Potential use of different lignocelullosic by-products for the cultivation of *Lentinula edodes*: effect on yield and total phenolic content

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Introduction

Lentinula edodes (Berk), Pegler, has traditionally been cultivated on hardwood logs, in order to obtain fruiting bodies for human consumption. However, this cultivation system represents a limiting factor and potential danger to the environment in Mexico as well as other countries due to the slow growth rate and the overuse of oak, jeopardizing the population of this important forest element. In Mexico, experimental cultivation of shiitake has been carried out using different lignocellulosic residues [1,2]. Furthermore, it has been observed that the composition of the substrate influences the chemical content of the harvested carpophores. Therefore, this study evaluated the productivity of four shiitake strains on different lignocellulosic by-products with the objective of correlating the composition of the substrate with the yield of carpophores and their polyphenol content

Materials and Methods

Strains

Four *Lentinula edodes* strains evaluated in this study were as follows: L35 from Hong Kong, CS.2 from USA, INRA V084 from USA, and strain FM009 from USA. The strains were deposited in the Fungi Strain Collection at the Ecology Institute (INECOL, Veracruz, Xalapa, Mexico) and are registered as IE-40, IE-105, IE-245, and IE-256, respectively.

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%), CaS04 (1.3%) and peat moss (1.3%), percentages are based on dry matter. The spawn reached a final moisture content of 70%. 300 g (fresh weight) of this mixture were placed in plastic bags and 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

Fungus was produced using vineyard pruning (*Vitis vinifera*) (VP), sorghum stubble (*Sorghum vulgare*) (SS), sugarcane bagasse (*Saccharum officinarum*) (CB) and oak shavings (*Quercus* sp.) (OS) (as control). The SS was chopped up using an electric chopper into small particle that ranged from 5 to 8 cm in length. All substrates were hydrated separately in a container for 12 h. After that, they were drained and were placed (1.2 kg wet weight) in 19.5 x 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 had completely covered the substrates, substrate bags were transferred to a production room. The relative humidity was maintained at 85-90% and the air temperature at 18±1°C. Production data were evaluated based on biological efficiency (BE), production rate (PR) and yield (Y) (fresh weight of harvested mushrooms/substrate fresh weight, expressed in percentages). Also considered was the production period and number of crops.

Changes in fiber content (NDF)

In order to determine the chemical composition of substrates the next analysis was conducted: Neutral Detergent Fiber (NDF), which is comprised of hemicellulose, cellulose, and lignin (cellulose content was calculated by the differences between ADF-lignin; hemicellulose by the difference between NDF-ADF) as determined by Goering & Van-Soest's technique [3] (FIBER ANALIZER ANKOM 200). All substrates were initially determined and subsequently at 13, 26, and 69 days after inoculation.

Total phenolic compounds

Fresh carpophores of each substrate tested were used. Carpophores of first crop were dried at 50°C for 24 hours and then the samples were pulverized with a power mill. The content of phenolic compounds was quantified spectrophotometrically, according to Singleton y Rossi method [4].

Statistical analysis. A completely random design with a factorial arrangement was applied to NDF analysis, total phenolic compounds, and production values. An analysis of variance was conducted for all values and comparison of means according to Tukey's test (p<0.05) using the statistical software Statistica (v. 7.0).

Results and Discussions

Carpophores production. During incubation period the samples showed dark-colored patches that eventually spread to cover the entire surface, this condition was considered to transfer the samples to fruiting area. The formation of sclerotia varied according to the substrate, with an average of 49 days on OS, 45 days on VP, 40 days on SS, and 50 days on CB. The SS was the best substrate in terms of early sclerotia formation. The total fresh mushrooms production varied from 113.02 g to 494.86 g. The number of crops obtained in the tested substrates were two or three, except on IE-105/OS and IE-256/CB. Production distribution displayed a similar pattern on CB, OS and VP, with more that 60% of the total obtained in the first crop (>61.17% on CB, >66.75% on OS and >67.57% on VP), but different on SS, with more that 45% (>47.18%) (Table 1).

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

Strain	Substrate	IP	Flushes	Total weight (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
	CB	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
	CB	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
	CB	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	
	CB	50	1	113.02±19.24[a]	100.00		

IP incubation period. Values are mean ± standard deviation. Means that do not have at least one letter in common of each strain in four substrates are significantly different (p<0.05, Tukey). [a]Fresh weight of mushrooms harvested from five replicates. [b]Distribution of total weight mushrooms obtained in each flush, estimated in percentage.

The largest BE was obtained for IE-256/SS (145.11%), IE-245/SS (142.61%) and IE-105/VP (110.23%). The highest PR was observed for IE-245/SS (1.69%), IE-256/ SS (1.57%) and IE-105/SS (1.34%), while the highest Y was recorded for IE-256/SS (41.96%), IE-245/SS (41.23%) and IE-105/SS (35.77%) (Table 2).

Table 2. Productivity of *Lentinula edodes* in Oak Shavings (OS), Vineyard Pruning (VP), Sorghum Stubble (SS) and Sugarcane Bagasse (CB)

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[d]	1.08±0.4[e]	32.97±12.24[d]
	IE-245	123.59±25.7[d]	1.26±0.26[e]	36.97±7.68[d]
	IE-256	97.34±4.97[d]	0.95±0.04[d]	29.12±1.48[d]
Means		102.01±36.49[b]	1.01±0.36[b]	30.51±10.91[c]
SS	IE-40	119.54±22.32[d]	1.19±0.22[e]	34.56±6.45[d]
	IE-105	123.7±26.57[d]	1.34±0.28[e]	35.77±7.68[d]
	IE-245	142.61±61.83[d]	1.69±0.73[e]	41.23±17.87[d]
	IE-256	145.11±21.26[d]	1.57±0.23[e]	41.96±6.14[d]
Means		132.7±35.87[c]	1.45±0.44[c]	38.38±10.37[d]
CB	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]

BE biological efficiency. *PR* production rate. *Y* yield. Values are mean ± standard deviation. Means that do not have at least one letter in common of each strain in four substrates and only among means are significantly different (p<0.05, Tukey).

The fiber results demonstrated that carpophores were significantly affected by growth period, strain, substrate and the selected combinations of strains and substrates. In general, it was observed that VP and SS had the highest percentage of biodegradation, where the highest hemicellulose and cellulose degradation occurred with IE-256 strain and the highest lignin degradation with IE-245 strain (Figures 1, 2, 3, 4).

The analysis of variance indicated that substrate (p<0.0001), strain (p<0.0001), and interaction substrate-strain (p<0.0001) had a significant effect on the content of phenolic compounds in the carpophores. The lowest and highest values were obtained with IE-256 strain on OS (1.5983 mg EAG*g-1) and SS (2.7197 mg EAG*g-1), respectively, which verifies that the interaction of substrate-strain was highly significant (Figure 5).

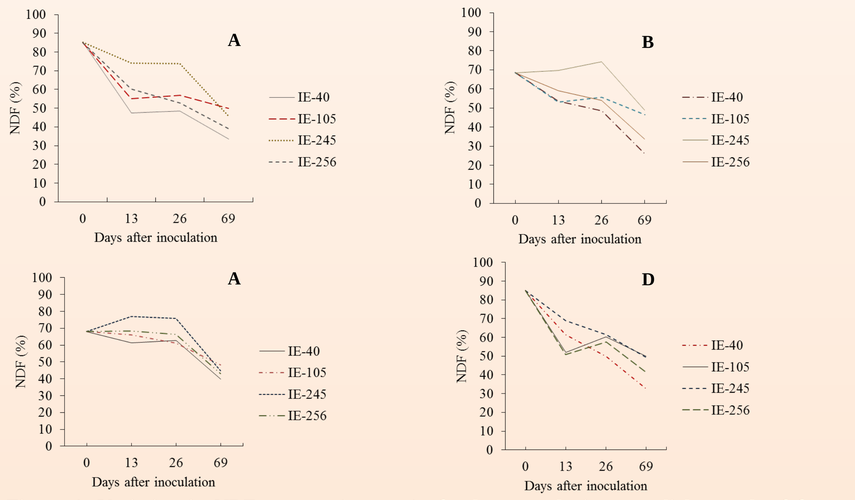

Figure 1. Variation in NDF content (average) of substrates during mycelial growth of four strains of *L. edodes*. OS (A), VP (B), SS (C), and CB (D).

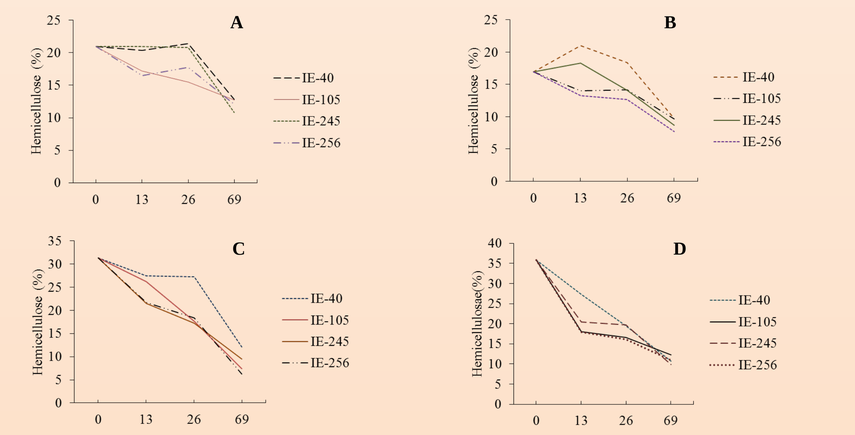

Figure 2. Variation in hemicellulose content (average) of substrates during mycelial growth of four strains of *L. edodes*. OS (A), VP (B), SS (C), and CB (D).

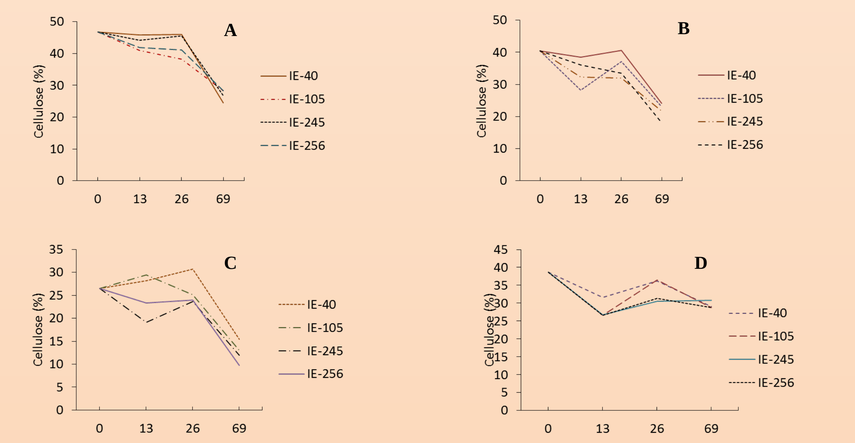

Figure 3. Variation in cellulose content (average) of substrates during mycelial growth of four strains of *L. edodes*. OS (A), VP (B), SS (C), and CB (D).

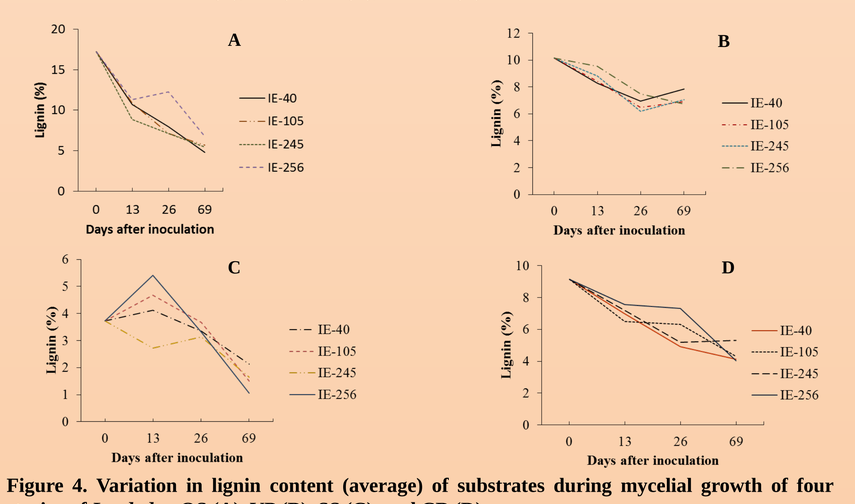

Figure 4. Variation in lignin content (average) of substrates during mycelial growth of four strains of *L. edodes*. OS (A), VP (B), SS (C), and CB (D).

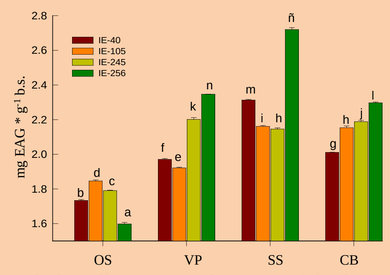

Figure 5. Variation of total phenolic compounds (average) in the *L. edodes* carpophores. *OS* oak shavings, *VP* vineyard pruning, *SS* sorghum stubble, *CB* sugar cane bagasse.

Conclusion

Sorghum stubble (SS) was the best substrate for producing carpophores, while the IE-245 strain presented the greatest values for BE, PR and Y. Thus, the carpophores of the IE-256 strain cultivated in SS could potentially present greater antioxidant activity due to highest polyphenols content.

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