were cultivated on four different lignocellulosic by-products with the objective of correlating the chemical composition of the substrates with the productivity and antioxidant potential (polyphenol content) of the carpophores.

MATERIALS AND METHODS

Strains

Four *Lentinula edodes* strains were evaluated in this study: L35 from Hong Kong and CS.2, INRA V084, and FM009 from the USA. All strains are deposited in the Fungal Strain Collection at the Institute of Ecology (INECOL, Veracruz, Xalapa, Mexico) and registered as IE-40, IE-105, IE-245, and IE-256, respectively. The strains were re-seeded on malt extract agar (Bioxon, USA) at 25 °C.

Spawn

The spawn was prepared as follows: Millet seeds (*Panicum miliaceum*) (88.5%) adjusted to ca. 55% moisture were mixed with oak wood powder (8.8%), $CaS0_4$ (1.3%), and peat moss (1.3%); the percentages of this mixture were based on dry matter. The spawn had a final moisture content of 55%. Three hundred grams (fresh weight) of this mixture was placed in a plastic bag and sterilized for 1.5 h at 121 °C. The sterile mixture was inoculated with 1 cm^2 of MEA containing *L. edodes* mycelia and incubated in complete darkness for 15 days at 25±1 °C. To multiply additional spawn, new bags were filled with the sterile mixture and inoculated with the first spawn developed for use in the substrate.

Substrate for fruiting

Fungi were produced on vineyard prunings (*Vitis vinifera*) (VP), sorghum stubble (*Sorghum vulgare*) (SS), sugarcane bagasse (*Saccharum officinarum*) (SB), and oak shavings (*Quercus* sp.) (OS) (as a control). The SS and VP were chopped into small particles ranging from 5 to 8 cm in length using an electric chopper. All substrates were hydrated separately in a container for 12 h and were then drained, reached 70% VP, 70% SS, 80% SB, and 60% OS moisture. The substrates were placed (1.2 kg wet weight) in 19.5 x 48 cm polypropylene bags with a micropore filter (Unicorn Import and Manufacturing Commerce, USA), and sterilized for 1.5 h at 121 °C. At following, the bags were cooled, inoculated with 5% (w/w) spawn, and incubated in a dark room at a controlled temperature of 25±1 °C.

During the incubation period, the samples showed dark-colored patches (sclerotia) that eventually spread to cover the entire surface. Once the mycelia had completely covered

the substrates, the substrate bags were transferred to a production room with favorable fruiting conditions, and the polypropylene bags were removed. Air recirculation was used as the cooling method and was also used to maintain uniform air distribution and low CO_2 levels. A photoperiod of 12 h was implemented, and light lamps with 350 lx illumination were turned on during the day to favor fruiting and to obtain fruiting bodies with normal morphology and pigmentation. The relative humidity was maintained at 85–90% and the air temperature at 18±1 °C. Production data were evaluated based on Gaitán-Hernández et al. (2017).

Changes in fiber content

To determine the chemical composition of the substrates during the mushroom crop cycles, neutral detergent fiber (NDF), detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van-Soest's et al. technique (1991), using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, NY, USA)). Cellulose content was calculated by the difference between ADF and ADL, and hemicellulose was calculated by the difference between NDF and ADF. The fiber content of all substrates was determined at 0, 13, 26, and 69 days after inoculation of the mushrooms spawn.

Compounds with antioxidant activity

Extract Preparation. Fresh basidiomes in the mature state from the first harvest were dried at 50 °C for 24 h (Riossa Digital, HCF-62-D, Mexico). Basidiomes under the same treatment (strain/substrate) were cut into small pieces of approximately 5 mm in length. These pieces were then homogenized (NutriBullet) with ethanol for approximately 15 seconds (4 g dry tissue in 40 mL ethanol [80% v/v]). The ethanol extract was centrifuged (Hettich, Tuttlingen, 32R, Germany) at 4000 rpm for 20 min and then the supernatant was used for quantification of total phenolic content.

Total Phenolics. Total phenolic content was measured using a spectrophotometer (Model 6305, Jenway, Staffordshire, UK) based on an oxidation-reduction reaction. Folin-Ciocalteu reagent was used according to the modified method described by Singleton and Rossi (1965). Quantification was conducted with a standard gallic acid curve (0.02–0.12 mg · mL^{-1}) and was reported as milligrams of gallic acid equivalents (GAE) per gram of dry weight (dw).

Statistical analysis

A completely random design with a factorial arrangement (4 strains x 4 types of substrates) was applied to fiber content, total phenolic compounds, and production values. Three samples were tested to fiber and phenolic compounds, and five to fruiting bodies production. An analysis of variance was conducted for all experimental values in addition to a comparison of means according to Tukey's test ($p<0.05$) in the statistical software Statistica (v. 7.0).

RESULTS AND DISCUSSION

Production of carpophores

During the incubation period, the samples showed dark-colored patches of mycelia that eventually spread to cover the entire surface of the substrates. The formation of sclerotia varied per substrate, taking 40 days on SS, 45 days on VP, 49 days on OS, and 50 days on SB. Therefore, SS was the best substrate in terms of early sclerotia formation. Total fresh mushroom production varied from 113.02 g to 494.86 g per sample. The treatments IE-105 RS and IE-245 RS had the highest production, whereas the treatments IE-256 BC and IE-105 ME had the lowest production. Two or three flushes were obtained from the tested substrates, except for the IE-105 OS and IE-256 SB treatments. The production patterns of the strains were similar on SB, OS, and VP, producing more than 60% of total mushrooms in the first crop, whereas SS produced more than 45% (>47.18%) (Table 1).

The majority of the strains developed mushrooms of the four size groups, showing variation between treatments. The size group with the most mushrooms was G2, including 212 mushrooms representing 44.91% of total mushrooms. The strain that produced the greatest quantity of mushrooms (93) was IE-245 on VP, whereas the treatment IE-256 on SB produced the lowest quantity (only 5). The strain IE-256 on SS produced the largest quantity (8) of G4 mushrooms. Considering all substrates, the G2 and G3 mushrooms represented 74.35% of total mushroom production (Table 2).

The BE was only significantly affected by substrate ($F=25.31$, $df=3$, $p=0.0001$), but not among strains ($F=2.48$, $df=3$, $p=0.0686$) and their interactions ($F=1.63$, $df=9$, $p=0.1233$). The highest BEs were obtained for IE-256 SS (145.11%), IE-245 SS (142.61%), IE-105 SS (123.7%), and IE-245 VP (123.59%), no statistical difference among four treatment were observed. On average, the highest BE values were found on SS (132.74%) and VP (102.01%) (Table 3), and the most productive strain was IE-245 (106.60%) (Table 4). These results are within the intervals cited in previous studies (18% to 130%), and some are even higher than those reported by previous authors (Gaitán-Hernández et al., 2006; 2017; Leifa et al, 1999; Morais et al., 2000; Pire et al., 2001; Rossi et al., 2003; Royse, 1996; Salmones et al., 1999; Sobal et al., 2010).

The PR was significantly affected by strain ($F=4.30$, $df=3$, $p=0.0079$) and substrate ($F=32.80$, $df=3$, $p=0.0001$). The

Table 1: Production of fresh *Lentinula edodes* in Oak Shavings (OS), Vineyard Pruning (VP), Sorghum Stubble (SS) and Sugarcane Bagasse (SB)

Strain	Substrate	IP	Flushes	Total weigth (g)[a]	Production by flush (%)[b]		
					1st	2nd	3rd
IE-40	OS	49	2	273.68±194.24[c]	85.29	14.70	
	VP	45	3	276.00±178.81[c]	67.57	25.57	6.85
	SS	40	3	414.82±77.46[c]	52.04	46.50	1.45
	SB	50	2	182.94±126.30[b]	84.43	15.56	
IE-105	OS	49	1	146.22±85.05[b]	100.00		
	VP	45	2	395.74±146.99[c]	80.65	19.35	
	SS	40	3	429.24±92.21[d]	47.18	46.89	5.92
	SB	50	2	150.74±89.12[b]	82.05	17.95	
IE-245	OS	49	3	408.52±134.06[c]	66.75	32.49	0.75
	VP	45	3	443.70±92.27[d]	79.62	20.12	0.25
	SS	40	3	494.86±214.55[e]	64.55	29.30	6.14
	SB	50	3	188.82±50.01[b]	61.17	35.96	2.86
IE-256	OS	49	3	159.24±175.31[b]	82.36	13.36	4.27
	VP	45	3	349.46±17.87[c]	71.94	21.74	6.31
	SS	40	2	386.42±223.61[c]	67.73	32.26	
	SB	50	1	113.02±19.24[a]	100.00		

Values are means ± standard deviation of five replicates. Values in a column with different superscripts are significantly different ($p<0.05$, Tukey). IP incubation period. [a]Fresh weight of mushrooms harvested from five replicates. [b]Distribution of total weight mushrooms obtained in each flush, estimated in percentage

Table 2: Mushroom size of *Lentinula edodes* harvested from Shavings (OS), Vineyard Pruning (VP), Sorghum Stubble (SS) and Sugarcane Bagasse (SB)

Strain	Substrate	G1[a]			G2			G3			G4		
		*	**	***									
IE-40	OS	13.68	4.99	5	77.18	28.20	12	100.2	36.61	6	82.62	30.18	3
	VP	8.08	2.92	3	47.26	17.12	5	106.52	38.59	8	114.14	41.35	4
	SS	8.8	2.12	3	69.26	16.69	10	165.74	39.95	12	171.02	41.22	6
	SB	3.8	2.07	1	16.78	9.17	3	105.22	57.51	6	57.14	31.23	2
IE-105	OS				17.64	12.06	2	62.84	42.97	4	65.74	44.95	2
	VP	2.54	0.64	1	29.62	7.48	10	217.1	54.85	14	146.48	37.01	5
	SS	11.4	2.65	4	27.2	6.33	5	182.96	42.62	10	207.68	48.38	6
	SB				26.72	17.72	4	21.24	14.09	2	102.78	68.18	4
IE-245	OS	5.64	1.38	3	79.9	19.55	18	188.44	46.12	15	134.54	32.93	4
	VP	13.54	3.05	20	267.74	60.34	60	162.42	36.60	13	-		
	SS	11.68	2.36	14	197.9	39.99	36	181.96	36.76	13	103.32	20.87	5
	SB	0.94	0.49	1	66.74	35.34	15	94.14	49.85	8	27	14.29	1
IE-256	OS	6.3	3.95	3	48.62	30.53	6	17.66	11.09	1	86.66	54.42	3
	VP	7.98	2.28	3	28.94	8.28	7	135.68	38.82	9	176.86	50.60	6
	SS				100.18	19.89	18	195.68	38.86	15	207.68	41.24	8
	SB				6.94	7.67	1	55.64	61.53	3	27.84	30.78	1

Means weight *, percentage ** number of mushrooms ***. [a]Groups of pileus size according to its diameter: G1, <5 cm; G2, 5–9.9 cm; G3, 10–14.9 cm; G4, >15 cm

highest PR was observed for IE-245 SS (1.69%), IE-256 SS (1.57%), and IE-105 SS (1.34%), with significant difference among the three treatment. In general, the highest PRs (1.45%) were found on SS and, on average, the strain IE-245 (1.39%) showed the highest PRs (Tables 3 and 4). The PRs obtained in this study are similar or even higher compared to those cited by Royse (1985) for shiitake mushrooms grown on sterilized enriched sawdust (0.29% to 0.79%).

Table 3: Productivity of *Lentinus edodes* in Oak Shavings (OS), Vineyard Pruning (VP), Sorghum Stubble (SS) and Sugarcane Bagasse (SB)

Substrate	Strain	BE (%)	PR (%)	Y (%)
OS	IE-40	54.19±38.46^{c}	0.52±0.37^{b}	22.80±16.18^{c}
	IE-105	36.19±4.65^{a}	0.34±0.04^{a}	15.23±1.96^{b}
	IE-245	80.89±26.54^{d}	0.81±0.26^{c}	34.04±11.17^{d}
	IE-256	39.41±29.9^{a}	0.35±0.27^{a}	16.58±12.58^{b}
Means		52.67±31.31^{a}	0.51±0.31^{a}	22.16±13.17^{b}
VP	IE-40	76.88±49.81^{d}	0.75±0.48^{c}	23.00±14.9^{c}
	IE-105	110.23±40.94^{e}	1.08±0.4^{e}	32.97±12.24^{d}
	IE-245	123.59±25.7^{g}	1.26±0.26^{f}	36.97±7.68^{d}
	IE-256	97.34±4.97^{d}	0.95±0.04^{d}	29.12±1.48^{c}
Means		102.01±36.49^{b}	1.01±0.36^{b}	30.51±10.91^{c}
SS	IE-40	119.54±22.32^{f}	1.19±0.22^{e}	34.56±6.45^{d}
	IE-105	123.7±26.57^{g}	1.34±0.28^{f}	35.77±7.68^{d}
	IE-245	142.61±61.83^{g}	1.69±0.73^{h}	41.23±17.87^{e}
	IE-256	145.11±21.26^{g}	1.57±0.23^{g}	41.96±6.14^{e}
Means		132.7±35.87^{c}	1.45±0.44^{c}	38.38±10.37^{d}
SB	IE-40	96.08±31.14^{d}	0.85±0.27^{c}	19.05±6.17^{b}
	IE-105	79.17±12.19^{d}	0.74±0.11^{c}	15.70±2.41^{b}
	IE-245	79.33±21.01^{d}	0.80±0.21^{c}	15.73±4.16^{b}
	IE-256	47.48±8.08^{b}	0.48±0.08^{b}	9.41±1.6^{a}
Means		75.51±25.83^{a}	0.72±0.22^{a}	14.97±5.12^{a}

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

Table 4 : Production of *Lentinula edodes* per strain evaluated

Strain	BE (%)	PR (%)	Y (%)
IE-40	86.67±41.87^{a}	0.83±0.4^{a}	24.85±12.42^{a}
IE-105	87.32±41.58^{a}	0.87±0.45^{b}	24.92±11.88^{a}
IE-245	106.60±44.4^{a}	1.14±0.54^{c}	31.99±14.46^{b}
IE-256	82.34±46.93^{a}	0.84±0.51^{a}	24.27±14.29^{a}

Values are means ± standard deviation of five replicates. Means in a column with different superscripts of each strain are significantly different (p<0.05, Tukey)

The Y was significantly affected by strain (F=2.86, df=3, p=0.0435) and substrate (F=21.89, df=3, p=0.0001). The highest Y was recorded for IE-256 SS (41.96%), and IE-245 SS (41.23%), no statistical difference between two treatment were observed (Table 3). Meanwhile, Delpech and Olivier (1991) obtained a Y of 11.9% to 15.9% on wheat straw that was vapor-pasteurized at 60 °C for 24 h. In this study, Y values of 14.97% (SB) to 38.38% (SS) were obtained. These values are similar or greater than those reported by Kilpatrick et al. (2000) for the cultivation of shiitake on sterile wheat (5% to 31%) supplemented with wheat bran and millet. Overall, based on the BE, PR, and Y, the best substrates were SS y VP and the best strains IE-105 and IE-245 (Tables 3 and 4).

The use of agro-industrial residues available at the local level (Philippoussis et al., 2002) can also reduce production costs (Martínez-Carrera and López Martínez, 2010) and represent one solution for the management of these wastes (Philippoussis, 2009).

Chemical analysis of the substrate

The NDF content significantly differed per strain (F=210.2, d.f.=3, p=0.001), substrate (F=52.6, d.f.=3, p=0.001), day (F=1570.4, d.f.=3, p=0.001), and their interactions (F=6.4, d.f.=27, p=0.001). The NDF content of the initial substrates was significantly different between VP and SS and significantly similar between OS and SB. At 69 days of growth, the treatments IE-105 OS (49.85%), IE-105 SB (49.83%), IE-245 SB (49.41%), and IE-245 VP (48.84%) presented the highest NDF content, whereas IE-40 VP (25.97%), IE-40 SB (32.62%), IE-40 OS (33.46%), and IE-256 VP (33.61%) presented the lowest content. On average, the strain that presented the greatest effect on the substrates was IE-40. Thus, the NDF content of the initial substrates differed statistically from that of end substrates (at 69 days) because of the strain effect (p<0.05) (Fig. 1).

The hemicellulose content was significantly affected by strain (F=113.14, d.f.=3, p=0.001), substrate (F=528.42, d.f.=3, p=0.001), day (F=2087.24, d.f.=3, p=0.001), and their interactions (F=8.41, d.f.=27, p=0.001). The hemicellulose content of the initial substrates statistically differed (p<0.05). Furthermore, SS and SB presented greater degradation in comparison to OS, and VP (Fig. 2). The strain IE-256 was associated with the highest degradation in SS, SB, and VP (,). However, in SS, the degradation of hemicellulose was notably higher because of the strain effect. The hemicellulose content of the initial substrates of VP, OS, SS, and SB was 16.92%, 20.93%, 31.36%, and 35.81%, respectively. The final percentages ranged from 7.68% to 9.73% for VP, 10.85% to 12.81% for OS, 6.26% to 12.06% for SS, and 9.87% to 12.25% for SB. The percentages of the initial substrates differed statistically from those of the end substrates because of the strain effect, and the percentages of the end substrates (69 days) also differed significantly (p<0.05). Our results suggested that the high concentration of hemicellulose and low concentration of cellulose detected in SS favored short periods of incubation and high rates of biodegradation, influencing high values of mushroom productivity. Leatham and Leonard (1989) mentioned that, during mycelial growth, *L. edodes* uses the most accessible sources of carbon, such as free sugars, starches, pectins, and hemicelluloses.

The cellulose content was also significantly affected by strain (F=23.77, d.f.=3, p=0.001), substrate (F=638.26, d.f.=3, p=0.001), day (F=543.05, d.f.=3, p=0.001), and their interactions (F=4.05, d.f.=27, p=0.001). The cellulose content of the control substrates of SS, SB, VP, and OS was 26.55%, 38.60%, 40.51%, and 46.78%,

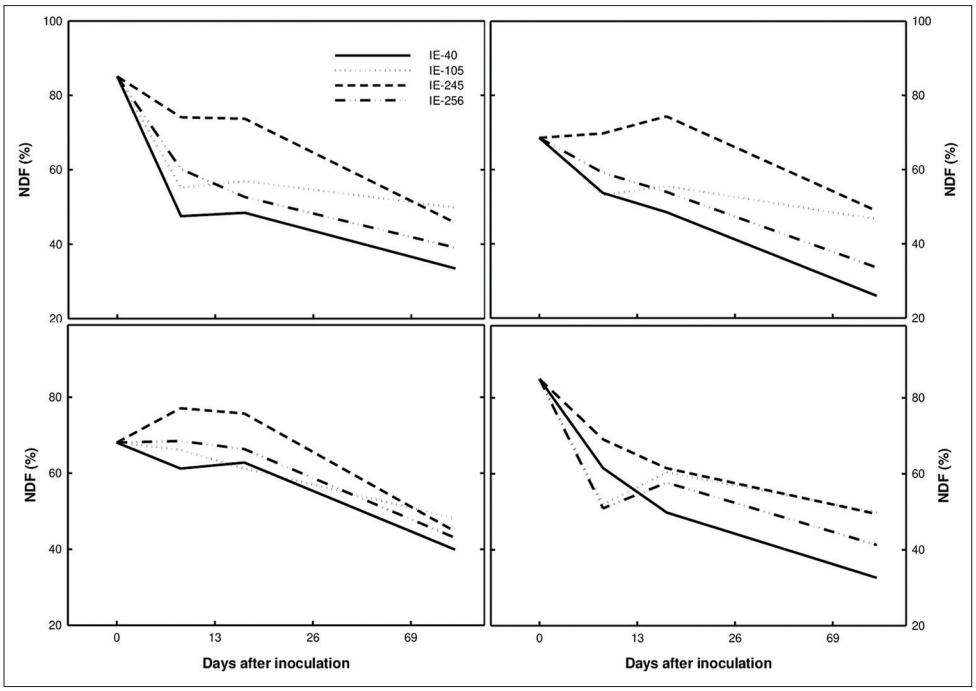

Fig 1. Variation in NDF content (average) of substrates during mycelial growth of four strains of *Lentinula edodes*. OS (a), VP (b), SS (c), and SB (d).

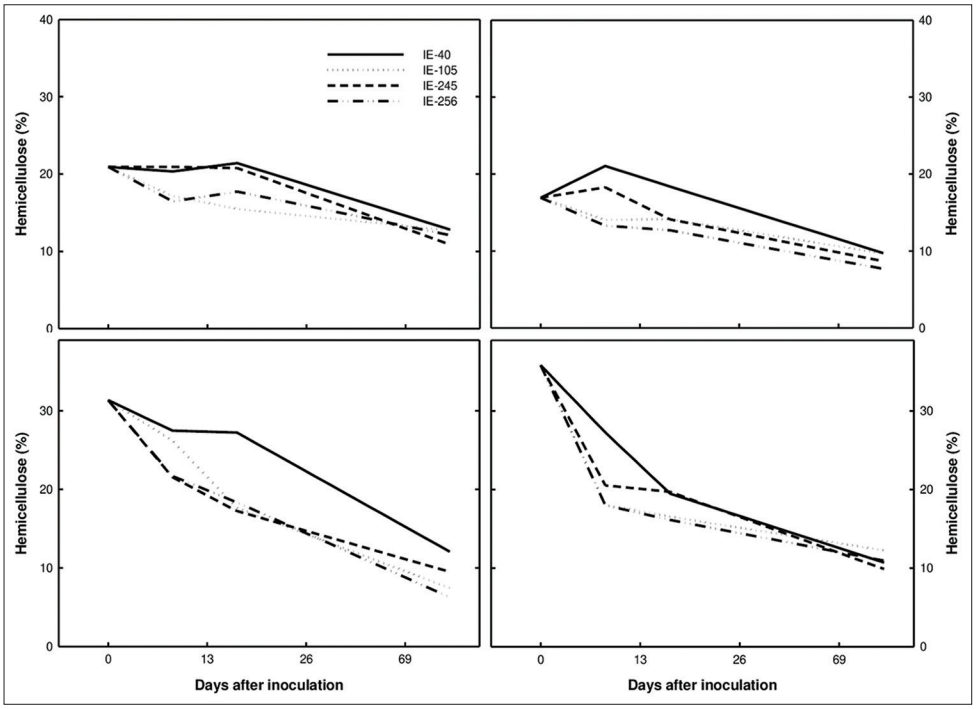

Fig 2. Variation in hemicellulose content (average) of substrates during mycelial growth of four strains of *Lentinula edodes*. OS (a), VP (b), SS (c), and SB (d).

respectively. The cellulose content was statistically similar between OS and VP but statistically different between SS and SB ($p<0.05$) (Fig. 3). The highest degradation occurred in the treatments IE105 OS (38.55%) IE-245 VP (31.61%) and IE-245 SS (20.32%). Meanwhile, in SB (31.34%), the highest degradation was related with IE-256 (Fig. 3).

In the present study, SS was the substrate with the lowest initial percentage of cellulose, and the four strains had the highest production on this substrate, even developing a greater percentage of mushrooms of size class 4. This confirms that the biosynthetic capacity of these studied strains was favored by the initial composition of SS, enabling the mushrooms to

efficiently use nutrients and develop fruiting bodies in less incubation time.

Finally, the lignin also significantly differed per strain (F=17.38, d.f.=3, p=0.001), substrate (F=1116.98, d.f.=3, p=0.001), day (F=625.61, d.f.=3, p=0.001), and their interactions (F=3.62, d.f.=27, p=0.001). The lignin content of the initial substrates of SS, SB, VP, and OS was 3.73%, 9.13%, 10.17%, and 17.24%, respectively (Fig. 4), showing statistical differences. Of the four strains utilized, the highest reduction in lignin by the end of the experiment was presented in SS (Fig. 4). The highest degradation was observed for the treatments IE-40 OS, IE-256 VP, IE-256 SS, and IE-256 SB (Fig. 4). The lignin content of the

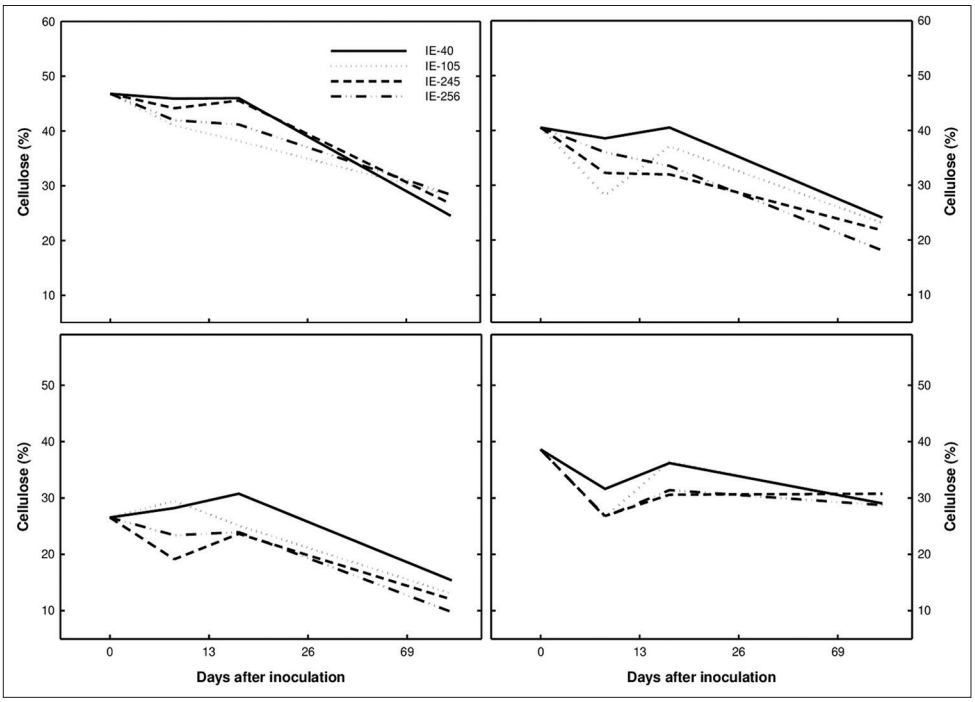

Fig 3. Variation in cellulose content (average) of substrates during mycelial growth of four strains of *Lentinula edodes*. OS (a), VP (b), SS (c), and SB (d).

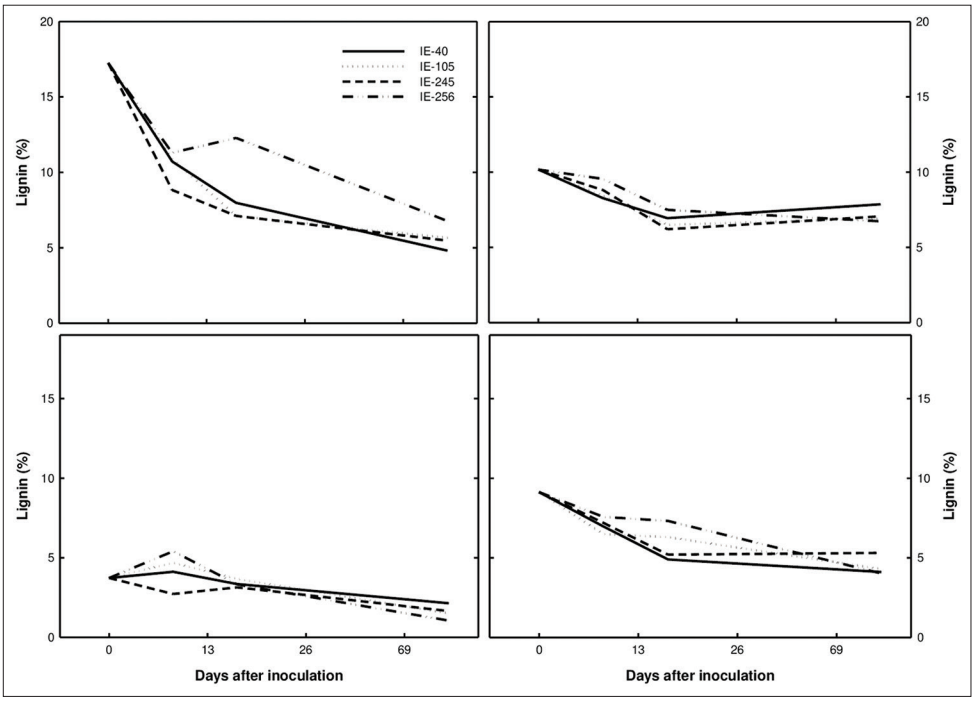

Fig 4. Variation in lignin content (average) of substrates during mycelial growth of four strains of *Lentinula edodes*. OS (a), VP (b), SS (c), and SB (d).

initial substrates statistically differed from that of the end substrates (69 days) because of the strain effect ($p<0.05$). In SS, a slight increase in the lignin content was observed at some of the evaluation points with respect to the control substrate (Fig. 4C). The increase in lignin after mushroom cultivation could be associated with high N content in the substrates, which causes the inactivation of the enzyme manganese peroxidase (Fu et al., 1997; Rüttimann-Johnson et al., 1994). Meanwhile, the variation in lignocellulose in the final substrate with respect to the initial substrate can be due to the use of lignocellulose by mushrooms for their growth. Finally, strains can act differently depending on the availability of the fiber fractions in the initial substrate and on the dynamic changes in digestion that occur during mushroom growth (Sánchez et al., 2002)

It was previously observed that supplements with high cellulose and hemicellulose contents, such as sugarcane molasses, do not increase mushroom growth (Rossi et al., 2003). This is due to the fact that when shiitake grows on non-woody substrates, cellulose degradation is lower (14%) than lignin degradation (40%-60%) (Salmones et al., 1999).

Quantification of total polyphenols in carpophores

Mushrooms synthesize a large variety of secondary metabolites, including polyphenols, which are well-known for their antioxidant capacity. Among these polyphenols, flavonoids, phenolic acids (hydroxycinnamic acid, hydroxybenzoic acid, caffeic acid, chlorogenic acid, gallic acid), tannins, chalcones, and coumarins stand out for their antioxidant capacity and constitute the polyphenolic fraction of various foods (Hirano et al, 2001; Martínez-Valverde et al., 2000). The analysis of variance indicated that the factors of substrate ($P<0.0001$), strain ($P<0.0001$), and their interaction ($P<0.0001$) affected the content of phenolic compounds in the shiitake carpophores. Fig. 5 shows the concentration of total phenols in the four strains of *L. edodes* cultivated on four different substrates. The lowest and the highest values were obtained for the strain IE-256 on OS (1.5983 mg GAE*g^{-1}) and SS (2.7197 mg GAE*g^{-1}), respectively, confirming that the interaction of both factors (substrate*strain) is highly significant for the phenol content of this type of mushroom. A negative low statistically correlation was observed between polyphenols of basidiomes and hemicellulose content in the substrate ($r=0.34$; $p=0.005$). The phenolic compound content of the shiitake fruiting bodies under the distinct treatments was within the interval of 1.59 and 2.71 mg GAE*g^{-1} (dw). This range is similar to that cited by Kitzberger et al. (2007) but lower than the values of 3.91, 4.79, and 6.27 mg GAE*g^{-1} (dw) and 1.88–5.83 g kg^{-1} (dw) reported by Sánchez et al. (2010), Cheung et al. (2003), Yang et al. (2002), and Zhang et al. (2013), respectively. Heleno et al. (2015a) reported the presence of *p*-hydroxybenzoic, protocatechuic, and cinnamic acids in concentration of 0.19, 0.14 y 0.07 mg/100 g, respectively in *L. edodes* marketed in Poland. Phenolic acids represent a significant importance due to antioxidant, antitumural, and antimicrobial activity (Heleno et al., 2015b). Also, given that existing reports have indicated that antioxidant activity is related with phenolic

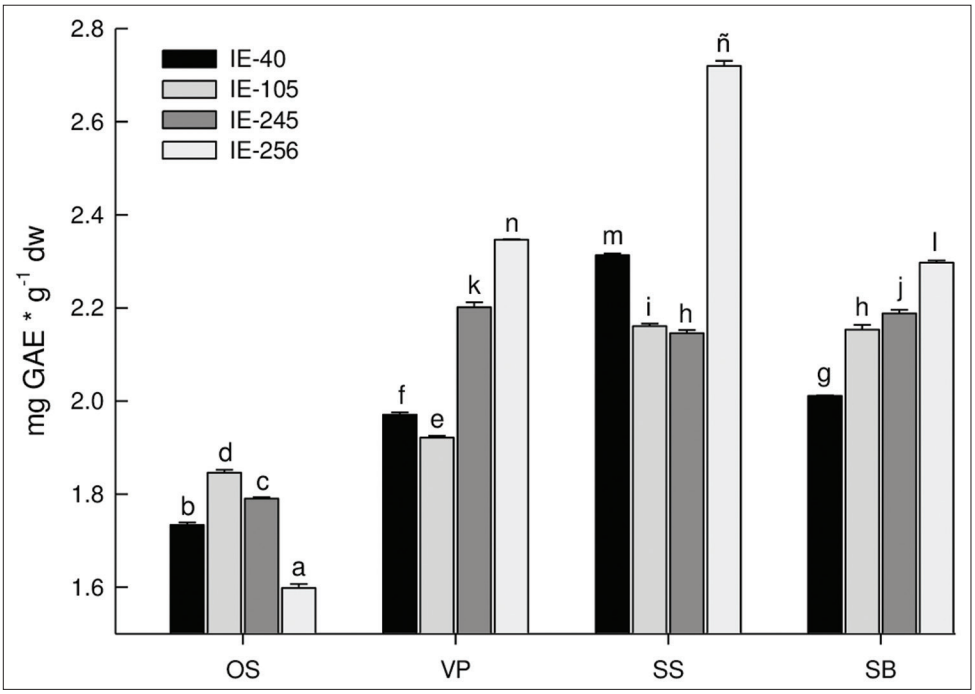

Fig 5. Total phenolics in basidiomes of four strains of *Lentinula edodes* cultivated in Oak Shavings (OS), Vineyard Pruning (VP), Sorghum Stubble (SS) and Sugarcane Bagasse (SB). The bars represent the mean ± standard error of four replicates. Different lowercase letters indicate significant differences ($p< 0.05$, Tukey).

compound content (Velioglu et al., 1989), it is important to identify the factors that promote the highest concentration of these compounds.

According our results, SS is the most recommendable for the cultivation of shiitake mushrooms because the strains cultivated on this substrate showed high productivity rates, even surpassing those of the traditionally employed substrate of OS. For at least three of the studied strains, SS influenced the high productivity rates, phenolic content and, consequently, the high antioxidant activity of the harvested carpophores.

CONCLUSIONS

The use of agricultural wastes for the cultivation of shiitake mushrooms is of particular interest for the edible and medicinal mushroom industry because it broadens the available production alternatives. In particular, the use of substrates that promote faster mushroom growth and have lower costs in regions where the traditional wood materials for cultivating this species are not readily available. Of the evaluated substrates, SS was the most appropriate substrate because it resulted in a high productivity rate and greater content of bioactive phenolic compounds in the harvested basidiomes. These topics should be further explored in future studies. The selection of substrates for producing edible mushrooms should be based on the productivity of the strains but also on the nutritional quality of cultivated mushrooms, which are currently in demand by consumers who wish to improve their health through consuming foods containing bioactive compounds.

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