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Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom

Funda Atila

Ahi Evran University, Faculty of Agriculture, Department of Horticulture, 40200, Kırşehir, Turkey

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ABSTRACT

The study assessed the changes in digestibility of some agricultural wastes such as chickpea straw (CPS), corn stalk (CS), alfalfa hay (AH) and sunflower head residue (SFH) during the spawn running period and fruitbody production of *Lentinula edodes* (Berk.) Pegler (shiitake) to better understand the nutritional needs of this mushroom species. In addition, the effects of these wastes on some productivity features of shiitake cultivated under laboratory conditions were evaluated. Oak awdust (OS) was used as a control substrate. The substrates were analyzed for nitrogen (N), cellulose, hemicellulose and lignin content at three different growth stages. Morever, the effect of agro-wastes on spawn run time, time to first primordia initiation, time to first harvest, yield, biological efficiency (BE) and average mushroom weight were evaluated during the cultivation cycle. Among the five substrates, the SFH exhibited maximum productivity, followed by CPS (233.7 g/kg and 228.1 g/kg, respectively). No correlation was found between the shiitake yield and the N or lignocellulosic content of the growing substrates. On the other hand, the shiitake consumed hemicellulose during the spawn running period, whereas the consumption of cellulose and lignin occured during the pinheading and fruiting formation stages. The hemicellulose, cellulose and lignin contents in the spent substrate were 36.5–51.9%, 5.3–27.0% and 24.0–36.8% lower, respectively, than in the initial substrates, while the N content in the mushroom substrate was increased by 10.3% (CS) − 97.1% (OS) after shiitake cultivation. In conclusion, shiitake mushrooms had a preference for substrates containing a moderate amount of N, hemicellulose and lignin, and having a low cellulose:lignin ratio. Morever, the chemical composition of the growing substrates changed during the life cycle of the mushroom and the protein content increased with time, while the amount of lignocellulosic content in the substrates was reduced, making it more digestible.

1. Introduction

Lentinula edodes (Berk.) Pegler, known as shiitake, is one of the most commonly produced mushroom species in the world. There has been a significant increase in shiitake production over the years. It is estimated that the total shiitake production was over 4.5 million tons in 2012 (Royse, 2014). The reason for this rise in production and consumption of shiitake mushrooms lies in their combined value as a food as well as a medicinal and nutraceutical source. Other than proteins, shiitake contain high levels of macronutrients, and are also high in sugars, tocopherols and polyunsaturated fatty acid (PUFA) levels and low in saturated fatty acid (SFA) content (Reis et al., 2012). Counted among their medicinal attributes are antitumor (Minato et al., 1999), antioxidant (Choi et al., 2006), antiviral (Rincão et al., 2012), antibacterial (Hatvani, 2001) and cholesterol-lowering (Fukushima et al., 2001) activities.

Shiitake is a type of white rot fungi (Oki et al., 1981), which are known to be the most efficient lignin degraders in nature. The lignocellulolytic enzymes produced by this white-rot fungus including cellulase, hemicellulase, ligninase, etc. have enabled them to be cultivated in a wide range of lignocellulosic substrates, including different agro-industrial residues.

Sawdust is the most popular basal ingredient used in the synthetic formulations of substrate for producing shiitake. Some additive materials (20–60% dry weight) such as wheat bran, rice bran, millet, rye, and maize may be added to the basal substrate to improve the production and quality (Royse and Sanchez, 2007). However, the availability of sawdust is limited in some areas. Consequently, some alternative materials such as wheat straw (Royse and Sanchez, 2007), hazelnut husk (Ozcelik and Peksen, 2007), barley straw and vineyard prunings (Gaitán-Hernández et al., 2011) and sun flower hulls (Curvetto et al., 2005) have been researched for use as growing substrates in shiitake cultivation.

At the end of mushroom harvests, the growing substrate is

E-mail addresses: fundacavuslar@hotmail.com, funda.atila@ahievran.edu.tr.

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considered spent and referred to as “spent mushroom substrate” (SMS). Although many countries have no data on SMS discharge, it is believed that about 5 kg of waste substrate are generated from the production of 1 kg of mushrooms (Medina et al., 2012). Disposal of SMS is one of the major environmental problems in the mushroom-producing countries. The SMS needs heat treatment before being removed from the growing chamber, which involves. extra cost and presents an economic problem for the mushroom industry. Zadrazil (1997) reported that throughout the growth of *Pleurotus* spp. on cereal straw, the chemical composition of the substrate changed continuously. The changes in digestibility of mushroom growing substrates make recycling of this material a possibility and thus enable waste disposal problems to be reduced by recycling. Morever, recycling of SMS for different uses such as animal feed or soil conditioner may increase sustainability in addition to and also benefitting help farm economy. However, the properties of the SMS must be known in order to determine the purposes for which it can be used.

Corn, sunflower, chickpea and alfalfa are abundantly produced in Turkey. Significant quantities of agro-waste accumulate each year, depending on the production of these crops. Crop residues are usually disposed of by burning, while some are used as livestock feed. This study examined the changes in the digestibility of chickpea straw, corn stalk, alfalfa hay and sunflower head residue substratesduring the spawn running period and fruitbody production of shiitake and investigated the relationship between the nitrogen and lignocellulosic contents of the substrates and their productivity. In addition, some of the chemical properties of shiitake SMS were determined.

2. Material and methods

The study was carried out at the Ahi Evran University Agriculture Faculty Mushroom Production Unit in Kırşehir, Turkey. The shiitake strain Sylvan-4312 was used in this study. The cultures were maintained on potato dextrose agar (PDA) medium (Merck) at 4 °C. Spawn was prepared on wheat grain inoculated with actively growing shiitake mycelia and incubated at 25 ± 2 °C for mycelial growth until the mycelium fully covered the grains.

2.1. Substrate preparation

The corn stalks (CS) were ground in a Wiley mill and screened with sieves to obtain particles ranging from 20 to 48 in mesh size. The chick pea straw (CPS), sunflower head residue (SFH) and alfalfa hay (AH) were chopped into small pieces (2–3 cm). No treatment was applied to the oak sawdust (OS) (60–80 mesh size) used as the control substrate. Substrates were prepared using the OS, CS, CPS, SFH and AH and water was added to raise the final moisture content to 55% (Shen et al., 2008). Polypropylene bags were filled with 1 kg of growing substrate and then autoclaved at 121 °C for 90 min. The sterilized subtrates were spawned using 3% grain spawn on a w/w wet weight basis.

The spawn running phase was carried out at 25 ± 2 °C until the full mycelial colonization was achieved, forming a dark brown-colored crust. During the browning process, light was provided throughout the spawn running period for 4 h (Royse, 1997) and the bags were sliced open and watered lightly once or twice per day to maintain continuous surface moisture. At the end of the spawn running period, the bags were moved to the production room (temperature: 15 ± 2 °C; relative humidity (RH): 90%; light: 300 lux for 12 h daily) to induce fructification. Sufficient air exchanges were made to keep the CO_2 concentration below 1200 ppm.

Mushroom fruiting bodies were harvested from the substrates when the veil had broken and the gills were fully exposed. The yield of mushrooms and their various quality parameters were recorded regularly.

2.2. Evaluation of the cultivation parameters

Spawn run time (days), time to first primordia initiation (days), time to first harvest (days) yield (g/kg), biological efficiency (%) and average mushroom weight (g) were evaluated during the shiitake cultivation on different substrates. Yields were obtained from three flushes and expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrate (w/w). Biological efficiency (BE) was calculated as the percentage ratio of the fresh weight of harvested mushroom per gram of dry substrate (Royse, 1985). The average mushroom weight (AMW) of the basidiomata was determined as the rate of the total weight of fresh mushrooms harvested by their number. Results were obtained from ten replicates for the shitake grown on each tested substrate.

2.3. Substrate analysis

Substrates were oven-dried at 60 °C for 48 h and ground to pass through a 1 mm sieve. The ash, moisture and pH were determined by standard procedures (Kacar and İnal, 2008). Carbon (C) content was assessed according to Sanchez and Royse (2007). The Kjeldhal method was used to determine the total nitrogen (N) content of each substrate. The carbon/nitrogen (C:N) ratio of each substrate was then calculated.

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) methods were used to determine hemicellulose, cellulose and lignin contents of the substrates (Vansoest et al., 1991). The hemicellulose was calculated using NDF and ADF and the cellulose was determined using ADF and ADL (hemicellulose = NDF − ADF; cellulose = ADF − ADL) (Zadrazil and Brunnert, 1982). Samples were taken on Day 0 (non inoculated substrates), after spawn running period and after harvest.

2.4. Statistical analysis

The experiments were set up in a completly randomized design to test ten replicates of five types of substrate. The data obtained from the experiments were subjected to variance and means analyses. The statistically significant differences were compared employing Tukey’s test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%. Pearson's correlation was used to analyze the relationship between chemical constituents of the substrates and spawn run time, yield, BE (%) and AMW.

3. Results

3.1. Proximate analysis of the initial substrates

The main physicochemical properties of the initial substrates are presented in Table 1. No significant difference was found among the substrates regarding moisture content ($P > 0.05$), although the pH, ash, C and N content and C:N ratio were affected by the substrates ($P < 0.01$). The moisture content of the substrates changed between 54.2% and 57.9%. The highest pH was determined in the AH substrate (6.1), while the lowest was observed in the OS substrate (4.5). All agro-wastes contained ash, with an average of 4.8–6.7%, while the N content of the substrates varied between 0.34% (OS) and 2.13% (AH) (Table 1). However, the C:N ratio of OS was higher than in the other substrates, whereas the C:N ratio of the AH was lower than in the others.

Significant differences were found among the substrates regarding the concentration of hemicellulose, cellulose and lignin ($P < 0.01$). The hemicellulose, cellulose, lignin and cellulose:lignin ratio also presented a wide variability, as 6.3–30.4%, 20.7–41.9%, 4.8–26.2% and 1.60–10.2 dw, respectively. As regards the lignocellulosic constituents, the CS exhibited the highest values for hemicellulose and the lowest for lignin, while the OS exhibited the highest values for cellulose and lignin and the lowest for hemicellulose contents.

Table 1
Chemical composition of growing substrates tested in the study.

	Moisture (%)	pH	Ash (%)	C (%)	N (%)	C:N ratio	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Cellulose:Lignin
OS	55.3^{ns}	4.5^{**c}	4.8^{**c}	55.2^{**a}	0.34^{**e}	160.8^{**a}	6.3^{**d}	41.9^{**a}	26.2^{**a}	1.60^{**e}
CPS	54.9	5.8^{a}	4.6 ^{c}	55.4 ^{a}	0.82 ^{c}	67.5 ^{c}	18.5 ^{b}	34.8 ^{c}	11.4 ^{b}	3.05 ^{b}
SFH	54.2	5.1^{b}	6.7 ^{a}	54.1 ^{c}	1.12 ^{b}	48.4 ^{d}	17.2 ^{b}	20.7 ^{e}	9.7 ^{d}	2.13 ^{d}
AH	57.9	6.1^{a}	5.4 ^{b}	54.9 ^{b}	2.13 ^{a}	25.8 ^{e}	11.7 ^{c}	26.4 ^{d}	10.4 ^{c}	2.53 ^{c}
CS	56.3	5.2^{b}	6.3 ^{a}	54.3 ^{c}	0.58 ^{d}	93.2 ^{b}	30.4 ^{a}	38.7 ^{b}	4.8 ^{e}	8.13 ^{a}

OS:oak sawdust; CPS:chickpea straw; SFH:sunflower head residue; AH: alfalfa hay; CS: corn stalk. Asterisks indicate significance at *P < 0.05, **P < 0.01,^{ns} not significant; values within the same column followed by the same letter are not significantly different by Tukey's test.

Table 2
Effect of different substrates on production of shiitake.

Substrates	Cultivation period (days)	Spawn running time (days)	Day to primordia initial (days)	Day to first harvest (days)	Yield			Total Yield (g/kg)	BE (%)	AMW (g)
					Flush 1 (g)	Flush 2 (g)	Flush 3 (g)			
OS	116.8^{**b}	44.0^{**a}	54.6^{**a}	72.2^{**b}	42.2	74.7	19.7	136.6^{**c}	30.6^{**c}	12.9^{**c}
CPS	95.0^{d}	37.8 ^{b}	46.2 ^{bc}	58.0 ^{d}	72.5	86.4	69.2	228.1 ^{a}	50.6 ^{a}	18.7 ^{a}
SFH	98.0^{d}	36.2 ^{b}	44.8 ^{c}	60.2 ^{d}	67.3	104.7	61.7	233.7 ^{a}	51.0 ^{a}	19.4 ^{a}
AH	103.2^{c}	32.4 ^{c}	48.0 ^{b}	64.4 ^{c}	54.3	54.0	48.6	156.9 ^{b}	37.3 ^{b}	15.8 ^{b}
CS	124.4^{a}	46.0 ^{a}	57.0 ^{a}	76.0 ^{a}	34.2	43.3	10.4	87.9 ^{d}	20.1 ^{d}	14.1 ^{bc}

AMW: Average mushroom weight; BE: Biological efficiency. Asterisks indicate significance at *P < 0.05. **P < 0.01; values within the same column followed by the same letter are not significantly different. by Tukey's test.

3.2. Effect of substrates on mycelial growth and yield

The fresh weight of different flushes (g), total yield (g), BE (%) and AMW (g) of shiitake mushrooms cultivated on different treatments are presented in Table 2. Spawn run time, yield, BE and AMW were all affected by the substrates ($P < 0.01$).

The spawn running period was completed in the different substrates within a period of 32.4–46.0 days. The AH was found to be the best substrate for mycelial growth in the five different agro-wastes. The longest spawn running period was 46.0 days in CS, followed by the OS at 44.0 days.

The mean time from inoculation to pinning on CPS, SFH and AH was 46.2, 44.8 and 48.0 days, respectively, while the longest period of primordia formation was 57.0 days for the CS substrate, followed by the OS substrate at 54.6 days. The CPS substrate took less time to start harvest, with an average of 58.0 days, while on CS and OS the fructification process started after 72.2 and 76.0 days, respectively.

Cultivation continued for 95–124.4 days, depending on the substrates, and three flushes were harvested in all substrates tested. There was no significant difference between the SFH and CPS substrates in total fresh weight or BE. The greatest yield and the highest BE were found on SFH substrate (233.7 g/kg and 51.0%), followed by CPS substrate (228.1 g/kg and 50.6%). Other substrates also demonstrated satisfactory performances with the exception of the CS. Yield distribution per flush showed significant variation among the substrates. Although the highest yield was obtained in the second flush on CPS substrate, the yield distribution of the flushes was similar. Moreover, there was no significant difference between flushes on the AH substrate regarding yield. Generally, the yield of the second flush was clearly higher than that of the first and third flushes for CS, SFH and OS, whereas the BE (%) of the third flush was clearly lower than that of the other flushes for CS and OS substrates. The CS and OS yielded 88.2% and 85.6% of the total yield in the first two flushes, respectively, while 73.6% of the total yield was obtained in first two flushes for the SFH substrate. Furthermore, the CPS and SFH substrates supported the production of significantly heavier mushrooms (18.7 g and 19.4 g, respectively) than the other substrates.

The results of this study showed a negative correlation between spawn run time and substrate N content (r^2 = -0.883) (Table 3).

Table 3
Correlations between chemical content of substrates and production of shiitake.

	Spawn run time (days)	Yield (g/kg)	BE (%)	Average mushroom weight (g)
Moisture	-0.223	-0.579	-0.511	0.543
pH	-0.715	0.263	0.327	0.325
Ash	0.019	-0.072	-0.098	0.045
C	-0.070	0.118	0.146	0.004
N	-0.883*	0.225	0.295	0.287
CN	0.821	-0.450	-0.495	-0.552
Hemicellulose	0.361	-0.257	-0.284	-0.150
Cellulose	0.815	-0.624	-0.645	-0.703
Lignin	0.207	-0.020	-0.029	-0.156
Sellulose/Lignin	0.566	-0.634	-0.652	-0.548

Asterisks indicate significance at *P < 0.05. **P < 0.01.
(+) positive correlation (−) negative corelation.

However, there was no correlation between the lignicellulosic content of the substrates and shiitake productivity.

3.3. Changes in compositions of substrates during shiitake cultivation

Significant differences were observed in the chemical content of the substrates for the three sampling periods ($P < 0.01$) (Tables 4 and 5). The substrate pH values and moisture content in the initial period differed from those of the colonized and spent substrates. The highest reduction in pH was observed on the CPS substrate (from 5.8 to 4.4) while the highest decrease in moisture was observed on CS substrate (from 54.2% to 39.6%). During the production cycle, the substrate ash concentration increased steadily relative to the initial substrate, with the ash content of samples varying between 5.7% and 7.8%, while these amounts increased to between 5.8% and 8.5% at the end of the spawn running period. Samples taken after the spawn running period showed that the N content of the substrate had substantially increased. After shiitake cultivation, the N content in the mushroom substrates was increased by 10.3% (CS) − 97.1% (OS). The C:N ratio varied between 20.9–118.5 and 19.8–80.6 at the end of the spawn running period and after harvest, respectively. There were also significant differences in the lignocellulosic content of the substrates for the three sampling periods

Table 4
Changes of compositional contents of substrate tested during shiitake cultivation period.

	Moisture (%)	pH	Ash (%)	C (%)	N (%)	C:N	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Cellulose:Lignin
OS										
Initial composition	55.3^{**a}	4.5^{ns}	4.8^{**b}	55.2^{**a}	0.34^{**c}	160.9^{**a}	6.3^{**a}	41.9^{**b}	26.2^{**b}	1.6^{ns}
After spawn run	54.3^{a}	4. 5	6.2^{a}	54.4^{b}	0.46^{b}	118.5^{b}	4.6^{b}	44.6^{a}	28.5^{a}	1.6
After harvest	44.7^{b}	4.1	6.5^{a}	54.2^{b}	0.67^{a}	80.6^{c}	4.0^{c}	30.6^{c}	19.4^{c}	1.6
CPS										
Initial composition	54.9^{**a}	5.8^{**a}	4.6^{**c}	55.3^{**a}	0.84^{**c}	65.7^{**a}	18.5^{**a}	34.8^{**a}	11.4^{**b}	3.1^{**b}
After spawn run	48.1^{b}	4.9^{b}	6.1^{a}	54.5^{b}	1.03^{b}	53.1^{b}	12.1^{b}	34.2^{a}	14.4^{a}	2.4^{c}
After harvest	40.2^{c}	4.4^{c}	5.8^{b}	54.6^{b}	1.15^{a}	47.5^{c}	8.9^{c}	26.8^{b}	7.2^{c}	3.7^{a}
SFH										
Initial composition	54.2^{**a}	5.1^{**a}	6.7^{**c}	54.1^{**a}	1.22^{**c}	44.5^{**a}	17.2^{**a}	20.7^{**c}	9.7^{**b}	2.1^{**c}
After spawn run	49.5^{b}	4.8^{a}	7.8^{b}	53.5^{b}	1.61^{b}	33.2^{b}	11.7^{b}	26.4^{a}	10.6^{a}	2.5^{b}
After harvest	39.6^{c}	4.1^{b}	8.5^{a}	53.1^{b}	1.86^{a}	28.6^{c}	9.2^{c}	21.8^{b}	6.4^{c}	3.4^{a}
AH										
IInitial composition	57.9^{**a}	6.1^{**a}	5.4^{**b}	54.8^{**a}	2.13^{**c}	25.8^{**a}	11.7^{**a}	26.4^{**a}	10.4^{**a}	2.5^{ns}
After spawn run	51.9^{b}	6.0^{a}	5.7^{b}	54.7^{a}	2.62^{b}	20.9^{b}	6.8^{b}	26.8^{a}	10.9^{a}	2.5
After harvest	44.5^{c}	5.4^{b}	6.6^{a}	54.2^{b}	2.73^{a}	19.8^{b}	5.0^{c}	20.4^{b}	7.9^{b}	2.6
CS										
Initial composition	56.3^{**a}	5.2^{**a}	6.3^{**c}	54.3^{**a}	0.58^{**c}	93.2^{**a}	30.4^{**a}	38.7^{**a}	4.8^{**a}	8.1^{**b}
After spawn run	49.2^{b}	5.2^{a}	7.6^{b}	53.6^{b}	0.62^{b}	86.5^{b}	21.4^{b}	40.1^{a}	4.1^{b}	9.7^{a}
After harvest	44.6^{c}	4.6^{b}	8.3^{a}	53.2^{c}	0.64^{a}	82.8^{b}	19.0^{c}	34.5^{b}	3.6^{c}	9.6^{a}

Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$ ^{,ns} not significant; values within the same column followed by the same letter are not significantly different. by Tukey's test.

($P < 0.01$). Samples taken after the spawn running period showed that the hemicellulose content of the substrates had substantially degraded, while a slight increase was observed in the cellulose and lignin content of the substrates. In AH and SFH, the hemicellulose concentration decreased gradually from 11.7% (initial substrate) to 6.8% (after spawn running) and from 17.2% (initial substrate) to 11.7% (after spawn running) respectively. Lesser decreases were observed in the hemicellulose content of OS and CS (27% and 29.6%, respectively. After harvest, the decreased ratio in the hemicellulose content of the substrates rose to 51.9% on CPS and 57.3% on AH. It was observed that the cellulosic and lignin contents of the substrates increased slightly during the period of mycelial growth and browning. Cellulose in the samples taken after the spawn running was 1.5–27.5% higher than in the initial substrates, while lignin content was 4.8–26.3% higher. The cellulose and lignin contents measured in the spent substrates ranged from 20.4% (AH) to 34.5% (CS) and from 3.6% (CS) to 19.4% (OS), respectively. Relatively higher reductions were found in the rich performing substrates (CPS and SFH), whereas AH was the substrate with the lowest decrease in lignin after shiitake cultivation.

4. Discussion

A shortened spawn running period is important in mushroom cultivation because of the high risk of contamination. Elisashvili et al. (2015) reported that the spawn run time of shiitake was 24–29 days, while Ozcelik and Peksen (2007) confirmed that this varied between 38.83 days and 59.0 days in different growing substrates. Compared to previous findings, the times of the present study are longer than those of Elisashvili et al. (2015), but similar to those of Ozcelik and Peksen (2007). Mycelial growth was very thin and slow on the OS and CS substrates. Apart from the chemical content of the substrates, the small particle size of the substrate may have slowed down gas exchange. Oxygen depletion was the cause of reduced mycelial biomass development in substrates containing smaller-sized particles (Ohga, 1990). The small particule size of OS and CS may have reduced the degrading activity of the shiitake mycelium and the spawn running period may have been prolonged accordingly.

The greatest mushroom mycelial growth was obtained in the AH substrate, which contained 2.13% N and had a 25.8C:N ratio. This study demonstrated a significant correlation between the N content of the substrate and the vegetative growth of shiitake. As the amount of nitrogen in the substrate increased, the spawn running period was

Table 5
The percentage rates of change in chemical and lignocellulosic content of shiitake growing substrates during diffrerent stages of mushroom production.

	Moisture (%)	Ph (%)	Ash (%)	C (%)	N (%)	C:N (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Cellulose Lignin
OS										
After spawn run	(−) 1.8	(−) 1.1	(+) 28.5	(−) 1.4	(+) 35.3	(−) 26.4	(−) 27.0	(+) 6.4	(+) 8.8	(−) 1.9
After harvest	(−) 19.2	(−) 8.2	(+) 36.0	(−) 1.8	(+) 97.1	(−) 49.9	(−) 36.5	(−) 27.0	(−) 26.0	(−) 1.3
CPS										
After spawn run	(−) 12.4	(−) 16.5	(+) 31.1	(−) 1.4	(+) 22.6	(−) 19.2	(−) 34.6	(−) 1.7	(+) 26.3	(−) 22.0
After harvest	(−) 26.8	(−) 25.0	(+) 25.3	(−)1.3	(+) 36.9	(−) 27.7	(−) 51.9	(−) 23.0	(−) 36.8	(+) 21.9
SFH										
After spawn run	(−) 8.7	(−) 5.9	(+) 17.4	(−) 1.1	(+) 32.0	(−) 25.4	(−) 32.0	(+) 27.5	(+) 9.3	(+) 17.4
After harvest	(−) 26.9	(−) 18.5	(+) 27.0	(−) 1.8	(+) 52.5	(−) 35.7	(−) 46.5	(−) 5.3	(−) 34.0	(+) 61.0
AH										
After spawn run	(−) 10.4	(−) 1.6	(+) 5.0	(−) 0.18	(+) 23.0	(−) 19.0	(−) 41.9	(+) 1.5	(+) 4.8	(−) 2.8
After harvest	(−) 23.1	(−) 11.3	(+) 21.5	(−) 1.09	(+) 28.2	(−) 23.3	(−) 57.3	(−) 22.7	(−) 24.0	(+) 2.4
CS										
After spawn run	(−) 12.6	(+) 0.6	(+) 20.2	(−) 1.3	(+) 6.9	(−) 7.2	(−) 29.6	(−) 3.6	(−) 14.6	(+) 19.6
After harvest	(−) 20.8	(−) 11.0	(+) 31.3	(−) 2.0	(+) 10.3	(−) 11.2	(−) 37.5	(−) 10.9	(−) 25.0	(+) 18.1

shortened. Philippoussis et al. (2003) also reported that mycelial growth in shiitake mushrooms was related to the nitrogen content of the substrates. The number of days to first harvest found in this study (58.0–76.0 days) was similar to the values reported by Balazs and Kovacs-Gyenes (1993) (60–70 days), while the time was shorter than that reported by Gaitan-Hernandez and Mata (2004) (81–117 days) and Ozcelik and Peksen (2007) (77–129 days).

Each mushroom species has an optimum C:N ratio for growth, which ensures the highest yield in a short period of production (Zied et al., 2011). The present results are consistent with those of Zied et al. (2009), who reported that minimum and maximum C:N ratios varied between 25 and 55, while the optimun ratio was 30-35 for shiitake. Philippoussis et al. (2003) found that hemicellulose had a positive effect during the active growth phase; however, there was no correlation between hemicellulose or other lignocellulosic content of the substrates in the current study.

The mushroom yields obtained from the substrates prepared from different agro-waste varied between 87.9 g/kg and 233.7 g/kg. Among the substrates examined in the study, the CPS and SFH exhibited the best performances, while the poorest was seen in CS. The highest mushroom yield was obtained in the SFH substrate, which contained 1.22.% N and had a 44.5 C:N ratio, followed by the CPS substrate containing 0.84% N with a 65.7C:N ratio.

Previous studies using different agro-wastes such as sunflower seed hulls and cotton stalks (Levanon et al., 1993) and hazelnut husk (Ozcelik and Peksen, 2007) reported 46% and 43.7% BE, respectively. On the other hand, when supplemented, the respective values attainable were as high as 87.7% (Ozcelik and Peksen, 2007) and 80.4–98.9% (Royse and Sanchez, 2007). The fresh mushroom yields obtained in this study were generally lower than those reported in previous studies. The lower BE values obtained in the current study may have been related to the lack of additive material in the growing medium. The results in the study demonstrated that the shiitake mushroom yield was not related to the nitrogen content of the substrates. The yield of the highest N-containing substrate (AH- 2.13%) did not increase in proportion to the nitrogen content. Kurt and Buyukalaca (2010) reported that although *Pleurotus ostreatus* yields and the N content of the substrates were positively correlated, high N values over a certain threshold could cause a reduction in yields. High levels of N may facilitate the presence of Trichoderma, the main competitive threat in shiitake cultures (Zied et al., 2009). İlbay (1994) also reported that contamination risk was higher in substrates with higher N content (1.5–2.0%) compared to those with 1.0% N content. Similarly, Doğan (2000) reported that the yield of *Pleurotus sajor-caju* was decreased on substrates containing N in amounts of more than 1.68%. In the study, contamination on AH during the fructification period caused lower mushroom yield in the substrate. Although Obodai et al. (2003) reported that the *P. ostreatus* mushroom yield was correlated with the lignin and hemicelluloses content of other substrates, the data from the present experiment demonstrated that the yield of shiitake was not directly correlated with the lignin or cellulose content or cellulose:lignin ratio of the substrate. This finding was corroborated by Wang et al. (2001), who reported there was no correlation between the yield and chemical composition of the substrate.

The moisture content of substrate tested in the present study was suitable according to Royse and Sanchez (2007), who observed that the moisture content of the substrate should be 55–60%. However, the substrates lost approximately 19.2–26.9% of their moisture during the shiitake cultivation period due to mushroom production and water evaporation. After the spawn running period, N and ash content of the substrates increased relative to the initial substrate. These increases were higher at the end of the production process than during the spawn running period. Adamoviç et al. (1998) and Zhang et al. (2002) also reported an increase in the ash content during the mushroom cultivation cycle. The increase in the amount of ash in the substrates may have been due to the decrease in the organic matter content (Escalona et al., 2001; Singh, 2000). On the other hand, increase in the N content has been related to the high protein content of the heavily grown mycelium in the substrate (Jonathan and ve Adeoyo, 2011). In contrast to the increase in N and ash content, the pH values and C:N ratios of the substrates were decreased during the production cycle. The decrease in C:N during the production cycle can be explained by the decrease in the amount of C due to the breakdown of the organic matter in the subatrate and the increase in the amount of N in the substrate over time. Jahromi et al. (2011) reported that the pH of fermented substrates was reduced as a result of biological applications. Morever, Adenipekun and Okunlade (2012) found that the change in pH value may be related to the presence of metabolic waste products in the substrate and the increase in its amino nitrogen content.

On the basis of the changes in lignocellulosic content detected, shiitake mushrooms appeared to have a preference for the hemicellulose component during the spawn running period. The present findings support the conclusion of Li et al. (2001) in that the hemicellulose was more degraded than the cellulose and lignin. In the case of mycelial growth, the percentage of biodegradation of the hemicellulose in AH and CPS was generally greater than that of other substrates. The number of days to primordia initaition was also fewer in these substrates. These results may indicate that the assimilation capacity of shiitake mushrooms is greater on AH and CPS substrates. On the other hand, the lowest percentage of hemicellulose biodegradation was observed in OS and CS substrates, where mycelial growth was the slowest. These results indicated that there was a direct relationship between the mycelial growth and the degradability of the hemicellulose in the substrate.

Singh (2000) reported that considerable amounts of lignin were degraded during spawn running; however, the rate of degradation slowed down during fructification. On the other hand, results from the present study indicated that hemicellulose was substantially degraded during the spawn running period, with a slight increase in the cellulose and lignin content of the substrates in this period. This can be explained by the cellulose and lignin not having been consumed during the mycelial growth period. In parallel with the decrease in the content of hemicellulose in the substrate, the cellulose:lignin ratio in the substrate increased

Although the degradation of cellulose and lignin was not observed during the spawn running period, there was a tendency for all lignocellulosic content to decrease in all substrates during the fructification period. By cultivating shiitake on wheat straw, Myoson and Verachter (1991) observed that lignin was degraded only after several weeks. Morever, Li et al. (2001) also reported that the cellulose degradation rate in the first flush period was faster than in the spawn running and primordia periods. According to these results, it can be said that where there has been a significant decrease in the substrates, the degradation of cellulose and lignin can be associated with the fruiting formation stages.

It was observed that the cellulose degradation was lower than that of lignin. At the end of the mushroom production, the shiitake mushrooms degraded between 24.0% and 36.8% of the initial lignin, while the initial cellulose was depleted by between 5.3% and 27.0% in the different substrates. This finding was supportedby Gaitán-Hernández et al. (2011), who reported that lignin degradation by shiitake was higher than that of cellulose during the primordium formation stage.

According to the present results, the lignocellulosic content degradation capacity of shiitake also differed on all the substrates. The degradation of lignin in CPS, SFH and AH substrates was significantly higher than that in OS. This result was corroborated by Villas-Bôas et al. (2003), who noted that lignin degradation was extremely dependent on the presence of oxygen. Morever, Gaitán-Hernández et al. (2011) reported that the lignocellulosic enzyme activities of shiitake during mycelial growth were higher when the oxygen concentration of the substrate was higher. On the other hand, Cullis et al. (2004) reported that smaller substrate particle size can positively affect lignocellulose degradation. However, findings in the present study showed the opposite.

Differences in the composition of the lignocellulosic content may affect the relation of the lignocellulosic enzymes with other chemical contents such as N. In parallel with this,Lin et al. (2015) reported that nitrogen-rich additive materials increased enzymatic activity and digestibility. However, the positive effect of N was observed up to a certain level. As mentioned previously, infection withgreen mold contamination was observed on the AH substrate, which had the highest N content (2.13%). Contaminating fungus could also have degraded the lignocellulosic content in the substrates and caused comparable lignocellulose degradation and low mushroom production (Lin et al., 2015). The highest yield and BE were obtained on the substrate with 3.1 (CPS) and 2.1 (SFH) cellulose:lignin ratios. The CS substrate had a much higher cellulose:lignin ratio (8.1). The low yield and BE of shiitake mushroom in the CS substrate could have been related to its high cellulose:lignin ratio.

5. Conclusion

The yield of shiitake mushroom is not directly correlated with the N, lignin, hemicellulose or cellulose content or cellulose:lignin ratio of the substrates. However, it appeared that shiitake mushrooms had a preference for substrates containing a moderate amount of N, hemicellulose and lignin, and having a low cellulose:lignin ratio. The results of the present study demostrated support for the efficient production of shiitake mushrooms on sunflower head residue and chickpea straw as alternatives to sawdust. Although alfalfa hay may sustain yield and BE, the contamination risk is very high in this substrate. On the other hand, corn stalk is not recommended as a basal substrate for shiitake mushroom cultivation because of its low yield and BE. The chemical composition of the growing substrates changed during the life cycle of the mushroom and the protein content increased with time, while the amount of lignocellulosic content in the substrates was reduced, makingit more digestible. Accordingly, these results indicate that the recycling of spent shiitake mushroom substrate may be recommended as a feed for livestock to increase sustainability and support farm economy.

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